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FACULTÉ DES SCIENCES ET DES TECHNIQUES

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Obésité et insulinorésistance chez des rats consommant des régimes enrichis en matières grasses ou en fructose. Intervention nutritionnelle avec de l'EPA ou des flavanones.

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THÈSE DE DOCTORAT

Discipline : Sciences de la vie et de la santé

Spécialité : Physiologie

Présentée
et soutenue publiquement par

Muhammad-Quaid ZAMAN

Le 30 mars 2012, devant le jury ci-dessous

Madame le Professeur Khadija OUGUERRAM, Université de Nantes, Présidente

Madame le Docteur Marianne DIEZ, Université de Liège, Rapporteur

Madame le Docteur Véronique LERAY, Oniris, Nantes, Co-encadrante de thèse

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Monsieur le Professeur Patrick NGUYEN, Oniris, Nantes, Directeur de thèse

Dedicated to

Pakistan

Word of thanks

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Insulin sensitivity and lipid profile in rats fed high-fat diet supplemented with polyunsaturated fatty acids.
Zaman M.-Q., Leray V., Le Bloc'h J., Ouguerram K. and Nguyen P.

Waltham International Nutritional Sciences Symposium (Cambridge, England, 16-18 September, 2010)
Omega 3 fatty acids supplementation improves insulin sensitivity and increases EPA and DHA tissue content in obese and insulin resistant dogs.
Le Bloc'h J., Leray V., Zaman M.-Q., Ouguerram K. and Nguyen P.

European Society of Veterinary and Comparative Nutrition. Vienna, 2008.
Kinetics aspects of energy intake, body weight gain and insulin sensitivity in dogs given a high-fat high-energy diet.
Le Bloc'h J., Serisier S., Zaman M.-Q., Leray V., Martin L., Dumon H., Magot T., Ouguerram K. and Nguyen P.

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Experimental Biology (San Diego, CA, USA, 21-25 April, 2012)
Adipose tissue gene expression in adult rats fed with high-fructose diet. Effects of EPA supplementation.
Leray V., Jonchere C., Zaman M.-Q. and Nguyen P.

Experimental Biology (San Diego, CA, USA, 21-25 April, 2012)
Effects of high-fat high-fructose diet and EPA supplementation on adipose tissue gene expression in adult rats.
Leray V., Jonchere C., Zaman M.-Q. and Nguyen P.

Experimental Biology (Washington, DC, USA, 9-13 April, 2011)
Effect of hesperidin and naringin on plasma lipid and insulin sensitivity in rats fed high-fat or/and high-fructose diet.
Zaman M.-Q., Leray V., Le Bloc'h J., Ouguerram K. and Nguyen P.

Experimental Biology (Washington, DC, USA, 9-13 April, 2011)
mRNA expression of genes involved in insulin sensitivity, carbohydrate and lipid metabolism in obese rats. Effects of EPA supplementation.
Leray V., Jonchere C., Zaman M.-Q., Le Bloc'h J. and Nguyen P.

Waltham International Nutritional Sciences Symposium (Cambridge, England, 16-18 September, 2010)
Lipid profile and insulin sensitivity in rats fed high-fat or high-fructose diets.
Zaman M.-Q., Leray V., Le Bloc'h J., Ouguerram K. and Nguyen P.

French Society of Biochemistry and Molecular Biology, 6th lipidomics meeting (Rennes, France, 1-3 July, 2009)
Nicotinic acid impacts on reverse cholesterol transport in obese insulin resistant dogs.
Le Bloc'h J., Leray V., Nazih H., Serisier S., Zaman M.-Q., Chétiveaux M., Magot T., Krempf M., Nguyen P. and Ouguerram K.

Experimental Biology (San Diego, CA, USA, 5-9 April, 2008)
Effect of nicotinic acid on plasma lipid and hepatic gene expression on obese insulin resistant dogs.
Le Bloc'h J., Leray V., Zaman M.-Q., Serisier S., Ouguerram K. and Nguyen P.

French Nutrition Society, 3rd meeting (Lille, France, 6-7 December, 2007)
L'acide nicotinique diminue les concentrations en triglycérides et cholestérol total sans affecter le cholestérol-HDL chez le chien obèse et insulinorésistant.
Le Bloc'h J., Zaman M.-Q., Leray V., Serisier S., Ouguerram K. and Nguyen P.

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ABC	ATP binding cassette transporter
ACAT	Acyl CoA cholesterol acyltransferase
ALA	α-linoleic acid
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
Apo	Apolipoprotein
BMI	Body mass index
CETP	Cholesteryl ester transfer protein
CVD	Cardiovascular diseases
DHA	Docoshexaenoic acid
EPA	Eicosapentaenoic acid
FATP	Fatty acid transport protein
HDL	High density lipoprotein
HL	Hepatic lipase
HSL	Hormone-sensitive lipase
IDF	International Diabetes Federation
IDL	Intermediate density lipoprotein
IR	Insulin resistance
IRS	Insulin receptor substrate
JK2	Janus kinase-2
LCAT	Lecithin: cholesterol acyltransferase
LDL	Low density lipoprotein
LDL-R	LDL receptor
LepR	Leptin receptor
LPL	Lipoprotein lipase
NCEP	National Cholesterol Education Program
NEFA	Non-esterified fatty acids
PI3K	Phosphatidylinositol 3 kinase
PKC	Protein kinase C
PLTP	Phospholipid transfer protein
PPAR	Peroxisome proliferator-activated receptor

PUFA	Polyunsaturated fatty acids
RCT	Reverse cholesterol transport
ROS	Reactive oxygen species
SREBP	Sterol regulatory element binding protein
TNFα	Tumor necrosis factor α
VLDL	Very low density lipoprotein
WC	Waist circumference
WHR	Waist-hip ratio
WHO	World health organization

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- *INTRODUCTION*

Scientific literature provides very rich experimental data for pathophysiological relationship between obesity and its associated disorders. Studies, by carrying a transversal approach in humans have allowed to characterize the endocrine, metabolic and cardiovascular changes in the obese people. However, these studies are not enough explicative to provide the relationship between obesity and low insulin sensitivity. The knowledge of the processes linking obesity with its associated disorders (including the low insulin sensitivity and dyslipidemia) is therefore extrapolated using the conflicting data that are often fragmentary and sometimes contradictory. These ambiguities could be based on the difficulty to form perfectly homogeneous groups of obese and insulin resistant patients, particularly due to their overweight history. A large amount of data is derived from the longitudinal studies conducted during weight loss. The utilization of these experimental results to understand the processes, involved in the induction of obesity, postulate that development of the disorders is mirrored in their reversibility. For obvious ethical reasons, it is impossible to study in humans the pathophysiological phenomena occurring during the induction of obesity and the progression to the insulin resistance. To better understand the links among the elements of metabolic syndrome, a longitudinal approach appears to be more promising.

In the scientific literature, a large number of different rodent models have been studied, such as rats, mice, hamsters. Nevertheless, the selection of a genetically mutated model does not seem the most sensible choice of a model, because human obesity is usually related to high calories intake. Unlike genetically-induced obesity, the obesity induced by nutritional manipulation seems closer to the etiology of human obesity. The animal model should allow the iterative investigation. The rats with dietary-induced obesity and insulin resistance appear to meet the criteria for selection of the model, mimic to that in humans, and to be suitable for the study of this syndrome. Rat model has already been explored in the context of studies on obesity and its associated disorders, but comparison of different diets related to the development of insulin resistance have so far been poorly described in this species.

The unit deals with animal and human health around two themes. The first relates to obesity and its consequences, including type 2 diabetes. A second theme concerns the dog's digestive functions and composition of their diet for better digestive tolerance. Dog has been used as an animal model in our laboratory since past some years for the study of insulin resistance and dyslipidemia with reference to lipid metabolism. This model of dietary induced

obesity and insulin resistance has not only been established successfully but the nutritional supplementations (such as nicotinic acid, green tea, omega-3 fatty acids) have also been effectively studied in our department. Since the cost and ethical issues in this model, the need to establish another animal model was always there. For this purpose, we aimed to study rats in our department. In the beginning, our concern was to establish this animal model of dietary induced obesity and insulin resistance in our laboratory. We aimed to compare the high-fat and the high-fructose diets for the development of obesity, insulin resistance and dyslipidemia. Moreover, we studied the nutritional supplementations in this new model in our laboratory.

The first part of our study was to characterize an animal model for the development of obesity and insulin resistance. As in humans, the obesity and its related disorders are an effect of diet, so we aimed to put the rats on high-fat or high-fructose diet. We followed the plasma parameters, likely to be associated with low insulin sensitivity and dyslipidemia at the start and at the end of the experiment. Such approach allowed us to keep into account the basal values of the animals in our study that is not the case in the other studies related to obesity and insulin sensitivity (assessment by euglycemic-hyperinsulinemic clamp technique) in rats.

Most of the plasma disturbances result from transcriptional changes of genes responsible for metabolic activity. The study of these anomalies could explain the molecular mechanism, altered in insulin resistance. In order to characterize as completely as possible the rat model, we studied the changes in the expression of genes in the hepatic and adipose tissues during obesity and insulin resistance. We measured the expression of several genes, which appears to have a key role in the development of obesity and insulin resistance by the real-time PCR.

The second part of our study was to observe the effects of nutritional supplementation to prevent obesity, and its associated metabolic disorders. Many nutritional and therapeutic agents including polyphenols, statins, nicotinic acid, fibrates, flavonoids, omega-3 polyunsaturated fatty acid (mostly in the form of fish oil) have been studied for their anti-inflammatory and hypolipidemic effects. These agents have been often used in the studies in their raw forms, and in recent years, many researchers have focused on the use of pure/active component in the studies. Indeed, using of raw products could not help to find out the potential role of any active ingredients and there could also be the side effects of other ingredients present in the raw product. We therefore preferred to study the effects of pure substances to determine the exact effects of that agent. We supplemented eicosapentaenoic

acid (EPA), and hesperidin and naringin to the rats fed with the high-fat or high-fructose diet to study their effects on low insulin sensitivity and dyslipidemia. Plasma parameters and gene expression in different tissues have been studied to explain the activity of these nutritional supplementations on obesity and insulin resistance.

- THEORATICAL ASPECTS

1 Definition of obesity

Obesity is a chronic disease consisting of an increase in body fat stores (a state of excess body fat storage) and contributes directly to the morbidity and the mortality. In developed countries, obesity has reached epidemic level and is increasing at an alarming rate. Moreover, it correlates with economic, social and life style changes.

Obesity can be considered as the result of a positive energy balance between energy intake and energy expenditure. The extra calories are stored in the form of fats, leading to overweight and then obesity. The genetics, the low physical activity, quantity and quality of the food, etc. are few important factors for the development of obesity. Westernization of the diet with high-caloric intake has led to worsen this epidemiology. According to the World Health Organization report of 2010, there are more than 400 million obese adults worldwide, and the projected figure is 700 million, by 2030 (Travers and McCarthy, 2011). The highest frequency of obesity is observed in the United States, Europe and the Middle East, and lowest in the sub-Saharan Africa and East Asia. In United States, obesity has reached to 25-30 % proportion in 2008, with certain states over 30 %. In 2007-2008, its prevalence in US population was 32.2 % among men and 35.5 % among women (Flegal *et al.*, 2010). About 150 billion dollars are spent per year to control obesity only in United States. In 2009, 14.5 % of the French population is found obese and 31.9 % were overweight (Obépi, 2009).

Obesity can be defined on the basis of body mass index (BMI) in humans, as shown in the table 1. It is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2). BMI is simple index that is commonly used to classify underweight, overweight and obesity in adults. A report by Prospective Studies Collaboration (PSC) shows reduction of 8-10 years in the median survival rate for morbidly obese individuals (BMI at 40-45 kg/m2) compared to those with normal BMI (Whitlock *et al.*, 2009).

The BMI is not a predictor for body composition and distribution of adiposity. For the same BMI, the obesity-associated risk mainly depends on fat depots in the upper body. Over the last twenty years, numerous epidemiological and metabolic studies have re-emphasized a notion introduced in mid 40 by Vague (1947), who has reported that obesity-related metabolic

Table 1: Classification of obesity on the basis of BMI.

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal range	18.5 - 24.9
Overweight	25.0 - 29.9
Obese	≥ 30.0
Obese class I	30.0 - 34.9
Obese class II	35.0 - 39.9
Obese class III	≥ 40.0

BMI: body mass index. (WHO, 2004; 2000; 1995).

disorders were more closely associated to the regional distribution of body fat than high body weight (Vague, 1947). Thus, it is recognized that a high proportion of visceral fat is a major risk factor for coronary heart disease, type 2 diabetes, and mortality associated with these disorders, independently of BMI (Haffner *et al.*, 1990; Donahue *et al.*, 1987; Ducimetiere *et al.*, 1986). Measure of central obesity, such as waist circumference (WC) and waist-hip ratio (WHR), might be helpful to better assess body fat distribution in cardiovascular disease patients. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III defined high WC as cutoffs of > 88 cm for women and > 102 cm for men (NCEP, 2001); and WHO defined high WHR as ≥ 0.85 for women and ≥ 0.90 for men (Alberti and Zimmet, 1998). On the other hand, the accumulation of fat in gluteofemoral region, called as gynoid obesity, does not seem to be associated with major metabolic complications (Vague, 1947).

In rodents, body mass indexes are generated by calculating a ratio of length to mass but can be inaccurate reflections of actual composition (Krebs and Singleton, 1993). Recently, Hickman and Swan have characterized body condition score technique to assess the health status in rat model of polycystic kidney disease (Hickman and Swan, 2010). They demonstrated rats as emaciated, under conditioned, well-conditioned, over conditioned and obese on the basis of palpation findings. However, this technique needs to be studied further to validate its application in rats. Moreover, whole-body carcass composition analysis is one of the traditional methods of determining body fat and lean mass, but it is time consuming and terminal procedure and preclude the longitudinal study. Different other methods are in use to determine body fat in laboratory animals, such as computed tomography, dual-energy x-ray absorptiometry, bioelectrical impedance analysis, magnetic resonance imaging system, infra-red spectrophotometry.

2 Lipid storage and circulation

2.1 Adipose tissue: Lipogenesis and lipolysis

The catalytic process of lipolysis leads to the breakdown of triglycerides into non-esterified fatty acids (NEFA) and glycerol in the circulation and the adipose tissue. The lipoysis is highly regulated to adjust the level of non-esterified fatty acids available for tissue oxidation. The non-esterified fatty acids in the blood circulation can be the main energy substrates for a large number of tissues (muscle, heart, liver, renal cortex), specially during

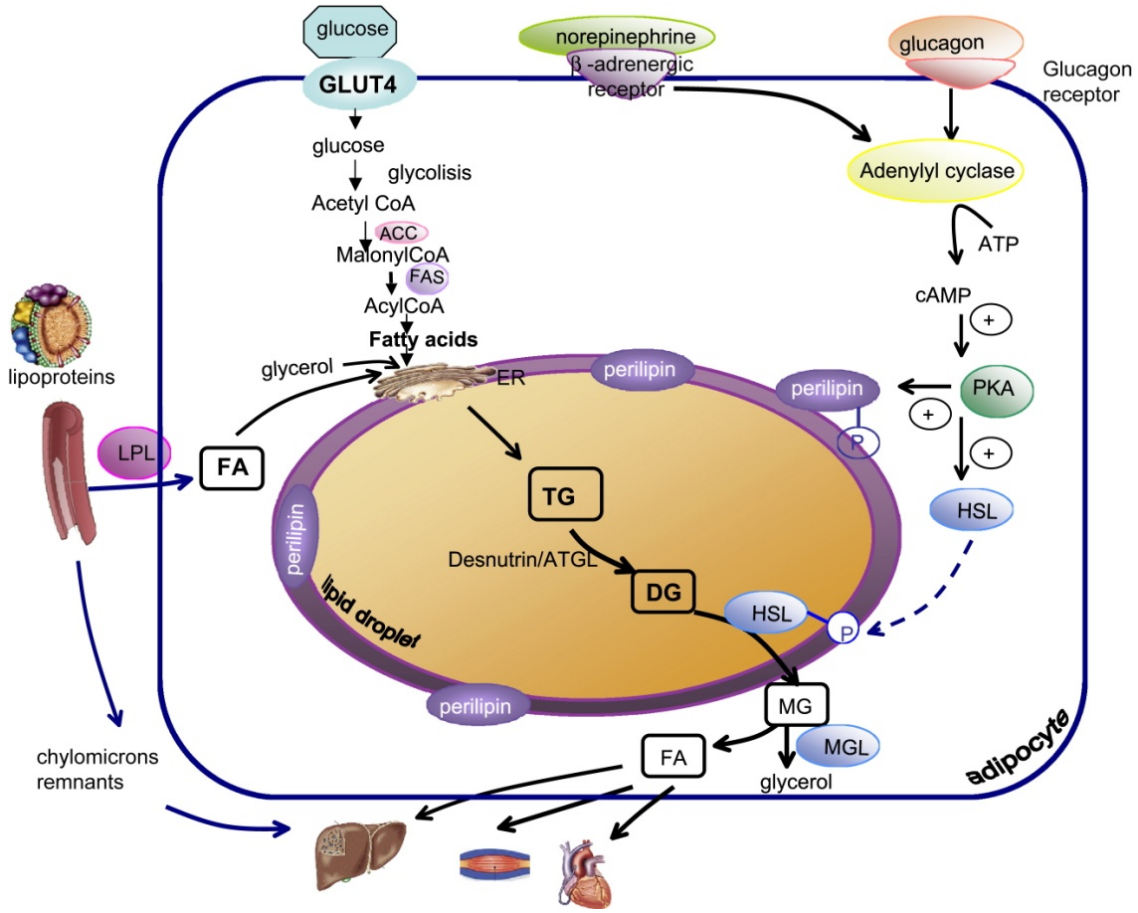

Figure 1: The diagrammatic representation of lipogenesis and lipolysis in the adipocyte.

ACC: acetyl-CoA carboxylase, FAS: fatty acid synthase, ATGL: adipocyte triglyceride lipase, MGL: monoglyceride lipase, LPL: lipoprotein lipase, HSL: hormone-sensitive lipase, FA: non-esterified fatty acids, PKA: protein kinase A, TG: triglycerides, DG: diacylglycerides, MG: monoacylglycerides (Vazquez-Vela *et al.*, 2008).

the interprandial periods. The plasma concentration of non-esterified fatty acids is determined by the balance between production (lipolysis) and the utilisation (lipogenesis or β-oxidation) of the non-esterified fatty acids. The sketch for the storage (lipogenesis) and breakdown (lipolysis) of triglycerides in the adipocytes has been presented in the figure 1.

When the dietary source was not sufficient to meet the energy need of the body, triglycerides of the adipose tissue are hydrolysed. Moreover, triglycerides (in the form of chylomicrons from intestinal absorption or in the form of the lipoproteins from hepatic synthesis) are hydrolysed into glycerol and non-esterified fatty acids. The main enzymes involved in the lipolysis include hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), adipose triglyceride lipase, monoglyceride lipase, triacylglycerol hydrolase. Perilipin A, an isoform of perilipins, normally prevents lipolysis by surrounding the lipid droplet, preventing the access of lipases (Brasaemle *et al.*, 2000). β-adrenergic stimulation of the adipocytes and subsequent phosphorylation of HSL triggers the translocation of HSL to the lipid droplet for lipolysis (Egan *et al.*, 1992). The plasma concentration of non-esterified fatty acids has been reported to be linearly correlated with the LPL-dependent VLDL-triglyceride hydrolysis (Pruneta *et al.*, 2001). The lipolysis in adipose tissue is regulated by different hormones (Jaworski *et al.*, 2007) that have direct or indirect effect on the enzymes of lipolysis. In humans, as in other species, catecholamines are known to be important agents of adipocytic lipolysis. In humans, thyroid hormones, cortisol, growth hormone, some natriuretic peptides as well as TNFα stimulate the lipolysis whereas insulin and adenosine inhibit the lipolysis (Jaworski *et al.*, 2007). The lipolysis, in the adipocytes and in the triglycerides-rich lipoproteins (VLDL), is the major source of plasma non-esterified fatty acids. The non-esterified fatty acids combine with CoA to form a thioesters and then stepwise re-esterification process leads to triglycerides synthesis. Glucose is the primary source of glycerol for this re-esterification.

The lipogenesis, the process of synthesis of fatty acids and triglycerides, takes place in the adipose tissue and the liver. Lipogenesis is regulated by nutritional and hormonal factors (including insulin, growth hormone, leptin), whose effects are mediated by various factors of transcription (SREBP-1, PPARγ in adipose tissue). High-carbohydrate diet stimulates lipogenesis, whereas fasting and polyunsaturated fatty acids inhibit it (Kersten, 2001). Polyunsaturated fatty acids (PUFA) suppress gene expression of fatty acid synthase and stearoyl-CoA (Jump *et al.*, 1994), and hence PUFA decrease lipogenesis. Kersten, *et al.* (1999) reported the diverse effects of the non-feeding/ fasting according to the tissues: fasting

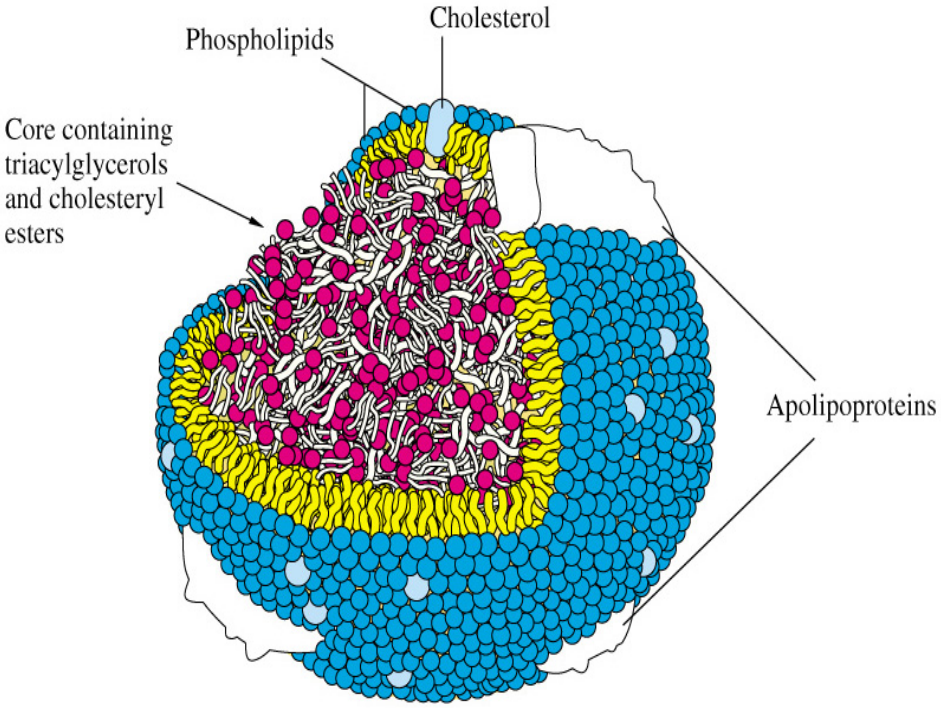

Figure 2: Structure of a lipoprotein showing components of outer envelope and inner core.

reduces adipocytic lipogenesis, associated with stimulation of the lipolysis in the adipose tissue, and it favours lipid accumulation in the liver. Insulin increases the glucose uptake in the adipocytes, and hence an important factor regulating lipogenesis. In fact, glucose is the key substrate for lipogenesis, and insulin regulates the glucose metabolism. Growth hormone could also reduce lipogenesis in the adipose tissue by inhibiting the expression of fatty acid synthase (Mildner and Clarke, 1991). Moreover, leptin may also be involved in the lipogenesis. It inhibits the food intake and inhibits lipogenesis (Bai *et al.*, 1996). Leptin downregulates the expression of the genes (fatty acid synthase, FAD-linked glycerol-3-phosphate dehydrogenase) involved in the biosynthesis of fatty acid and triglycerides (Soukas *et al.*, 2000). Finally, the acylation stimulating protein (ASP), produced by the adipose tissue, increases the triglycerides synthesis and reduces lipolysis simultaneously (Van Harmelen *et al.*, 1999).

Hormonal and nutritional factors, such as insulin and glucose, induce the expression of lipogenic genes through sterol regulatory element binding proteins (SREBP) in the liver (Shimano *et al.*, 1999). These transcriptional factors regulate gene expression involved in the metabolism of cholesterol and fatty acids (Hua *et al.*, 1993), such as LDL receptor, farnesyl pyrophosphate synthase, HMG-CoA reductase genes. In adipose tissues, the PPARγ is an important transcription factor, which regulates the genes encoding the adipocyte fatty acid binding protein, lipoprotein lipase, fatty acid transport protein, acyl-CoA synthetase and phosphoenol pyruvate carboxykinase.

2.2 Lipoproteins: classification and metabolism

As lipids are hydrophobic, so for their transport in the circulation, the lipids must be incorporated in the complex structures, called lipoproteins, which are amphiphilic in nature. The hydrophobic core of the lipoproteins contains cholesterol esters and triglycerides, and the peripheral envelope is made up of proteins (apolipoproteins), phospholipids and cholesterol, ensuring the hydrophilicity of the macromolecule. Figure 2 represents the structure of a typical lipoprotein.

Lipoprotein particles are polydisperse and have ready access to cells in various tissues. These globular molecules transport lipids from the intestine to the liver, from the liver to the peripheral tissues and also from peripheral tissues to the liver. In humans, there are five main classes of the lipoproteins: chylomicrons, very low-density lipoproteins (VLDL),

Table 2: Identification, tissue expression, distribution and functions of the major plasma apolipoproteins in human.

Apo	Tissue	Distribution	Functions
Apo AI	Liver, intestine	CM, HDL	Structure, activator of LCAT, efflux of cholesterol
Apo AII	Liver, (intestine)	HDL	Structure, activator/inhibitor of HL, efflux of cholesterol
Apo AIV	Liver, intestine	CM, HDL	RCT, activator of LCAT, metabolism of triglycerides-rich lipoproteins
Apo AV	Liver		Metabolism of triglycerides-rich lipoproteins
Apo B100	Liver	VLDL, IDL, LDL	Structure, synthesis and secretion of VLDL, ligand of LDL-receptor
Apo B48	Intestine	CM	Structure, synthesis and secretion of CM, ligand of receptor
Apo CI	Liver, (intestine)	CM, VLDL, HDL	Inhibitor of CETP, activator of LCAT, inhibitor of linkage with receptor
Apo CII	Liver, (intestine)	CM, VLDL, HDL	Activator of LPL
Apo CIII	Liver, (intestine)	CM, VLDL, HDL	Inhibitor of LPL, inhibitor of hepatic uptake of triglycerides-rich lipoproteins
Apo D	Liver, intestine, spleen, brain, pancreas,	HDL, LDL, VLDL	RCT?
Apo E	Liver, brain, macrophage	CM, VLDL, IDL, HDL	Ligand of receptors LDL-receptor and LDL receptor related protein
Apo F	Liver	LDL, (VLDL, HDL)	Inhibitor of CETP
Apo G	?	HDL	?
Apo H	?	HDL	?
Apo J	?	HDL	Anti-inflammatory
Apo M	Liver, kidney	HDL	Efflux of cholesterol, RCT

CETP: cholesteryl ester transfer protein, CM: chylomicrons, HL: hepatic lipase, LCAT: lecithin: cholesterol acyltransferase, LPL: lipoprotein lipase, RCT: reverse cholesterol transport.

intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The increase in the density of the lipoproteins is as an effect of depletion of triglycerides along with enrichment of cholesterol. The non-covalent molecular linkages in the lipoproteins make it possible to transform from one form to another. The lipoproteins differ profoundly from each other by the nature and proportion of their constituents, their site of synthesis, their biological role and metabolism. Chylomicrons and VLDL carry mainly the triglycerides, and LDL and HDL carry mainly the cholesterol. Chylomicrons are responsible for transport of lipids from the intestine to the liver. The VLDL are synthesized in the liver and transport triglycerides to the peripheral tissues. The hydrolysis of VLDL-triglycerides leads to formation of IDL, which are involved in the transport of lipids to the peripheral tissues. The depletion of IDL-triglycerides leads to the formation of LDL that carry lipids to the peripheral tissues. The HDL are the only lipoproteins allowing the transport of cholesterol from the peripheral tissues to the liver.

After lipid absorption, enterocytes re-esterify all the triglycerides and some part of dietary cholesterol. The addition of apolipoproteins (apo B48, apo AI, apo AII), synthesized in part by the intestine, allows the formation of nascent chylomicrons, which are then exported into the lymph where they exchange apolipoproteins (apo E and apo C) with HDL. The chylomicrons undergo hydrolysis of triglycerides into fatty acids, by the lipoprotein lipase. Moreover, some surface components (cholesterol, phospholipids, apo C and all the apo A) from the chylomicrons join the pool of HDL. All these processes lead to the formation of small size chylomicrons, called as chylomicrons remnants, which are taken up mainly by the liver, and also by the other tissues, such as muscle, soft bones.

The apolipoproteins are amphiphilic proteins with the hydrophobic part penetrated into the core of lipoprotein and the hydrophilic part located on the surface of the particle. The apolipoproteins have, therefore important structural function (involved for the overall cohesion of lipoprotein molecule), and they also allow the recognition of lipoproteins by different receptors and enzymes that play a role in the metabolism of lipoproteins. Initially, the apolipoproteins were divided into three distinct groups: A, B and C (Gustafson *et al.*, 1966), mainly the apo A are associated with HDL, the apo B with LDL, and the apo C with VLDL and HDL. Although, still commonly used, this nomenclature has evolved considerably over the years. Table 2 lists the main characteristics of these apolipoproteins.

The apo B100 is the principal apolipoprotein of VLDL and LDL, and is synthesized by the liver. There is only one molecule of apo B100 per molecule of VLDL and LDL, which

helps to determine their plasma concentration (Ganda, 2009). The structure of apo B100 is composed of five parts: a N-terminal globular $\beta\alpha_1$ domain of both α-helix and β-sheet, two β-domains of β-sheets and two domains of α-helices, one between the two β-sheets and other at the C-terminal of the protein (Segrest *et al.*, 2001). The N-terminal domain is of great importance for the metabolism of VLDL, as it interacts with microsomal triglyceride transfer protein (MTP) that allows the association of lipids with apo B containing particles, VLDL and LDL (Dashti *et al.*, 2002). The apo B100 is also the ligand for LDL-receptor and therefore, allows the uptake of LDL-cholesterol. The apo B48 is synthesized by the small intestine, and is a component of chylomicrons. The same gene encodes apo B48 and apo B100. A post-transcriptional modification of RNA of the apo B100 leads to formation of a protein of 48 % of apo B100, apo B48 (Chen *et al.*, 1987; Powell *et al.*, 1987).

The apo AI is present in the majority of HDL, constituting 70 % of the protein fraction; hence plasma concentration of apo AI is perfectly correlated with that of HDL. A small proportion of apo AI is also found in chylomicrons. The apo AI is primarily synthesized in the liver and intestine, and is associated with cholesterol and phospholipids to form pre-β-HDL. The expression of apo AI gene is regulated by a myriad of nutritional, hormonal and pharmacological factors, such as lipid, alcohol, estrogens, androgens, glucocorticoids, fibrates, statins (Malik, 2003). The apo AI is an activator of lecithin: cholesterol transferase (LCAT), an enzyme for esterification of plasma cholesterol (Frank and Marcel, 2000) and a ligand of scavenger receptor B1, a receptor for selective uptake of cholesterol ester in the liver (Lagrost *et al.*, 1995). The apo AII is second (in abundance, 25 % of protein fraction) apolipoprotein of HDL, and is synthesized primarily by the liver. Apo AII inhibits hepatic lipase, and therefore prevents the hydrolysis of triglycerides and phospholipids in the HDL (Zhong *et al.*, 1994). It has been shown that despite an increase in HDL-cholesterol, the apo AII promotes the development of atherosclerosis (Schultz *et al.*, 1993; Warden *et al.*, 1993).

The apo C (CI, CII and CIII) are synthesized by the liver and intestine and are found in chylomicrons, VLDL and HDL. Apo C have the ability of redistribution among different lipoproteins, as between chylomicrons (secreted by the intestine) and VLDL (synthesized in the liver). During the hydrolysis of triglycerides by LPL, the released apo C are transferred to HDL. Apo C activates lecithin: cholesterol transferase (LCAT) (Soutar *et al.*, 1975), and inhibits binding of lipoproteins to receptors as well as CETP (Gautier *et al.*, 2000). The apo CII is an essential cofactor of LPL (Catapano, 1982), and therefore is directly related to plasma triglycerides level. Apo CII can inhibit, to a lesser extent than apo CIII, the uptake of

triglycerides-rich lipoproteins by hepatic receptor (Havel, 1986). The apo CIII is the most atherogenic apo C, which has been shown by a strong correlation between concentration of apo CIII and triglycerides (Lee *et al.*, 2003), and also with the progression of atherosclerosis (Hodis, 1999; Alaupovic *et al.*, 1997). Apo CIII is the principal inhibitor of lipolysis of VLDL.

Apo CIII inhibits LPL (Brown and Baginsky, 1972), and decreases hepatic uptake of remnants of chylomicrons and VLDL *in vitro* (Windler and Havel, 1985; Quarfordt *et al.*, 1982), by inhibiting the binding of apo B containing lipoproteins with LDL receptor (Clavey *et al.*, 1995). Metabolic regulation of apo CIII could be a new therapeutic approach for the treatment of dyslipidemia and atherosclerosis in patients with metabolic syndrome (Ooi *et al.*, 2008).

The apo E is synthesized primarily in the liver, and it constitutes 10-20 % of the protein fraction of VLDL, and it is also found in chylomicrons and HDL. Its important role is to ensure the recognition of lipoproteins by LDL receptor (apo B / E receptor), LDL receptor-related protein (apo E receptor) and VLDL receptor. The apo E, along with LCAT, plays a role in cholesterol efflux to HDL (Matsuura *et al.*, 2006).

Except the postprandial periods, the hepatic VLDL replace the role of chylomicrons for the transport of fatty acids. The VLDL formation results from the assembly of triglycerides and cholesterol esters when secreted by the liver. During their transport through the blood circulation, the VLDL exchange one to one the molecules of triglycerides against the molecules of cholesterol esters with the HDL. The lipolysis of VLDL resembles that of chylomicrons. The VLDL are hydrolysed by lipoprotein lipase (LPL) and then by hepatic lipase (HL) to release fatty acids. The action of LPL on the VLDL leads to the formation of triglycerides-depleted particles, the IDL, and then to the LDL that are enriched in cholesterol esters. These three classes of the lipoproteins (VLDL, IDL and LDL) are, therefore, linked by a delipidation cascade leading to the formation of more dense particles. In humans, the LDL transport 60 % to 65 % of the total plasma cholesterol.

The metabolism of HDL is complex, because it exists in form of various sub-classes with different metabolic roles. The first evidence that HDL is not a homogeneous set of lipoproteins, has been put in 1979, when the existence of a pre-β migrating subclass of apo A-I-containing particles was found in the human amniotic fluid (Gebhardt *et al.*, 1979). Later, similar particles have been reported in peripheral lymph of dogs (Lefevre *et al.*, 1988; Sloop *et al.*, 1987), and in human lymphedema fluid (Reichl *et al.*, 1985). After esterification of the

pre-β HDL cholesterol, the cholesteryl esters are pooled into the core of the lipoprotein particle, eventually making spherical α-HDL. In humans, HDL are synthesized by the liver and the intestine and secreted in form of nascent particle (pre β1-HDL) (Kunitake *et al.*, 1992). During their circulation, they capture free cholesterol and gradually enriched in cholesterol, which is then esterified. This enrichment of cholesterol esters increases the size of the HDL. Some cholesteryl esters from HDL are transferred to LDL, VLDL or chylomicrons in exchange for triglycerides and phospholipids (Ihm *et al.*, 1982; Marcel *et al.*, 1980). Following the exchange of cholesterol esters and triglycerides by the cholesterol ester transfer protein (CETP), and exchange of phospholipids by the phospholipid transfer protein (PLTP) (Chung *et al.*, 1998; Tall *et al.*, 1983), the HDL are enriched in triglycerides and apo E and further increase their size. The hydrolysis of triglycerides and phospholipids on the surface of these new particles converts them back into the original HDL or pre β1-HDL. Rats do not express CETP, which might be a possible reason for the high plasma HDL, compared to humans. PLTP has not only been involved in the transfer of phospholipid to HDL, but also in HDL conversion process (Settasatian *et al.*, 2001), in which PLTP mediates fusion of intermediate sized α-HDL particles to generate larger HDL particles with a concomitant release of lipid-poor apo A-I. These actions result in an enhanced capacity to take up cellular cholesterol. The study of plasma transport of cholesterol is important because of implication of cholesterol in the cardiovascular diseases, particularly in atherosclerosis. In fact, in case of hypercholesterolemia, bulk of LDL particles is taken up by the macrophages of the blood vessel wall. These macrophages, when enriched with lipids, become foam cells and contribute to the development of atherosclerotic plaque that can block the blood vessels leading to cardiovascular disorders (myocardial infarction, stroke, etc.).

In humans, the metabolism of lipoproteins is regulated at the cellular level by transcriptional factors belonging to PPAR family. It is also regulated indirectly by lipolytic enzymes and transfer proteins. The LPL and HL are key enzymes among others involved in the hydrolysis of VLDL-triglycerides, transformation of IDL into LDL (Connelly, 1999) and and the lecithin cholesterol acyltransferase (LCAT) is the key enzyme for the esterification of free cholesterol in the HDL (Yamamoto *et al.*, 1980). LCAT plays an important role in reverse cholesterol transport (RCT). In fact, the esterification of cholesterol in the HDL increases the concentration gradient of free cholesterol between the cells and HDL, and favours cholesterol efflux (Czarnecka and Yokoyama, 1996). Moreover, cholesteryl esters are less likely to return to the cells after migration into the HDL that also enhances the efficacy of

RCT. The activity of LCAT therefore, appears to be beneficial in reducing the risk of CVD. However, high LCAT activity does not predict low incidence of CVD (Dullaart *et al.*, 2009), and also low LCAT activity is also not associated with increased atherosclerosis (Holleboom *et al.*, 2009).

In rats, besides the lower levels of the LDL, the HDL are predominantly larger particles with more apolipoprotein E than those in human. The metabolism of lipoproteins in rats differs from that in humans in several respects. Two of the most important species differences are the remnant removal pathway and the cholesterolyl ester transfer reaction. The rat has an efficient mechanism for clearance of chylomicron and VLDL remnants from the circulation. Reverse cholesterol transport (RCT) is one of the major mechanisms by which excess cholesterol from extrahepatic tissues is removed, transported to the liver and secreted into the bile, and the HDL serve as a shuttle for this concept of RCT. The term RCT has been introduced in early 1970s (Glomset, 1973). Both free cholesterol and the esterified form are involved in RCT. The cell membrane of hepatocytes presents receptors that recognize HDL (Bachorik *et al.*, 1982). In rats, the rate of uptake of HDL cholesteryl esters by the liver is several times greater than that of HDL apolipoprotein A-1, which suggests that cholesteryl esters dissociate from HDL particles at the surface of the hepatocytes. This process might be facilitated by transient binding of HDL to the cell membranes. A similar process has been reported in cultures of human hepatoma cells (Rinninger and Pittman, 1988). Briefly, the efflux of cholesterol from the peripheral tissues to apo-A-I and HDL are mediated by the ABCA1, ABCG1 and / or ABCG4, and the subsequent uptake of HDL cholesterol by liver involves the role of scavenger receptor, class B, type I (SR-BI) (van der Velde, 2010).

The scavenger receptor, class B, type I (SR-BI) was identified as an HDL receptor in 1996. It is primarily expressed in the liver and nonplacental steroidogenic tissues, and binds HDL with high affinity and mediates the selective transfer of cholesteryl esters from the HDL to the liver and the non-placental steroidogenic tissues (Acton *et al.*, 1996). SR-BI also mediates the bidirectional flux of nonesterified cholesterol and phospholipids between HDL and cells, depending on concentration gradient (Connelly and Williams, 2004; Yancey *et al.*, 2003; Krieger, 2001). In genetically modified SR-BI-/- mice, the plasma total cholesterol was approximately two times elevated, and most of it was in the abnormally large, heterogeneous, apo-E-enriched HDL-like particles (Rigotti *et al.*, 1997). Conversely, overexpression of SR-BI in the liver of mice results in the virtual disappearance of plasma HDL and a substantial increase in biliary cholesterol (Kozarsky *et al.*, 1997).

2.3 Lipoprotein lipases

2.3.1 Lipoprotein lipase (LPL)

LPL belongs to the lipases family, including particularly hepatic lipase, pancreatic lipase and LPL itself. The in situ hybridization studies revealed the presence of mRNA of LPL in many cell types, and the site of physiological action of LPL is the surface of endothelial cells of capillaries (Camps *et al.*, 1991; Camps *et al.*, 1990). LPL is expressed in many tissues, but is mainly found in the muscle and adipose tissue. It plays leading role in the transport and lipid metabolism by catalyzing triglycerides hydrolysis in the triglyceride-rich lipoproteins, such as chylomicrons and VLDL, releasing non-esterified fatty acids into the blood (Cryer, 1981). Its triglycerides lipase activity is about 100 times higher than its phospholipid lipase activity. The enzyme acts through a specific interaction with an apolipoprotein, the apo CII (Miller and Smith, 1973).

The hydrolysis of chylomicrons and VLDL by LPL results in the release of cholesterol, apolipoproteins and phospholipids, which are then captured by the HDL. Therefore, LPL has a role in the HDL metabolism. In fact, there is a positive correlation between the LPL activity and the level of HDL-cholesterol (Tornvall *et al.*, 1995). Homozygous or heterozygous LPL deficiencies in humans lead to a significant reduction in plasma HDL level. Genetically modified mice have been shown that the hypotriglyceridemic effect of LPL is observed only in the presence of CETP that decreases the exchange of cholesterol esters and triglycerides among the lipoproteins (Clee *et al.*, 1997).

In addition, other functions of LPL were then identified. LPL is able to promote endothelial cell proliferation of smooth muscles (Mamputu *et al.*, 2000). Moreover, the LPL induces the expression of TNFα, and could promote inflammation and atherosclerosis (Renier *et al.*, 1994).

LPL is regulated according to the different physiological states, and the variation in its activity as well as its expression can be adapted to use fatty acids for metabolic needs. In rats and humans, glucocorticoids and insulin increase the expression and/ or activity of LPL in the physiological conditions (Fried *et al.*, 1993; Ong *et al.*, 1988). Studies conducted on the rat adipose tissues have revealed that catecholamines, growth hormone, prolactin and thyroxin have a stimulatory role on the LPL, while parathyroid hormone and estrogens inhibit its expression and/ or activity (Homma *et al.*, 2000; Querfeld *et al.*, 1999). The LPL activity is directly modulated by its active cofactor, apo CII (Kinnunen *et al.*, 1977), whereas the apo E

and apo CIII inhibit its activity (Wang *et al.*, 1985). The mechanisms by which these regulators act on the LPL are still little known, but several studies have shown that they also depend on transcription factors of the PPAR family (Gbaguidi *et al.*, 2002; Sartippour and Renier, 2000; Schoonjans *et al.*, 1996b).

2.3.2 Hepatic lipase (HL)

Hepatic lipase is a lipolytic enzyme, synthesized by the liver that causes the hydrolysis of the triglycerides and phospholipids present in plasma lipoproteins (chylomicron remnants, IDL and HDL), and is thereby, involved in the conversions of VLDL into IDL, and IDL into LDL and in the remodelling of the HDL. Its triglycerides lipase activity is 20 times greater than its phospholipids lipase activity. Major substrates for hepatic lipase are the chylomicron remnants, the IDL and the large size, triglycerides-rich HDL.

The relation between hepatic lipase and cardiovascular diseases has not been clearly defined. It has been shown that the hepatic lipase reduced the size of the LDL (Baynes *et al.*, 1991). Patients with hepatic lipase deficiency have been found with hypercholesterolemia or hypertriglyceridemia and increased chylomicron remnants, VLDL, IDL, triglycerides-rich LDL and HDL (Jansen *et al.*, 1997; Brand *et al.*, 1996; Knudsen *et al.*, 1996; Hegele *et al.*, 1993; Carlson *et al.*, 1986; Breckenridge *et al.*, 1982). In addition, an inverse correlation between its activity and plasma HDL concentration has been reported and a positive correlation with insulin resistance (Baynes *et al.*, 1991). The hepatic lipase, therefore, has pro-atherogenic effects. However, on the other hand, the hepatic lipase also seems to possess anti-atherogenic effects. In fact, it stimulates reverse transport of cholesterol by an increase in the capture of cholesterol esters (Lambert *et al.*, 2000; Lambert *et al.*, 1999), mainly through the hydrolysis of the triglycerides of the HDL. Finally the anti- or pro-atherogenic effects of the hepatic lipase have not yet been clearly elucidated. However, in a review based on clinical observations, the effect of hepatic lipase on lipoprotein metabolism during hypertriglyceridemia may be interpreted as pro-atherogenic (formation of small, dense LDL, lowering of HDL), whereas the effect of hepatic lipase during hypercholesterolemia appears to be anti-atherogenic (stimulation of reverse cholesterol transport, clearance of IDL) (Jansen, 2004).

In humans (as in all animal models), the hepatic lipase activity is greatly increased during insulin resistance (Riemens *et al.*, 1999; Baynes *et al.*, 1991). This increased activity could enhance catabolism of triglyceride-rich HDL. In addition to lipolytic role, hepatic lipase

Table 3: Protein and non-protein factors secreted by adipose tissue.

Substance	Main biological effect
Leptin	Signals to the CNS about body's energy status, stimulation of lipolysis, autocrine regulation of leptin expression.
Adiponectin	Increases insulin sensitivity, anti-inflammatory, attenuates the progression of atherosclerosis.
Resistin	Increases insulin resistance, acts on glucagon receptors.
TNF-α	Stimulation of lipolysis, reduces insulin sensitivity, regulation of leptin secretion, inhibition of adipocyte differentiation.
IL-6	Inhibition of LPL activity, induction of lipolysis, pro-inflammatory, reduces insulin sensitivity.
Acylation stimulating protein	Stimulates TG synthesis in adipose tissue.
Angiotensinogen	Angiotensin II precursor, regulation of arterial vasoconstriction, increases lipogenesis.
Plasminogen activation inhibitor-1 (PAI-1)	Blocks fibrinolysis.
Tissue factor	Initiates coagulation cascade, acts on TNF-α receptors.
Vascular endothelial growth factor (VEGF)	Stimulates angiogenesis in adipose tissue.
Visfatin	Imitates insulin, predominantly produced by visceral fat, glucose lowering effect.
Monobutyrin	Vasodilator, induces angiogenesis.
Transforming growth factor-β (TGF-β)	Regulates differentiation and proliferation in pre-adipocytes, development and apoptosis of adipocytes.
Insulin-like growth factor-1 (IGF-1)	Stimulates proliferation and differentiation in adipocytes, mediates growth hormone.
Hepatocyte growth factor	Stimulates development and differentiation of adipocytes.
Macrophage migration inhibitory factor (MIF)	Paracrine action on adipose tissue, pro-inflammatory processes and immunoregulation.
LPL	Regulates hydrolysis of TG in lipoproteins
Cholesterol ester transfer protein (CETP)	Transfers cholesterol esters between lipoproteins.
Prostaglandins	Inflammation, blood coagulation.
Glucocorticoids	Transforms cortisone into cortisol in adipose tissue.
Estrogens	Produced by action of aromatase, controls white adipose tissue distribution.
Adipsin	Activates alternative complement pathway.
Apelin	Energy metabolism.

CNS: central nervous system, TG: triglycerides. Adapted from (Stehno-Bittel, 2008)

acts as a ligand that facilitates the uptake of lipoproteins and lipoprotein lipids by cell surface receptors or proteoglycans. *In vitro*, hepatic lipase enhances the binding or uptake of chylomicrons, chylomicron remnants, VLDL, LDL (Komaromy *et al.*, 1996; Krapp *et al.*, 1996; Choi *et al.*, 1994; Diard *et al.*, 1994; Ji *et al.*, 1994; Shafi *et al.*, 1994), and HDL cholesterol (Lambert *et al.*, 1999; Marques-Vidal *et al.*, 1994) into a variety of cell types. For the binding or uptake of lipoproteins by the action of hepatic lipase, the cell surface receptors, including the LDL receptor (Komaromy *et al.*, 1996), LDL receptor-related protein (Krapp *et al.*, 1996), scavenger receptor B1 (Lambert *et al.*, 1999; Marques-Vidal *et al.*, 1994) and proteoglycans (Ji *et al.*, 1994) have been implicated.

3 Adipose tissue as an endocrine organ

Adipose tissue has been recognized as an endocrine organ in addition to fat storage depots (Kershaw and Flier, 2004). White adipose tissue secretes hundreds of biological active molecules, the adipokines and cytokines. These are implicated in many physiological and pathological processes, such as appetite and satiety, glucose and fat metabolism, blood pressure regulation, inflammation, insulin sensitivity, cardiovascular diseases and immune functions (Li *et al.*, 2011). These are a group of bioactive proteins, secreted mainly by adipose tissue, such as adiponectin, monocyte chemoattractant protein (MCP)-1, leptin, resistin, visfatin, adipsin, serum amyloid A3, omentin, tumor necrosis factor-α (TNFα), interleukin (IL)-1, IL4, IL-6, IL8, IL10, IL18. The levels of adipokines could affect lipid metabolism, insulin sensitivity, development of cardiovascular diseases and type 2 diabetes mellitus. Table 3 lists some of the protein and non-protein factors secreted by adipose tissue with their main biological effect.

3.1 TNFα

TNFα is a cytokine, identified originally as a pro-inflammatory molecule. It is synthesized as a transmembrane protein on the surface of monocytes (Kriegler *et al.*, 1988). TNFα is mainly synthesized by the macrophages, T and B lymphocytes, natural killer cells, dendritic cells, and it is reported to be produced in the white adipose tissue (Hotamisligil *et al.*, 1993). TNFα is expressed as a 26 kDa plasma membrane-bound monomer, and then its proteolytic cleavage by the TNFα converting enzyme (TACE) releases a 17 kDa soluble or circulatory TNFα. TNFα is recognized by two types of receptors, TNFR1 and TNFR2 present

in different proportions. These receptors differ in their cellular expression profiles, ligand affinities and signaling pathways. The TNFR1 have an important role in pro-inflammatory and cytotoxic responses, whereas TNFR2 only modulate the responses generated by TNFR1 (Peschon *et al.*, 1998). These receptors mediate the TNFα signal by forming protein complexes with cytoplasmic adaptor proteins. The expression of TNFR2 in adipose tissue has been correlated with BMI and the TNFα expression in adipose tissue (Hotamisligil *et al.*, 1997), and hence have a role in human obesity.

Role of TNFα:

TNFα was initially described as a serum factor causing tumor necrosis and its secretion is induced by the inflammatory reactions, such as inflammations triggered by the bacterial infections (Carswell *et al.*, 1975). Later, it was reported that TNFα is similar to the cachectin, secreted by the macrophages exposed to the endotoxin (Beutler *et al.*, 1985). It is capable to induce cachexia in the animals (Tracey *et al.*, 1987) and inhibit LPL activity in the cell culture of adipocytes (Kawakami and Cerami, 1981), and hence increase triglycerides level. TNFα exerts pleiotropic functions in immunity, inflammation, control of cellular proliferation, differentiation and apoptosis, cell survival, cytotoxity, production of other cytokines, such as interleukin 1 (IL-1) and IL-6 (Nieto-Vazquez *et al.*, 2008; Wallach *et al.*, 1999).

3.2 Adiponectin

The adiponectin is isolated independently by several researchers in humans as in the rodents (Nakano *et al.*, 1996; Scherer *et al.*, 1995). This 244-amino acid protein is described with various names in the literature; adipoQ, Acrp30 (adipocyte complement-related protein of 30 kDa) or GBP28. In humans, this metabolically active protein is encoded by a gene expressed exclusively in the adipocytes. The adiponectin gene is located on chromosome 3q26, a region associated with susceptibility to developing metabolic syndrome and type 2 diabetes (Ruan and Lodish, 2003).

Adiponectin circulates in the form of multimers (full-length or high molecular weight), hexamers (medium molecular weight) or trimers (low molecular weight) (Ziemke and Mantzoros, 2010). Adiponectin is recognized by the receptors present on the cell surface. Two subtypes of adiponectin receptors, AdipoR1 and AdipoR2, have been identified, containing seven transmembrane domains (Yamauchi *et al.*, 2003). The binding of adiponectin to AdipoR1 and AdipoR2 leads to the activation of adenylate cyclase, then of AMP-activated

protein kinase (AMPK) and PPARα respectively (Kadowaki and Yamauchi, 2005) that has been confirmed in knockout mice. Targeted disruption of AdipoR1 results in abrogation of adiponectin-induced activation of AMPK, whereas disruption of AdipoR2 inhibited adiponectin-stimulated PPARα signaling in mice (Yamauchi *et al.*, 2007). Disruption of both AdipoR1 and AdipoR2 receptors inhibits the binding and actions of adiponectin, and leads to insulin resistance and decreased glucose tolerance (Yamauchi *et al.*, 2007). It may also partially act via receptors of the fraction C1q of the complement, with which it has the strong structural similarities (Yokota *et al.*, 2000).

The synthesis and secretion of the adiponectin is regulated by several mechanisms. For example, the insulin stimulates gene expression and/ or secretion of the protein in the 3T3-L1 line adipocytes (Scherer *et al.*, 1995), as IGF1 does on isolated human adipocytes from visceral adipose tissue (Halleux *et al.*, 2001). PPAR also seem to be involved in the regulation of the gene expression of the adiponectin. In db/db obese mice, PPARα agonists decreased expression of adiponectin (Moore *et al.*, 2001). Other studies *in vitro* and in humans and rodents have shown a stimulatory effect of rosiglitazone, an anti-diabetic agent and PPARγ agonist, on the expression of the adiponectin (Maeda *et al.*, 2001; Yamauchi *et al.*, 2001). On the other hand, TNFα inhibited the gene expression and secretion of adiponectin in a dose-dependent manner (Maeda *et al.*, 2001). According to the clinical observations, there is a sexual dimorphism on the secretion of the adiponectin, which is decreased by the androgens and has higher level in women.

Role of adiponectin:

Adiponectin plays an important role in the regulation of glucose and lipid metabolism and modifies insulin sensitivity and energy balance. It promotes fatty acid oxidation, decreases lipid synthesis and suppresses hepatic glucose production (Combs *et al.*, 2001). The AMP kinase phosphorylation promotes fatty acids oxidation, glucose uptake in the muscle and reduces hepatic gluconeogenesis, by regulating acetyl-CoA carboxylase and FAS (Ziemke and Mantzoros, 2010). Adiponectin increases the oxidation of non-esterified fatty acids in the muscles (Fruebis *et al.*, 2001), and decreases the triglycerides content in the muscle and liver and decreases plasma concentration of non-esterified fatty acids (Yamauchi *et al.*, 2001). In skeletal muscles, it also induces the expression of the proteins involved in the metabolism of the fatty acids, such as acyl CoA oxidase and UCP2. However, it has no effect on the fatty acid oxidation at hepatic level and on the intestinal absorption of fatty acids. Moreover, it has no effect on the HSL. Therefore, it is suggested that adiponectin decreases

plasma level of non-esterified fatty acids by enhancing their capture and not via the inhibition of the lipolysis (Fruebis *et al.*, 2001). It could also increase the fatty acid catabolism by stimulating the expression of PPARα, which controls the expression of the enzymes involved in lipid metabolism (Yamauchi *et al.*, 2001). Moreover, it also increases the uptake of glucose by the muscle and decreases its production by the liver. It was shown that adiponectin stimulates AMP kinase (Yamauchi *et al.*, 2002), so it is capable to increase glucose uptake by muscle cells (Mu *et al.*, 2001) and inhibit the expression of enzymes involved in the gluconeogenesis. Other studies suggest an anti-inflammatory role of the adiponectin on the vascular wall. It accumulates in the injured vessel wall and exerts an inhibitory effect on TNFα production by the macrophages (Yokota *et al.*, 2000). At the same time, adiponectin reduces the inflammatory response of endothelial cells by inhibiting the signals produced by the NFκB factor and reducing the adhesion and the expression induced by the TNF-α (Ouchi *et al.*, 2000). Recently, a modulatory effect of vascular remodelling was also suggested by the suppressive activity of adiponectin on proliferation and migration of human aortic smooth muscle cells. These results were confirmed by studies in Knock-out mice, deficient in adiponectin (Kubota *et al.*, 2002). These mice showed the neointimal formation twice as large as that observed in the normal mice in response to external vascular injury (Kubota *et al.*, 2002). Many studies have shown that adiponectin, via its anti-inflammatory actions and in the lipoprotein metabolism, would also be a cytokine with anti-atherosclerosis effect. In addition to these peripheral roles of adiponectin, it may act centrally to modulate food intake and energy expenditure (Kubota *et al.*, 2007).

3.3 Leptin

Leptin, an adipocyte-derived hormone, signals the status of body energy stores to the central nervous system to regulate appetite and energy expenditure. The studies in the rodents with autosomal recessive mutations ob (obese), and/ or db (diabetes) (Coleman, 1978; Hummel *et al.*, 1966) have revealed the existence of an anorexigenic substance in the circulation, leptin, which is encoded by the gene ob. Leptin is member of the helical cytokine family. In mammals, leptin is produced by the adipose tissue, particularly subcutaneous adipose tissue (Montague *et al.*, 1997; Masuzaki *et al.*, 1995b) and to a lesser extent by the gastric epithelium (Bado *et al.*, 1998) or the placenta (Masuzaki *et al.*, 1997). It circulates mainly bound to transport proteins (Houseknecht *et al.*, 1996). Leptin acts through a cell-surface leptin receptor (LepR). A single *Lepr* gene produces multiple LepR isoforms;

however a single isoform, LepRb appears to account for all the actions of leptin (Chua *et al.*, 1997; Tartaglia, 1997). A series of signaling events initiates on binding of leptin to LepRb, beginning with the activation of the constitutively receptor-associated Janus kinase-2 (Jak2), a tyrosine kinase (Villanueva and Myers, 2008). Moreover, the Jak2 activation (autophosphorylation of Jak2) stimulates the phosphorylation of multiple residues on the intracellular domain of LepRb (Gong *et al.*, 2007). These phosphorylated residues recruit the other enzymes (SH2-containing tyrosine phosphatase-2; extracellular signal-regulated kinase; signal transducer and activator of transcription-3, (STAT3); STAT5) (Villanueva and Myers, 2008). In addition, leptin regulates the PI3-kinase (Plum *et al.*, 2006; Xu *et al.*, 2005) that remains to be clarified. The elimination of leptin mainly occurs by the kidneys, which are actively involved in the degradation of the hormone (Cumin *et al.*, 1996).

The expression of leptin and its activity is regulated by a multitude of factors. The availability of energy metabolites, such as glucose (Grinspoon *et al.*, 1997) and non-esterified fatty acids (Wang *et al.*, 1999b), insulin and cortisol (Wabitsch *et al.*, 1996) are the main stimulators for the expression of leptin. Catecholamines and the fasting state are capable to inhibit this expression (Kosaki *et al.*, 1996). Corticosteroids inhibit the hypothalamic production of corticotrophin-releasing hormone, the effect which is opposite to that of the leptin (Schwartz *et al.*, 1999) and it has been shown that glucocorticoid deficiency increased the anorexigenic action of leptin (Woods *et al.*, 1998). In addition, the balance of the effects of leptin and neuropeptide Y (NPY) also appears to be modulated by glucocorticoids. NPY induced hyperphagia, increased body weight and hypertriglyceridemia in rats (Sainsbury *et al.*, 1997). Glucocorticoids limit the effects induced by leptin and favour those of NPY (Zakrzewska *et al.*, 1999; Zakrzewska *et al.*, 1997).

Leptin is characterised as signal of energy sufficiency because it reduces food intake and increases energy expenditure, thus improves insulin sensitivity (Webber, 2003).

Role of leptin:

- Regulation of the appetite:

In humans, the decrease in plasma leptin concentration is associated with an increased desire to eat, while high leptin levels are related to reduction in hunger, regardless of the variation in body weight and body fat (Keim *et al.*, 1998).

- Action on the basal metabolism and energy expenditure:

The ob/ob mice exhibit defective thermogenesis (Bray and York, 1979). Recombinant leptin treatment increases basal metabolism, body temperature and level of activity, and normalizes metabolic status as well (Pelleymounter *et al.*, 1995). Unlike mice, humans with leptin deficiency (Farooqi *et al.*, 1999; O'Rahilly, 1998) or its receptor (Clement *et al.*, 1998) have a normal temperature and their metabolism is not lowered. Leptin increases the consumption of calories, particularly by its action on thermogenesis. It maintains or favours the mechanisms dissipating energy by induction of uncoupling proteins, probably secondary to its central action (Cusin *et al.*, 1998; Scarpace *et al.*, 1997). But this role in the regulation of energy expenditure seems less important in humans (having low amount of brown tissue) than in rodents.

- Actions on tissue-specific metabolism and hormonal interactions:

This is the fundamental component of the actions of leptin to potentially control the regulation of energy reserves. Leptin administration causes weight loss in a dose-dependent manner, by loss of adipose tissue and sparing lean body mass (Halaas *et al.*, 1995). On the other hand, leptin deficiency is associated with increased fat deposits (Friedman and Halaas, 1998; Coleman, 1978).

- Lipid metabolism:

Lipolysis may be induced in an adrenergic way, but there are other forms of lipolysis (independent of catecholamines), in which leptin acts directly on the tissues (Muoio *et al.*, 1997). It induces a rapid change of the energy sources available to the body: the transition of catabolism using carbohydrate to the lipolysis in adipose tissue would be one of the earliest metabolic effects of leptin (Hwa *et al.*, 1997). According to Wang, *et al.* (1999b) leptin reduces fat reserves without increasing non-esterified fatty acids or ketone bodies. Leptin converts the adipocytes, storage cells, into fat-burning cells. Leptin could also induce apoptosis of the adipocytes (Qian *et al.*, 1998). Finally, according to some authors, leptin limits the storage of triglycerides in the adipocytes and protects non-adipocyte cells (including β-pancreatic cells) from lipotoxicity (Unger *et al.*, 1999; Nakamura, 1995). In skeletal muscle, leptin increases fatty acid oxidation and decreases the synthesis of triglycerides (Muoio *et al.*, 1997).

- Carbohydrate metabolism:

Administration of recombinant leptin corrects hyperglycemia and hyperinsulinemia in ob/ob mice, but not in db/db mice (Schwartz *et al.*, 1996; Pelleymounter *et al.*, 1995). These hypoglycemic effects are mainly independent of the decrease in appetite or of the weight loss. Administration of leptin appears to increase glucose metabolism independently of insulin (Haque *et al.*, 1999; Kamohara *et al.*, 1997). It increases the glucose uptake by the heart, brown adipose tissue and skeletal muscle but not by white adipose tissue (Haque *et al.*, 1999), and inhibits hepatic glucose production (van den Hoek *et al.*, 2008; Pocai *et al.*, 2005). These stimulatory effects of leptin are not due to direct peripheral action on the glucose transport in insulin-sensitive tissues (Zierath *et al.*, 1998). It therefore would act at the central level, in synergy with the insulin in peripheral tissue for glucose uptake. However, leptin could oppose other functions of insulin. It was shown that leptin treatment attenuated the ability of insulin to stimulate glycogen synthesis, lipogenesis and uptake of amino acids in the liver (Cohen *et al.*, 1996). Leptin also suppresses insulin secretion via a receptor present in β-pancreatic cells (Ookuma *et al.*, 1998; Emilsson *et al.*, 1997) and acting on the ATP-dependent potassium channels. Thus, this hormone is capable to activate and inhibit glucose-stimulated insulin secretion, by producing a membrane hyperpolarisation of β-cells (Kieffer *et al.*, 1997). Finally, leptin is able to inhibit gene transcription of preproinsulin, by altering binding of the transcription factors at the promoter (Seufert *et al.*, 1999).

4 Insulin & Glucose transporters

Obesity related disorders are placing a considerable strain on healthcare system of the whole world, especially in the developed countries. Obesity is one of the principal factors behind many clinical disorders, like respiratory diseases, renal problems, cardiovascular problems, hypertension, insulin resistance, type 2 diabetes, atherosclerosis, dyslipidemia and cancer. However, we focussed on insulin resistance and dyslipidemia in our study.

4.1 Insulin

Insulin is an important hormone of glucose metabolism in normal healthy individuals. It helps to maintain normal blood sugar levels in various physiological conditions. Insulin is a peptide hormone, synthesized, stored and secreted by the β-cells of the islets of Langerhans, which are dispersed in the exocrine parenchyma of the pancreas.

4.1.1 Insulin secretion

The hormone is synthesized and stored in the form of inactive proinsulin, which after maturation split at the time of secretion into active insulin. The major characteristic of the pancreatic β-cells is to act like 'metabolic detectors' (Henquin *et al.*, 1994). In fact, the pancreatic β-cells adapt insulin secretion according to fluctuations in the levels of glucose and other nutrients in the blood. The β-cells translate the variations of the blood concentrations of the nutrients into signals, which control the enzymes required for the release of insulin. The glucose penetrates into the β-cells by facilitated diffusion, by the help of GLUT2. When concentration of glucose increases, the metabolism of the β-cells accelerates and high ATP/ADP ratio cause the closure of ATP-sensitive K^+ channels. This closure leads to the depolarization of the membrane, the opening of the calcium channels, influx of Ca^{+2}, an increase of cytoplasmic Ca^{+2} concentration, and stimulation of insulin secretion. In addition to this main route of signaling triggered by the high level of Ca^{+2}, the glucose metabolism may also influence the calcium channels and amplify the efficacy of Ca^{+2} on the insulin secretion.

After its secretion, insulin disappears rapidly from the blood by binding to its receptors on all target cells, including hepatocytes. Insulin induces cascade of enzymatic reactions leading to various physiological effects. It is then degraded in the cells. The half-life of insulin is less than few minutes in the blood (Matthews *et al.*, 1985).

4.1.2 Insulin signaling

The metabolic effects of the insulin results from the hormone binding to a specific receptor, present on the surface of all the body cells, and predominantly on its three target tissues, the liver, the adipose tissue and the muscle.

Insulin receptor belongs to the family of the receptors for the growth factors, which possess tyrosine kinase activity in their intracellular domain (i.e., transfer of phosphate group from ATP to tyrosine residues on intracellular target proteins). Like the other protein hormone receptors, the insulin receptor is embedded in the cell membrane. It is composed of the two α-subunits connected with the two β-subunits, linked by disulfide bridges. The α-chains are entirely extracellular and contain insulin binding domains, and the linked β-chains penetrate through the plasma membrane. Insulin binding with high affinity on the two α-subunits, allows the activation of the two β-subunits of the receptor. Each β-subunit has an intracellular tyrosine kinase activity domain in the form of a regulatory loop, which occludes the catalytic site of tyrosine kinase and keeps it in an inactive state. At the time of receptor activation, the

binding of ATP to its site allows the opening of the loop and the phosphorylation of the tyrosine residues. When the tyrosine kinase domain is completely activated, it can phosphorylate other tyrosine residues present on the β-chains, leading to the autophosphorylation of not only the receptor, but also other cellular enzymes, leading to their activation or inactivation, thereby generating or inhibiting a biological response. Insulin receptor exists in two isoforms, IR-A and IR-B, differing by the absence or presence of 12 amino acids at carboxyl terminus of the α-subunit respectively. The IR-B is more abundant isoform in muscle, liver and adipose tissue (Siddle, 2011).

Insulin signaling in the cells involves regulatory subunits, called 'substrates'. Several intracellular proteins have been identified as phosphorylation substrates for the insulin receptor. The IRS-1 and IRS-2 (insulin receptor substrate) are the main representatives of the IRS family, which are capable to link with the activated receptor. The stable link leads to the tyrosine phosphorylation the IRS proteins by the insulin receptor β-subunit (Chang *et al.*, 2004). These IRS proteins play complementary role in insulin signaling (White, 2002). One of the major insulin signaling pathways is that of phosphatidylinositol 3 (PI3) kinase. The generation of phosphatidylinositol 3,4,5-triphosphate patches in the cell membrane could act as docking sites for protein kinase B (also known as Akt) (Cheatham and Kahn, 1995). Three isoforms of Akt are known with AKt1 contributing to the growth and Akt2 contributing to insulin-mediated Glut4 redistribution (Zhou *et al.*, 2004). The activation of Akt is crucial for the insulin signaling to GLUT redistribution (Welsh *et al.*, 2005). The effect of insulin on the transport of glucose illustrates the complexity of the insulin signaling. There is induction of the translocation of the vesicles containing GLUT4 towards the plasma membrane in the muscle cells and adipocytes (Bryant *et al.*, 2002; Kido *et al.*, 2001). Another important insulin signaling pathway is the mitogen-activated protein (MAP) kinase pathway for the activation of the gene expression and the cellular proliferation. Then, the insulin receptor complex is taken up by the endosomes and insulin signaling ends after the degradation of the hormone. The majority of the receptors are recycled to the membrane, while others are degraded. The dephosphorylation of tyrosine residue of the receptor and IRS proteins is accomplished by the help of tyrosine phosphatases. In addition to tyrosine kinase pathway, insulin receptor regulates cell growth-related gene expression via the Ras/MAP kinase pathway (Avruch, 2007).

4.1.3 Metabolic effects of insulin

The insulin has an immediate hypoglycaemic action; it stimulates the transport of ingested glucose into the skeletal muscle and adipose tissue. It also decreases the glucose production by the liver by reducing glycogenolysis and gluconeogenesis. Insulin stimulates the cellular uptake of glucose and also storage of carbohydrates, fats and proteins, and it suppresses the release of non-esterified fatty acids from adipose tissue by reducing lipolysis.

It also decreases the release of amino acids and glucose. Insulin also has a delayed action on the synthesis of nucleic acids and proteins. These effects result in the cellular differentiation and proliferation, similar to that induced by the growth factors. Insulin has an active role in all body cells; however dependence of the cells on insulin for glycemia is variable. Most of the brain cells, red blood cells and cells of the medulla of the kidney only depend on the concentration gradient for glucose supply, while the other cells (liver, adipose tissue and muscle) are insulin dependent.

Insulin may promote synthesis of fatty acids from excess glucose in the liver. When glycogen accumulates to high levels (roughly 5 % of liver mass), further synthesis is strongly suppressed, and any additional glucose taken up by hepatocytes is shunted into pathways leading to synthesis of fatty acids, which are exported from the liver as lipoproteins. The lipoproteins are ripped apart in the circulation, providing non-esterified fatty acids for use in other tissues, including adipocytes, which use them to synthesize triglycerides. Insulin has also a role to inhibit lipolysis, the hydrolysis of triglycerides to fatty acids. Insulin facilitates entry of glucose into adipocytes, and there, glucose can be used to synthesize glycerol. This glycerol, along with the fatty acids delivered from the liver, is used to synthesize triglycerides within the adipocyte. So by these mechanisms, insulin is involved in further accumulation of triglycerides in fat cells.

In addition to its well-defined peripheral role of glucose clearance and utilization insulin also has a key role in the control of appetite and feeding. It is involved in afferent (and efferent) hypothalamic pathways controlling energy intake, and also limbic system controlling pleasurable responses to food. Insulin is known to store energy reservoirs in liver, fat and muscle, however in central nervous system, its role is to decrease energy intake by an elegant instance of negative feedback. First, insulin decreases the desire to eat and secondly it decreases the pleasurable and motivating aspects of food (Isganaitis and Lustig, 2005).

Insulin plays a major role in the anabolism of the body in the reserve and the utilisation of the energy substrates, carbohydrates or fats, i.e., glucose uptake, glycogenesis and lipogenesis, inhibition of glycogenolysis, gluconeogenesis and lipolysis. Figure 3 represents some classical actions of insulin. In addition, insulin also has pleiotropic role on the protein metabolism (increase of the synthesis and inhibition of the proteolysis), growth, apoptosis and development.

4.2 Glucose transporters

The facilitated transport of glucose is ensured by the GLUT family members, twelve members have so far been largely described. The primary function of these transporters is to ensure the transport of glucose from the blood into the cytoplasm. Insulin stimulates glucose transport in muscles and adipose tissue by at least two ways; eliciting the translocation of the glucose transporters from an intracellular pool to the cell membrane via exocytic-like process, and enhancing transporter intrinsic activity (moles of glucose transported per transporter/unit time) (Simpson and Cushman, 1986). Previous molecular cloning studies revealed that glucose transport is mediated by a group of facilitated diffusion glucose transporters encoded by distinct genes (Bell *et al.*, 1990; Mueckler, 1990; Thorens *et al.*, 1990).

The GLUT2, a bidirectional glucose transporter is expressed by renal tubular cells, small intestinal epithelium, hepatocytes and pancreatic β-cells. The GLUT3 is expressed in the neurons and placenta. GLUT4 is one of the 13 sugar transporter proteins (GLUT1-GLUT12, and HMIT) that catalyzes hexose transport across cell membranes through an ATP-independent, facilitative diffusion mechanism (Huang and Czech, 2007). GLUT5 and GLUT11 are likely fructose transporters. The GLUT4 transporter is specific for the adipocytes and muscle cells (Birnbaum, 1989). The adipose tissue and muscle are insulin sensitive, as they respond to insulin signal by a rapid and reversible increase in glucose transport. GLUT1 is expressed in many tissues (erythrocytes, endothelia cells of blood-brain barrier) including those in which glucose transport is not acutely stimulated by insulin. Although adipose tissue and muscle express other isoforms, including GLUT1, the GLUT4 seems to be the principal responsible for the transport stimulated by insulin, while GLUT1 plays a more minor role (Calderhead *et al.*, 1990; Zorzano *et al.*, 1989; Oka *et al.*, 1988). In fact, GLUT4 is the most abundant in these target tissues (Marette *et al.*, 1992; Holman *et al.*, 1990). In addition, its expression is correlated during cell differentiation of adipocytes with the beginning of the transport stimulated by insulin (Tordjman *et al.*, 1989). Its level of

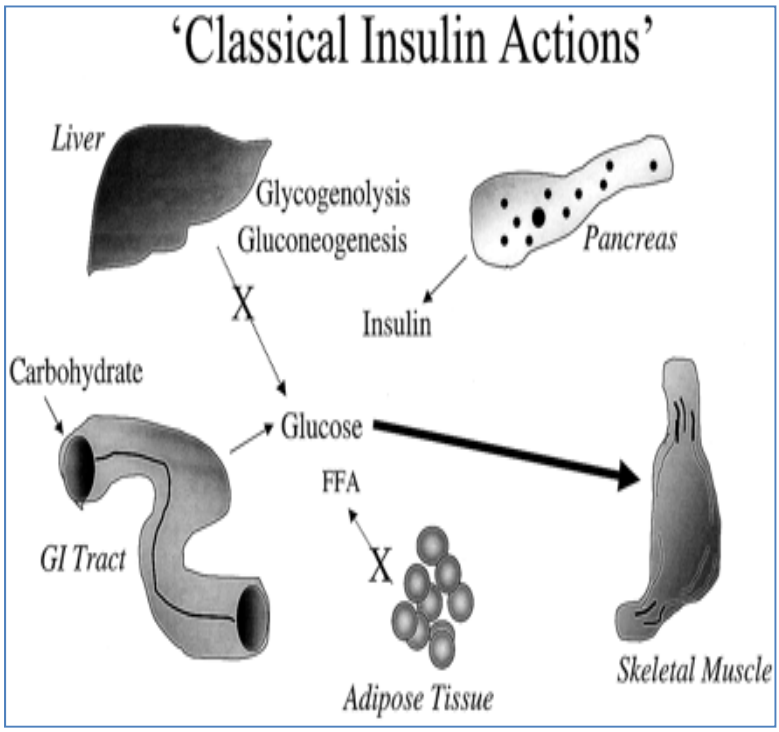

Figure 3: Summary of insulin actions.

(Bessesen, 2001)

expression appears to be associated to the variations in glucose available, as a result of insulin stimulation (James *et al.*, 1989). Finally, another study also reported GLUT4 over expressed cell lines with an increase in glucose transport stimulated by insulin (Lawrence *et al.*, 1992). GLUT4 is a continuously recycling protein (Dugani and Klip, 2005) with faster endocytosis than re-exocytosis, resulting in a net intracellular retention. It is well-known that insulin primarily promotes GLUT4 exocytosis. In addition to GLUT4, GLUT1, GLUT5 and GLUT12 significantly contribute to sugar uptake in the skeletal muscle (Stuart *et al.*, 2006; Stuart *et al.*, 2000), and GLUT8, GLUT12 and HMIT are expressed in the adipose tissue (Wood *et al.*, 2003; Wood and Trayhurn, 2003). The GLUT4 transporter has been extensively studied, because 20 to 95 % of the glucose reserves in the muscle in humans, depending on physiological conditions (Baron *et al.*, 1988). These results were confirmed by other studies that have reported a direct correlation between the level of muscle GLUT4 and the amount of glucose available across the whole body (Eriksson *et al.*, 1992; Koranyi *et al.*, 1991). In addition, skeletal muscle is the site of more than 80 % of insulin-mediated glucose uptake *in vivo* (DeFronzo *et al.*, 1981), and in the adipose tissue, relatively little glucose uptake even in case of increased adiposity (Kraegen *et al.*, 1986; Storlien *et al.*, 1986). However, GLUT4 mRNA and protein levels in skeletal muscle from genetically obese Zucker rats (Yamamoto *et al.*, 1991; Friedman *et al.*, 1990), mice with genetic (Koranyi *et al.*, 1990) or acquired obesity (Le Marchand-Brustel *et al.*, 1990), obese humans (Garvey *et al.*, 1992; Pedersen *et al.*, 1990), are similar to those in lean controls. Thus, the changes in the GLUT4 levels in skeletal muscle do not necessarily parallel to those in the adipose tissue in obesity; GLUT4 levels in muscle seems to be less susceptible to regulation than in the adipose tissue.

The expressions and the mechanism of action of GLUT4 transporters are mainly regulated by insulin (hence, its old name insulin regulatable glucose (IRG) transporter). Insulin, after being linked to its receptors, causes translocation of glucose transporters from an intracellular pool to the plasma membrane. Cyclic AMP (Kelada *et al.*, 1992), some esters (Holman *et al.*, 1990), and muscle contraction (Douen *et al.*, 1990) are capable to enhance the glucose transport via the mechanism of translocation. Recently, Berenguer *et al.*, (2010) reported that an unidentified serum factor, in addition to insulin, contributes to GLUT4 regulation, and it still functions in insulin resistance (Berenguer *et al.*, 2010).

5 Low insulin sensitivity (Insulin resistance [IR])

5.1 Definition & Main characteristics

5.1.1 Definition

According to the International Diabetes Federation (IDF), there are almost 366 million people (20-79 years old) with type 2 diabetes worldwide in 2011, and the projected figure is 552 million, by 2030 (IDF, 2011). The metabolic syndrome is cluster of insulin resistance (or impaired glucose tolerance), visceral obesity, dyslipidemia and hypertension. WHO proposed that metabolic syndrome includes in addition to insulin resistance or glucose intolerance, at least two of the following anomalies: hypertension, hypertriglyceridemia, low plasma HDL-cholesterol concentration, high waist to hip ratio, obesity or microalbuminuria (Alberti and Zimmet, 1998). Metabolic syndrome is defined by the National Cholesterol Education Program in its Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), National Heart, Lung, Blood Institute, National Institute of Health, USA in May 2001; as the presence of at least three of the following criteria: waist > 102 cm for men and > 88 cm for women; TG > 1.6 mmol/L; HDL-cholesterol < 1.04 mmol/L for men and > 1.29 mmol/L for women; arterial blood pressure ≥ 130/85 mm Hg; and basal glycemia ≥ 6.1 mmol/L. The IDF defines metabolic syndrome as the presence of visceral obesity (waist circumference ≥ 94 cm for Europid men and ≥ 80 cm for Europid women) and any two of the four factors: elevated circulating triglycerides level (≥ 1.7 mmol/l), reduced HDL-cholesterol (< 1.03 mmol/l in males and < 1.29 mmol/l), high blood pressure (systolic BP ≥ 130 or diastolic BP 85 mm Hg) and raised fasting glycemia (≥ 5.6 mmol/l) (IDF, 2006). The IDF eventually recommends other criteria to diagnose metabolic syndrome that include adipose tissue biomarkers, increased levels of circulating inflammatory and/or thrombotic markers or reduced levels of anti-inflammatory molecules, such as adiponectin, leptin, TNFα, IL6 (IDF, 2006). Other definitions of similar conditions have been developed by the World Health Organization and the Association of Clinical Endocrinologists. The NCEP ATP III defined metabolic syndrome as presence of three or more of the five criteria: abdominal obesity, hypertriglyceridemia, low HDL, hypertension and high fasting glucose (NCEP, 2001). In fact, the syndrome includes resistance to insulin-stimulated glucose uptake, glucose tolerance, hyperinsulinemia, hypertension, dyslipidemia characterized by high triglycerides level and low HDL level. In

addition, central obesity; small, dense LDL; increased uric acid concentrations; high level of plasminogen activator inhibitor and decreased level of adiponectin contribute to this syndrome.

Low insulin sensitivity is simply defined as diminution of biological effects of insulin or decrease in insulin sensitivity of tissues (muscle, fat and liver cells). This is a worldwide metabolic disorder and is associated with some of the most common disorders including diabetes, hypertension, obesity and cardiovascular diseases. It is a silent condition that increases chances of developing diabetes and cardiovascular diseases. The cause of insulin resistance is multifactorial, including age, genetics and eating behaviour. Certain body conditions, like obesity may also lead to develop and deteriorate this problem. Excess fat interferes with the ability of muscles to use insulin.

The derangements in insulin signaling have widespread and devastating effects on many organs and tissues. The negative control of insulin signaling may happen as a result of the degradation of the hormone or by the dephosphorylation of the receptor. Under the physiological conditions, the synthesis of receptors maintains a sufficient number of receptors on the cell. However, in the presence of persistent hyperinsulinemia, the cycle of intake/ recycle may lead to the decreased number of receptors on the cell surface, also called 'down regulation' as a secondary participant for the induction of insulin resistance. The increased activity of the tyrosine phosphatase, observed in the muscles of the diabetic patients, could be involved for the insulin resistance in this tissue or could be a compensatory process in response to reduced signaling. The phosphorylation of serine or threonine residues with respect to receptor and IRS proteins appears to play the antagonistic role to that of tyrosine residues, and hence mainly involves in the mechanism of insulin resistance. The serine or threonine phosphorylation would end the physiological activation of the receptor, and its severity may further induce the resistance to insulin in disease. Several studies have recently been interested in the serine or threonine phosphorylation of IRS proteins, which decouples the receptor and thus stops insulin signaling (Le Roith and Zick, 2001). Many factors appear to be capable to induce this phosphorylation, such as non-esterified fatty acids, diacylglycerol, acyl CoA, glucose, and inflammatory cytokines, like TNFα and IL-1. Finally, the regulation of the amount of IRS degraded after ubiquitination will determine the metabolic effects of insulin. Insulin resistance is usually associated with hyperinsulinemia. In fact, moderate increase in the insulinemia might be tolerated in the short term, whereas chronic hyperinsulinemia worsens insulin resistance and contributes directly to pancreatic β-cells

failure and diabetes (White, 2003). In addition, the intracellular accumulation of lipids (diacylglycerol) in the muscle and liver impairs insulin signalling and cause insulin resistance.

Another factor in the pathogenesis of low insulin sensitivity relates to the oxidative stress. The reactive oxygen species (ROS) are chemically reactive molecules and their cellular accumulation induces inflammation and mutagenesis which has been linked with many diseases (including cancer, Alzheimer's disease, cardiovascular diseases, diabetes). The accumulation of visceral fat, a key element in the installation of insulin resistance is correlated with systemic oxidative stress (Evans *et al.*, 2002). The high level of fatty acids is involved in this increased production of ROS through the β oxidation and mitochondrial respiration (Shibata *et al.*, 2010). Conversely the restoration of the balance between ROS and antioxidants in humans as in animals improves insulin sensitivity (He *et al.*, 2010).

5.1.2 LPL, obesity and insulin resistance

Keeping in view of the major role of LPL in the control of non-esterified fatty acids supply to the different tissues, it is not surprising that its regulation could be disturbed in metabolic disorders, such as obesity. Studies in rodents and humans have shown that increased LPL activity was associated with obesity (Goldberg and Merkel, 2001; Eckel, 1989; Greenwood, 1985). Moreover, *ob/ob* obese and leptin-deficient mice, with no LPL in adipose tissue, have decreased fat mass (Weinstock *et al.*, 1997). However, type 2 diabetic or insulin resistant patients show a decrease in LPL activity in the adipose tissue (Knudsen *et al.*, 1995; Taylor *et al.*, 1979; Nikkila *et al.*, 1977; Pykalisto *et al.*, 1975) that results in an increase of plasma VLDL. Indeed, the LPL is an insulin sensitive enzyme in humans (Taskinen, 1987). This decreased LPL activity could be explained by decreased activity of PPARα resulting on the one hand, into a decrease in the concentration and activity of LPL, and on the other hand, into an increase of apo CIII. An increase in the concentration of apo CIII could contribute to develop hypertriglyceridemia (Li *et al.*, 1995). In addition, the expression of LPL is increased as a result of antidiabetics (Simsolo *et al.*, 1992), such as thiazolidinediones, which are known to act via PPAR. In type 2 diabetes and insulin resistance, the increased production of certain cytokines by adipose tissue contribute to the reduced expression of LPL (Halle *et al.*, 1998).

5.1.3 Dyslipidemia

One of the major consequences of the insulin resistance is the development of dyslipidemia, leading to markedly increase the cardiovascular risk. Dyslipidemia enhances 2

to 4 times risk of cardiovascular disease in patients with type 2 diabetes mellitus compared with non-diabetic individuals. Atherogenic dyslipidemia (also called dyslipidemia of insulin resistance or the atherogenic lipoprotein phenotype) is characterised by the high levels of triglycerides, VLDL, LDL-cholesterol, smaller and denser plasma LDL than the normal ones, and low HDL-cholesterol concentration.

The hypertriglyceridemia, observed in the patients with insulin-resistant could be explained by an increased lipolysis, which leads to an increase in plasma non-esterified fatty acids, leading to an overproduction of triglycerides and VLDL. In fact, the microsomal triglyceride transfer protein (MTP), which allows transfer of lipids to apo B100, is highly expressed and active in insulin-resistant patients (Qiu *et al.*, 2005). In addition, the high level of non-esterified fatty acids stimulates the hepatic production of triglycerides and imposes transfer of lipids on the apo B100 as well as its stability.

The hypertriglyceridemia is also linked to the reduced activity of LPL. In case of insulin resistance, the LPL is less activated by the insulin. Therefore, there is less hydrolysis of VLDL, resulting in the increased plasma concentration of VLDL-triglycerides. This could also be linked to the apo CIII, which is known to suppress LPL activity (Florez *et al.*, 2006). Indeed, the promoter of the gene for apo CIII is capable to respond to the insulin, resulting in the reduction of its expression (Dallinga-Thie *et al.*, 2001). The decreased insulin sensitivity could promote the expression of gene for the apo CIII, and therefore, inhibition of the LPL.

In addition, the LDL enriched with esterified cholesterol and HDL with the triglycerides due to exchange by the help of CETP. These triglycerides-rich HDL are sensitive to the action of HL, and thus their catabolism is accelerated. In addition, esterified cholesterol and triglycerides rich-LDL are hydrolysed by the LPL or the HL and become smaller and denser (Tan *et al.*, 1995), and promotes the atherosclerosis.

Plasma triglycerides plays a role on the esterification of cholesterol (Murakami *et al.*, 1995), as evident by high activity of LCAT in hypertriglyceridemic patients (Tato *et al.*, 1997), in insulin resistent patients (Riemens *et al.*, 1998). Moreover, the activity of CETP also increase in hypertriglyceridemia (Guerin *et al.*, 2001; Riemens *et al.*, 1998), leading to cholesterol-rich VLDL, and small and dense LDL (triglycerides and cholesterol rich LDL) that favours atherosclerosis. In addition, a decrease in the expression of SR-B1 was observed in the liver of *ob/ob* mice (Lundasen *et al.*, 2003), but an increase in insulin resistant rats (Roberts *et al.*, 2004).

5.2 Contribution of adipose tissue to low insulin sensitivity

5.2.1 Visceral vs. subcutaneous adipose tissue

Previous studies have made evident the contribution of subcutaneous and visceral fat depots for the development of obesity-related disorders (Lemieux and Despres, 1994; Pouliot *et al.*, 1992). They reported that glucose tolerance found in the obese individuals with low level of visceral fat has been similar to that found in the healthy individuals (Pouliot *et al.*, 1992). In contrast, the obese individuals characterized by high level of visceral fat showed significant higher glycemic response to oral glucose administration than the obese individuals with low visceral fat and the healthy individuals. The significant difference was also observed for insulinemic response to oral administration of glucose, and the obese individuals with high level of visceral obesity showed high glycemic and insulinemic responses. Studies showed severe insulin resistance in the patients with visceral obesity (Banerji *et al.*, 1995; Cefalu *et al.*, 1995). In fact, the quantity of visceral fat is a critical element for the induction of obesity-induced disorders, including insulin resistance, which is the major component of metabolic syndrome.

The position of the adipose tissue also play important role in the development of insulin resistance. In fact, the visceral adipose tissue is more harmful than subcutaneous adipose tissue (Bergman *et al.*, 2006). Indeed, obesity is also considered as chronic inflammatory condition. The inflammatory signals affect cellular pathways that intersect with insulin actions (Shoelson *et al.*, 2006). Obesity and its link with inflammatory and metabolic complications may be due to two factors. Firstly, transport of the non-esterified fatty acids and inflammatory mediators from the visceral fat depots into the portal circulation and secondly significant differences in expression of several genes in different body fat deposits. Portal theory, i.e., non-esterified fatty acids flow to the liver from the lipid turn over in viscera, has significant role in the development of insulin resistance. Adipocytes secrete several cytokines (TNFα, IL-6, leptin, resistin, visfatin and adiponectin), responsible for determining the hepatic and muscular insulin sensitivity. In a review, Shimamoto and Ura reported that TNFα may be associated with the development of insulin resistance in patients with type 2 diabetes or obesity and in animal models with genetic or diet-induced obesity by the suppression of insulin signal transduction (Shimamoto and Ura, 2006). An increase in cytokines or reduction in adiponectin release is important factor in the pathogenesis of obesity-induced insulin resistance. Briefly, number of molecular factors, such as non-

esterified fatty acids, TNFα, and different cytokines secreted by adipose tissue, are implicated in the pathogenesis of low insulin sensitivity. Hypoxia seems to play a role as well. It is obvious that all these factors develop insulin resistance, and they can also be caused by insulin resistance. Molecular and cellular disturbances may be, therefore both cause and consequence of insulin resistance.

5.2.2 Role of non-esterified fatty acids

In 1963, Randle, *et al.* reported the presence of glucose-fatty acid cycle by using *in vitro* isolated rat heart perfusion experiments (Randle *et al.*, 1963). They found that an increase in the concentration of non-esterified fatty acids in the perfusion medium led to the reduction in glucose uptake and utilisation by the myocardium. Later, they generalized these observations to the skeletal muscles, and suggested that an excess of non-esterified fatty acids may contribute to decrease the glucose utilisation *in vivo* and develop type 2 diabetes (Randle *et al.*, 1988). Subsequent studies in humans have confirmed that intravenous administration of the non-esterified fatty acids inhibits glucose uptake in the muscle in a dose-dependent manner (Roden *et al.*, 1996; Boden *et al.*, 1994). In contrast, the reduced non-esterified fatty acids concentration stimulated the utilisation of glucose (Reaven, 1988; Balasse and Ooms, 1973) and decreased hyperinsulinemia in patients with type 2 diabetes (Boden *et al.*, 1998). Some years after the discovery of Randle, it was shown that fatty acids derived from the hydrolysis of the intracellular triglycerides may also be as important for the glucose-fatty acids cycle, as the fatty acids from the blood stream (Zierler, 1976). The glucose-fatty acid cycle is related to the competition between glucose and fatty acids for their oxidation in muscle and adipose tissue. In muscle, excessive oxidation of the fatty acids leads to an increase in the ATP / ADP and acetyl CoA / CoA ratios, which inhibit glucose metabolism (by inhi biting pyruvate dehydrogenase and the glycolysis) (Randle *et al.*, 1963). Increased non-esterified fatty acids affect insulin action, decreases glucose uptake, glycolysis, glycogen synthesis and carbohydrate oxidation. In addition, excess fatty acids in the muscle would disrupt the insulin signaling mechanism (Shulman, 2000) via the accumulation of acyl CoA and diacylglycerol that activate protein kinase C leading to phosphorylation of serine and threonine residues of IRS (Griffin *et al.*, 1999). The serine/ threonine phosphorylation limits the phosphorylation of tyrosine residues and therefore, leads to inhibition of series of reactions, normally induced by insulin (Wilcox, 2005). The insulin signaling pathway is therefore reduced which leads to reduced glucose uptake. The same type of mechanism exists in the liver, leading to inhibition of key glycolytic enzymes along with the activation of

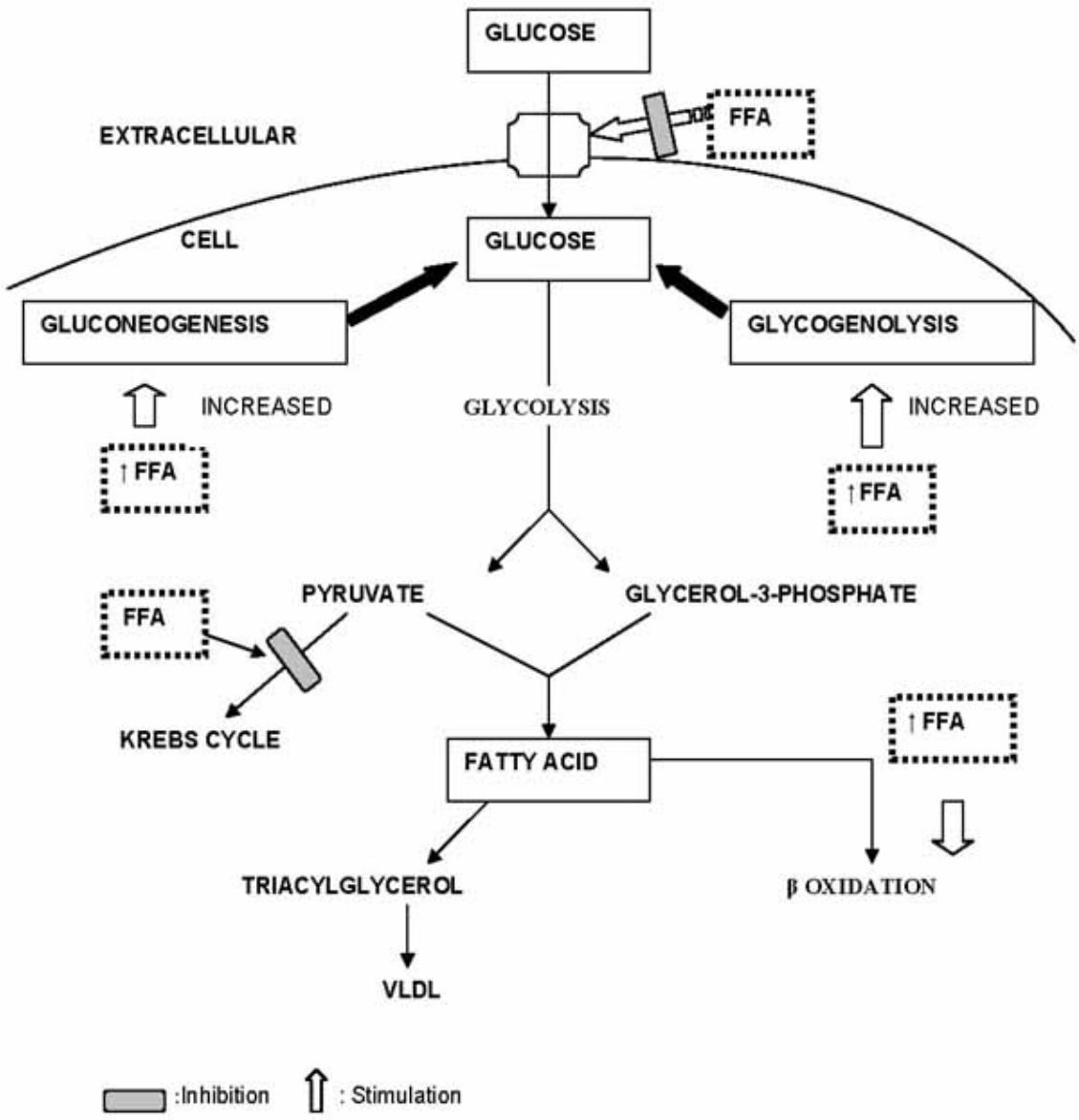

Figure 4: Role of non-esterified fatty acids in insulin resistance.

(Choudhury and Sanyal, 2005).

gluconeogenesis. The phosphoenolpyruvate carboxylase (PEPCK) favours the gluconeogenesis, and important for glucose homeostasis in the body. Figure 4 represents the effect of non-esterified fatty acids in insulin resistance by increasing glycogenolysis, gluconeogenesis and β-oxidation of fatty acids, and reducing glucose uptake by impairing insulin signaling.

In fact, the oxidation of non-esterified fatty acid in the liver provides the necessary co-factors (ATP, NADH, acetyl CoA) for certain key steps of gluconeogenesis, leading to increased hepatic glucose production (Boden, 2003; Girard, 1995). Mice deprived of adipose tissue were very insulin-resistant due to defect in the insulin signaling in the liver and muscle, and they had an accumulation of triglycerides in these tissues (Kim *et al.*, 2000b). Transplantation of the adipose tissues in these mice has been effective against these abnormalities (Gavrilova *et al.*, 2000), suggesting that obesity-induced insulin resistance is induced by alternation (disturbance) in the distribution of non-esterified fatty acids among the adipocytes, liver and muscle. Finally, it was shown that fatty acids were also involved in the regulation of insulin secretion. In fact, when the islets of Langerhans were chronically exposed to high concentration of fatty acids, as in diabetic patients, triglycerides accumulate in the β-cells and inhibit insulin secretion in response to glucose. The lipotoxicity leads to the destruction of cells by increasing apoptosis (Girard, 2000). In short, fatty acid induced insulin resistance could be characterized by impaired insulin signaling, leading to decreased expression of GLUT4 in the muscle cells and increased gluconeogenesis and decrease glycogen synthesis in the liver, shown in figure 5.

The level of non-esterified varies according to feeding intervals and also day time or night time. It is found that nocturnal increase in plasma non-esterified fatty acids may account for obesity-induced insulin resistance (Bergman *et al.*, 2007).

5.3 GLUT4 transporter and low insulin sensitivity

Insulin resistance is characterized by the low sensitivity of the main target tissues (muscle cells, fat cells, and liver cells) to the action of insulin by saturation and decrease in number and/or the effect of specific insulin receptors, and this will induce the hyperinsulinemia ineffective. Muscle accounts for 60-70 % of whole-body insulin mediated glucose uptake, adipose tissue accounts for about 10 % whole-body insulin-dependent glucose uptake, and the liver for about 30 % (Smith, 2002). One of the early events in the

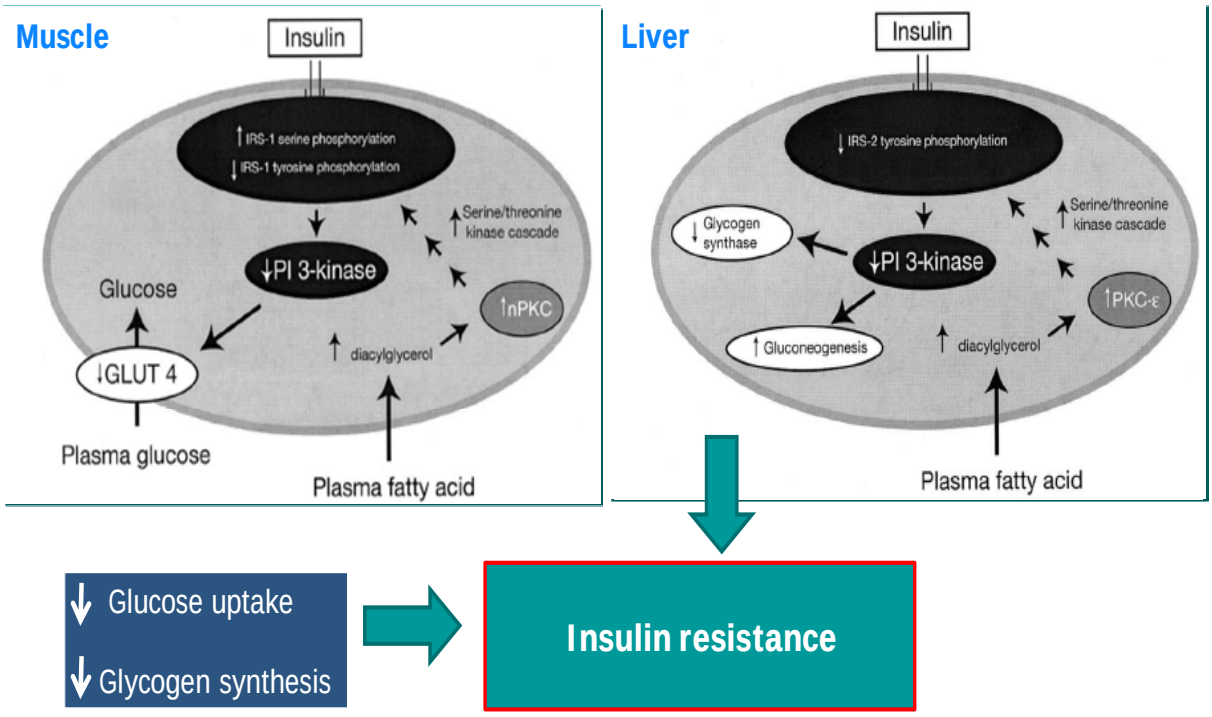

Figure 5: Fatty acid induced insulin resistance.

GLUT4: Glucose transporter 4, IRS: insulin-receptor substrate, PI 3-kinase: phosphatidylinositol 3-kinase, nPKC: novel protein kinase C. Adapted from (Petersen and Shulman, 2006).

development of type 2 diabetes appears to be the inhibition of insulin-mediated GLUT4 redistribution to the cell surface of GLUT4 expressing tissues. Many teams have examined the effect of metabolic diseases, including insulin resistance, on the expression of GLUT4. In fact, the tissue-specific GLUT4 has profound effects in other tissues. Mice with muscle-specific GLUT4 deficiency display decreased insulin responsiveness in adipose tissue and liver (Zisman *et al.*, 2000), and adipose-tissue specific GLUT4 depletion exhibited muscle and liver insulin resistance (Abel *et al.*, 2001).In a recent clinical study, Type 2 diabetic patients with severe insulin resistance showed reduced GLUT4 expression in skeletal muscle and adipose tissue (Kampmann *et al.*, 2011). The treatment with streptomycin, which destroys the pancreatic cells in rats, leading to diabetes is accompanied in adipose tissue or adipocytes in culture by a decrease in mRNA and GLUT4 protein (Berger *et al.*, 1989; Sivitz *et al.*, 1989). The same findings were observed in the adipocytes of diabetic patients (Garvey *et al.*, 1989). The overexpression of GLUT4 in the adipose tissue of muscle-specific GLUT4 deficient mice overcomes the glucose intolerance and diabetes (Carvalho *et al.*, 2005). Moreover, it appears that leptin administration to the insulin resistant rats improves insulin sensitivity in muscles by normalizing the concentration of GLUT4 transporters (Yaspelkis *et al.*, 2001). However, insulin resistance in the muscle is not always associated with lower levels of GLUT4 proteins. In a recent study, liver cirrhosis patients had lower insulin sensitivity index than normal control group, but unchanged contents of GLUT4 protein and mRNA (Shan *et al.*, 2011). Certain genetically obese animal models (Friedman *et al.*, 1990; Koranyi *et al.*, 1990) and diabetic patients (Lund *et al.*, 1993) have normal concentration of GLUT4 in the muscles but have impairment in the translocation of glucose transporters. The insulin resistance results from an alteration in the insulin signaling pathway between the insulin receptor and GLUT4 vesicles (Okamoto *et al.*, 1992). The inhibition of GLUT4 translocation is one of the most important reasons for poor glucose uptake. Translocation is the process by which GLUT4-vesicles move to and fuse with the plasma membrane under insulin stimulation. Malfunctioning of GLUT4-vesicles translocation can lead to insulin resistance (Peck *et al.*, 2009). In addition to the fat level, the main cause of insulin resistance would be decreased GLUT4 protein (Ezaki *et al.*, 1992), via a decrease in gene transcription (Gerrits *et al.*, 1993).

5.4 Adipokines and low insulin sensitivity

5.4.1 TNFα, obesity and insulin resistance

TNFα has been proposed a link between adiposity and the development of insulin resistance. In the mid 90s, Hotamisligil *et al.* (1993, 1995) had reported higher levels of mRNA of TNFα in the adipose tissue in obese animals and individuals than healthy subjects. These high amounts of mRNA of TNFα were also positively correlated with the degree of obesity or hyperinsulinemia, and decreased with weight loss (Kern *et al.*, 1995). Further studies revealed that the concentration of plasma TNFα could be correlated with the abdominal adiposity. TNFα is an important contributor to systemic insulin resistance by impeding insulin's actions in liver and skeletal muscle (Hotamisligil and Erbay, 2008). Other studies have shown the contribution of the TNFα for the inhibition of insulin stimulated glucose uptake in the cell culture of adipocytes (Hotamisligil *et al.*, 1994b) or induction of insulin resistance in the rodents (Lang *et al.*, 1992). In addition, the neutralization of the TNFα improves insulin sensitivity and reduces hyperinsulinemia in fa/fa Zucker rats (Hotamisligil *et al.*, 1994a), and decreased IL-6 expression but no beneficial effects on insulin sensitivity in humans (Rosenvinge *et al.*, 2007). In fact, the absence of TNFα or its receptors significantly increases insulin sensitivity in mouse model of obesity (Uysal *et al.*, 1997). However, this effect has not been demonstrated in humans; the administration of a TNFα neutralizing antibodies for four weeks in obese type 2 diabetes patients did not improve insulin sensitivity (Ofei *et al.*, 1996). The molecular mechanism by which TNFα induces insulin resistance has been investigated in many studies. TNFα deficient mice exhibited lower plasma non-esterified fatty acids and triglycerides levels than wild-type animals (Uysal *et al.*, 1997). The role of TNFα could be indirect, by increasing the concentration of non-esterified fatty acids. However, the progression of insulin resistance in rats fed with a high-fat diet is closely related to plasma non-esterified fatty acids rather than TNFα and IL-6 levels (Jiao *et al.*, 2008). It has been reported that TNFα inhibits the expression of genes encoding enzymes for the regulation of fatty acids uptake or lipogenesis, such as LPL and acetyl CoA carboxylase (Cheung *et al.*, 1998; Torti *et al.*, 1985), and stimulates adipocytic lipolysis. The TNFα could also induce insulin resistance directly, by inhibiting the tyrosine kinase activity or the insulin receptor or by blocking the signaling pathway. Many studies have shown that chronic exposure of the cell culture of adipocytes to TNFα resulted in the inhibition of autophosphorylation of the insulin receptor and its major substrate, IRS-1 (Guo and Donner,

1996; Hotamisligil *et al.*, 1994b, 1996). Moreover, *in vitro* study has showed that the IκB kinase β (IKKβ) phosphorylates serine in response to the TNFα (Lee *et al.*, 2004; Sakurai *et al.*, 1999). TNFα also blocks the glucose uptake by inhibiting the gene expression of glucose transporter, GLUT4 (Stephens and Pekala, 1991). Moreover, TNFα exerts deleterious effects on the functions of β-cells of the pancreas by increasing their sensitivity to the glucotoxic and lipotoxic effects through the production of free radicals (Bolaffi *et al.*, 1994). The TNFα may indirectly affect thermogenesis and food intake (Grunfeld *et al.*, 1996), probably via an action of leptin or IL-1. TNFα acts in different target tissues, such as liver, skeletal muscle and adipose tissue and its role in metabolic syndrome can be little doubtful. However, it is not clear whether the increased level of TNFα is the cause or the consequence of obesity-induced insulin resistance. The deleterious effects of TNFα occur when the animal starts to develop obesity, while the overproduction of TNFα by the adipocytes of the obese individual inhibits the triglycerides synthesis in the adipocytes, limiting adipocytic hypertrophy.

5.4.2 Adiponectin, insulin resistance and obesity

As opposed to other known adipocytokines, which are stimulated in the obesity, the expression of the adiponectin in the adipose tissue and its plasma concentration are reduced in overweight and obese subjects. This has been observed in different animal models of obesity, such as ob/ob leptin-deficient mice (Hu *et al.*, 1996), db/db leptin-resistant mice (Yamauchi *et al.*, 2001), very high-fat diet fed mice, obese humans (Arita *et al.*, 1999), and rhesus monkeys spontaneously induced obesity and type 2 diabetes (Hotta *et al.*, 2001). In humans, it has been shown that the plasma concentration of adiponectin was negatively correlated with body mass index and basal insulinemia (Weyer *et al.*, 2001), triglyceridemia (Comuzzie *et al.*, 2001), and insulin resistance and metabolic syndrome (Yatagai *et al.*, 2003). Adiponectin is closely associated with visceral fat than with subcutaneous fat (Cnop *et al.*, 2003). However, according to the knowledge available about the metabolism of lipoproteins associated with obesity, adiponectin was positively correlated with the plasma level of HDL-cholesterol, and negatively associated with plasma triglycerides and apo B100 (Schulze *et al.*, 2005).

Moreover, weight loss due to a low calorie diet and surgical treatments to reduce body fat have increased the concentration of adiponectin (Monzillo *et al.*, 2003). The findings made in the obese patients or in the animal models of obesity suggest that the adiponectin concentrations were inversely correlated to glycemia (Hotta *et al.*, 2001). An epidemiological

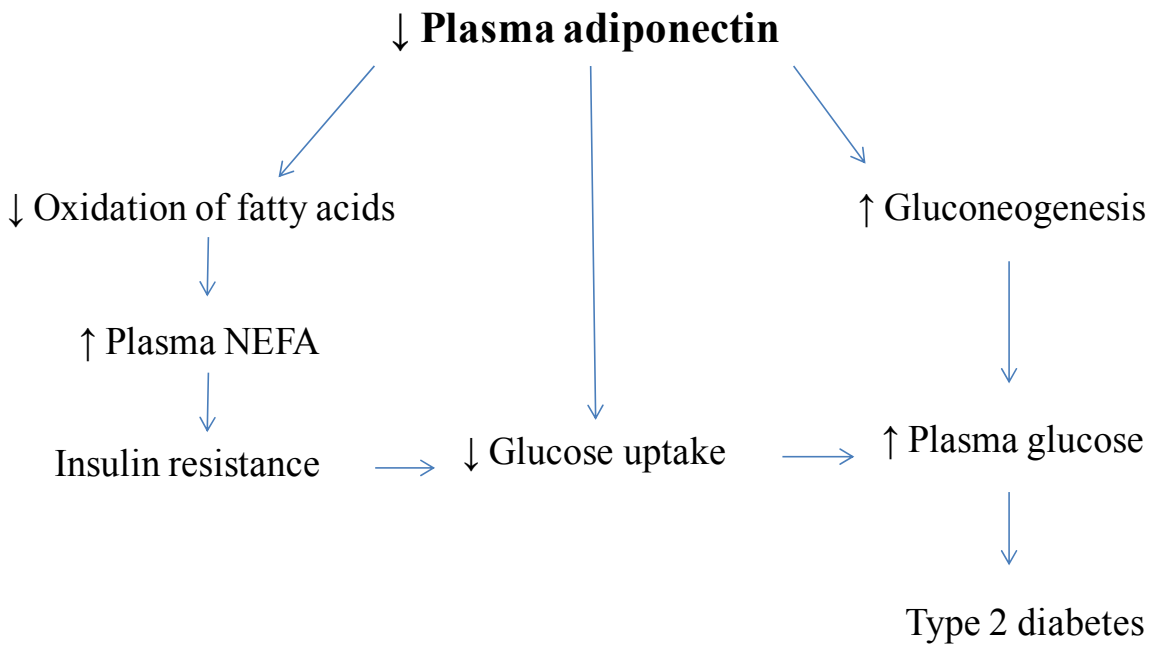

Figure 6: Schematic diagram of mechanism of adiponectin to develop type 2 diabetes.

↓: decrease, ↑: increase, NEFA: non-esterified fatty acids. (Sheng and Yang, 2008)

study has reported that low plasma adiponectin in middle-aged individuals is a predictor for the development of impaired glucose regulation (Jalovaara *et al.*, 2008). By taking into account the multiple actions of adiponectin on the metabolism of carbohydrates and fats, and due to the modulation of its secretion by insulin, the close relationship between insulin resistance and adiponectin is not surprising. In pathological conditions of insulin resistance in type 2 diabetes and obesity, the plasma adiponectin is decreased. Hypoadiponectinemia causes a decrease of fatty acid oxidation that increases non-esterified fatty acids and leads to insulin resistance and ultimately type 2 diabetes (Sheng and Yang, 2008) as in figure 6. Clinical studies have reported an improvement in the insulin sensitivity resulted in an increase of plasma adiponectin concentration (Stefan *et al.*, 2002). Moreover, the insulin-sensitizing effects of the anti-diabetic agents, such as PPARγ agonists, are accompanied by an increase in the adiponectin. However, the increase in plasma adiponectin as an effect of changes in life style in obese children was not necessarily associated with changes in body weight (Cambuli *et al.*, 2008). Adiponectin is one of the adipokines with favourable effects on insulin sensitivity and cardiovascular function, and has been proposed as a promising therapeutic target for combating obesity-associated disorders (Berg and Scherer, 2005). Adiponectin administration in the lipodystrophic or obese mice cured their insulin resistance (Yamauchi *et al.*, 2001). The studies on animals have shown that the adiponectin reduces hyperglycemia in different mice models of obesity or type 2 diabetes (Berg *et al.*, 2001). They found that insulinemia was reduced simultaneously with glycemia, suggesting that the hypoglycaemic effect of adiponectin was not associated with the stimulation of insulin secretion, but with an increase in insulin sensitivity. Thus, adiponectin may improve insulin sensitivity by increasing fat oxidation, by acting directly on the signal transmission of insulin at both receptor and post-receptor on the phosphorylation of the IRS-1 and on the protein kinase B (Yamauchi *et al.*, 2001). Hypoadiponectinemia can cause decreased glucose uptake in skeletal muscle cells and increased gluconeogenesis in hepatic cells, leading to hyperglycemia (Sheng and Yang, 2008). Adiponectin inhibits the gluconeogenesis by reducing the expression of enzymes involved (Combs *et al.*, 2001), and it also inhibits the TNFα signaling in the adipose tissue in the same manner as in the macrophages or the endothelial cells. The variation in the plasma levels of adiponectin is closely related to insulin sensitivity in humans, and lower adiponectin level appears to precede the onset of insulin resistance. This could 'predict' the progression to type 2 diabetes (Stefan *et al.*, 2002).

5.4.3 Leptin, obesity and insulin resistance

Many publications have revealed that obese individuals are often hyperleptinemic and become almost leptin resistant. Relatively low leptin levels were found in Pima Indians prone to weight gain or obesity (Ravussin *et al.*, 1997). This leptinemia predisposes an individual to the development of obesity. In obesity, a decrease in sympathetic nervous activity also appears that could be related to the lowered leptinemia and/or leptin resistance (Ravussin *et al.*, 1997). On the other hand, a study (of nearly 500 Americans of Japanese origin subjects) showed that the accumulation of fat could rather be preceded by increased leptinemia (Chessler *et al.*, 1998). The insulin resistance could contribute to the variability in leptin levels in patients with a similar body fat (Maffei *et al.*, 1995). However, it is possible that other factors affect leptin; therefore the increase of leptin could vary prior to the weight gain. The concentration of leptin is positively correlated with total weight of adipose tissue (Fernandez-Galaz *et al.*, 2002; Ahima and Flier, 2000). It has also been shown that obese women with no family history of obesity, the predictive value of leptinemia is better (Lindroos *et al.*, 1998). If increase in the adipose tissue mass contributes a large share of hyperleptinemia in the obese, the intervention of other factors is likely possible. Insulin and cortisol are two stimulators of leptin expression in adipose tissue and may contribute to the hyperleptinemia found in some obese subjects (Wabitsch *et al.*, 1996). In the *fa/fa* rats, a rise in leptinemia occurs during the development of obesity due to progressive hyperinsulinemia (Cusin *et al.*, 1996). But when obesity is well established, a normalization of insulin by fasting does not allow reducing the gene expression of leptin by adipose tissue. Only the stimulatory effect of insulin remains functional in the obese animals. However, it seems that in obese humans, the hyperleptinemia is not always due to hyperinsulinemia (Segal *et al.*, 1996). In addition, the increase in TNFα is linked to that of leptin; it is possible that these increases are dependent on each other and independent of the body fat.

Thus, the hyperleptinemia could be a link between obesity and diabetes (Emilsson *et al.*, 1997; Kieffer *et al.*, 1997). The inability of leptin to inhibit pancreatic secretion of insulin may explain somehow the development of hyperinsulinemia, insulin resistance and the progression to type 2 diabetes. It was suggested that other abnormalities of the mode of action of leptin could contribute to sympathetic and cardiovascular modifications, associated with obesity (Dunbar *et al.*, 1997; Haynes *et al.*, 1997).

Leptin resistance:

Since leptin reduces body weight and food intake, the increase in leptin level along with obesity is interpreted as leptin resistance. Leptin resistance is referred to the reduced ability of leptin to suppress appetite and weight gain, and to promote energy expenditure. It results from the defects in leptin transport into the brain, leptin signaling and/or the hypothalamic neural circuitry that regulates energy homeostasis (Morris and Rui, 2009). In obese individuals, hyperleptinemia may be associated with leptin resistance (Bluher and Mantzoros, 2009). Leptin likely to enters the brain via the blood-brain barrier (Banks, 2008; Hileman *et al.*, 2000), and impaired blood-brain barrier leptin transport during development of the obesity (Levin *et al.*, 2004; Banks and Farrell, 2003), suggest the primary cause of leptin resistance. Obesity attenuates leptin signaling and induces weight gain by genetic and environmental factors (Myers *et al.*, 2010). In the condition of leptin resistance, fat storage capacity is increased and oxidizing rate is diminished. Hyperleptinemia associated with leptin resistance leads to accumulation of lipids in skeletal muscle, heart and pancreas (Kershaw and Flier, 2004).

5.4.4 Other adipokines

Resistin, identified in 2001 (Steppan *et al.*, 2001) and is expressed in the adipocytes and its plasma level increases with obesity and insulin resistance (McTernan *et al.*, 2002). Resistin induced severe hepatic insulin resistance (Rajala *et al.*, 2003) and increased expression of resistin induced insulin resistance in normal rats (Satoh *et al.*, 2004). Thus, resistin appears to link between obesity and insulin resistance, which is confirmed in mice (Steppan *et al.*, 2001), but not in humans (Utzschneider *et al.*, 2005; Meier and Gressner, 2004). Visfatin is recently identified. Its expression in the visceral adipose tissue leads to obesity (Fukuhara *et al.*, 2005). In another study in rats, visfatin improved insulin sensitivity by upregulation of the tyrosine phosphorylation of IRS-1 protein in the liver and adipose tissue, and it had also hypocholesterolemic effects by upregulation of expressions of PPARγ and SREBP-2 in the liver and adipose tissue (Sun *et al.*, 2009). The changes in the obesity-induced insulin resistance are described in figure 7.

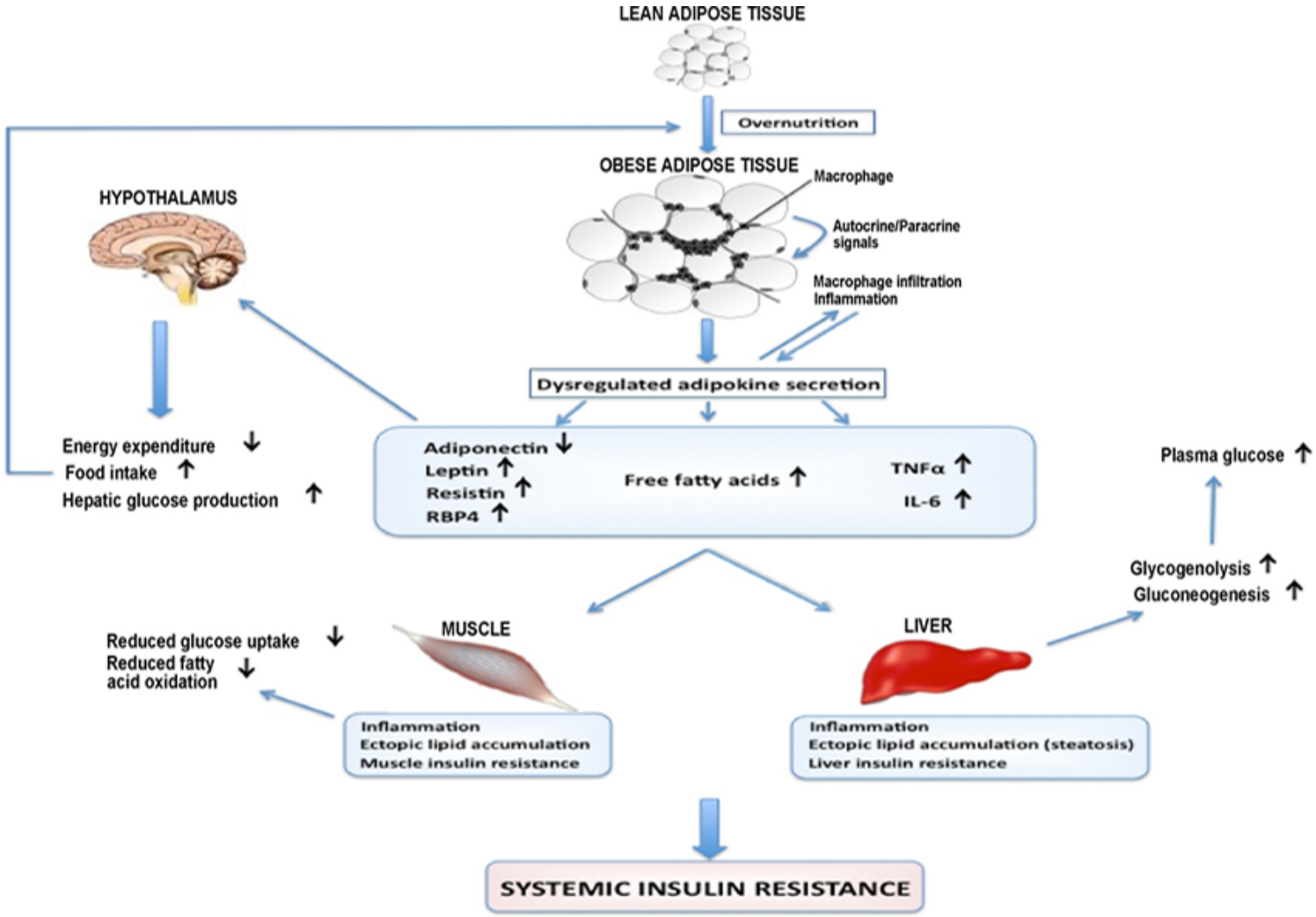

Figure 7: Obesity induced changes in adipokine secretion in development of insulin resistance.

(Galic *et al.*, 2010)

5.5 Nuclear receptors & Transcription factors

5.5.1 Peroxisome Proliferator Activated Receptor (PPAR)

PPAR belong to the subfamily of the superfamily of nuclear receptors, which are activated by fatty acids or fatty acid derivatives (Kletzien *et al.*, 1992). These receptors are transcription factors, whose activity is modulated by interaction with a specific ligand. PPAR are composed of a binding domain to DNA and a domain of interaction with the ligand. They bind to a specific region of DNA located in the promoter region of target genes, called as peroxisome proliferator response element (PPRE). The receptor activation by its ligand results in the association between PPAR and retinoic acid receptor, retinoid X receptor (RXR). This dimer binds to PPRE and causes the activation of transcription of the target gene. Three isoforms of PPAR have been described: PPARα, PPARδ (β) and PPARγ (Lemberger *et al.*, 1996). They mainly differ in their sites of expression and specific role related to the need of the tissues, in which they are present. These factors are involved in regulating many physiological processes, such as reproduction, development (Barak *et al.*, 2002) or even inflammation (Blanquart *et al.*, 2002). Moreover, numerous studies established the importance of these receptors in controlling the cellular proliferation and differentiation in various metabolisms, including lipid and glucose homeostasis (Mangelsdorf *et al.*, 1995). Thus, they have become a major pharmaceutical target and have essential metabolic effects.

5.5.2 PPARα

PPARα is expressed in the tissues with potential catabolic fatty acids, particularly in the liver. However, it is also found in the kidney, muscle, heart and intestine. PPARα activation results in lipid oxidation leading to decrease in plasma triglycerides. Several studies have shown that PPARα directly regulates certain genes involved in the penetration of fatty acids, β-oxidation or even ω-oxidation, such as the gene for FABP (fatty acid binding protein), acyl-CoA oxidase and cytochrome P450 respectively. The studies on PPARα-deficient mice have indeed shown that PPARα was required for the stimulation of these genes, particularly during fasting (Kersten *et al.*, 1999; Leone *et al.*, 1999) and during the action of synthetic ligands, such as fibrates (Aoyama *et al.*, 1998; Peters *et al.*, 1997). Following a period of fasting, these mice showed severe hypoglycemia and increased plasma NEFA concentration, indicating an alteration of the uptake and oxidation of fatty acids resulting from deregulation of genes involved in these mechanisms. Moreover, the PPARα is also involved in the regulation of

lipoprotein metabolism. Studies conducted in humans, have shown that fibrates increase plasma levels of HDL by inducing the expression of genes coding for apolipoproteins AI and AII, the two major apolipoproteins of HDL (Berthou *et al.*, 1996; Vu-Dac *et al.*, 1995; Vu-Dac *et al.*, 1994). In addition, fibrates induce the expression of LPL in the liver, increased lipolysis and the rate of pre β-HDL (Schoonjans *et al.*, 1996b). Finally, by inducing the expression and production of HDL receptors, such as ATP-binding cassette transporter A1 (ABCA1) (Chinetti *et al.*, 2001) and scavenger receptor type 1 (SR-B1) (Ji *et al.*, 1997), the PPARα modulates the reverse transport of cholesterol. PPARα has a direct role in the regulation of gluconeogenesis via stimulation of expression of pyruvate dehydrogenase kinase 4 (Wu *et al.*, 2001), which favours the utilization of pyruvate for gluconeogenesis.

5.5.3 PPARβ

Unlike PPARα and PPARγ, the PPARβ is widely distributed in tissues and is expressed in the small intestine and placenta. The PPARβ (also called as PPARδ) is abundant in muscles (Muoio *et al.*, 2002). The target gene of PPARβ is adipose differentiation-related protein (ADRP), a lipid droplet coating protein. It regulates genes involved in glucose homeostasis and fatty acid synthesis/storage and catabolism (Reilly and Lee, 2008). It may have hypolipidemic role by increasing lipid oxidation in the skeletal muscle (Karpe and Ehrenborg, 2009). It is an important agonist in the treatment of dyslipidemia, and also acts in the differentiation of central nervous system cells (Kersten *et al.*, 2001). In a review, the activation of PPARβ reduces weight gain, increases skeletal muscle metabolism and improves insulin sensitivity in animal models (Reilly and Lee, 2008).

5.5.4 PPARγ

PPARγ is predominantly expressed in the adipocytes, but also in other cell types, such as enterocytes and macrophages (Braissant *et al.*, 1996), or even kidney, liver, muscle and pancreas (Auboeuf *et al.*, 1997). Most of its transcriptional effects have been demonstrated on the genes expressed in the fat cells (Way *et al.*, 2001). The adipocyte is an active cell that participates largely in control of energy homeostasis in the body. It is shown that PPARγ was able to promote adipogenesis in non-adipongenic cells, such as fibroblasts (Tontonoz *et al.*, 1994), and PPARγ2 (one of the three isoforms of PPARγ) favours synthesis of small adipocytes. The role of PPARγ was subsequently confirmed *in vivo* through the development of mouse models (Rosen *et al.*, 1999). PPARγ also regulates the expression of genes involved in the glucose uptake, such as GLUT4 transporter (Young *et al.*, 1995), in the storage and

uptake of lipids, such as LPL, the FATP and acyl-CoA synthase (Martin *et al.*, 1997; Schoonjans *et al.*, 1996a), or even in the mechanisms of energy expenditure, such as uncoupling proteins, including UCP2 and 3 (Aubert *et al.*, 1997). PPARγ also modulates the expression of adipocytokines: it inhibits the expression of the leptin and TNFα (Miles *et al.*, 1997; De Vos *et al.*, 1996) and stimulates the expression of adiponectin (Berg *et al.*, 2001). In addition to these actions on adipose tissue, administration of PPARγ ligands in rodent models, has allowed to observe the actions of this nuclear receptor in other tissues. The PPARγ increases glucose oxidation in muscle and decreases hepatic gluconeogenesis (Way *et al.*, 2001). Few studies on PPARγ activity, particularly in macrophages, have shown that it regulates the transcription of certain HDL receptors, suggesting that PPARγ is also involved in the mechanisms of cholesterol transport (Chawla *et al.*, 2001; Nagy *et al.*, 1998). Several studies revealed that PPARγ activation could protect or restore the function of pancreatic β-cells during the development of type 2 diabetes. It would inhibit the intracellular accumulation of triglycerides and stimulate the expression of the GLUT2 transporter, thus improving glucose uptake into β-cells (Kim *et al.*, 2000a; Shimabukuro *et al.*, 1998).

5.5.5 PPAR, obesity and insulin resistance

The involvement of PPARγ in insulin resistance is now obvious. A large number of studies have shown that PPARγ was the molecular target of pharmacological agents, the thiazolidinediones, improving the insulin sensitivity. This is confirmed by the fact that the discovery of new PPARγ ligands has potentially strong affinity, *in vivo*, for the insulin sensitivity of Zucker diabetic rats (Brown *et al.*, 1999). In addition, mutations of gene for PPARγ have been identified in patients with severe insulin resistance. Clinical studies have also shown an association between the human mutant allele and decreased receptor activity, a lowering of the body mass index, attenuation of obesity, improved insulin sensitivity and a decreased risk of type 2 diabetes (Altshuler *et al.*, 2000; Deeb *et al.*, 1998). The reduction in insulin resistance (or improvement in insulin sensitivity) is via the regulation of key genes in the adipocyte. Thus, PPARγ promotes the flow of fatty acids and triglycerides to the adipose tissue, stimulating the renewal of adipocytes and increases expression of genes, involved in glucose uptake and in the insulin signaling pathway. This results in to potentiate the peripheral glucose utilization. Moreover, in the adipose tissue, it mitigates the effect of cytokines involved in obesity-induced insulin resistance and improves function of pancreatic β-cells. The PPARβ agonists reduce adiposity and improve glucose tolerance and insulin sensitivity in mouse models of obesity (Tanaka *et al.*, 2003). Moreover, PPARβ agonists

increase glucose uptake independent of insulin level via stimulating AMPK-dependent signalling pathway in cultured human muscle cells (Kramer *et al.*, 2007). PPARβ was shown to reduce hepatic glucose secretion in mouse liver by increasing the glucose flux through the pentose phosphate pathway and enhancing fatty acid synthesis (Lee *et al.*, 2006a). In the high-fat diet fed rats and cultured adipocytes, PPARβ agonists increased adiponectin secretion and decreased resistin secretion (Choi *et al.*, 2007).

For the major role of PPARα in the metabolism of lipids and lipoproteins, fibrates have primarily been used in patients with dyslipidemia. However, several studies in the literature have shown that PPARα agonists could also improve insulin sensitivity. Thus, in rat model (Matsui *et al.*, 1997) and in patients with type 2 diabetes, activation of PPARα appears to have a beneficial effect on insulin resistance and reduce adiposity (Guerre-Millo *et al.*, 2000; Ogawa *et al.*, 2000). One study on the physiological and transcriptional responses to activation of PPARα in rodent model of insulin resistance, showed that the gene for the protein tyrosine phosphatase, one of the first molecules involved in signal transmission of the insulin receptor, was regulated by PPARα agonists, suggesting that activation of this receptor could improve the insulin sensitivity (Frederiksen *et al.*, 2003). The study carried out on knockout mice has shown a decrease in gene expression of the enzymes involved in the gluconeogenesis and glycolysis (Xu *et al.*, 1999a). Moreover, PPARα null mice are more insulin-sensitive and are protected from insulin resistance when on a high-fat diet (Guerre-Millo *et al.*, 2001); however, in another study, the insulin sensitivity does not show any change (Haluzik *et al.*, 2004).

5.5.6 Sterol regulatory element binding proteins (SREBP)

Sterol regulatory element binding proteins are nuclear transcription factors, expressed in most tissues but with a high level in macrophages, liver, adipose tissue and brain. The SREBP family includes three transcription factors, SREPB-1a, SREBP-1c and SREBP-2. The SREBP-1a and SREBP-1c are encoded by the same gene, but they possess different promoters and transcriptional start sites, and SREBP-2 is expressed from a separate gene (Horton *et al.*, 2002). The SREBP-1c regulates expression of the genes involved in fatty acid metabolism such as FAS, stearoyl-CoA desaturase (for conversion of stearate to oleate) (Horton *et al.*, 2002), and also the genes involved in cholesterol metabolism such as ABCG1 (Ecker *et al.*, 2007). The SREBP-1c is expressed in liver cells, and the activity and expression of SREBP-1c are regulated by different fatty acids. The SREBP-1c regulates the enzymes

involved in fatty acid and triglycerides synthesis, including acetyl-CoA carboxylase (ACC), FAS, stearoyl-CoA desaturase (SCD) and glyceraldehyde-3-phosphate acyltransferase (GPAT) (Horton *et al.*, 1998a, 2002; Shimomura *et al.*, 1998). PUFA inhibit the transcription of the genes involved in lipogenesis by inhibiting the expression of SREBP-1c. Unlike PUFA, saturated and monounsaturated fatty acids have no effect on SREBP-1c. SREBP-2 preferentially regulates expression of the enzymes involved in the cholesterol biosynthesis (Horton *et al.*, 1998b, 2003).

The acetyl-CoA carboxylase (ACC) transforms the product of glycolysis, acetyl-CoA into malonyl-CoA, and fatty acid synthase (FAS) then converts malonyl-CoA into fatty acids (Marin *et al.*, 1992). The genes for FAS and ACC are under the control of SREBP (Osborne, 2000; Sul *et al.*, 2000).

6 Animal models

6.1 Interest

High prevalence of obesity and insulin resistance in humans accounts for the need of an animal model. There are numerous limits to study the problems associated with obesity and insulin resistance in humans. Important ones are homogeneity of patient groups, no history of overweight, no control on diet, ethical obstacles and methods to develop insulin resistance and to correct it. Therefore, an appropriate animal model is greatly needed for studies on the pathogenesis and therapy of these obesity-related metabolic disorders.

The use of animal models allows researchers to investigate progression of disease in a physiologically relevant state to humans. From the scientific and ethical point of view, not only the phenotype, but also the pathogenesis of the animal model mimics the human disease examined. Moreover, animal models have been used to test preventive and therapeutic agents, allowing large scale clinical trials to be based upon the data generated from animal models, especially rodents. Moreover, the animals enable researchers to obtain answers in a short duration of time, since 10 days in the life of a rat are approximately 1 year in human life, when comparing changes in body weight. Experimental models are important to study specific causes and effects of obesity and its related disorders, but the study and extrapolation to human is limited, since mostly human obesity is the result of specific characteristics of individuals and interactions with the environment.

The scientific literature is full of experimental models of obesity and/or insulin resistance (Wang and Liao, 2012; Costa *et al.*, 2011; Zhao *et al.*, 2008; Afonso *et al.*, 2007; Kavanagh *et al.*, 2007; Gayet *et al.*, 2004). These models can be spontaneous, obtained after selective breeding or as a result of various nutritional or genetic experimental manipulations. The rodents (rat, mouse, hamster, etc.) have been used as animal models; however, some other animal species (rabbit, dog, monkey, etc.) have been also studied. The occurrence and severity of these disorders varies according to rodent species, strain and diet. Most commonly, the obesity can be induced in animals by genetic and nutritional changes, which includes feeding on hypercaloric diets and genetic manipulations for obesity.

6.2 Genetic models

A number of genes have been identified that, after mutation, can develop the condition of obesity. The scientific interest of the monogenic models is multiple. In recent years, these models led to the identification of new proteins, including their malfunctioning or abnormal secretion to increase fat depots. The study of these models and the characterization of the products formed from the mutated genes help in the identification of new pathophysiological processes, which are potentially involved for the induction of obesity and its complications. However, these models of simple monogenic obesity have certain disadvantages, including their reductionist aspect. In addition, the epidemiological studies conducted in humans show that the share of genetics in the obesity is not dominant (about 25 %), even if it is a significant risk factor (Bouchard, 1991). The environment in which the genetic mutation is expressed, influence potentially the phenotype of obesity and obesity-related metabolic disorders. The first five monogenic models of obesity were diabetic (*db/db*), obese (*ob/ob*), Tubby (tub), 'Agouti' (A^y) and fat (fat) (Von Diemen *et al.*, 2006).

The influence of genetics for the induction of obesity and its related disorders in humans cannot be bound to few mutations, and it is obviously polygenic. Other genetically complex and heterogeneous models are necessary to determine the role of genetics in comparison to the environment, including nutrition in the development of this syndrome. The great similarity and homology between genomes of rodents and humans make these animal models a major tool to study obesity and its related disorders.

The use of genetic models to study obesity increased in the 1990s because of cloning and identification of different genes causing obesity. The development of mapping by

'quantitative trait loci' (QTL) (Pomp, 1997) has increased the genetic importance of these models, as it allows to locate the gene controlling the phenotype of interest. Moreover, genetically modified or knockout animals have been produced to study new genes that can influence the rate of obesity. The knowledge of the chromosomal regions of interest and synteny in human or animal make it possible to determine the loci in humans potentially associated with, for example, a genetic predisposition to the metabolic syndrome. In addition to their genetic advantages, these models are widely used for the study of metabolic and endocrine effects of obesity.

6.3 High-fat and/or high-fructose nutritional models

The increased prevalence of insulin resistance is linked to the western diet and reduced physical activity. The diet-induced animal models over-consume a diet and the excess calories lead to overweight state and eventually obesity. Either ad libitum or administered by gavage, the most commonly used experimental diets are based on high-fat, high-carbohydrate, mixed or 'cafeteria' (a mixture of cookies, candy, cheese and processed meats). These dietary-based models are very close to the most common cause of human obesity. In fact, this is the simplest model of obesity-induction, and possibly the one that closely resembles the reality of obesity in humans, since the increase in the dietary energy is the major cause of the energy imbalance leading to obesity in humans. The ultimate experimental outcome of these diets is generally associated with obesity, insulin resistance (or type 2 diabetes), and sometimes hyperlipidemia and hypertension, providing a good reflection of the metabolic syndrome of humans. Obesity can be reversed by nutritional manipulation, i.e., by return to balanced diet. This finding helped in numerous studies associated with metabolic disorders along with their reversibility. The rodents have been used as nutritional models to study in detail the pathophysiological effects of diet on obesity. Sometimes, rabbits, chickens, dogs, pigs, dogs and monkeys have also been used as such models.

Different diets (mostly high-fat) have been used to induce obesity in rodent models of obesity. Like humans, different rat strains show different degrees of susceptibility to the development of obesity, and composition of the diet may influence the results. Therefore, inconsistent findings of the development of obesity and low insulin sensitivity have been obtained in different studies. High-fat diet may develop insulin resistance and dyslipidemia in rats, though with large variability in their severity. It does not seem possible to define an ideal high-fat diet with exact diet composition. Different high-fat diets have been used with 20 % to

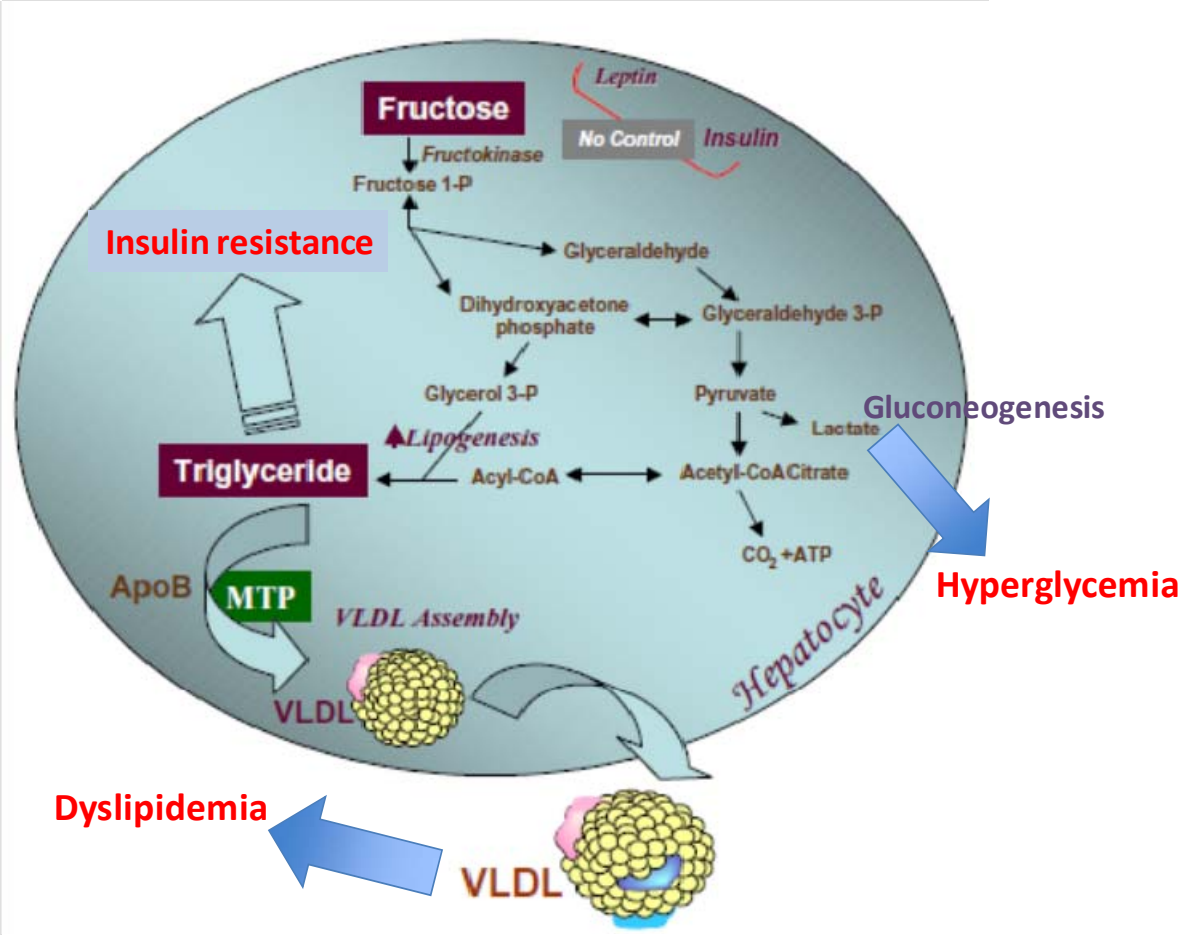

Figure 8: Hepatic fructose metabolism (lipogenic pathway).

(Basciano *et al.*, 2005)

70 % energy as fat, and the basic fat source varies between animal-derived fats (lard, beef-tallow) and plant oils (corn or safflower oil). High-fat diets may (Naderali and Williams, 2003) or may not (Morin *et al.*, 1997) induce dyslipidemia (high non-esterified fatty acids and triglycerides) within days or weeks. The development of insulin resistance in the high-fat fed model is dependent on the type of the dietary fat. Increase in body weight and the accumulation of intra-abdominal fat occur soon thereafter (Mittendorfer *et al.*, 1998; Morin *et al.*, 1997; Akiyama *et al.*, 1996). Diets rich in saturated fats take weeks to induce glucose intolerance and insulin resistance, which is not followed by hyperinsulinemia. In fact, hyposecretion of insulin has been observed (Ahren *et al.*, 1999), in contrast to the situation in obese Zucker rats and in human obesity (Moller and Kaufman, 2005; van Zwieten, 1999). Increased plasma insulin level has been reported after 4 weeks in an experiment using gastric cannulation (Akiyama *et al.*, 1996) but plasma insulin, glucose and leptin were unchanged after 12 weeks in a study using oral high-fat feeding. However, leptin and insulin levels were increased after 32 weeks of high-fat feeding in that study (Tulipano *et al.*, 2004). Thus, high-fat feeding induces obesity after a relatively-short period of time, whereas the associated syndrome of obesity takes longer time to develop, if it develops. In a review, Buettner, *et al.* (2007) reported normoglycemia, slight hyperglycemia and type 2 diabetes development with the high-fat diet. Defects in insulin signaling in the peripheral tissues are reported during a high-fat diet (Bell *et al.*, 2000). High-fat diet reduced the IRS-1 and IRS-2 proteins in the adipose tissue, however in the liver, IRS-1 and IRS-2 proteins and their phosphorylations were not affected, and phosphatidylinositol 3-kinase activity was increased (Taouis *et al.*, 2002; Anai *et al.*, 1999; Yaqoob *et al.*, 1995). The expression of GLUT4 in the adipose tissue of rats presented variable pattern with different types and stages of obesity. In high-fat induced obese rats GLUT4 levels in the adipose tissue are depressed (Sevilla *et al.*, 1997; Pedersen *et al.*, 1991), while in genetically obese Zucker *fa/fa* rats, GLUT4 levels are increased at 5 and 10 weeks of age, but decreased at 20 weeks of age (Pedersen *et al.*, 1992). The major difference was the levels of postprandial insulinemia, which reduced in high-fat fed rats and increased in the genetically obese rats at all ages. Some other studies have reported the hyperinsulinemic states associated with increased GLUT4 levels in the adipose tissue (Kahn *et al.*, 1989; Kahn *et al.*, 1987). The gene expression of leptin was increased in the adipose tissue of high-fat diet fed rats (Rousseau *et al.*, 1997; Masuzaki *et al.*, 1995a).

Fructose is naturally occurring monosaccharide, an epimer of glucose, found in fruits and vegetables and it makes up about 50 % of honey and sucrose. For past few decades,

fructose consumption has increased several folds above the quantity found in natural foods, because of the extensive use of high-fructose corn syrup and corn-derived crystalline fructose in pharmaceuticals and mainstream foods (carbonated beverages, baked food, canned fruits, jams and dairy products). High intake of refined carbohydrates may also contribute to the risk of developing insulin resistance. The high-fructose diet induces metabolic syndrome by reducing insulin action and increasing dyslipidemia. In animal models, the high-fructose diets contribute to a metabolic disturbance leading to insulin resistance. The fructose fed rats produce insulin resistance and dyslipidemia, and growing evidence suggests the role of inflammation in this model (Kannappan *et al.*, 2010; Delbosc *et al.*, 2005). The high-fructose diet fed rats provide an animal model of insulin resistance associated with hyperinsulinemia and hypertriglyceridemia (Olatunji and Soladoye, 2007; Okada *et al.*, 2000). High-fructose (≥ 60 % by weight) diet increased total cholesterol / HDL-cholesterol ratio along with impaired glucose (Okada *et al.*, 2000) and increased plasma glucose, insulin resistance (assessed by the homeostasis model assessment, HOMA), and total cholesterol (Olatunji and Soladoye, 2007). The production of non-esterified fatty acids through hepatic fructose metabolism could play important role for development of insulin resistance and dyslipidemia. Fructose metabolism can provide glycerol and acyl parts for hepatic lipogenesis, leading to increased hepatic triglycerides production and ultimately increased VLDL concentration, as shown in figure 8. Leptin and insulin has no control on the fructokinase, a key enzyme in fructose metabolism for the phosphorylation of fructose. Increased levels of triglycerides and NEFA could lead to increased serine/threonine phosphorylation of IRS-1, and hence to reduce insulin signaling (Tappy and Lê, 2010).

7 Eicosapentaenoic acid (EPA) & Citrus flavonoids

Due to increased prevalence of cardiovascular diseases, various pharmacological molecules were used to reduce the obesity, insulin resistance and the cardiovascular risk factors, including dyslipidemia. The role of nutritional substances in the management of obesity and insulin sensitivity requires further elaboration; however available information suggests some substances positively influence insulin sensitivity. Many nutritional and therapeutic agents including polyphenols, statins, nicotinic acid, fibrates, flavonoids, omega-3 polyunsaturated fatty acid (mostly in the form of fish oil) have been studied for their anti-inflammatory and hypolipidemic effects. We focussed on the supplementation of eicosapentaenoic acid (EPA), and the supplementation of flavanones (hesperidin and

naringin) in the rats fed with the high-fat or high-fructose diet to study their effects on obesity, insulin sensitivity and dyslipidemia.

7.1 Eicosapentaenoic acid (EPA)

7.1.1 General aspects

Polyunsaturated fatty acids (PUFA) are the long-chain carboxylic acids having carbon-carbon double bonds. PUFA are composed of four families. Palmitoleic (n-7) and oleic (n-9) families are non-essential while linoleic (omega-6) and linolenic (omega-3) families are essential, as these cannot be synthesized *de novo* by animal cells and hence must be taken in the diet. All the members of essential fatty acids derive by alternate desaturation-elongation reactions from their respective precursors, α-linolenic acid (LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6). Long-chain PUFA are essential for foetus growth and brain development (EPA and DHA increase growth rate). LNA is important, especially in the diet of pregnant and nursing women as a source of DHA for neural and visual tissues during development of the embryo and the neonate (Kinsella, 1991).

Essential fatty acids provide not only energy but also the important structural component, such as cell membrane and promote normal growth. These must be provided to tissues in appropriate and equilibrated amounts. Their dietary intake is the most critical factor likely to affect their availability to the tissues. An intake of 3 % to 6 % of essential fatty acids is generally recommended for the better biological functions (Bezard *et al.*, 1994). The ratio of intake between omega-6 and omega-3 polyunsaturated fatty acids (PUFA) ranges from 4-6:1 (Neuringer *et al.*, 1988) or 2-4:1 is recommended (Simopoulos, 2002). Recommended omega-3 PUFA requirement is 2 g/day; however its consumption does not exceed 0.8 g/day.

Eicosapentaenoic acid (EPA), a PUFA found in fish oil, belongs to omega-3 polyunsaturated a-linolenic acid family. It is an important component of cell membrane and is essential for normal growth and development. Chemically, EPA is cis-5,8,11,14,17-eicosapentaenoic acid (structure is shown in the figure 9), and therapeutically it has been categorized as a hypolipidemic, antiplatelet agent and cardioprotectant. EPA has been found to exert beneficial effects in obesity and insulin resistance.

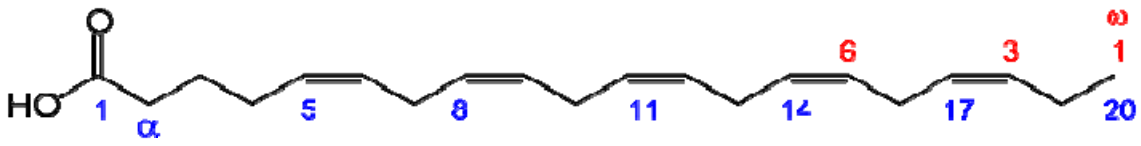

Figure 9: Structure of eicosapentaenoic acid (EPA).

7.1.2 Effect on obesity, IR and dyslipidemia

The omega-3 PUFA could play a key role in the prevention and management of several diseases such as coronary heart disease, type 2 diabetes, insulin resistance, dyslipidemia, and hypertension. There is a growing body of evidence suggesting an inverse association between omega-3 PUFA and insulin resistance. The effects of fish oil on human body composition and insulin resistance vary depending on the health status of the individuals and the nature of the study (Anderson and Ma, 2009). As a result, it has become difficult to evaluate the effects of omega-3 PUFA on obesity and insulin resistance related parameters. Fish oil found to decrease body fat mass and concurrently stimulate lipid oxidation in healthy volunteers (Couet *et al.*, 1997), and 12-week EPA+DHA supplementation reduced inflammatory markers in overweight women (Browning *et al.*, 2007), which may be involved in the insulin-sensitizing effect of omega-3 PUFA. However, in type 2 diabetes patients, no correlation was identified between dietary EPA and DHA, and insulin resistance, measured by HOMA (Kusunoki *et al.*, 2007). The omega-3 PUFA potentially contribute to favourable effects on obesity and insulin resistance, possibly by increasing insulin sensitivity, decreasing inflammatory mediators, or altering lipid metabolism in lean subjects, however, their beneficiary effects does not seem to extend to obese or type 2 diabetic individuals. In other words, the omega-3 PUFA may have preventive role against obesity and insulin resistance, but not a curative role in humans.

Many researchers have reported that EPA improves insulin sensitivity in several rat models of obesity and diabetes (Nobukata *et al.*, 2000; Mori *et al.*, 1997, 1999). EPA-ethyl ester (0.3 g/kg/day) improved insulin sensitivity (assessed by higher glucose infusion rate in the euglycemic hyperinsulinemic clamp test) in rats after 17-18 weeks (Mori *et al.*, 1997). EPA and DHA prevented alloxan-induced diabetes (Suresh and Das, 2003) and were reported to be more effective than α-linolenic acid at lowering glycemia and insulinemia, and improving insulin sensitivity (Andersen *et al.*, 2008). Moreover, fish oil corrected the inhibitory effect of a high-sucrose diet on the antilipolytic action of insulin, reduced the *in vitro*-enhanced basal lipolysis and normalized isoproterenol-stimulated lipolysis in the hypertriglyceridemic and insulin resistant rats (Soria *et al.*, 2002). The EPA+DHA increased systemic insulin-sensitizing adiponectin (Gonzalez-Periz *et al.*, 2009; Perez-Matute *et al.*, 2007; Flachs *et al.*, 2006) and improved response to a glucose load (Ikemoto *et al.*, 1996) in high-fat diet fed mice. However, Mustad, *et al.* (2006) reported no effect of EPA+DHA on insulin sensitivity or basal insulinemia in male ob/ob mice. Fish oil feeding attenuated

peripheral insulin resistance, hyperglycemia, and fat pad mass (Pighin *et al.*, 2003; Soria *et al.*, 2002) and also increased insulin-stimulated glucose transport (Peyron-Caso *et al.*, 2002) in sucrose-diet fed rats.

EPA as well as DHA are preferentially incorporate into the cell membranes, thus increase membrane fluidity that, in turn, has been reported to increase the number of insulin receptors on the cell membrane and also their insulin binding affinity (Das, 2005a). The upregulation of the insulin receptors favourably modifies the glycemic response and decreases insulin resistance of an individual that could potentially delay or prevent onset of type 2 diabetes.

The omega-3 PUFA serve as key mediators of gene expression, working via peroxisome proliferator-activated receptors (PPAR) controlling the expression of genes involved in lipid and glucose metabolism (Jump, 2002). EPA and DHA act as ligands for PPAR, and hence may have anti-diabetic effect. However, EPA seems to be more potent ligand than DHA (Pawar and Jump, 2003). In rats, dietary fish oil increased the fatty acid capacity of their liver through its ability to function as ligand activator to PPARα, and hence induces the transcription of several gene-coding proteins affiliated with fatty acid oxidation (Neschen *et al.*, 2002). PPARγ has been implicated in the onset of insulin resistance (Park *et al.*, 1997). The omega-3 PUFA (especially EPA) activates the expression of PPARγ2 (PPARγ isoform) in the adipose tissue. PPARγ2 activation increases number of genes involved in lipogenesis and fatty acid transport, fatty acid storage and fatty acid oxidation in the adipose tissue (Rousseau *et al.*, 1997). Stimulation of PPARγ inhibits the expression of cytokines, which promote insulin resistance, along with increasing adiponectin (Gross and Staels, 2007). It is possible that EPA increases the expression and translocation of GLUT1 and GLUT4, thus facilitating glucose uptake in adipose tissue and muscle. EPA-ethyl ester supplemented rats showed significant higher GLUT4 mRNA in the skeletal muscle, compared with the control rats (Mori *et al.*, 1997). However, in high-sucrose fed rats supplemented with fish oil, an increase of insulin-stimulated glucose transport in adipocytes is reported to be associated with an increase of GLUT4 protein and mRNA levels but no change in glycemia and no effect on muscle GLUT4 protein level (Peyron-Caso *et al.*, 2002). EPA administration enhances adipocytes ability to secrete adiponectin, which could improve insulin sensitivity. An increase in adiponectin production has been also associated with an up-regulation of the transcriptional factor PPARγ (Lorente-Cebrian *et al.*, 2006). The increase in adiponectin results in decreasing plasma glucose levels by improving insulin sensitivity and decreasing hepatic glucose

production (Yamauchi *et al.*, 2001). EPA and DHA have been shown *in vivo* to be more potent activators of PPARα than other fatty acids (Schmitz and Ecker, 2008), suggesting a preferential of EPA and DHA in PPAR pathway.

Increased intake of omega-3 PUFA decreases the production of inflammatory factors, and hence decreases the susceptibility of an individual to develop chronic inflammatory disorders. EPA+DHA supplementation for 12 weeks reduced inflammatory markers in overweight women (Browning *et al.*, 2007), which may be involved in the insulin-sensitizing effect of omega-3 PUFA. EPA and DHA can inhibit the production of IL-1β and TNFα by monocytes (Zhao *et al.*, 2004; Chu *et al.*, 1999). The consumption of EPA and DHA decrease the levels of TNFα, associated with a decrease in insulin resistance (Das, 2005a; 2005b). However, many other studies reported no impact of EPA and DHA on plasma levels of IL-6 or TNFα or their related basal glycemia management (Klein-Platat *et al.*, 2005; Jellema *et al.*, 2004; Mori *et al.*, 2003). EPA and DHA have been suggested to reduce plasma level of leptin (Reseland *et al.*, 2001a, 2001b), and hence improve insulin sensitivity. However, no change in mRNA of leptin in adipose tissue was found in high-fat diet rich in omega-3 PUFA fed rats (Takahashi and Ide, 2000). Moreover, EPA and DHA reduced gene expressions of inflammatory mediators including TNFα, IL-1B and IL-6 in various animal studies (Robinson *et al.*, 1996; Chandrasekar and Fernandes, 1994; Renier *et al.*, 1993).

Increased consumption of omega-3 PUFA protects against the development of obesity in animals exposed to obesogenic diet and reduce body fat when already obese (Li *et al.*, 2008). Fish oil found to decrease body fat mass and concurrently stimulate lipid oxidation in healthy volunteers (Couet *et al.*, 1997). This could be due to appetite-suppressing effects (Lawton *et al.*, 2000), apoptosis of adipocytes (Perez-Matute *et al.*, 2007) and changes of gene expression in several tissues (Al-Hasani and Joost, 2005). The omega-3 PUFA reduced the levels of triglycerides and VLDL-triglycerides in the rats, but this effect disappears when cholesterol-rich diet is used (Lin *et al.*, 2005). EPA and DHA decrease plasma triglyceride and cholesterol in the rats (Spady, 1993), however Berge, *et al.* (1999) reported EPA, not DHA, as the principle component for lowering triglycerides.

Increase in EPA intake has been closely related to positive health benefits associated to insulin sensitivity and glucose metabolism, and with reduction of triglycerides and inhibition of VLDL production. EPA+DHA are well-known to reduce triglycerides and increase HDL-cholesterol (Balk *et al.*, 2006). Fish oils reduce triglycerides (Cottrell *et al.*, 2003; Szapary and Rader, 2001) and more precisely, EPA+DHA induced a decrease in plasma triglycerides

level of 24 % and 35 % in normolipidemic and hypertriglyceridemic subjects respectively (Harris, 1997a). Pure EPA found to lower plasma level of triglycerides in type 2 diabetes (Nomura *et al.*, 2003), however with same dose (1800 mg/day of EPA-ethyl ester), there was no change in the level of triglycerides (Nakamura *et al.*, 1998; Westerveld *et al.*, 1993). Denke suggested the consumption of 6-12 grams per day of EPA and DHA in the form of fish oils or 9-12 ounces of salmon per day to combat hypertriglyceridemia (Denke, 2002). In carbohydrate-fed rats, fish oils decrease plasma and hepatic triglycerides levels and VLDL-triglycerides secretion (Herzberg and Rogerson, 1988). Fish oil supplementation prevented hyperlipidemia that occurred in non-supplemented fructose fed rats (Nyby *et al.*, 2005). The omega-3 fatty acids suppress triglycerides synthesis, VLDL secretion and lower triglycerides levels by impaired VLDL assembly and secretion (reviewed in (Jump and Clarke, 1999) and direct suppression of VLDL apolipoprotein B (Baker and Gibbons, 2000). The hypotriglyceridemic effect is indicated to be due to low triglycerides synthesis in hepatocytes (Surette *et al.*, 1992; Wong and Marsh, 1988; Marsh *et al.*, 1987), likely by the impairment of SREBP pathway (Le Jossic-Corcos *et al.*, 2005), and increased triglycerides clearance. In fact, diets rich in linoleic acid, EPA or DHA decrease the expression of SREBP-1c (Xu *et al.*, 1999b). Fish oil could downregulate the hepatic mRNA level of the SREBP-1, which also controls several lipogenic genes (Takahashi *et al.*, 2002; Clarke, 2001; Kim *et al.*, 1999). Moreover, fish oil feeding downregulated hepatic SRE-dependent genes such as LDL receptor, 3-hydroxy-methyl glutaryl-CoA reductase, 3-hydroxy-methyl glutaryl-CoA synthase and ACC (Kim *et al.*, 1999). Adipose tissue lipoprotein lipase (LPL) activity was increased more than two-fold, where as hepatic FAS (not adipose tissue) activity was significantly lower in high-sucrose induced insulin resistant rats, in which fish oil substituted with standard vegetable oil (Peyron-Caso *et al.*, 2003). Moreover, EPA found to downregulate HMG-CoA reductase in Reuber H35 hepatoma cells (Garcia-Pelayo *et al.*, 2004). The mRNA levels of FAS, hormone-sensitive lipase, LPL, phosphoenolpyruvate carboxykinase and leptin are decreased in the retroperitoneal fat tissue of high-fat diet fed rats supplemented with omega-3 PUFA (Raclot *et al.*, 1997).

Fish oil administration (2.7 g EPA and 1.7 g DHA per day) resulted in a significant decrease in non-esterified fatty acids but no change in plasma glucose and insulin sensitivity (Rivellese *et al.*, 1996), suggesting these changes are regulated by a mechanism other than that plasma non-esterified fatty acids level-mediated insulin sensitivity. However, very high dose of fish oil (10 g/day) did not affect plasma non-esterified fatty acids and glucose levels

(Annuzzi *et al.*, 1991), suggesting that high-dose of omega-3 PUFA may not have desirable effects on insulin sensitivity.

Administration of fish oils resulted in no change in cholesterol concentrations (Friedberg *et al.*, 1998; Nakamura *et al.*, 1998; Rivellese *et al.*, 1996; Annuzzi *et al.*, 1991); however Popp-Snijder, *et al.* (1987) reported a decrease in plasma cholesterol using Super EPA fish oil. The already elevated LDL-cholesterol in metabolic syndrome is further mildly increased with fish oil consumption (Szapary and Rader, 2001), but Jain, *et al.* (2002) reported a decrease in LDL-cholesterol, suggesting a redistribution of LDL to HDL. Fish oils also increased HDL-cholesterol (Barrett and Watts, 2003; Pedersen *et al.*, 2003; Jain *et al.*, 2002). Few studies reported an increase in LDL-cholesterol and no change in HDL-cholesterol with the consumption of fish oils (Rivellese *et al.*, 1996; Annuzzi *et al.*, 1991).

In short, several possible mechanisms of the hypolipemic action of fish oils (omega-3 PUFA) could include intestinal lipolysis and absorption, chylomicron clearance and hepatic VLDL secretion.

7.2 Citrus flavonoids: Hesperidin and naringin

Flavonoids are natural components in our diet and, with the burgeoning interest in alternative medicine, are increasingly being ingested by the general population. Several beneficial properties have been attributed to these dietary compounds, including antioxidant, anti-inflammatory and anticarcinogenic effects. Flavonoids preparations are marketed as herbal medicines or dietary supplements for a variety of alleged nontoxic therapeutic effects (Galati and O'Brien, 2004).

Dietary flavonoids represent the largest class of naturally occurring polyphenol compounds found in common food items derived from plants. The flavonoids are characterized by common structure of diphenylpropanes (C6-C3-C6), consisting of two aromatic rings (A and B) linked through three carbons, as shown in the figure 10 (Ross and Kasum, 2002). Depending on the variations in the C-ring (as shown in the figure 10, flavonoids can be further subdivided into six major subclasses: flavones (apigenin, luteolin), flavonols (quercetin, myricetin), isoflavones (genistein, daidzein), flavanones (naringenin, hesperidin), flavanols or catechins (epicatechin, gallocatechin) and anthocyanidins (cyaniding, pelargonidin) (Ross and Kasum, 2002; Middleton *et al.*, 2000). The biological activities of flavonoids are structure dependent. Flavonoids provide much of the flavour and colour of

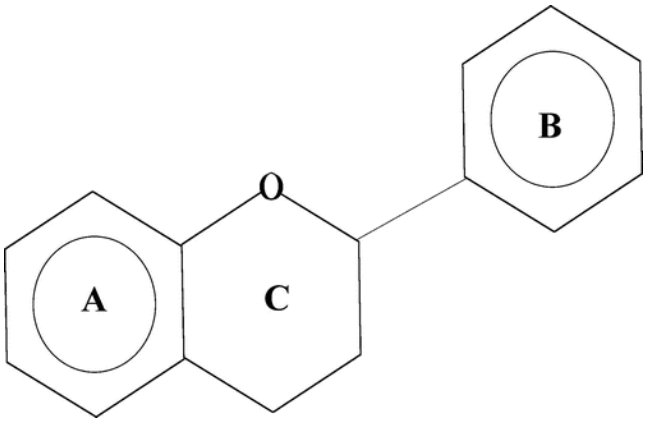

Figure 10: Basic flavonoids structure.

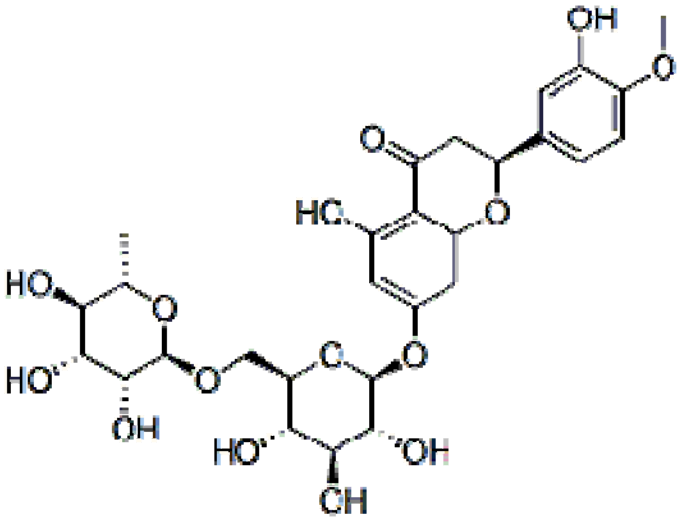

Figure 11: Structure of hesperidin ($C_{27}H_{32}O_{14}$).

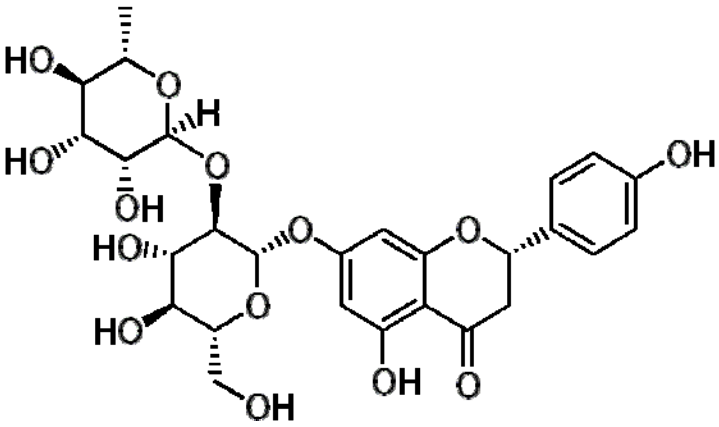

Figure 12: Structure of naringin ($C_{28}H_{34}O_{15}$).

fruits and vegetables. More than 8000 compounds with a flavonoids structure have been identified (Benavente-Garcia and Castillo, 2008), and more than 5000 subclasses of flavonoids have been described by 1990 (Harbourne, 1993).

7.2.1 General aspects

Plant polyphenols were considered important in plant physiology for their role in plant pigmentation and flavour. These are involved in plant growth and reproduction, provide resistance against pathogens and predators, and protect crops from disease and preharvest seed germination (reviewed in (Ross and Kasum, 2002). Polyphenols are frequently found attached to sugars (glycosides) and occasionally as aglycones. Citrus fruits contain various bioflavonoids. Hesperidin and naringin are flavanone glycosides, comprising of an aglycones, hesperetin and naringenin respectively, attached to a disaccharide, rutinose. Hesperidin is hesperetin 7-O-β-rutinoside or hesperetin-7-rhamnoglucoside, also known as vitamin P (Miyake *et al.*, 1998), and naringin is naringenin-7-rhamnoglucoside (structures are shown in the figures 11 and 12).

The biological activities of flavonoids were first published in 1936 (Rusznyák and Szent-Györgyi, 1936). Plant flavonoids may attenuate inflammatory and immune responses. Certain flavonoids are potent inhibitors of the production of prostaglandins, a group of powerful pro-inflammatory signaling molecules.

In 1970s, the daily intake of flavonoids in the United States was estimated to be approximately 1 g/day (Kuhnau, 1976). However, in 1990s the estimated intake of flavonols and flavones in the Netherlands to be 23 mg/day (Hertog *et al.*, 1993) and that of flavonols, flavones and flavanones in Denmark to be 28 mg/day (Leth and Justesen, 1998).

7.2.2 Effects on obesity, IR and dyslipidemia

Hesperidin and naringin have been used widely for their anti-oxidant, anti-inflammatory and anti-cancerous effects. These have been recently used for the prevention of obesity-induced disorders, like insulin resistance and dyslipidemia. These are thought to be potential hypolipidemic and hypoglycemic agents.

Dietary hesperidin (10 g/kg diet) decreased plasma glucose by altering the activity of glucose-regulating enzymes in diabetic rats (Akiyama *et al.*, 2010). Hesperidin might inhibit the damage to the pancreatic islet by its antioxidative efficacy, resulting in decrease in plasma glucose and alter glycogen contents in the diabetic tissues by improving glycolytic and

gluconeogenic enzymes (Stanley Mainzen Prince and Kamalakkannan, 2006). Moreover, hesperidin and naringin increase mRNA level of glucose kinase (glycolytic enzyme) and decrease the level of glucose-6-phosphatase (gluconeogenic enzyme) in type 2 diabetic mouse liver (Jung *et al.*, 2006). Similar findings have been observed for the activities of both enzymes in the liver of diabetic rats supplemented with hesperidin (Akiyama *et al.*, 2010). Flavonoids stimulate glucose uptake in the peripheral tissues, regulate the activity and expression of the rate-limiting enzymes in the glucose metabolism and also influence insulin signaling to improve diabetes condition (Cazarolli *et al.*, 2008). Moreover, hesperidin has been reported to increase plasma adiponectin level in the diabetic rats (Akiyama *et al.*, 2010). Consistently, hesperetin and naringenin upregulated the expression of adiponectin and also activate PPARγ in a dose-dependent manner in the adipocytes (Liu *et al.*, 2008).

Citrus flavonoids are promising bioactive compounds against hyperlipidemia and lipid biosynthesis. Hesperidin and naringin increase HDL and lower plasma cholesterol, LDL and triglycerides levels in rats (Miceli *et al.*, 2007; Monforte *et al.*, 1995). However, the mechanism of lipid lowering effect by flavonoids remains unknown. Although flavonoids have been studied for about 50 years, the cellular mechanisms involved in their biological actions are still completely unknown. It is suggested to reduce risk of cardiovascular diseases through their radical scavenging function and hypocholesterolemic action (Miceli *et al.*, 2007). The HMG-CoA inhibitors are known to be very effective in lowering plasma cholesterol. Acyl CoA: cholesterol O-acyltransferase (ACAT) catalyzes the intracellular esterification of cholesterol and also involved in cholesterol absorption, hepatic VLDL-cholesterol secretion and cholesterol accumulation in the vascular wall by catalyzing cholesterol esterification. Hesperidin and naringin are potent agents for inhibition of the plasma and hepatic activities of HMG-CoA reductase and ACAT, and also beneficial for lowering plasma cholesterol levels in cholesterol-fed rats (Choi *et al.*, 2004; Kim *et al.*, 2003; Bok *et al.*, 1999). Moreover, hesperidin (5 % and 10 %) has been reported to decrease plasma triglycerides level in rats (Kawaguchi *et al.*, 1997), and even 0.5 % hesperidin has been reported to decrease plasma triglycerides and cholesterol in sham operated female rats (Horcajada *et al.*, 2008). In a recent study, hesperidin suppressed the increase in the plasma triglycerides, total cholesterol and VLDL+LDL-cholesterol, and increased plasma HDL-cholesterol level in diabetic rats (Akiyama *et al.*, 2010). Furthermore, hesperidin has been reported to enhance expression of the gene encoding the LDL receptor, and reduce the activity and expression of microsomal triglyceride transfer protein (Wilcox *et al.*, 2001).

- OBJECTIVE

Obesity is a major factor leading to many clinical disorders including insulin resistance and dyslipidemia. Since study limitation in the human, the use of an 'appropriate' animal model is evident. In our laboratory, dog has been an established animal model of obesity and insulin resistance. However, the development of new animal model has always been there to meet the new challenges. We aimed to characterise rat as animal model of obesity and insulin resistance. As in humans, the development of obesity and insulin resistance is an effect of dietary habits, so we used dietary intervention for induction of obesity and insulin resistance in our study. We preferred the use of longitudinal approach to the transversal approach that, to our knowledge, has never been done in rats for the study of insulin resistance. In later part of the study, nutritional supplementations were made for the preventive effects on dietary-induced obesity and related metabolic disorders in this model. In short, we set two main objectives for our study. First relates to the characterization of the rat model of obesity and insulin resistance by a longitudinal approach, and secondly to observe the effects of nutritional interventions of eicosapentaenoic acid or flavanones.

For the purpose of characterization of model, rats were given different diet that could induce obesity and / or insulin resistance, i.e., a high-fat diet and / or a high-fructose diet. The high-fat diets (different levels of fat content in the diet) have been used to develop these metabolic disorders. The high-fructose diets have been used to develop insulin resistance with mild-to-severe dyslipidemia. However, differences in diet composition, interventional duration, strain of the rat, dietary source and age of the animals have complicated the comparisons of these studies. The use of the high-fat (65 % by energy) diet, or the high-fructose (65 % by energy) diet for 10 weeks in our study helped us to compare the parameters subjected to affect in the development of obesity, dyslipidemia and insulin resistance. The parameters studied were body weight, body composition, glycemia, insulinemia, insulin sensitivity and plasma lipid profiles (triglycerides, total cholesterol and non-esterified fatty acids). Moreover, the plasma lipoprotein profile and the expression of genes of interest in insulin target tissues (liver and adipose tissue) helped to understand the mechanism behind these metabolic disorders.

Various nutritional interventions (omega-3 polyunsaturated fatty acids, PUFA; flavonoids; etc.) have been used to prevent the development of obesity, insulin resistance and

dyslipidemia with variable effects and various modes of actions. The omega-3 PUFA have controversial effects to decrease plasma triglycerides and / or total cholesterol concentrations, and to improve insulin sensitivity. The flavanones were used in research for their anti-oxidant, anti-cancerous and anti-inflammatory properties, however in recent years, these flavanones has been used in the prevention of type 2 diabetes (to reduce hyperglycemia) and dyslipidemia. We aimed to study two nutritional interventions, i.e., supplementation of eicosapentaenoic acid and supplementation of hesperidin and naringin (flavanones) in rats fed with the high-fat (65 % by energy) diet, or the high-fructose (65 % by energy) diet.

The study of expressions of genes in liver and visceral adipose tissues helped us to understand the mechanisms for the development of dyslipidemia and insulin resistance, and also for the prevention of these disorders. The genes, we have chosen to study in liver and visceral adipose tissue, relate to the different proteins involved expansion of body fat (leptin), the insulin signalling, glucose uptake, insulin resistance and glucose metabolism (insulin receptor, IRS-1, GLUT4, PEPCK), lipogenesis and lipolysis (FAS, ACC, LPL, HSL, hepatic lipase, perilipin), transcriptional factors involved in lipid metabolism (PPAR and SREBP-1c) and the inflammation (TNFα).

- *EXPERIMENTAL ASPECTS*

The experimental aspects of the study could be divided in three parts.

I- Characterization of rat model of obesity and insulin resistance by a high-fat or a high-fructose diet.

II- Effects of the supplementation with eicosapentaenoic acid (EPA) in rats.

III- Effects of the supplementation with hesperidin and naringin (HN) in the presence of the high-fat or the high-fructose diet in rats.

1 Material and methods

In the first part of the study, five groups have been made: control, high-fat, high-fructose, high-fat EPA and high-fructose EPA. In the second part of the study, three groups have been made: control HN, high-fat HN and high-fructose HN. The study design is presented in figures 13 and 14.

1.1 Animals

Thirty male Wistar rats (Janvier, Le Genest Saint-Isle, France) were used in the study. The rats were housed in individual cages, with free access to the feed and water. They were maintained under a 12-h light−12-h dark cycle and a temperature of 22 ± 2 °C. They were housed at Oniris (National College of Veterinary Medicine, Food Science and Engineering, Nantes, France), according to the regulations for animal welfare of the French Ministry of Agriculture. The experimental protocol adhered to the European Union guidelines and was approved by the local animal use and care advisory committee.

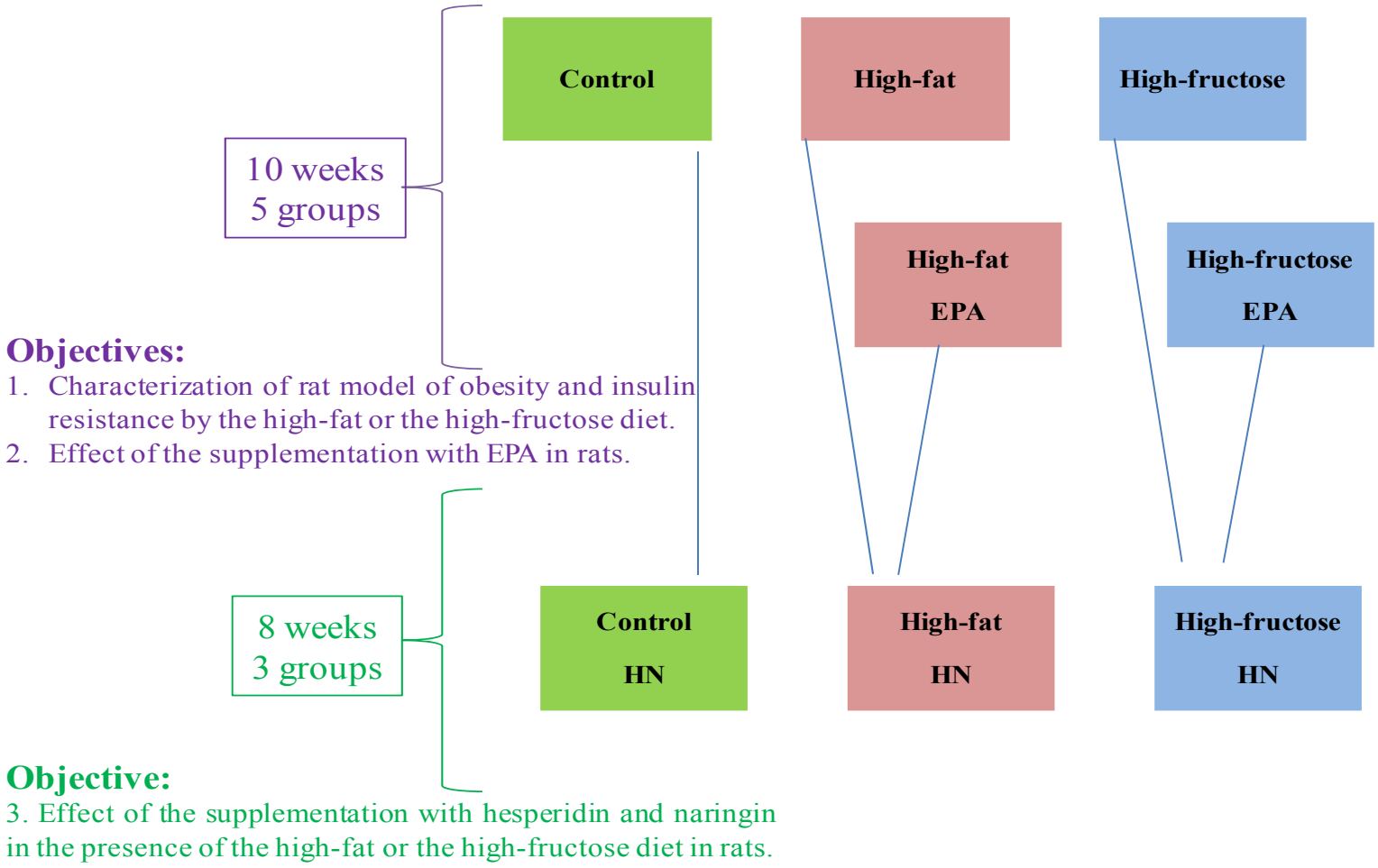

Figure 13: Groups of the rats used in the study of dietary indued-obesity and -insulin resistance, and nutritional interventions with EPA or flavanones.

(EPA: eicosapentaenoic acid; HN: hesperidin and naringin, n = 6 for each group.)

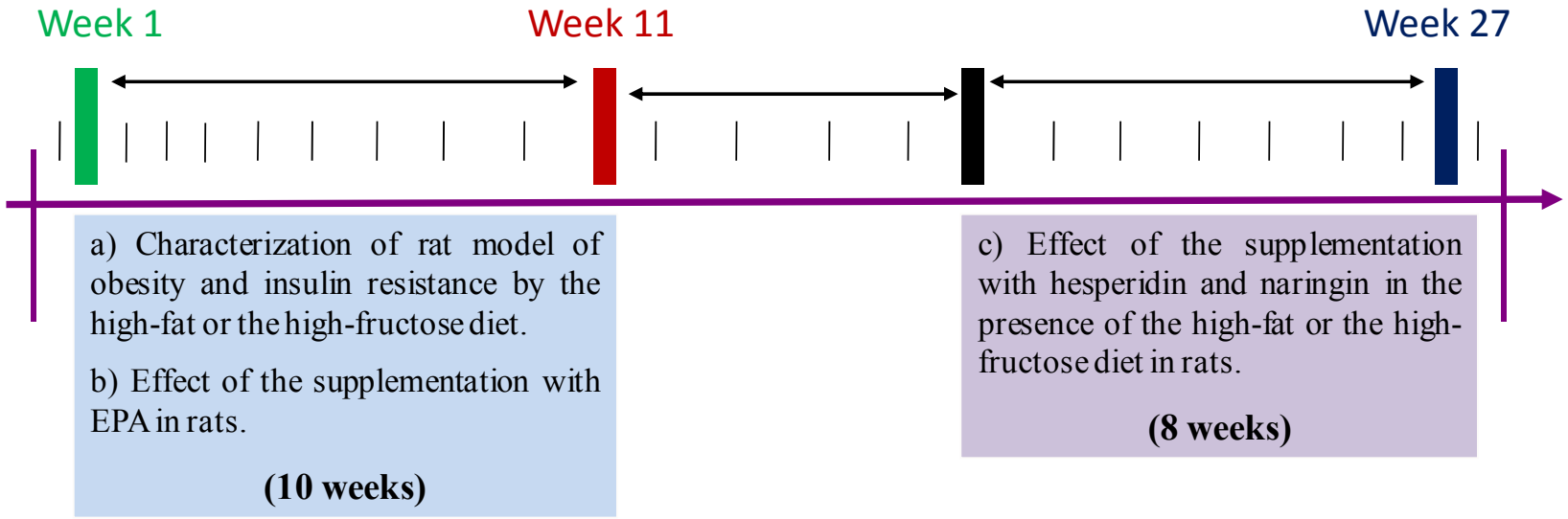

Figure 14: The study design of dietary indued-obesity and -insulin resistance, and nutritional interventions with EPA or flavanones.

Week 1 and week 11 indicate the time (in weeks), when the parameters were studied for '*characterization of rat model of obesity and insulin resistance by the high-fat or the high-fructose diet*', and '*effects of the supplementation with EPA in rats*'. Week 27 indicates the time (in weeks), when the parameters were studied for '*effects of the supplementation with hesperidin and naringin in the presence of the high-fat or the high-fructose diet in rats*'.

(EPA: eicosapentaenoic acid)

Table 4: Composition of the control, the high-fat, and the high-fructose diets.

a) Energy values of the control, the high-fat, and the high-fructose diets.

	Control	**High-fat**	**High-fructose**
Protein calories	20	20	20
Lipid calories	15	65	15
Carbohydrate calories	65	15	65
Energetic value (kcal/kg)	**3488**	**4859**	**3488**

Energy values have been presented in % energy (% kcal).

b) Dry matter composition (percentage by weight) of the control, the high-fat, and the high-fructose diets.

	Control	**High-fat**	**High-fructose**
Dietary casein	20.5	28.6	20.5
Corn starch	39.7	12.65	-
Dextrose / glucose	20	6.5	-
Fructose	-	-	59.7
Cellulose	6	6	6
Sunflower oil	5.8	3.85	5.8
Lard	-	31.25	-
Minerals[a]	7	9.75	7
Vitamins[b]	1	1.4	1
	100	**100**	**100**

Dietary components have been presented in g per 100 g of the diet.

[a]: Minerals mix SAFE; [b]: Vitamin mix SAFE.

1.2 Diets and supplementation

1.2.1 Characterization of rat model of obesity and insulin resistance by a high-fat or a high-fructose diet

Twelve-week old male adult Wistar rats were randomly separated into three groups (six per group): control, high-fat, or high-fructose. The rats were fed with a control diet, a high-fat diet, or a high-fructose diet (SAFE diets, Scientific Animal Food & Engineering, Augy, France). The composition of the control, the high-fat, and the high-fructose diets has been listed in the table 4. The duration of the study was 10 weeks.

1.2.2 Effects of the supplementation with eicosapentaenoic acid (EPA) in rats

Twelve-week old male adult Wistar rats were randomly separated into five groups (six per group): control, high-fat, high-fructose, high-fat EPA, or high-fructose EPA. The control, the high-fat, and the high-fructose groups (without supplementation with EPA) were similar, as discussed above. The EPA-supplemented groups were given 120 mg/rat/day or 200 - 300 mg/kg/day EPA (KD-Pharma Bexbach GmbH, Bexbach, Germany). The EPA was mixed with the cheese (Schmelzkäse Leicht, aro, Düsseldorf, Germany) to avoid oxidation. The groups without EPA-supplementation were also given the cheese without EPA. The cheese was stored at -20 °C and the box of cheese in use was kept at 4 °C. Cheese was weighed daily for each rat. The duration of the study was 10 weeks.

1.2.3 Effects of the supplementation with hesperidin and naringin (HN) in the presence of the high-fat or the high-fructose diet in rats

Thirty-one-week old male adult Wistar rats were randomly divided into three groups (six per group): control HN, high-fat HN, or high-fructose HN. The composition of the diets has been listed in the table 4. The HN-supplemented groups were given 8.75 mg/kg$^{0.75}$/day of each hesperidin and naringin (Natural Orange Extract, and Citroflavonoides, Zoster, S. A., Beniel, Spain through Affinity Petcare, Barcelona, Spain). Both hesperidin and naringin are mixed with the diet. The duration of the study was 8 weeks.

1.3 Parameters studied

1.3.1 Body weight and body composition

Body weight was recorded weekly. The body fat mass was determined by the method of isotope dilution (^2H_2O; Eurisotop, Gif-sur-Yvette, France). Blood samples (1 mL) were collected prior to and 2 h after a ^2H_2O injection (500 mg/kg body weight). Plasma ^2H_2O concentrations were measured using Fourier-transformed infrared spectroscopy (Brüker SA, Wissembourg, France). The principle of the technique is based on the fact that the diluted isotope, injected to an individual, is distributed uniformly throughout the aqueous compartments of the body. By measuring the concentration of the isotope in one of these compartments, one can determine the volume of total body water, in which the isotope dispersed. The average hydration of lean mass in rats is equal to 73 % (Spray and Widdowson, 1950). From the estimation of total body water, the body lean mass can be calculated, and ultimately body fat mass.

1.3.2 Insulin sensitivity (Euglycemic-hyperinsulinemic clamp technique)

The euglycemic-hyperinsulinemic clamp technique is the standard method to assess insulin sensitivity (DeFronzo *et al.*, 1979). This technique allows to determine the required quantity of glucose perfused to maintain basal glycemia in an overnight not fed rat, perfused with constant perfusion of insulin. A catheter (PE 10, Intramedic polyethylene tubing, Becton Dickinson and Company, Sparks, MD, USA) was inserted under anaesthesia into the jugular vein. Insulin (Actrapid, Novo Nordisk, Danemark) was perfused (72 mU/kg for 1 min, then 18 mU/kg/min for 3 h), and glycemia was measured every 5 min. Glucose (Glucose 20 %; Braun Medical SAS, Boulogne Cedex, France) was perfused at a variable rate through the same catheter. The glucose infusion rate (GIR; mg/kg/min) was adjusted to attain and maintain the basal glycemia, and it directly reflected the whole body insulin sensitivity. Glycemia was measured using the glucose oxidase method (Accu-Chek® Active; Roche Diagnostics, Mannheim, Germany). Insulin was measured using the radioimmunoassay (RAT INSULIN RIA kit, Millipore Corporation, Billerica, USA).

1.3.3 Plasma lipid profiles

Blood samples of 1 ml were collected in EDTA tubes, centrifuged (5000 g for 10 minutes at 4 °C) and stored at -20 °C. The basal plasma concentrations of total cholesterol, triglycerides and non-esterified fatty acids were assayed using enzymatic methods (Cholestérol RTU, Triglycérides enzymatique TG PAP150, BioMérieux, Marcy-l'Etoile, France; and NEFA C, Wako, Oxoid, Dardilly, France).

The plasma samples from the rats, not fed overnight, were pooled for each group, and the plasma lipoproteins were separated using a fast-protein liquid chromatography system (UNICORN 520, GE Healthcare, Pittsburgh, USA). The cholesterol and triglycerides concentrations were measured in each fraction using enzymatic methods. Each group presented one value, so mean could not be taken for statistical analysis and comparison.

1.3.4 mRNA expression of genes in the liver and visceral adipose tissue

Biopsies of the liver and visceral adipose tissue were performed in the rats that were not fed overnight. About 100 mg of the hepatic or visceral adipose tissue was cleaned in normal saline. TRIzol® reagent (Invitrogen, Carlsbad CA, USA) was added, and the tissue was stored immediately at -80 °C.

RNA was extracted using TRIzol® reagent, according to the manufacturer's instructions. The total RNA concentration was quantified using spectrophotometer absorbance at 260 nm. The 260-280 nm absorption ratio of all preparations was between 1.5 and 2.0.

Total RNA (1 µg) was reverse-transcribed in a 20 µl reaction volume using random primers (Pharmacia, Saclay, Orsay, France) and SuperScript III Reverse Transcriptase (invitrogen, Carlsbad CA, USA), according to the manufacturer's instructions. Real time PCR analysis was performed using the 7000 sequence Detection System with MESA GREEN qPCR MasterMix Plus for SYBER® Assay (Eurogentec, Angers, France). A large number of copies, called amplicons, of target gene were produced from cDNA by the PCR and specific primers. MESA GREEN qPCR MasterMix Plus for SYBER® Assay is a non-specific double-stranded DNA intercalating dye, that fluoresces once bound to the DNA. The emitted fluorescence can be detected and related to the amount of generated amplicons. Table 5 lists the primers used in the study of mRNA expression of genes. The sequence of primers were obtained from the literature or determined by the Primer3 website. The mRNA levels were

Table 5: Sense / antisense primers used.

Genes	Sens primers (5'→ 3')	Antisens primers (5'→ 3')	Amplicon size
GAPDH	TCCCATTCTTCCACCTTTGATGCT	ACCCTGTTGCTGTAGCCATATTCAT	104
Insulin receptor	GTGCTGCTCATGTCCTAAGA	AATGGTCTGTGCTCTTCGTG	269
IRS-1	GCCAATCTTCATCCAGTTGC	CATCGTGAAGAAGGCATAGG	336
GLUT4	GCTTCTGTTGCCCTTCTGTC	TGGACGCTCTCTTTCCAACT	166
Resistin	AAGGCACAACCGTCACTAGC	AGGGCAAGCTCAGTTCTCAA	170
Leptin	TTGTCACCAGGATCAATGACATTT	GACAAACTCAGAATGGGGTGAAG	105
Perilipin	TGCGCAAGAAGAGCTGAGTA	GTGGGTCTCGAGAACATCGT	169
SREBP-1c	GGCATGAAACCTGAAGTGGT	TGGGCTTTCACCTGGTTATC	164
PPAR-α	GAGACCCTCGGGGATCTTAG	CGTCTTGTGTCCTGAGCTTG	102
PPARγ	CTGACCCAATGGTTGCTGATTAC	GGACGCAGGCTCTACTTTGATC	79
TNFα	GCAGAGCCTTCCAAGCCTACC	GTTACCCAGCCCACCTCCTTTG	134
FAS	CGCCGTGGTGCTGGAGATTG	CTTGCCGAGGTTGGTGAGGAAG	141
HSL	CTCCTCATGGCTCAACTCCTTTCC	GTGGGTATCAGTTCTTGGGGA	434
LPL	CAGCTGGGCCTAACTTTGAG	AATGGCTTCTCCAATGTTGC	197
Hepatic lipase	GAACACAGTGCAGACCATAATGCT	TTCAGGTCACATTTCACGAAGACTT	180
PEPCK	AGTCACCATCACTTCCTGGAAGA	GGTGCAGAATCGCGAGTT	107
ACC	CGATTCCCATCCGCCTCTTCC	GGTCCCTGCTTGTCTCCATACG	126

normalized using GADPH as a housekeeping gene, and the mRNA level measured at week 1 (samples of hepatic tissue) or in the control group (samples of adipose tissue) were set at 100 %.

1.4 Statistical analysis

Data were expressed as the mean with the standard error of the mean (SEM). In the studies of '*characterization of rat model of obesity and insulin resistance by the high-fat or the high-fructose diet*' and '*effects of the supplementation with the EPA in rats*', data analysis for the body weight, body fat mass, basal insulinemia, basal glycemia, glucose infusion rate during euglycemic-hyperinsulinemic clamp technique, basal plasma lipid profiles was performed using R software (version 2.10, lme4 package, R Foundation for Statistical Computing, Vienna, Austria). A linear mixed effects model has been performed in order to study the effect of time, type of diet and the interaction between them for each variable in the study of '*characterization of rat model of obesity and insulin resistance by the high-fat or the high-fructose diet*'. A linear mixed effects model has been performed in order to study the effect of time, EPA-supplementation and the interaction between them for each variable in the study of '*effects of the supplementation with the EPA in rats*'. The mixed effects models are the most efficient way to analyse repeated measurements data. A multiple comparison of means procedure with Tukey contrasts, adapted to the mixed effects models, has been used, when the interaction between time and the type of diet or EPA-supplementation was significant.

For mRNA expression study, significance was established using the Mann-Whitney test using Statview software (version 5.0; SAS Institute Inc., Cary, NC, USA). The comparison was made between week 1 and week 11 for the expression of genes of the hepatic tissue, and between the groups at week 11 for the expression of genes of the visceral adipose tissue. A significant difference has been considered for P-value < 0.05.

In the third part of the study, data analysis was performed using xlstat. Data were expressed as the mean with the standard error of the mean (SEM). Analysis of variance (ANOVA) for one factor along with Fisher (LSD) post-hoc test was performed. A significant difference has been considered for P-value < 0.05.

2 Characterization of the animal model

2.1 Lipid profile and insulin sensitivity in rats fed with high-fat or high-fructose diets

2.1.1 Article

Lipid profile and insulin sensitivity in rats fed with high-fat or high-fructose diets.

Muhammad-Quaid Zaman, Véronique Leray, Jérôme Le Bloc'h, Chantal Thorin, Khadija Ouguerram and Patrick Nguyen

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Lipid profile and insulin sensitivity in rats fed with high-fat or high-fructose diets

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Abstract

The occurrence and severity of obesity- and insulin resistance-related disorders vary according to the diet. The aim of the present longitudinal study was to examine the effects of a high-fat or a high-fructose diet on body weight (BW), body fat mass, insulin sensitivity (IS) and lipid profiles in a rat model of dietary-induced obesity and low IS. A total of eighteen, 12-week-old male Wistar rats were divided into three groups, and were fed with a control, a high-fat (65% lipid energy) or a high-fructose diet (65% fructose energy) for 10 weeks. BW, body fat mass (^2H_2O dilution method), IS (euglycaemic–hyperinsulinaemic clamp technique), plasma glucose, insulin, NEFA, TAG and total cholesterol were assessed before and at the end of 10-week period. Cholesterol was measured in plasma lipoproteins separated from pooled samples of each group and each time period by using fast-protein liquid chromatography. All rats had similar BW at the end of the 10-week period. Body fat mass was higher in the high-fat group compared to the control group. There was no change in basal glycaemia and insulinaemia. The IS was lower in the high-fat group and was unchanged in the high-fructose group, compared to the control group. Plasma TAG concentration and cholesterol distribution in lipoproteins did not change over time in any group. Plasma NEFA concentration decreased, whereas plasma TAG concentration increased over time, regardless of the diet in both cases. The 10-week high-fat diet led to obesity and low IS, whereas rats fed with the high-fructose diet exhibited no change in IS and lipidaemia. The high-fat diet had more deleterious response than high-fructose diet to induce obesity and low IS in rats.

Key words: insulin resistance: obesity: dyslipidemia: high-fat diet: high-fructose diet: rat

There is a growing prevalence of obesity and low insulin sensitivity (IS), often called insulin resistance, in human subjects. Thus, there is a need for an animal model to study the time course of these metabolic disturbances as well as their unhealthy consequences. Different animal models have been used to study obesity and IS, notably the rat, in which obesity can be caused by genetic mutations or induced by nutritional interventions. As human obesity is mainly due to nutritional habits, animal models of obesity and low IS induced by specific diets may be preferable to genetic models.

Various nutritional interventions have been used to induce obesity, low IS and dyslipidaemia in rats. High-fat diets have been shown to cause these metabolic disorders in previous studies, but there has been a large variability in the intensity of the metabolic changes[1–5]. High-fructose diets have also been shown to lower IS and promote mild-to-severe dyslipidaemia[6–9]. The differences in nutritional interventions, such as diet composition and interventional duration, have complicated the comparisons of these studies. Therefore, it is difficult to define the best nutritional intervention to induce obesity in an animal model that closely mimics the human disease. Longitudinal studies could be useful in determining the best diet to induce obesity and related disorders. To our knowledge, this type of approach has never been conducted in rats. Here, we aimed to perform a longitudinal study to compare the effects of a high-fat diet and a high-fructose diet on body weight (BW), IS and plasma lipid profiles in rats.

Abbreviations: BW, body weight; IS, insulin sensitivity.

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Table 1. Body weight, body fat mass, insulin sensitivity and plasma lipid profiles of rats in the control, high-fat and high-fructose groups
(Mean values with their standard errors, *n* 6)

	Control				High fat				High fructose						
	Week 1		Week 11		Week 1		Week 11		Week 1		Week 11				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Interaction	Time	Type of diet
BW (g)	401	6	526	14	383	19	534	26	399	12	529	16	NS	†††	NS
Body fat mass (g)	35·6	3·3	63·3	9·5	25·6	4·7	90·0	12·2	34·6	5·7	78·9	11·8	*	‡	‡
Basal glycaemia (mmol/l)	5·51	0·11	4·93	0·25	5·60	0·15	5·31	0·19	5·35	0·19	5·52	0·31	NS	NS	NS
Basal insulinaemia (pmol/l)	402	8·4	400	17·2	415	24·8	388	9·9	386	12·4	402	11·0	NS	NS	NS
GIR (mg/kg per min)§	19·0	1·4	18·5	1·2	19·3	1·6	12·9	2·0	18·2	1·5	15·3	1·4	*	‡	‡
Total cholesterol (mmol/l)‖	1·81	0·11	2·04	0·14	1·46	0·06	1·99	0·17	1·59	0·09	1·83	0·14	NS	†	NS
TAG (mmol/l)	1·89	0·15	1·64	0·10	1·86	0·12	1·76	0·12	1·78	0·11	2·0	0·30	NS	NS	NS
NEFA (mmol/l)	0·46	0·07	0·30	0·04	0·45	0·06	0·31	0·04	0·42	0·04	0·41	0·04	NS	†	NS

BW, body weight; GIR, glucose infusion rate.
* Mean values were significantly different among groups for the interaction between time and the type of diet (fixed-effects model analysis revealed that values in the high-fat group is significant at week 11, compared to the control group; $P<0·05$).
† Mean values were significantly different among groups for the effect of time (significant for week 11 *v.* week 1): † $P<0·05$ and ††† $P<0·0001$.
‡ Mean values were significantly different for the interaction between time and the type of diet; the effect of time and type of diet were not considered ($P<0·05$).
§ *n* 5 for GIR (in hyperinsulinaemic conditions, GIR measures the insulin-mediated glucose uptake and is considered as a good reflection of insulin sensitivity).
‖ At week 1, plasma total cholesterol concentration was different among groups.

Materials and methods

Animal groups and diets

Male Wistar rats (12 weeks old; Janvier, Le Genest Saint-Isle, France) were randomly separated into three groups (six per group): control, high fat or high fructose. According to their groupings, the rats were fed with a control diet (39·7% maize starch, 20% dextrose, 5·8% sunflower oil and 20·5% casein by weight), a high-fat diet (12·7% maize starch, 6·5% dextrose, 3·9% sunflower oil, 31·3% lard and 28·6% casein by weight) or a high-fructose diet (59·7% fructose, 5·8% sunflower oil and 20·5% casein by weight) for 10 weeks. The rats were housed in individual cages, with free access to the feed and water. The rats were maintained under a 12-h light–12-h dark cycle and a temperature of 22 ± 2°C. The animals were housed at Oniris (National College of Veterinary Medicine, Food Science and Engineering, Nantes, France), according to the regulations for animal welfare of the French Ministry of Agriculture. The experimental protocol adhered to the European Union guidelines and was approved by the local animal use and care advisory committee.

Body weight and body fat mass

BW was recorded weekly. The body fat mass was determined by isotope dilution (^2H_2O; Eurisotop, Gif-sur-Yvette, France) on week 1 (before the dietary intervention) and at the end of the 10-week diet period (week 11). Blood samples (1 ml) were collected before and 2 h after a ^2H_2O injection (500 mg/kg BW). Plasma ^2H_2O concentrations were measured using Fourier-transformed IR spectroscopy (Brüker SA, Wissembourg, France).

Euglycaemic–hyperinsulinaemic clamp technique

The euglycaemic–hyperinsulinaemic clamp technique was performed before and at the end of the 10-week diet period. The catheter was inserted under anaesthesia into the jugular vein of an animal that was not fed overnight. Insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was perfused [72 mU/kg (500 pmol/kg) for 1 min, then 18 mU/kg per min (125 pmol/kg per min) for 3 h] and glycaemia was measured at every 5 min. Glucose (20%; Braun Medical SAS, Boulogne Cedex, France) was perfused at a variable rate. The glucose infusion rate (mg/kg per min) was adjusted to attain and maintain the basal glycaemia. In hyperinsulinaemic conditions, glucose infusion rate measures the insulin-mediated glucose uptake and is considered as a good reflection of IS. Glycaemia was measured using the glucose oxidase method (Accu-Chek Active; Roche Diagnostics, Mannheim, Germany). Insulin was measured using ELISA (Rat Insulin; Shibayagi Company Limited, Gunma, Japan).

Plasma lipid profiles

The basal plasma concentrations of total cholesterol, NEFA and TAG were assayed before and at the end of the 10-week diet period using enzymatic methods (Cholestérol

RTU, BioMérieux, Marcy-l'Etoile, France; Triglycérides enzymatique TG PAP150, BioMérieux; and NEFA C, Wako, Oxoid, Dardilly, France).

The plasma samples were pooled for each group and each time period (weeks 1 and 11), and the plasma lipoproteins were separated using a fast-protein liquid chromatography system (UNICORN 520; GE Healthcare, Pittsburgh, PA, USA). The cholesterol concentration was measured in each fraction.

Statistical analysis

Data analysis was performed using Statview software (version 5.0; SAS Institute Inc., Cary, NC, USA) and R software (version 2.10, lme4 package; R Foundation for Statistical Computing, Vienna, Austria). Data were expressed as mean values with the standard error of the mean. A linear mixed-effects model has been performed in order to study the effect of time, type of diet and the interaction between them for each variable. The mixed-effects models are the most efficient way to analyse repeated measurements data[10]. A multiple comparison of means procedure with Tukey contrasts, adapted to the mixed-effects models, has been used when the interactions between time and the type of diet were significant. A significant difference has been considered for P value $<0{\cdot}05$.

Results

Body weight and body fat mass

Table 1 gives the values of BW and body fat mass of the control, the high-fat and the high-fructose groups before and at the end of the 10-week period. The BW gain was similar for all groups. At week 11, the high-fat group had significantly higher ($P<0{\cdot}05$) body fat mass compared to the control group. At week 11, compared to initial values from week 1, the high-fat diet caused a 393 (SEM 170)% increase in body fat mass and high-fructose diet caused a 139 (SEM 23)% increase.

Insulin sensitivity

No differences were observed in basal glycaemia and insulinaemia among groups. The high-fat group had lower IS compared to the control group at week 11, assessed by significantly lower ($P<0{\cdot}05$) glucose infusion rate value. The high-fructose group had no difference in IS compared to the control group (Table 1).

Plasma lipid profiles

Plasma TAG concentration was not different among groups at any time. Plasma NEFA concentration decreased over time in all groups, regardless of the diet.

At week 1, the plasma total cholesterol concentration was different among groups. Indeed, it was higher in the control group than in others. There was a 38 (SEM 12)% increase in total cholesterol concentration in rats fed with high-fat diet and a 15 (SEM 8)% increase in rats fed with high-fructose diet, but the difference did not reach level of significance (Table 1). Nevertheless, plasma total cholesterol concentration increased over time, regardless of the diet.

On the basis of a pooled sample from each of the groups, there is a preliminary indication that there was no difference due to diet (data not shown) in the plasma lipoprotein fractions, but these results must be considered to be very preliminary.

Discussion

The aim of the present study was to compare the effects of a high-fat and a high-fructose diet on obesity-related disturbances in rats by using a longitudinal approach. In the present study, all the basal values were in the range of normal values of rats. In the literature, various concentrations of plasma lipids have been measured in control rats. However, we did not find any study of obesity and IS in rats with the baseline values of all variables. Therefore, we used a longitudinal approach and composed the groups randomly. The plasma total cholesterol concentration was significantly different among groups at baseline level (week 1). The randomisation process used to compose the groups had been expected to abolish all the differences among groups, and all cholesterol concentrations have been measured at the same time, i.e. at the end of the study, to guarantee same analytical conditions. Unfortunately, the randomisation process failed to reach its objectives, at least in this regard (there was no other difference among the groups at week 1). The baseline values were then taken into account in the statistical analysis in order to nullify the pre-existing differences. Another factor is the age at which exposure to a high-fat or high-fructose diet started. It has been shown that adult rats fed with high-fructose diet produce signs of metabolic syndrome, but young rats do not[11]. We therefore chose adult rats to conduct the present experiment.

We observed similar BW at the end of the 10-week period in all groups. However, rats fed with high-fat diet had higher body fat mass compared to the control rats. Increase in adiposity has previously been described in rats fed with a high-fat diet[2–5,12], and it was associated with increase in weight gain. An increase in BW alone does not necessarily represent obesity, but other factors have to be taken into account, such as changes in body composition. In the present study, the increase in body fat mass reflected the obesogenic property of the high-fat diet, which was due to lard being used as the source of dietary fat. This excess fat was stored in the form of adipose tissue in the body. In rats fed with high-fructose diet, we observed no change in BW and body fat mass compared to the control rats. Previous studies showed that administering high-fructose (60% by weight) diet for about 10 weeks increased BW[6,8,13] and epididymal and retroperitoneal fat depots[13]. However, de Moura *et al.*[11] observed no difference in BW in adult rats administered fructose, despite higher retroperitoneal, mesenteric and subcutaneous fat depots weights. This suggests that high-fructose diet does not necessarily lead to obesity.

The rats fed with high-fat diet had lower IS (assessed by glucose infusion rate, which is considered as a good reflection of

IS, as it measures the insulin-mediated glucose uptake under hyperinsulinaemic conditions) compared to the control group, as previously shown in several studies[14,15]. Moreover, low IS has been shown to be associated with an increase in epididymal fat[3,4], and the present results of higher body fat mass in rats fed with high-fat diet are consistent with the previous results. However, basal glycaemia and insulinaemia were unchanged. Some studies using high-fat diet (lard or safflower oil) reported low IS, associated with hyperinsulinaemia[3–5,12,16] and hyperglycaemia[16]. We think that we could have assessed low IS by euglycaemic–hyperinsulinaemic clamp technique before the appearance of hyperglycaemic and hyperinsulinaemic states, and that the present experiment could have been long enough to develop insulin resistance but not diabetes.

On the other hand, we found no change in IS, basal glycaemia and insulinaemia in rats fed with high-fructose diet. Many previous studies have reported hyperglycaemia[7–9,13], but Nakagawa *et al.*[6] did not show any modification in glycaemia after administering high-fructose diet. The higher hepatic glycogen content that has been described in rats fed with high-fructose diet[17] could prevent hyperglycaemia. Previous studies have also shown that the high-fructose diet developed low IS and was associated with increased plasma TAG and NEFA concentrations[6–8]. These increased concentrations in response to high-fructose diet could have an important role in the development of low IS by reducing insulin signalling pathway (reviewed in Tappy & Le[18]). However, we did not find dyslipidaemia, which is in accordance with the unchanged IS. Moreover, in a study in rats, high-sucrose diet has been shown to induce low IS in the liver before muscle[19]. We assessed the IS by using the euglycaemic–hyperinsulinaemic clamp technique, which is the gold standard method for direct assessment of the whole-body IS[20]. We suggest that high-fructose diet could have caused minor impairment of insulin action in the liver, but could not have caused whole-body low IS.

We observed no difference in plasma total cholesterol concentration between the groups, but there was a significant increase over time, regardless of the diet. This increase could have reflected an effect of age, whereas the absence of dietary cholesterol could be a possible explanation for the absence of any specific effect of the diet. Indeed, additional dietary fat, whatever its nature, had no effect on plasma cholesterol concentration in the absence of dietary cholesterol in a study conducted in hamsters[21]. In the present study, the non-significant increase in plasma total cholesterol in rats fed with high-fat diet could be due to small amount of dietary cholesterol in lard. On the other hand, previous studies reported variable responses of the high-fructose diets on plasma total cholesterol concentration[7,8,13,22], and even the same high-fructose diets behaved differently[7,13]. Thus, it is difficult to conclude about the effects of fructose diet on cholesterol concentration. Moreover, the dietary cholesterol and amount of fat in a high-fructose diet could affect plasma cholesterol concentration. We also observed no difference in cholesterol concentrations in plasma lipoproteins, as reported by Sinitskaya *et al.*[2]. Conversely, Mohamed Salih *et al.*[8] reported an increase in VLDL-cholesterol and LDL-cholesterol, and a decrease in HDL-cholesterol at the end of a high-fructose diet. The present results for lipoprotein-cholesterol concentrations are in accordance to unchanged plasma total cholesterol concentration, and absence of dietary cholesterol could possibly be the cause for unchanged cholesterol concentration.

Plasma basal NEFA concentration was not different among groups. Variable changes in plasma NEFA concentration have been described in response to a high-fat diet[1,2,12]. The present results in rats fed with high-fructose diet were not consistent with some previous reports[8,13]. We hypothesise that the liver could have higher hepatic TAG storage[23], by capturing a bulk amount of plasma NEFA, which would explain that no difference in plasma NEFA concentration was observed.

We found no change in plasma basal TAG concentration in any group. Some studies showed an increase in plasma TAG concentration at the end of a high-fat diet[3,4], whereas others with nearly the same fat content did not[1,2,5]. Variable response on plasma basal TAG concentration has also been reported in rats fed with high-fructose diet[6–8,13,22,24]. A few studies have reported higher postprandial TAG concentration in rats fed with high-fructose diet, but no change in the basal TAG concentration for a short period (2 weeks)[25] or for a long period (11 months)[26]. In the present study, we measured only the basal TAG concentration. We suggest that the rats in our study could have had higher postprandial TAG concentration that could not have been observed in overnight unfed rats.

On the basis of the present findings, feeding 12-week-old rats a high-fat diet for 10 weeks induces obesity and low IS. In contrast, the high-fructose diet produced no change in obesity-related disorders. In the future, it will be useful to study the specific effects of different fats, oils or fatty acids that account for the discrepancy between these studies.

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References

1. Morens C, Sirot V, Scheurink AJ, *et al.* (2006) Low-carbohydrate diets affect energy balance and fuel homeostasis differentially in lean and obese rats. *Am J Physiol Regul Integr Comp Physiol* **291**, R1622–R1629.

2. Sinitskaya N, Gourmelen S, Schuster-Klein C, *et al.* (2007) Increasing the fat-to-carbohydrate ratio in a high-fat diet prevents the development of obesity but not a prediabetic state in rats. *Clin Sci (Lond)* **113**, 417–425.
3. Sridhar MG, Vinayagamoorthi R, Arul Suyambunathan V, *et al.* (2008) (2008) Bitter gourd (*Momordica charantia*) improves insulin sensitivity by increasing skeletal muscle insulin-stimulated IRS-1 tyrosine phosphorylation in high-fat-fed rats. *Br J Nutr* **99**, 806–812.
4. Vinayagamoorthi R, Bobby Z & Sridhar MG (2008) Antioxidants preserve redox balance and inhibit c-Jun-N-terminal kinase pathway while improving insulin signaling in fat-fed rats: evidence for the role of oxidative stress on IRS-1 serine phosphorylation and insulin resistance. *J Endocrinol* **197**, 287–296.
5. Matveyenko AV, Gurlo T, Daval M, *et al.* (2009) Successful versus failed adaptation to high-fat diet-induced insulin resistance: the role of IAPP-induced beta-cell endoplasmic reticulum stress. *Diabetes* **58**, 906–916.
6. Nakagawa T, Hu H, Zharikov S, *et al.* (2006) A causal role for uric acid in fructose-induced metabolic syndrome. *Am J Physiol Renal Physiol* **290**, F625–F631.
7. Liu IM, Tzeng TF & Liou SS (2011) A Chinese herbal decoction, Dang Gui Bu Xue Tang, prepared from Radix Astragali and Radix *Angelicae sinensis*, ameliorates insulin resistance induced by a high-fructose diet in rats. *Evid Based Complement Alternat Med*, vol. 2011, Article ID 248231, 11 pages. doi:10.1093/ecam/nep004.
8. Mohamed Salih S, Nallasamy P, Muniyandi P, *et al.* (2009) Genistein improves liver function and attenuates non-alcoholic fatty liver disease in a rat model of insulin resistance. *J Diabetes* **1**, 278–287.
9. Kannappan S, Palanisamy N & Anuradha CV (2010) Suppression of hepatic oxidative events and regulation of eNOS expression in the liver by naringenin in fructose-administered rats. *Eur J Pharmacol* **645**, 177–184.
10. Davidian M & Giltinan DM (1995) *Nonlinear Models for Repeated Measurement Data*. New York, NY: Chapman & Hall/CRC.
11. de Moura RF, Ribeiro C, de Oliveira JA, *et al.* (2009) Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols. *Br J Nutr* **101**, 1178–1184.
12. Posey KA, Clegg DJ, Printz RL, *et al.* (2009) Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *Am J Physiol Endocrinol Metab* **296**, E1003–E1012.
13. Shih CC, Lin CH, Lin WL, *et al.* (2009) Momordica charantia extract on insulin resistance and the skeletal muscle GLUT4 protein in fructose-fed rats. *J Ethnopharmacol* **123**, 82–90.
14. Kraegen EW, Clark PW, Jenkins AB, *et al.* (1991) Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* **40**, 1397–1403.
15. Oakes ND, Cooney GJ, Camilleri S, *et al.* (1997) Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. *Diabetes* **46**, 1768–1774.
16. Vikram A, Jena GB & Ramarao P (2010) Increased cell proliferation and contractility of prostate in insulin resistant rats: linking hyperinsulinemia with benign prostate hyperplasia. *Prostate* **70**, 79–89.
17. Yadav H, Jain S, Yadav M, *et al.* (2009) Epigenomic derangement of hepatic glucose metabolism by feeding of high fructose diet and its prevention by Rosiglitazone in rats. *Dig Liver Dis* **41**, 500–508.
18. Tappy L & Le KA (2010) Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev* **90**, 23–46.
19. Pagliassotti MJ, Prach PA, Koppenhafer TA, *et al.* (1996) Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. *Am J Physiol* **271**, R1319–R1326.
20. DeFronzo RA, Tobin JD & Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* **237**, E214–E223.
21. Sessions VA & Salter AM (1994) The effects of different dietary fats and cholesterol on serum lipoprotein concentrations in hamsters. *Biochim Biophys Acta* **1211**, 207–214.
22. Stark AH, Timar B & Madar Z (2000) Adaptation of Sprague–Dawley rats to long-term feeding of high fat or high fructose diets. *Eur J Nutr* **39**, 229–234.
23. Benhizia F, Hainault I, Serougne C, *et al.* (1994) Effects of a fish oil-lard diet on rat plasma lipoproteins, liver FAS, and lipolytic enzymes. *Am J Physiol* **267**, E975–E982.
24. Rizkalla SW, Boillot J, Tricottet V, *et al.* (1993) Effects of chronic dietary fructose with and without copper supplementation on glycaemic control, adiposity, insulin binding to adipocytes and glomerular basement membrane thickness in normal rats. *Br J Nutr* **70**, 199–209.
25. Koo HY, Wallig MA, Chung BH, *et al.* (2008) Dietary fructose induces a wide range of genes with distinct shift in carbohydrate and lipid metabolism in fed and fasted rat liver. *Biochim Biophys Acta* **1782**, 341–348.
26. Lee YC, Ko YH, Hsu YP, *et al.* (2006) Plasma leptin response to oral glucose tolerance and fasting/re-feeding tests in rats with fructose-induced metabolic derangements. *Life Sci* **78**, 1155–1162.

2.1.1.1 Introduction

The prevalence of obesity and its related disorders are growing alarmingly all over the world, especially in the developed countries. The main factor is our life style that includes feeding habits and low physical activity. Insulin resistance and dyslipidemia are also associated with obesity. Scientific literature shows numerous studies on obesity and its related disorders in humans and animal models. Different animal models have been used to study for this purpose, notably the rat. Obesity can be caused by genetic manipulations and nutritional interventions in these models. Since human obesity is mainly due to nutritional habits, animal models of obesity and low insulin sensitivity induced by specific diets may be preferable to the genetic models.

High-fat and high-fructose diets have been used to induce obesity and low insulin sensitivity. Longitudinal approach could be useful to study diet-induced obesity and low insulin sensitivity. This type of approach has never been conducted in rats to study obesity and low insulin sensitivity (assessment of insulin sensitivity by euglycemic-hyperinsulinemic clamp technique).

In this part of study, we aimed to perform longitudinal study to compare the effects of a high-fat diet and a high-fructose diet on body weight, insulin sensitivity and plasma lipid profiles in rats. The work is divided in two parts: one is explained in the form of an article, and the other is related to the study of the mRNA expression of genes in the liver and adipose tissue, involved in the insulin sensitivity, and metabolisms of glucose and lipid.

2.1.1.2 Results (article)

Weight gain was similar for all the groups. The rats consuming the high-fat diet had a body fat significantly higher ($P < 0.05$) compared to the rats consuming the control diet, at the end of the protocol. No difference in basal glycemia or insulinemia was observed in any group. The rats consuming the high-fat diet showed lower ($P < 0.05$) glucose infusion rate during hyperinsulinemic-euglycemic clamp test, compared to the rats consuming the control diet, at the end of the protocol. The rats consuming the high-fructose diet had no change in the insulin sensitivity compared to rats consuming the control diet. Plasma concentrations of total cholesterol, triglycerides and non-esterified fatty acids were similar in all the groups at the end of the protocol. The concentrations of cholesterol and triglycerides in plasma lipoproteins have not changed in any group. The values of the parameters studied were presented in the table 6.

Table 6: Body weight, body fat mass, insulin sensitivity, and plasma lipid profiles of rats in the control, the high-fat, and the high-fructose groups at week 1 and 11 of the experiment.

	Control				High-fat				High-fructose						
	Week 1		Week 11		Week 1		Week 11		Week 1		Week 11				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*Interaction*	*Time*	*Type of diet*
Body weight (g)	401	6	526	14	383	19	534	26	399	12	529	16	*NS*	‡‡	*NS*
Body fat mass (g)	35.6	3.3	63.3	9.5	25.6	4.7	90.0	12.2	34.6	5.7	78.9	11.8	*	-	-
Basal glycemia (mmol/l)	5.51	0.11	4.93	0.25	5.60	0.15	5.31	0.19	5.35	0.19	5.52	0.31	*NS*	*NS*	*NS*
Basal insulinemia (pmol/l)	402	8.4	400	17.2	415	24.8	388	9.9	386	12.4	402	11.0	*NS*	*NS*	*NS*
GIR (mg/kg/min)	19.0	1.4	18.5	1.2	19.3	1.6	12.9	2.0	18.2	1.5	15.3	1.4	*	-	-
Total cholesterol (mmol/l)	1.81	0.11	2.04	0.14	1.46	0.06	1.99	0.17	1.59	0.09	1.83	0.14	*NS*	‡	*NS*
Triglycerides (mmol/l)	1.89	0.15	1.64	0.10	1.86	0.12	1.76	0.12	1.78	0.11	2.0	0.30	*NS*	*NS*	*NS*
NEFA (mmol/l)	0.46	0.07	0.30	0.04	0.45	0.06	0.31	0.04	0.42	0.04	0.41	0.04	*NS*	‡	*NS*

SEM, standard error of the mean; NS, non significant; GIR, glucose infusion rate during euglycemic-hyperinsulinemic clamp technique. * $P < 0.05$, for comparisons among groups for the interaction between time and the type of diet (Fixed effects model analysis revealed that values in the high-fat group is significant at week 11, compared to the control group), ‡ $P < 0.05$ and ‡‡ $P < 0.0001$ for comparisons among groups for the effect of time (significant for week 11 vs. Week 1) and - when $P < 0.05$ for comparison for the interaction between time and the type of diet, the effect of time and type of diet were not considered. Data were represented as the mean values and SEMs (n = 6).

2.1.1.3 mRNA expression of genes in hepatic tissue

The mRNA expression of genes in hepatic tissue was presented in the figures 15-18. The mRNA expressions of genes of involved in insulin signalling (insulin receptor and IRS-1) were not changed in liver, at week 11 compared to week 1 in any group. The mRNA expression of the GLUT4 was decreased in the liver in the high-fat and the high-fructose groups at the end of the experiment, compared to week 1. There was no change in the mRNA expressions of FAS, LPL, PEPCK and HSL in liver at the end of the experiment, and that of hepatic lipase was increased in liver at week 11, compared to week 1 in the high-fructose group. The mRNA expression of hepatic SREBP-1c was increased compared to week 1 in the high-fat group. The mRNA expression of hepatic PPARα was increased compared to week 1 in the high-fructose group.

2.1.1.4 mRNA expression of genes in visceral adipose tissue

The mRNA expression of genes in visceral adipose tissue was presented in the figures 19-23. The mRNA expressions of genes of involved in insulin signalling (insulin receptor and IRS-1) in the visceral adipose tissue have not been different in any group. The mRNA level of GLUT4 in the visceral adipose tissue was lower in the rats fed with the high-fat or the high-fructose diets compared to the control group at the end of the experiment. The mRNA expressions of genes of leptin and TNFα in the adipose tissue were higher in the rats fed with the high-fat diet compared to the control group. The mRNA expressions of FAS and ACC in the adipose tissue were higher in the rats fed with the high-fat diet compared to the control group, and that of LPL was similar in all the groups. The mRNA expression of perilipin in the visceral adipose tissue was higher in the rats fed with the high-fat diet compared to the control group, and that of HSL was similar in all the groups. The mRNA expression of SREBP-1c in the visceral adipose tissue was not different in any group. The mRNA expression of PPARγ in the visceral adipose tissue was tended to be lower ($P = 0.1023$) in the rats fed with the high-fat diet compared to the control group.

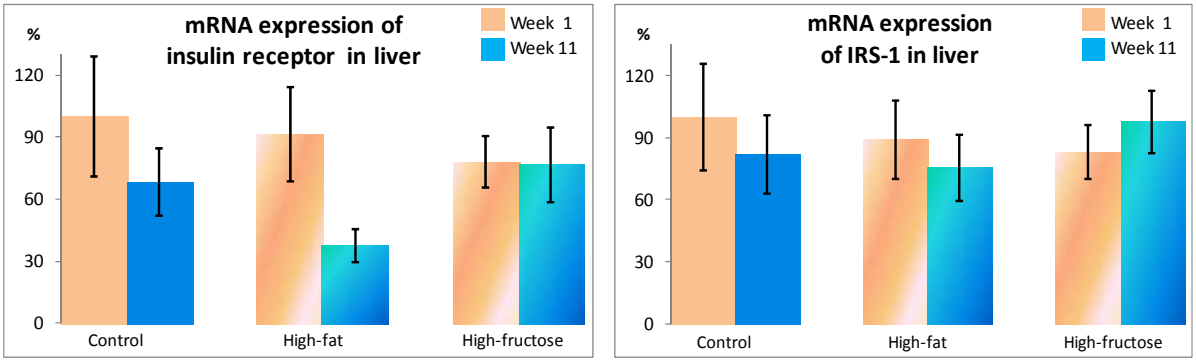

Figure 15: The mRNA expressions of insulin receptor and IRS-1 in the liver of rats in the control, the high-fat, and the high-fructose groups at week 1 and 11 of the experiment.

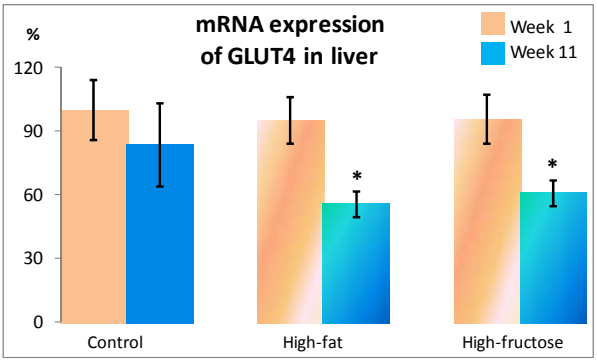

Figure 16: The mRNA expressions of GLUT4 in the liver of rats in the control, the high-fat, and the high-fructose groups at week 1 and 11 of the experiment.

* $P < 0.05$ compared to week 1 of same group.

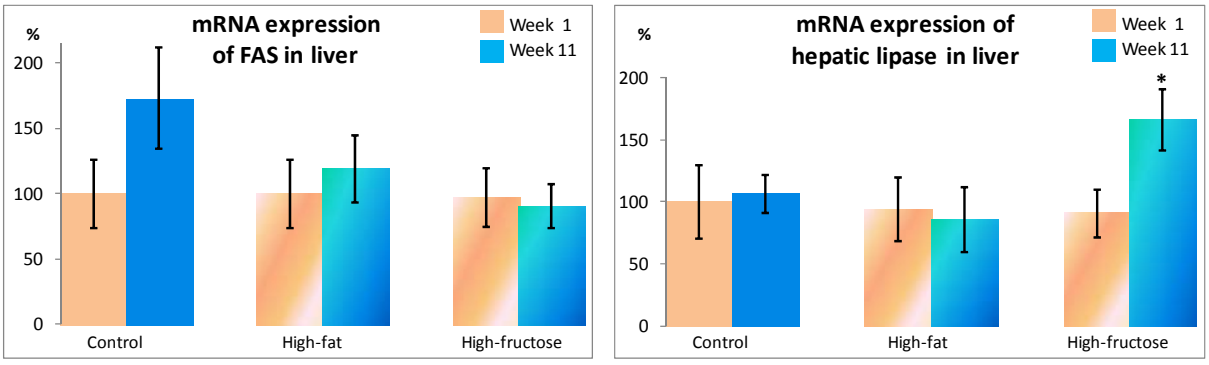

Figure 17: The mRNA expressions of FAS and hepatic lipase in the liver of rats in the control, the high-fat, and the high-fructose groups at week 1 and 11 of the experiment.

* $P < 0.05$ compared to week 1 of same group.

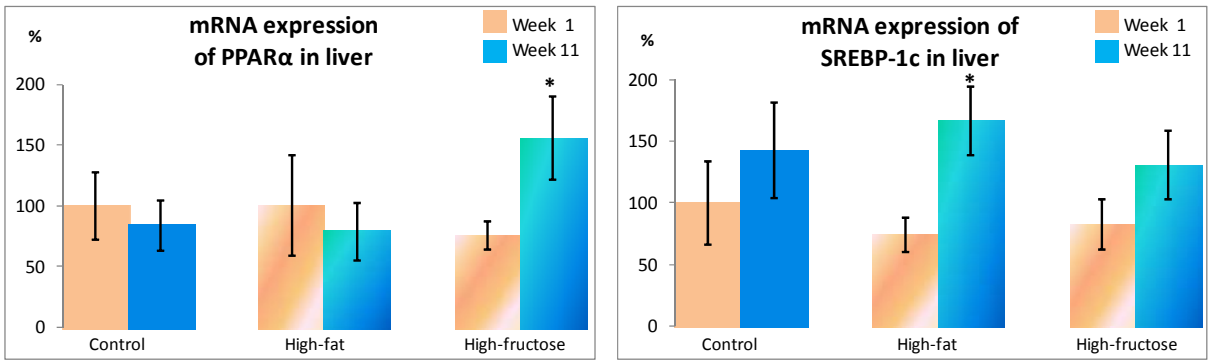

Figure 18: The mRNA expressions of PPARα and SREBP-1c in the liver of rats in the control, the high-fat, and the high-fructose groups at week 1 and 11 of the experiment.

* P < 0.05 compared to week 1 of same group.

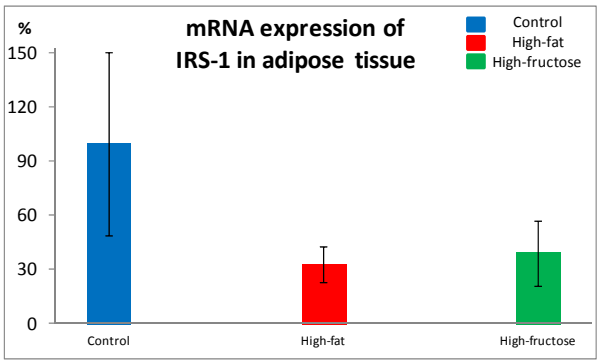

Figure 19: The mRNA expressions of IRS-1 in the visceral adipose tissue of rats in the control, the high-fat, and the high-fructose groups at the end of experiment.

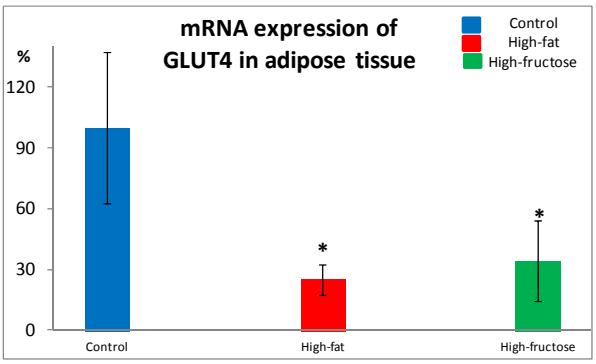

Figure 20: The mRNA expressions of GLUT4 in the visceral adipose tissue of rats in the control, the high-fat, and the high-fructose groups at the end of experiment.

* P < 0.05 compared to the control group at the end of the experiment.

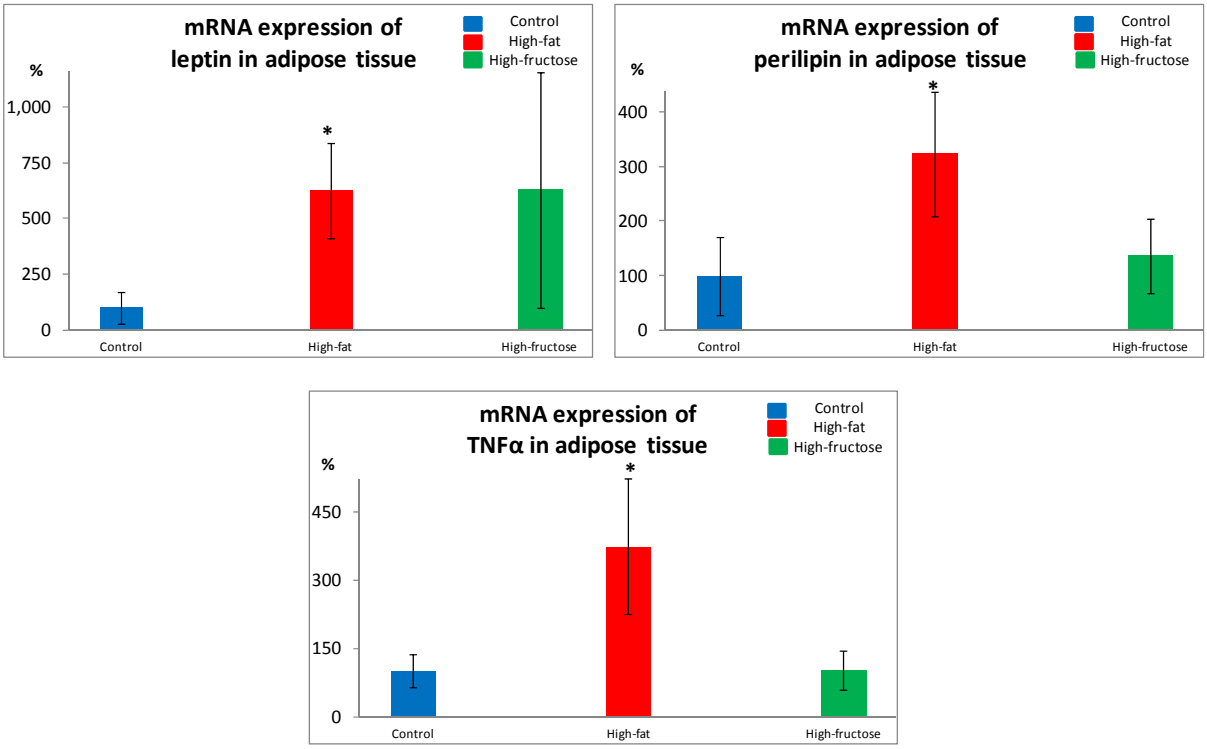

Figure 21: The mRNA expressions of leptin, perilipin and TNFα in the visceral adipose tissue of rats in the control, the high-fat, and the high-fructose groups at the end of experiment.

* P < 0.05 compared to the control group at the end of the experiment.

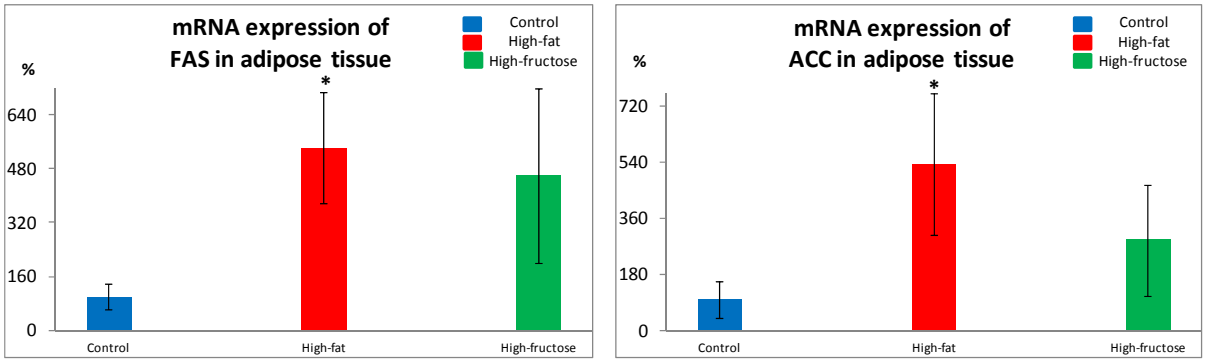

Figure 22: The mRNA expressions of FAS and ACC in the visceral adipose tissue of rats in the control, the high-fat, and the high-fructose groups at the end of experiment.

* P < 0.05 compared to the control group at the end of the experiment.

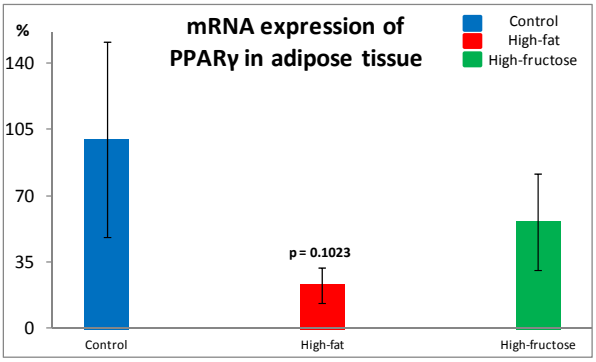

Figure 23: The mRNA expressions of PPARγ in the visceral adipose tissue of rats in the control, the high-fat, and the high-fructose groups at the end of experiment.

* $P < 0.05$ compared to the control group at the end of the experiment.

3 Supplementation with eicosapentaenoic acid (EPA)

Lipid profile and insulin sensitivity in rats fed with the high-fat or the high-fructose diet supplemented with EPA.

3.1 Introduction

Various pharmacological and nutritional interventions have been studied to prevent obesity-induced insulin resistance and dyslipidemia. Over the years, omega-3 polyunsatured fatty acids have been used for this purpose with variable effects. Fish oil (rich in EPA and DHA) has has been suggested to decrease plasma triglycerides level and with controversial effect on the insulin sensitivity.

In this study, eicosapentaenoic acid (EPA) has been supplemented to the rats fed with the high-fat, or the high-fructose diet. The aim was to study the effects of the supplementation with EPA on insulin sensitivity, plasma lipid profile and on the genes involved in the insulin sensitivity, and in lipid and glucose metabolism.

3.2 Results

3.2.1 Supplementation with EPA with the high-fat diet

3.2.1.1 Body weight and body composition

The results of body weight and body composition were presented in the table 7. All groups showed significant effect of time on body weight. There was significant effect of interaction between time and EPA-supplementation on body fat mass. The high-fat groups (supplemented with or without EPA) showed increased body fat mass, compared to week 1.

3.2.1.2 Insulin sensitivity

The results of insulin sensitivity were presented in the figure 24. There was no change in basal glycemia and insulinemia. All the groups showed significant effect of interaction between time and EPA-supplementation on glucose infusion rate in the euglycemic-

hyperinsulinemic clamp test. The high-fat group without EPA showed lower glucose infusion rate, compared to week 1.

3.2.1.3 Plasma lipids

The results of plasma lipids were presented in the table 8. All the groups showed significant effect of time on the concentrations of total cholesterol, triglycerides and NEFA. There was no change in the plasma lipoprotein cholesterol and triglycerides profile, and the supplementation with EPA decreased plasma VLDL-cholesterol in the high-fat diet fed rats compared to week 1.

3.2.1.4 mRNA expression of genes in hepatic tissue

The mRNA expression of genes in hepatic tissue was presented in the figures 25-28. The mRNA expressions of insulin receptor and IRS-1 in the liver were unchanged, and that of hepatic GLUT4 was unchanged in the EPA-supplemented group in the high-fat diet fed rats. The supplementation with EPA tended to decrease ($P = 0.0639$) the mRNA expression of hepatic LPL in the high-fat diet fed rats compared to week 1, and did not change the mRNA expressions of FAS and PEPCK in the liver. The mRNA expression of HSL in the liver was increased in the high-fat EPA group compared to week 1, and that of hepatic lipase was unchanged. The mRNA expression of hepatic SREBP-1c was increased compared to week 1 in the high-fat groups, supplemented with or without EPA; and that of hepatic PPARα was unchanged.

3.2.1.5 mRNA expression of genes in visceral adipose tissue

The mRNA expression of genes in visceral adipose tissue was presented in the figures 29-31. The mRNA expression of IRS-1 and GLUT4 were higher in the adipose tissue in the rats fed with the high-fat diet supplemented with EPA, compared to the high-fat non-supplemented group at the end of the experiment. The mRNA expressions of insulin receptor; leptin; TNFα; lipogenic key proteins (FAS, ACC and LPL); lipolytic key proteins (perilipin and HSL); and transcriptional factors (PPARγ and SREBP-1c) in the adipose tissue were not different at the end of supplementation with EPA in the rats fed with the high-fat diet.

Table 7: Body weight and body fat mass of rats in the control, the high-fat and the high-fat EPA groups at week 1 and 11 of the experiment.

	Control		**High-fat**		**High-fat EPA**				
(g)	**Week 1**	**Week 11**	**Week 1**	**Week 11**	**Week 1**	**Week 11**	***Interaction***	***Time***	***EPA-suppl.***
Body weight	401±6	526±14	383±19	534±26	385±19	540±21	*NS*	‡	*NS*
Body fat mass	35.6±3.3	63.3±9.5	25.6±4.7	90.0±12.2*	33.2±3.8	99.0±10.3*	*	-	-

EPA, eicosapentanoid acid; EPA-suppl., supplementation with EPA; NS, non significant. * $P < 0.05$, for comparisons among groups for the interaction between time and the EPA-supplementation (Fixed effects model analysis revealed that values in the high-fat and the high-fat EPA groups are significant at week 11, compared to the control group), ‡ $P < 0.05$ for comparisons among groups for the effect of time (significant for week 11 vs. Week 1) and - when $P < 0.05$ for comparison for the interaction between time and the EPA-supplementation, the effect of time and EPA-supplementation were not considered. Data were represented as the mean values and standard error of the means (n = 6).

Table 8: Plasma lipid profiles of rats in the control, the high-fat and the high-fat EPA groups at week 1 and 11 of the experiment.

	Control		**High-fat**		**High-fat EPA**				
(mmol/l)	**Week 1**	**Week 11**	**Week 1**	**Week 11**	**Week 1**	**Week 11**	***Interaction***	***Time***	***EPA-suppl.***
Total cholesterol	1.81±0.11	2.04±0.14	1.46±0.06	1.99±0.17	1.62±0.12	1.71±0.08	*NS*	‡	*NS*
Triglycerides	1.89±0.15	1.64±0.10	1.86±0.12	1.76±0.12	1.76±0.17	1.60±0.18	*NS*	‡	*NS*
NEFA	0.46±0.07	0.30±0.04	0.45±0.06	0.31±0.04	0.48±0.07	0.44±0.06	*NS*	‡	*NS*

EPA, eicosapentanoid acid; EPA-suppl., supplementation with EPA; NS, non significant; NEFA, non-esterified fatty acids. ‡ $P < 0.05$ for comparisons among groups for the effect of time (significant for week 11 vs. Week 1). Data were represented as the mean values and standard error of the means (n = 6).

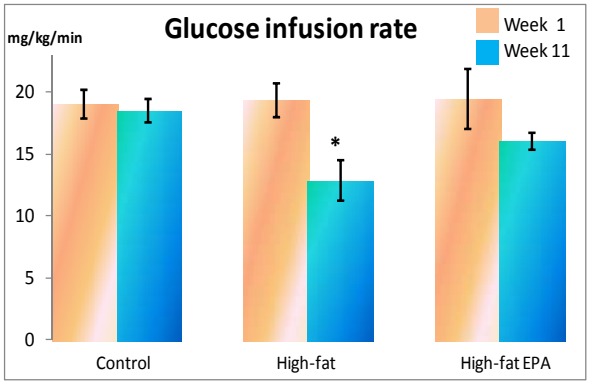

Figure 24: Glucose infusion rate during euglycemic-hyperinsulinemic clamp technique in rats in the control, the high-fat and the high-fat EPA groups at week 1 and 11 of the experiment.

* P < 0.05, for comparisons among groups for the interaction between time and the EPA-supplementation (Fixed effects model analysis revealed that value in the high-fat group is significant at week 11, compared to the control group).

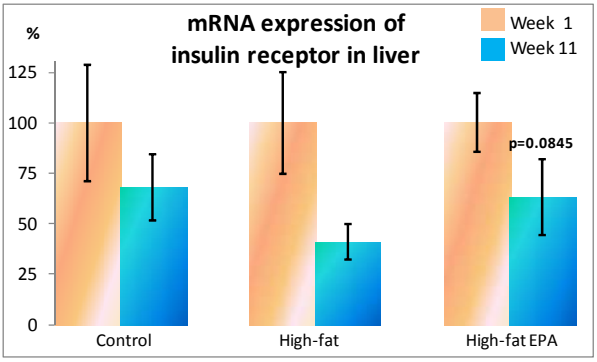

Figure 25: The mRNA expressions of insulin receptor in the liver of rats in the control, the high-fat and the high-fat EPA groups at week 1 and 11 of the experiment.

P = 0.0845 compared to week 1 of same group.

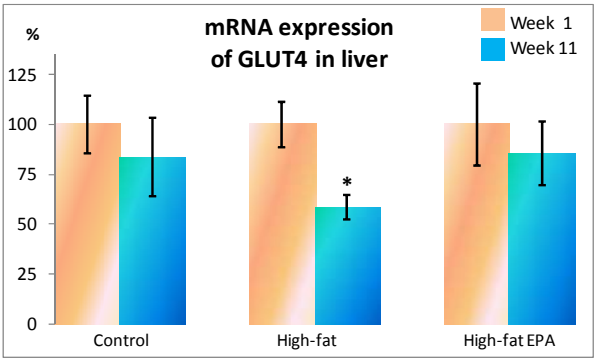

Figure 26: The mRNA expressions of GLUT4 in the liver of rats in the control, the high-fat and the high-fat EPA groups at week 1 and 11 of the experiment.

* P < 0.05 compared to week 1 of same group.

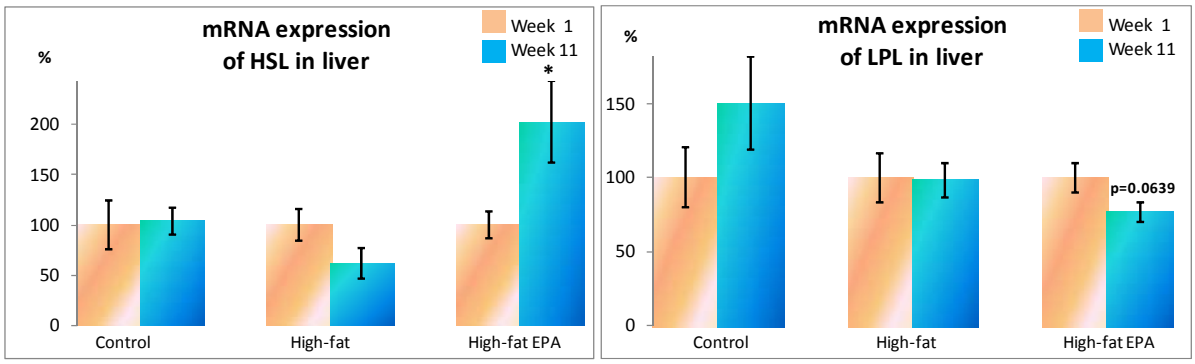

Figure 27: The mRNA expressions of HSL and LPL in the liver of rats in the control, the high-fat and the high-fat EPA groups at week 1 and 11 of the experiment.

* $P < 0.05$ and $P = 0.0639$ compared to week 1 of same group.

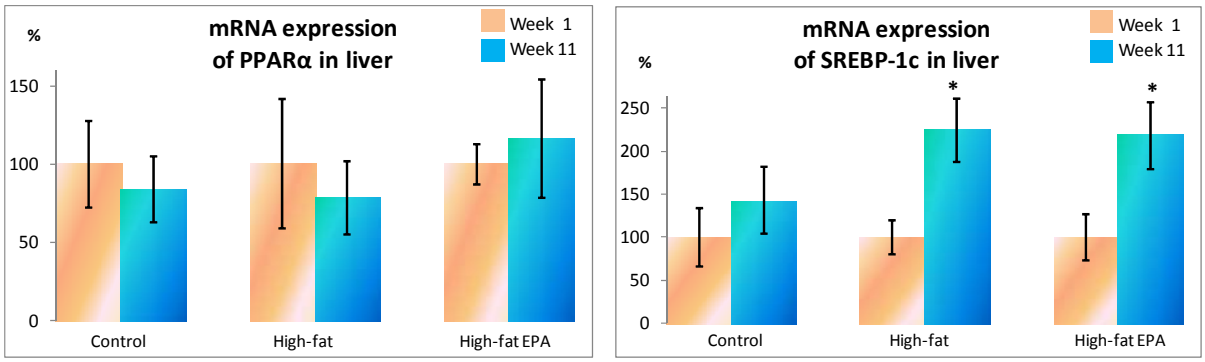

Figure 28: The mRNA expressions of PPARα and SREBP-1c in the liver of rats in the control, the high-fat and the high-fat EPA groups at week 1 and 11 of the experiment.

* $P < 0.05$ compared to week 1 of same group.

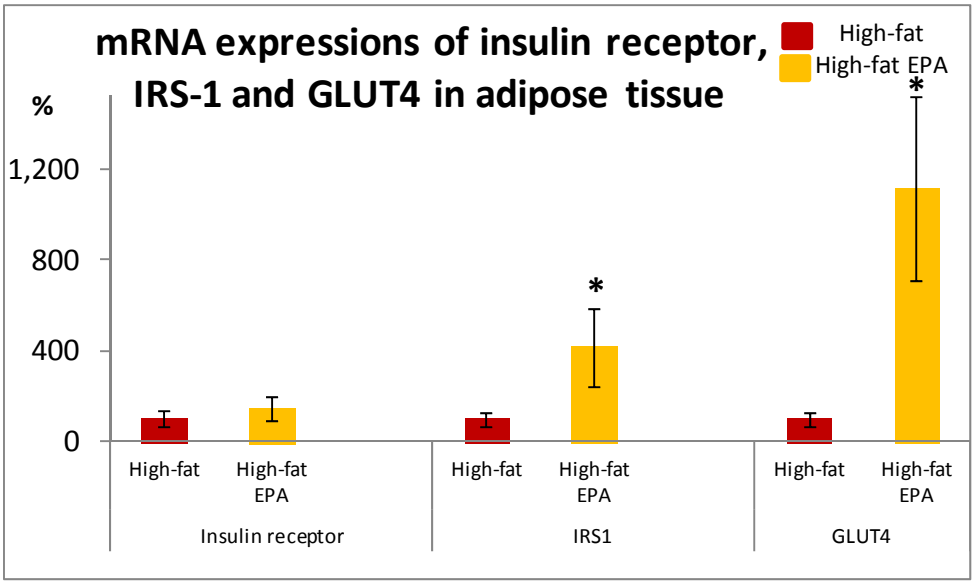

Figure 29: The mRNA expressions of insulin receptor, IRS-1 and GLUT4 in the visceral adipose tissue of rats in the high-fat and the high-fat EPA groups at the end of experiment.

* $P < 0.05$ compared to the high-fat group at the end of the experiment.

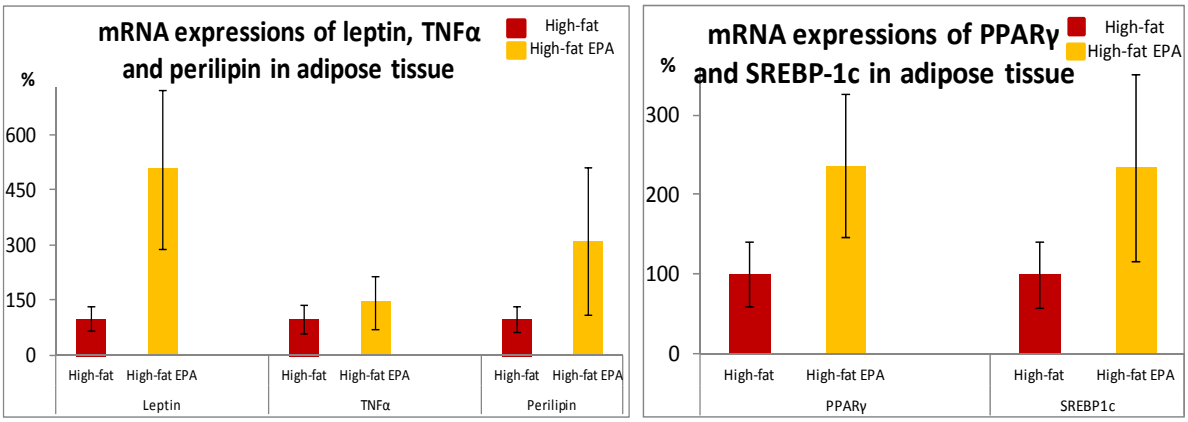

Figure 30: The mRNA expressions of leptin, TNFα, perilipin, PPARγ and SREBP-1c in the visceral adipose tissue of rats in the high-fat and the high-fat EPA groups at the end of experiment.

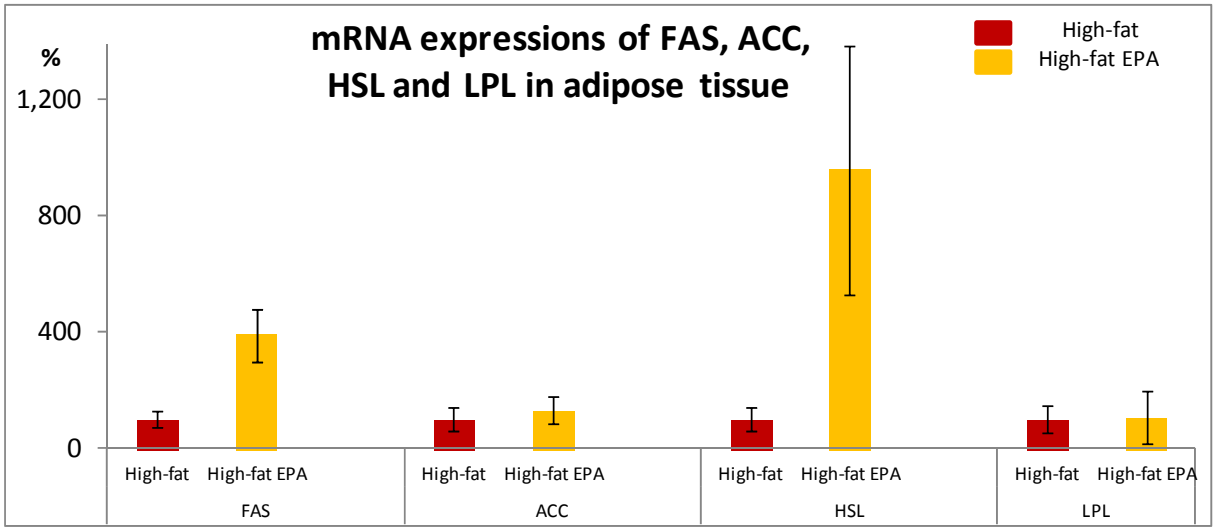

Figure 31: The mRNA expressions of FAS, ACC, HSL and LPL in the visceral adipose tissue of rats in the high-fat and the high-fat EPA groups at the end of experiment.

3.2.2 Supplementation with EPA with the high-fructose diet

3.2.2.1 Body weight and body composition

The results of body weight and body composition were presented in the table 9. All the groups showed significant effect of interaction between time and EPA-supplementation on body weight. The high-fructose group supplemented with EPA showed increased body weight, compared to week 1. All the groups showed significant effect of time on body fat mass.

3.2.2.2 Insulin sensitivity

The results of the insulin sensitivity were presented in the figure 32. There was no change in basal glycemia, insulinemia and glucose infusion rate in euglycemic-hyperinsulinemic clamp test in the high-fructose group supplemented or not with EPA.

3.2.2.3 Plasma lipids

The results of plasma lipids were presented in the table 10. All the groups showed significant effect of time on total cholesterol concentration. All the groups showed significant effect of the interaction between time and the EPA-supplementation on the concentrations of triglycerides and NEFA. The high-fructose EPA group showed tendency of decrease in triglycerides concentration ($P = 0.086$) and NEFA concentration ($P = 0.05$). There was no change in the plasma lipoprotein cholesterol and triglycerides profile, and plasma VLDL-cholesterol was increased in the EPA-supplemented group of the high-fructose diet fed rats at the end of the experiment, compared to week 1.

3.2.2.4 mRNA expression of genes in hepatic tissue

The mRNA expression of genes in hepatic tissue was presented in the figures 33-36. The mRNA expressions of major genes involved in the insulin signalling (insulin receptor and IRS-1) and glucose uptake (GLUT4) in the liver were unchanged in the high-fructose EPA group. The mRNA expression of LPL in the liver was increased in the high-fructose EPA group at the end of the experiment, compared to week 1, and those of FAS and PEPCK in the hepatic tissue were unchanged. The mRNA expression of HSL in the liver was increased in the high-fructose EPA group compared to week 1, and that of hepatic lipase was unchanged.

The supplementation with EPA did not change the mRNA expressions of the hepatic PPARα and hepatic SREBP-1c in the rats fed with the high-fructose diet.

3.2.2.5 mRNA expression of genes in visceral adipose tissue

The mRNA expression of genes in visceral tissue was presented in the figures 37-39. The mRNA expressions of major genes involved in the insulin signalling (insulin receptor and IRS-1) and glucose uptake (GLUT4) in the visceral adipose tissue were similar at the end of the supplementation with EPA in the rats fed with the high-fructose diet. The mRNA expressions of leptin and TNFα in the visceral adipose tissue were similar at the end of the supplementation with EPA in the rats fed with the high-fructose diet. The mRNA expression of FAS was higher in the visceral adipose tissue at the end of the experiment in the rats fed with the high-fructose diet supplemented with EPA compared to the non-supplemented group, and those of ACC and LPL were similar between the groups. The mRNA expressions of perilipin and HSL in the visceral adipose tissue were similar at the end of the supplementation with EPA in the rats fed with the high-fructose diet. The the mRNA expression of PPARγ in the visceral adipose tissue was higher at the end of the supplementation with EPA in the rats fed with the high-fructose diet, and that of SREBP-1c was similar at the end of the experiment.

Table 9: Body weight and body fat mass of rats in the control, the high-fructose and the high-fructose EPA groups at week 1 and 11 of the experiment.

	Control		**High-fructose**		**High-fructose EPA**				
(g)	**Week 1**	**Week 11**	**Week 1**	**Week 11**	**Week 1**	**Week 11**	***Interaction***	***Time***	***EPA-suppl.***
Body weight	401±6	526±14	399±12	529±16	395±18	553±17*	*	-	-
Body fat mass	35.6±3.3	63.3±9.5	34.6±5.7	78.9±11.8	30.8±3.6	79.5±11.7	*NS*	ǂ	*NS*

EPA, eicosapentanoid acid; EPA-suppl., supplementation with EPA; NS, non significant. * $P < 0.05$, for comparisons among groups for the interaction between time and the EPA-supplementation (Fixed effects model analysis revealed that values in the high-fructose EPA group are significant at week 11, compared to the control group), ǂ $P < 0.05$ for comparisons among groups for the effect of time (significant for week 11 vs. Week 1) and - when $P < 0.05$ for comparison for the interaction between time and the EPA-supplementation, the effect of time and EPA-supplementation were not considered. Data were represented as the mean values and standard error of the means (n = 6).

Table 10: Plasma lipid profiles of rats in the control, the high-fructose and the high-fructose EPA groups at week 1 and 11 of the experiment.

	Control		**High-fructose**		**High-fructose EPA**				
(mmol/l)	**Week 1**	**Week 11**	**Week 1**	**Week 11**	**Week 1**	**Week 11**	***Interaction***	***Time***	***EPA-suppl.***
Total cholesterol	1.81±0.11	2.04±0.14	1.59±0.09	1.83±0.14	1.59±0.08	1.83±0.24	*NS*	‡	*NS*
Triglycerides	1.89±0.15	1.64±0.10	1.78±0.11	2.00±0.30	2.49±0.19	1.73±0.11x	*	-	-
NEFA	0.46±0.07	0.30±0.04	0.42±0.04	0.41±0.04	0.62±0.02	0.30±0.03y	*	-	-

EPA, eicosapentanoid acid; EPA-suppl., supplementation with EPA; NS, non significant; NEFA, non-esterified fatty acids. * $P < 0.05$, for comparisons among groups for the interaction between time and the EPA-supplementation (Fixed effects model analysis revealed that values in the high-fructose EPA group are tended to decrease at week 11, x $P = 0.086$ and y $P = 0.05$ compared to the control group), ‡ $P < 0.05$ for comparisons among groups for the effect of time (significant for week 11 vs. Week 1) and - when $P < 0.05$ for comparison for the interaction between time and the EPA-supplementation, the effect of time and EPA-supplementation were not considered. Data were represented as the mean values and standard error of the means ($n = 6$).

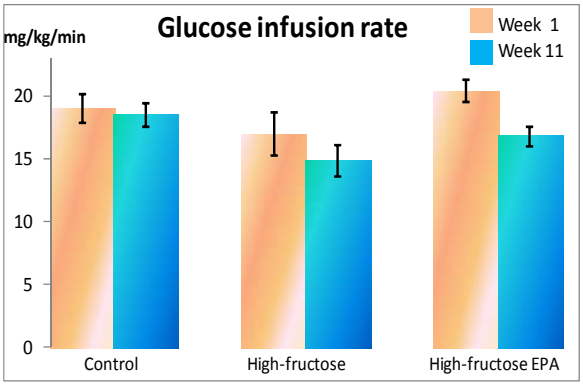

Figure 32: Glucose infusion rate during euglycemic-hyperinsulinemic clamp technique in rats in the control, the high-fructose and the high-fructose EPA groups at week 1 and 11 of the experiment.

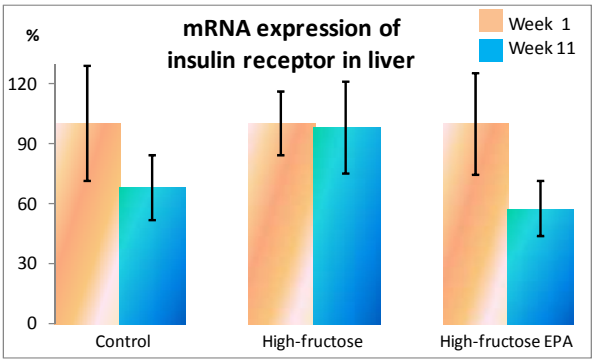

Figure 33: The mRNA expressions of insulin receptor in the liver of rats in the control, the high-fructose and the high-fructose EPA groups at week 1 and 11 of the experiment.

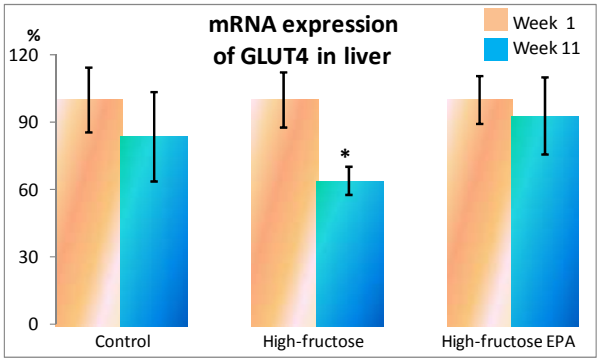

Figure 34: The mRNA expressions of GLUT4 in the liver of rats in the control, the high-fructose and the high-fructose EPA groups at week 1 and 11 of the experiment.

* $P < 0.05$ compared to week 1 of same group.

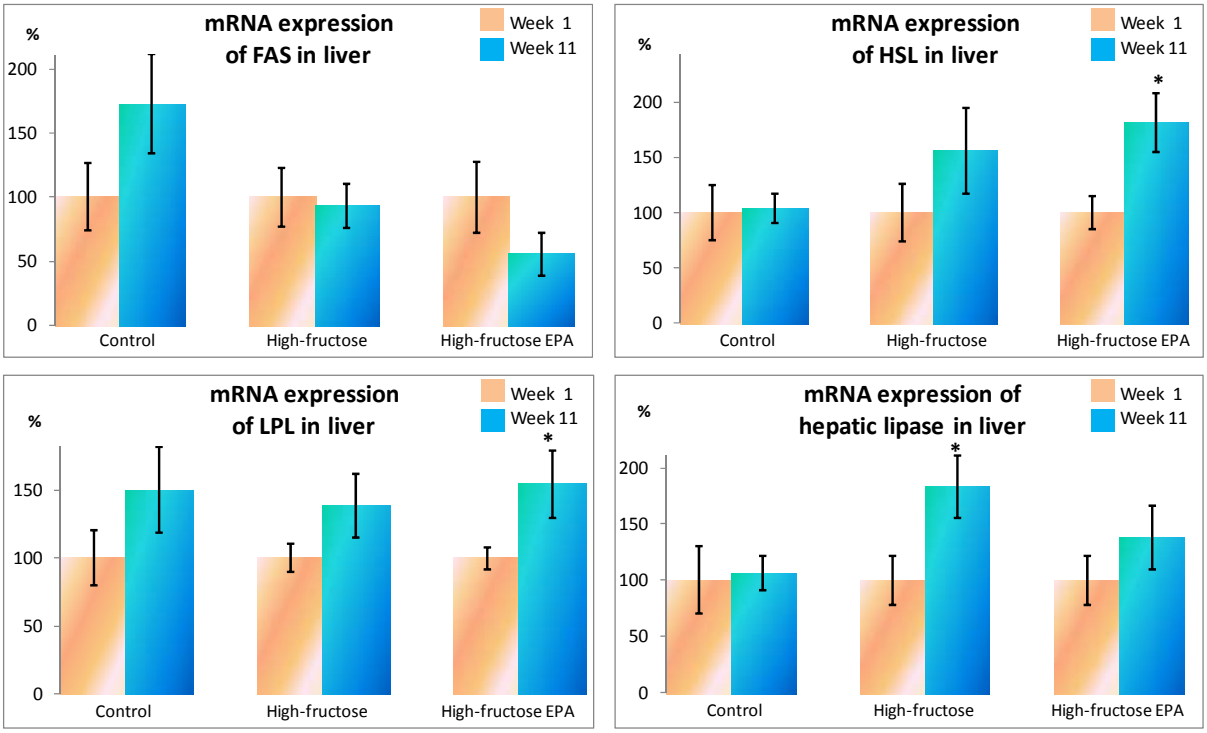

Figure 35: The mRNA expressions of FAS, HSL, LPL and hepatic lipase in the liver of rats in the control, the high-fructose and the high-fructose EPA groups at week 1 and 11 of the experiment.

* $P < 0.05$ compared to week 1 of same group.

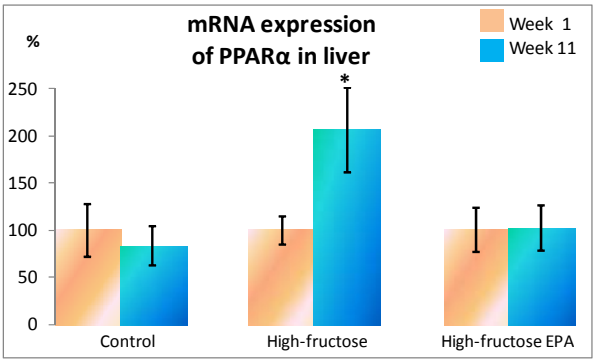

Figure 36: The mRNA expressions of PPARα in the liver of rats in the control, the high-fructose and the high-fructose EPA groups at week 1 and 11 of the experiment.

* $P < 0.05$ compared to week 1 of same group.

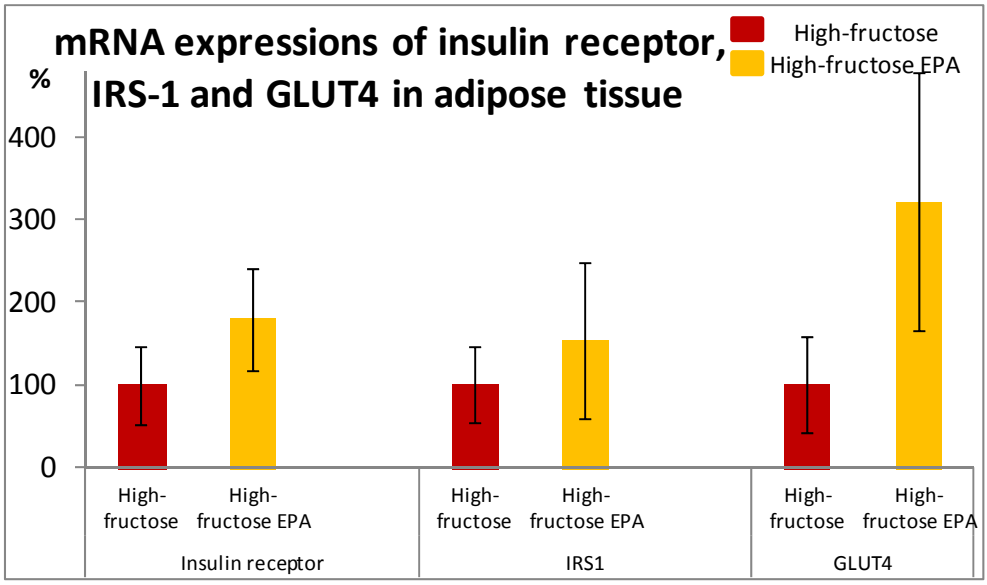

Figure 37: The mRNA expressions of insulin receptor, IRS-1 and GLUT4 in the visceral adipose tissue of rats in the control, the high-fructose and the high-fructose EPA groups at the end of experiment.

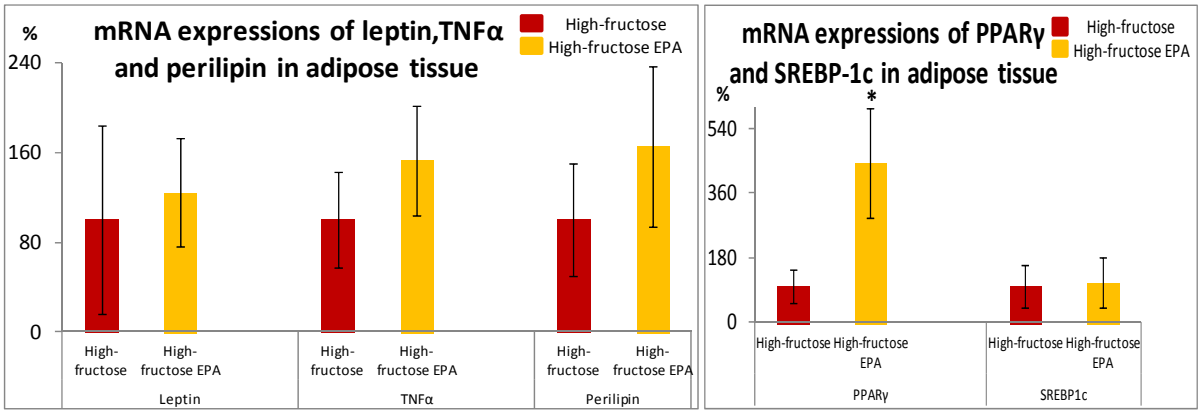

Figure 38: The mRNA expressions of leptin, TNFα, perilipin, PPARγ and SREBP-1c in the visceral adipose tissue of rats in the control, the high-fructose and the high-fructose EPA groups at the end of experiment.

* $P < 0.05$ compared to the high-fructose group at the end of the experiment.

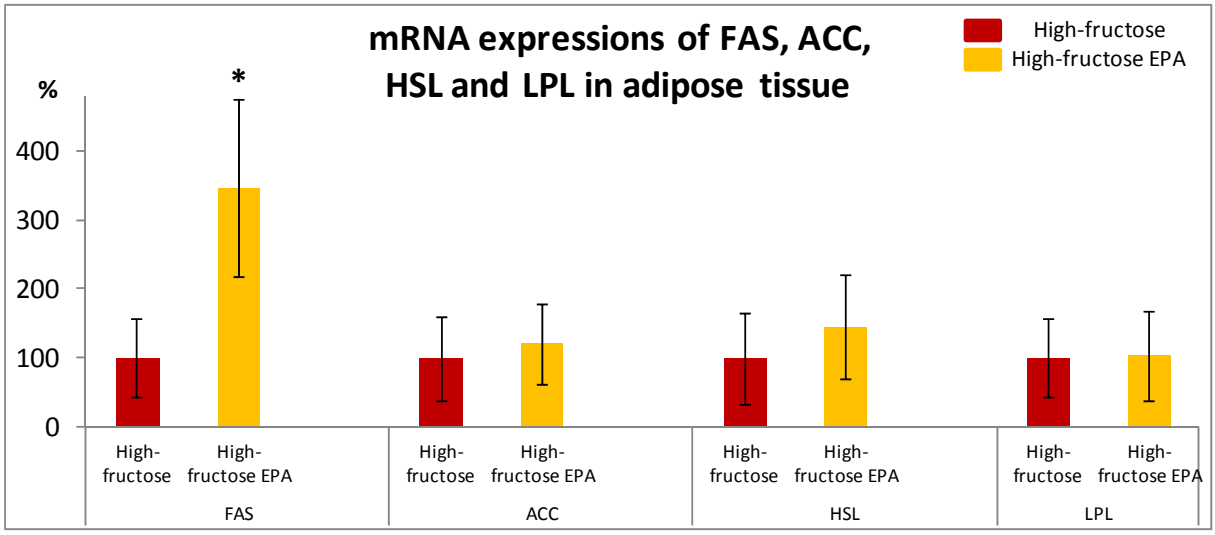

Figure 39: The mRNA expressions of FAS, ACC, HSL and LPL in the visceral adipose tissue of rats in the control, the high-fructose and the high-fructose EPA groups at the end of experiment.

* $P < 0.05$ compared to the high-fructose group at the end of the experiment.

4 Supplementation with hesperidin and naringin

Lipid profile and insulin sensitivity in rats fed with the high-fat or the high-fructose diet supplemented with hesperidin and naringin.

4.1 Introduction

Citrus flavonoids, a category of polyphenols, have been studied since years for their anti-oxidant and anti-cancerous effects. In the past some years, flavanones have been studied for their possible hypolipidemic and hypoglycemic actions.

In this study, hesperidin and naringin (flavanone glycosides) have been supplemented with the control, the high-fat, and the high-fructose diets in rats. The aim was to study the effects of hesperidin and naringin in the presence of the control, the high-fat, and the high-fructose diets on insulin sensitivity and plasma lipid profile in rats.

4.2 Results

4.2.1 Body weight and body composition

The results of body weight and body fat composition were presented in the table 11. Body weight and body fat percentage were similar among the groups at the end of experiment.

4.2.2 Insulin sensitivity

The results of basal glycemia, insulinemia and insulin sensitivity were presented in the table 12. There was significant effect on the basal glycemia among the groups at the end of the experiment. Basal insulinemia was similar among the groups at the end of the experiment. There was significant effect on glucose infusion rate in the euglycemic hyperinsulinemic clamp test among the groups at the end of the experiment. The high-fat HN, and the high-fructose HN groups had lower glucose infusion rate, compared to the control HN group at the end of the experiment.

Table 11: Body weight and body fat percentage of rats in the control HN, the high-fat HN, and the high-fructose HN groups at the end of the experiment.

	Control HN	**High-fat HN**	**High-fructose HN**	***ANOVA***
Body weight (g)	537 ± 38	571 ± 31	628 ± 21	*NS*
Body fat (g)	109.5 ± 14.5	133.2 ± 16.1	137.0 ± 18.1	*NS*

NS, non significant; HN, hesperidin and naringin. Data were represented as the mean values and standard error of the means (n = 6).

Table 12: Basal glycemia, basal insulinemia and glucose infusion rate during euglycemic-hyperinsulinemic clamp technique in rats in the control HN, the high-fat HN, and the high-fructose HN groups at the end of the experiment.

	Control HN	**High-fat HN**	**High-fructose HN**	***ANOVA***
Basal glycemia (g/l)	0.88 ± 0.05	0.77 ± 0.04	0.81 ± 0.05	*
Basal insulinemia (ng/ml)	2.04 ± 0.34	1.75 ± 0.22	3.14 ± 0.57	*NS*
GIR (mg/kg/min)	18.9 ± 1.4	10.7 ± 0.9*	12.6 ± 1.3*	*

NS, non significant; HN, hesperidin and naringin; GIR, glucose infusion rate during euglycemic-hyperinsulinemic clamp technique. * $P < 0.05$ compared to the control group at the end of the experiment. Data were represented as the mean values and standard error of the means (n = 6).

Table 13: Plasma lipid profiles of rats in the control HN, the high-fat HN, and the high-fructose HN groups at the end of the experiment.

(mmol/l)	**Control HN**	**High-fat HN**	**High-fructose HN**	***ANOVA***
Total cholesterol	2.11 ± 0.17	2.09 ± 0.12	2.19 ± 0.13	*NS*
Triglycerides	1.50 ± 0.38	2.68 ± 0.28*	2.63 ± 0.18*	*
NEFA	0.37 ± 0.08	0.33 ± 0.05	0.51 ± 0.05	*NS*

NS, non significant; HN, hesperidin and naringin; NEFA, non-esterified fatty acids. * $P < 0.05$ compared to the control group at the end of the experiment. Data were represented as the mean values and standard error of the means (n = 6).

4.2.3 Plasma lipids

The results of the plasma lipids were presented in the table 13. Plasma total cholesterol was similar among the groups at the end of the experiment. There was significant effect on the concentration of plasma triglycerides among the groups at the end of the experiment. The triglycerides concentration was higher in the high-fat HN and the high-fructose HN groups compared to the control HN group at the end of the experiment. Plasma non-esterified fatty acids concentration was not different among the groups at the end of the experiment. The high-fat HN and the high-fructose HN groups had higher VLDL-cholesterol and lower HDL-cholesterol compared to control HN group, at the end of the experiment.

5 Discussion

5.1 Characterization of rat model of obesity and insulin resistance induced by high-fat or high-fructose diet:

The development and characterization of the animal models used for obesity and insulin sensitivity studies are important. The objective of this part of the study was the development of diet induced-obesity and -insulin resistance in rats, and more precisely the characterization of rat model of obesity and insulin resistance by high-fat or high-fructose diet using a longitudinal approach.

We composed the groups randomly and used longitudinal approach. The determination of baseline values of biochemical variables was important in order to exclude existent differences. Nevertheless, the plasma total cholesterol concentration was significantly different among groups at baseline level (week 1); however, there was no difference for other variables (body weight, body fat mass, basal glycemia and insulinemia, plasma triglycerides and NEFA levels) among the groups at week 1. Variable concentrations of plasma lipids have been reported in rats, e.g. plasma total cholesterol range from 1.04 mmol/l to 3.38 mmol/l (Wesche, 2009) that is considered in normal range. In our study, the plasma concentration of total cholesterol was between 1.249 mmol/l and 2.109 mmol/l on week 1 that suggests the variation among the rats.

The randomisation process had been expected to abolish all the differences among the groups; however it failed to reach its objectives in this regard. The baseline values were then taken into account in the statistical analysis, which helped to nullify the pre-existent differences.

The induction of obesity and insulin resistance is possible using a well-defined, semi-purified or purified ingredient diet has been in use but standard rodent chow, added with fat or fructose, and ‘cafetria’ diet have also been in practice. However, all these diets are not satisfactory: the use of ‘cafeteria’ diet was considered inappropriate (Moore, 1987), the chow based diets could include extraneous components like plant-derived phytochemicals, and the chow based diets added with fat or fructose could unbalanced and lead to malnutrition. Phytoestrogens, an example of phytochemicals in rodent diets, could affect endocrine studies (Kanno *et al.*, 2002). We therefore, used the semi-purified diet in our study to eliminate or

reduce the effects of deficiency or imbalance in macro- and micronutriments by adjusting their amount.

Several characteristics of high-fat diets to induce obesity and insulin resistance should be taken into account: type, amount, source of the dietary-fat. In fact, the percentage of added fat influences the development and degree of obesity, dyslipidemia and insulin resistance. Ghibaudi, *et al.* (2002) reported that weight gain and the body fat mass were increased dose-dependently as effect of dietary fat in rats. The source of fat in the diet must also be taken into account, Buettner, *et al.* (2006) have shown that diet rich in saturated fat had more deleterious effect on development of insulin resistance and hepatic steatosis than diet rich in polyunsaturated fatty acids. In our study, we therefore, used lard that is rich in saturated fatty acids as the main source of dietary fat.

In addition to the high-fat diets, high-fructose diets with 60 % to 65 % by energy from fructose source have been widely used (Mohamed Salih *et al.*, 2009; Sanchez-Lozada *et al.*, 2007; Nakagawa *et al.*, 2006). Hence, we studied the development of obesity and insulin resistance as an effect of the high-fat (65 % by energy) and the high-fructose (65 % by energy) diets using a longitudinal approach for 10-week duration.

In our study, all the rats had similar body weight gain at the end of 10-week period. The increase in body fat mass, independent of an increase in body weight, could be a risk factor for the induction of insulin resistance and associated metabolic disorders. In our study, the increase in body fat mass in rats fed with high-fat diet reflects the obesogenic property of lard, containing high amount of saturated fatty acids, being used as the source of dietary fat. In the course of development of obesity, the adipocytes increase in size and number. The adipose tissue secretes certain adipokines, and leptin is one of the major proteins secreted by the adipocytes, and it acts on the central nervous system to regulate food intake and energy expenditure. Leptin stimulates its receptors in hypothalamus and decreases appetite, and the absence of leptin or its receptors or resistance to leptin lead to an unchecked feed intake and ultimately the obesity. In our study, the gene expression of leptin in the visceral adipose tissue was higher in the high-fat diet fed rats compared to the control rats that was consistent with the higher body fat mass. Li, *et al.* (2002) observed an increase in the expression of leptin in rats fed with a high fat diet, that was related to an expansion of adipose tissue. Our results related to the body weight and the body fat mass in the high-fructose diet fed rats reveal that the diet rich in fructose does not necessarily lead to obesity. In the study of Chicco, *et al.* (2003), a significant increase in the weight gain at the end of 15 and 30 weeks of a high-

sucrose diet, and not during the first 15 weeks of feeding that suggests a prolonged time period to develop excessive weight gain. In addition, the mRNA level of leptin in visceral adipose tissue was not changed in the high-fructose diet fed rats. This leads us to hypothesize that the development of obesity in rats could be leptin-dependent: the high-fat diet could enhance the expression of leptin in the adipose tissue, and this hyperleptinemia could develop leptin resistance, that lead to high energy intake and ultimately to obesity.

Our results of low insulin sensitivity and higher body fat mass in the high-fat diet fed rats are coherent with those reported in a high-fat diet fed rats (Sridhar *et al.*, 2008; Vinayagamoorthi *et al.*, 2008) showing a decrease in insulin sensitivity associated with an increase in epididymal fat. In our study, the high-fructose diet fed rats showed no change in insulin sensitivity and basal glycemia. A previous study has reported that a high-fructose diet increased hepatic glycogen (Yadav *et al.*, 2009) as an effect of inhibition of glycogenolysis rather than glycogen synthesis. In fact, fructose 1-phosphate, an intermediate of fructose metabolism in liver, inhibits the activity of the glycogen phosphorylase by depletion of inorganic phosphate (Kaufmann and Froesch, 1973), and hence, inhibits glycongenolysis. The increase in the amount of hepatic glycogen could involve in a mechanism to hinder the development of hyperglycemia.

The bulk production of non-esterified fatty acids by *de novo* lipogenesis could play important role in the development of insulin resistance and dyslipidemia. The conversion of fructose to triose-phosphates (glyceraldehydes-3-phosphate and dihydroxyacetone phosphate) is insulin independent and rapid process due to absence of negative feedback by ATP (Bruynseels *et al.*, 1999; Cortez-Pinto *et al.*, 1999) or citrate. Fructose metabolism can provide glycerol and acyl parts for hepatic lipogenesis, leading to increased hepatic triglycerides production. In addition, fructose inhibits the lipid oxidation in the liver, thus favours synthesis of hepatic VLDL (Topping and Mayes, 1972).

An increase in the concentrations of triglycerides and non-esterified fatty acids could lead to an increase in the phosphorylation of serine / threonine of the IRS-1, and hence reduce insulin signaling. In fact, intramyocytic lipid accumulation was associated with an increase in serine phosphorylation and decrease in actions of insulin (Morino *et al.*, 2005). In our study, the high-fructose diet fed rats did not develop dyslipidemia and no change in the mRNA levels of the insulin receptor and the IRS-1 in the liver. It is worth mentioning that a high-sucrose diet induced hepatic low insulin sensitivity before muscle (Pagliassotti *et al.*, 1996). We have assessed the insulin sensitivity using the euglycemic-hyperinsulinemic clamp

technique, which is the gold standard method for the direct assessment of the whole body insulin sensitivity (DeFronzo *et al.*, 1979), as it measures the insulin-mediated glucose uptake under hyperinsulinemic conditions. It is possible that the high-fructose diet could have caused impairment of insulin action in the liver or hepatic insulin resistance, as suggested by the decrease in mRNA level of GLUT4 transporter in this tissue, without causing whole body insulin sensitivity, and no change in mRNA levels of insulin receptor and IRS-1 in visceral adipose tissue. It has been shown that the decrease in glucose uptake in insulin resistance was not necessarily correlated with the expression of GLUT4 (Pedersen *et al.*, 1990), and in fact, this uptake also depends on the intrinsic activity of GLUT4 (Konrad *et al.*, 2002). The fluidity of the membrane also plays a major role in the translocation of GLUT4 to the membrane and glucose uptake (Sandra *et al.*, 1984). The defects in the cascade of insulin signaling in the development of insulin resistance reduce the efficacy of insulin, despite at normal or high insulin level. The insulin receptor is the first effector of this signaling. The mRNA expressions of this receptor in the liver and visceral adipose tissue did not differ among the groups, as did the mRNA expressions of IRS-1, indicating that insulin signalling seems little or not affected in the liver and adipose tissue. Our results suggest that the high-fructose diet could only cause mild impairment in insulin actions. In addition, the duration of our experiment could not have been long enough for the development of whole-body insulin resistance. We observed that the increase in mRNA expression of ACC was associated with insulin resistance in the high-fat diet fed rats. A decrease in the expression of ACC was positively correlated with the insulin sensitivity (Rosa *et al.*, 2003). The activation of ACC could inhibit fatty acids oxidation by increasing malonyl-CoA and increase intramyocytic triglycerides and leads to insulin resistance. It is also suggested that the PPARγ could play a role in the insulin sensitivity in adipose tissue by influencing the expression of certain genes: adiponectin (Iwaki *et al.*, 2003), resistin (Ghosh *et al.*, 2003; Patel *et al.*, 2003) and GLUT4 (Giron *et al.*, 1999), and could improve insulin sensitivity. The decrease in the expressions of PPARγ and GLUT4, in visceral adipose tissue in the high-fat diet fed rats in our study, confirms the link among these genes.

The relationship between the inflammation and the development of type 2 diabetes (Hotamisligil *et al.*, 1993), led to consider obesity as a chronic inflammatory condition and to explore the consequences of inflammation in the development of insulin resistance. The adipose tissue, particularly the vicseral adipose tissue produces pro-inflammatory cytokines, mainly TNFα, and this production increases with the expansion of body fat (Hotamisligil *et*

al., 1993, 1994a). In our study, the mRNA expression of TNFα in the visceral adipose tissue was increased in the high-fat diet-fed rats. Several studies have investigated the effect of a nutritional induction of obesity and insulin resistance on the production of TNFα and found conflicting results. Morin, *et al.* (1997) observed an increase in the expression of TNFα in visceral adipose tissue of rats fed a high fat diet, not correlated with insulin-resistant status, whereas Li, *et al.* (2002) found no change in the expression of TNFα. Our results suggest a positive relationship between the inflammation (increased expression of TNFα) and obesity, associated with the insulin resistance in rats fed with the high-fat diet.

Concerning the plasma lipids, no change in concentration of plasma total cholesterol or concentration of cholesterol in plasma lipoproteins in the high-fat diet or the high-fructose fed rats was observed. The absence of any effect in cholesterol level could be an effect of the absence of dietary cholesterol. Our results are in accordance with those of Sinitskaya, *et al.* (2007) showing no change in the ceoncentration of cholesterol in the plasma lipoproteins using a high-fat diet, but are contradictory to those of Mohamed Salih, *et al.* (2009), showing an increase in VLDL-cholesterol and LDL-cholesterol, and a decrease in HDL-cholesterol in a high-fructose diet fed rats. Most strains of the rats are resistant to develop atherosclerosis (Moghadasian, 2002), and possess the ability to maintain their cholesterol profile: low levels of total cholesterol, and LDL-cholesterol and high levels of HDL-cholesterol. Moreover, it is usually necessary to include dietary cholesterol and cholic acid in the diet to induce atheroscelorosis (Andrus *et al.*, 1956), since cholic acid promotes intestinal absorption of fat and cholesterol (Wang *et al.*, 1999a).

We found no change in the plasma concentrations of non-esterified fatty acids and triglycerides in any group in our study. In literature, contradictory results have been reported. Some authors have reported an increase of plasma non-esterified fatty acids (Posey *et al.*, 2009; Sinitskaya *et al.*, 2007) and triglycerides (Sridhar *et al.*, 2008; Vinayagamoorthi *et al.*, 2008) in the high-fat diet fed rats, whereas some other authors have reported no change in non-esterified fatty acids (Morens *et al.*, 2006) and triglycerides (Matveyenko *et al.*, 2009; Sinitskaya *et al.*, 2007; Morens *et al.*, 2006). Our results concerning plasma concentrations of non-esterified fatty acids in the high-fructose diet fed rats were not consistent with those reported in previous studies (Mohamed Salih *et al.*, 2009; Shih *et al.*, 2009). It is possible that liver could have higher triglycerides storage, by capturing the bulk amount of non-esterified fatty acids, as suggested by an increase in the hepatic expression of PPARα in the high-fructose diet fed rats. In fact, an increase of PPARα favours the uptake and oxidation of fatty

acids. Finally, it is possible that rats could have had higher postprandial plasma lipids levels that could not have been observed in rats unfed for 12h, as reported in some previous studies (Koo *et al.*, 2008; Lee *et al.*, 2006b).

It is evident that the increase in body fat was related to adipocytic lipogenesis. In the visceral adipose tissue, the mRNA expressions of FAS and ACC were increased in the rats fed with the high-fat diet compared to the control rats, suggesting the *de novo* lipogenesis in the adipocytes; however its expression was unchanged in the liver indicates an unchanged hepatic lipogenesis. The increase in the hepatic expression of SREBP-1c in rats fed with the high-fat diet could reflect an accumulation of triglycerides in the liver. In addition, the unchanged expression of SREBP-1c in the visceral adipose tissue of the high-fructose diet fed rats was consistent with normal triglyceridemia. A marked *de novo* hepatic lipogenesis and uptake and oxidation of plasma fatty acids for the synthesis of triglycerides in the liver and adipose tissue in the high-fructose diet fed rats was ruled out because of unchanged gene expression of FAS. A positive relationship between the insulin sensitivity and plasma LPL (Hanyu *et al.*, 2004), and the activity of LPL in muscle has been established; however in our study the hepatic expression of LPL was not modified in rats fed with the high-fat or the high-fructose diet. The high-fructose diet did not affect lipogenesis at both hepatic and adipocytic level that was consistent with the plasma lipid profile of triglycerides and non-esterified fatty acids, and the unchanged insulin sensitivity.

The relationship between the decrease in PPARγ and insulin resistance has been established (Kubota *et al.*, 1999). In our study, the lower mRNA levels of PPARγ, in the visceral adipose tissue in the high-fat diet fed rats than the control, was related to insulin resistance in these rats. The activation of PPARγ stimulates gene expression of LPL in adipose tissue of rats (Schoonjans *et al.*, 1996a). Our results concerning the expression of LPL in the adipose tissue were not consistent with this result.

The HSL is the key enzyme of lipolysis, subject to such negative regulation of insulin (Stralfors and Honnor, 1989). The gene expression of HSL was increased during the weight loss, associated with an increase in lipolysis (Sztalryd and Kraemer, 1994) and knock-out mice for its gene had an increased lipolysis (Martinez-Botas *et al.*, 2000). The paradoxical increase in lipolysis in obesity is mainly due to resistance of adipose tissue to the antilipolytic action of insulin. In adipocytes, perilipin stabilizes the lipid droplet and prevents the action of HSL. The expression of perilipin was increased in adipose tissue of obese individuals (Kern *et al.*, 2004), and decreased after weight loss in dogs (Leray *et al.*, 2008). In our study, mRNA

levels of HSL in the liver and adipose tissue were unchanged and those of perilipin were increased in the high-fat diet fed rats. This increase in the expression of perilipin could inhibit lipolysis, by inhibiting the action of HSL.

In conclusion, the high-fat diet developed obesity by an increase in the body fat mass and decreased insulin sensitivity in adult rats. The decrease in expression of GLUT4 in both liver and adipose tissue of rats fed with the high-fat or the high-fructose diet could induce an impairement in glucose uptake in target tissues. The high-fat diet increased the expressions of TNFα and leptin in adipose tissue, SREBP-1c in liver, perilipin in adipose tissue, FAS and ACC in adipose tissue, and decreased the expression of PPARγ. The high-fructose diet, on the other hand, increased the expressions of FAS and ACC in the visceral adipose tissue. Briefly, the 10-week high-fat diet led to obesity and insulin resistance, whereas rats fed with the high-fructose diet exhibited no change in insulin sensitivity and lipidemia. The high-fat diet had more deleterious response than the high-fructose diet, and seems more effective to induce obesity and insulin resistance in rats.

5.2 Effects of supplementation with EPA:

The objective of this part of the study was to observe the effects of supplementation with EPA, in the rats fed with the high-fat or the high-fructose diets, on the insulin sensitivity and the lipid profile. In addition, our objective was to study the role of EPA in the metabolism of lipids and carbohydrates.

In our study, all the rats gained body weight in a similar way. In fact, this weight gain could be an effect of aging and growth. The high-fat groups (supplemented with EPA or not) showed an increase in the body fat mass, compared to week 1, and EPA did neither reduce body weight nor body fat mass. The nature of dietary fat is considered important to develop obesity and insulin resistance. The higher body fat mass in the high-fat diet fed rats could be an effect of presence of lard, being used as the source for dietary fat in the high-fat diet. It is possible that such a diet induces the storage of fat depots from the extra calories, leading to obesity, but we did not measure feed intake. The previous studies have shown that substitution of 6 g/day of fish oil (EPA and DHA) for dietary fat (butter, olive oil, sunflower oil and pea-nut oil) decreased body fat mass in healthy individuals (Couet *et al.*, 1997). In addition, omega-3 PUFA seem to protect against obesity in healthy and also reduce body fat in obese rats (Shirouchi *et al.*, 2007; Minami *et al.*, 2002; Raclot *et al.*, 1997), as an effect of appetite-suppression (Lawton *et al.*, 2000). In our study, we did not measure food intake; however the free access of the animals to the feed could have led to an increase in intake of calories and ultimately an increase in body fat mass. Reseland, *et al.* (2001a) showed a decrease in circulating leptin levels at the end of supplementation with omega-3 PUFA in rats that was linked with a decreased expression of leptin in rat adipose tissue (Reseland *et al.*, 2001b; Raclot *et al.*, 1997). We did not find any change in the expression of leptin, body weight and body fat mass at the end of supplementation of EPA.

In our study, basal insulinemia and basal glycemia were not changed in any group. The rats fed with the high-fructose diet (supplemented with EPA or not) did not develop low insulin sensitivity compared to the control rats in our study. The high-fat group without EPA-supplementation showed low insulin sensitivity, and EPA-supplementation showed no change in insulin sensitivity in rats fed with the high-fat diet, hence we can say that EPA prevented development of low insulin sensitivity that was present in the rats without EPA-supplementation. Previous studies have shown a beneficial role of the omega-3 fatty acids on the insulin sensitivity in rats fed with a high fat diet (Andersen *et al.*, 2008; Lombardo *et al.*, 2007; Storlien *et al.*, 1987), but Gillam, *et al.* (2009) have not obtained any improvement of

insulin resistance in *fa/fa* Zucker rats. The increased mRNA expressions of IRS-1 and GLUT4 in the adipose tissue of the high-fat diet fed rats suggested that EPA could improve insulin signalling and glucose uptake, without any change of these genes in the liver. It has been reported that EPA supplementation facilitates glucose uptake and improve the insulin sensitivity by an increase in the expression of GLUT4 in skeletal muscle (Mori *et al.*, 1997). The study of expressions of genes involved in insulin signaling and glucose uptake in muscular tissue could allow understanding better the beneficial effect of supplementation of EPA on insulin sensitivity. Our experiment lasted for 10 weeks and we used the supplementation of EPA in Wistar rats, whereas the study of Mori *et al.* (1997) lasted for 17 to 18 weeks using a supplementation of fish oil in Otsuka Long-Evans Tokushima Fatty rats. The improvement of glucose uptake by supplementation of EPA could require more time in the liver and muscle than in the adipose tissue. The omega-3 PUFA protected development of hepatic insulin resistance induced by a high-fat diet, and decreased concentrations of diglycerides and triglycerides in the liver, in a PPARα-dependent manner in mice (Neschen *et al.*, 2007). The improvement of insulin sensitivity by a fish oil supplementation in high-sucrose diet fed rats was associated with an increase in plasma adiponectin level (Rossi *et al.*, 2005). It is possible that such a mechanism occurred in our study of the supplementation of EPA in rats fed with the high-fat or the high-fructose diet, but the improvement of insulin sensitivity did not reach the level of significance. The high-fructose diet did not induce insulin resistance; hence the potential effect of EPA-supplementation to improve the insulin sensitivity is not clear to establish.

The omega-3 PUFA seem to play a greater role to improve the dyslipidemia and lipoprotein profiles than insulin resistance. Raclot, *et al.* (1997) conducted a study comparing the efficacy of DHA and EPA, and found that DHA have shown a superior anti-adipogenic effect to EPA. In our study, the amount of EPA supplemented is relatively low compared to the amount of fat in the high-fat diet. The plasma total cholesterol level was not changed at the end of the high-fat or the high-fructose diet period. The dietary cholesterol could have a key role in the hypocholesterolemic effect of omega-3 PUFA, and in the absence of dietary cholesterol it could not induce hypocholesterolemic effect. An EPA-supplementation (300 mg/kg/day) for 5 weeks reduced plasma total cholesterol in cholesterol-diet fed rats (1 % of cholesterol and 1 % of cholic acid), but not in control diet fed rats (Ku *et al.*, 1999). Mizuguchi, *et al.* (1992) studied the effect of EPA in rats fed with a control diet for 7 days and a high-cholesterol (1 % of cholesterol and 1 % of cholic acid) diet for 4 weeks. The EPA reduced plasma total cholesterol, at 100, 300 and 1000 mg/kg of diet in hypercholesterolemic

rats, and at 300 and 1000 mg/kg of diet in rats fed with control diet (Mizuguchi *et al.*, 1992). In another study, a supplementation of EPA (1 g/kg body weight) or fish oil (3 g/kg body weight) reduced plasma total cholesterol in chow-fed rats (Cummings *et al.*, 2010) that suggest the hypocholesterolemic effect of EPA could be due to its high doses. In our study, it is possible that the low dose of EPA (200 - 300 mg/kg body weight/day) did not affect the plasma total cholesterol concentration in rats fed with the high-fat or the high-fructose diet. The EPA administration reduced plasma total cholesterol in 2-week period (Spady, 1993), but not in 17-18 weeks period (Mori *et al.*, 1997) that suggest the long term duration of EPA administration could not be beneficial on cholesterolemia.

In our study, plasma concentrations of triglycerides and non-esterified fatty acids were not changed at the end of the high-fat or high-fructose diet compared to the control rats. The supplementation of EPA showed a tendency to decrease plasma concentrations of triglycerides and non-esterified fatty acids in the high-fructose diet fed rats. The beneficial effect of EPA and DHA on insulin sensitivity could be due to reduction in the accumulation of triglycerides in muscle and adipose tissue (Storlien *et al.*, 1987) and changing the fatty acid composition of membrane phospholipids (Luo *et al.*, 1996). The long-chain omega-3 fatty acids and their derivatives are also ligands for certain transcription factors such as PPAR and could, therefore influence the regulation of genes involved in adipogenesis, insulin sensitivity and lipid metabolism (Banga *et al.*, 2009; Itoh *et al.*, 2008). An administration of EPA decreased plasma triglycerides level in 5 days (Aarsland *et al.*, 1990) or 2 weeks (Spady, 1993), but did not with an administration of 0.3 to 1 g/kg/day for 4 or 17 (Naba *et al.*, 2006; Mori *et al.*, 1997). In a study, a supplementation of fish oil (3 g/kg body weight) reduced plasma triglycerides at the end of 3-month and 4-month period, not before (1 or 2 month) and after (5, 6 or 7 month) of treatment (Cummings *et al.*, 2010). In our study, the supplementation of EPA had no effect on plasma concentrations of triglycerides and non-esterified fatty acids in rats fed with the high-fat diet, but decreased these lipid parameters in rats fed with the high-fructose diet. This was consistent with a study in rats fed with a diet rich in glucose or fructose (Herzberg and Rogerson, 1988). The omega-3 PUFA repress the genes of lipogenesis like FAS in the liver (Blake and Clarke, 1990). A study have shown a decrease in the activity of FAS in the liver of a high-sucrose diet fed rats (Peyron-Caso *et al.*, 2003) that could decrease plasma triglycerides and non-esterified fatty acids. In our study, the decrease in the expression of FAS in the liver could not reach level of significance as an effect of EPA supplementation in the high-fructose diet fed rats. In our study, an increase in the expression of FAS in the adipose tissue of the high-fructose diet fed rats suggested the *de*

novo lipogenesis in the adipocytes. Raclot, *et al.* (1997) showed that omega-3 PUFA decreased the expression of HSL. An administration of omega-3 PUFA enhanced mRNA expression of HSL in the liver and adipose tissue (Sun *et al.*, 2011), involved in lipolysis, and in our study, we also found an increase in the mRNA expression of HSL in the liver of the high-fat or the high-fructose diet fed rats, that suggest the ability of EPA to induce lipolysis in the liver, but not in the adipose tissue. An increase in the expression of PEPCK was observed in the adipocytes as an effect of fatty acids (Antras-Ferry *et al.*, 1994) in favour of increased lipogenesis. We found no change in PEPCK expression in the liver. There appears the differences between tissues for modulation of the expression of these genes (FAS, HSL, PEPCK), and also regional differences between different types of fat, i.e. subcutaneous or visceral (Raclot *et al.*, 1997). Dietary fish oil could have greater triglycerides-lowering effect than other polyunsaturated diets, owing to decreased triglycerides caused by an inhibition of acyl-CoA: diacylglycerol acyltransferase (Rustan *et al.*, 1992). An increase in peroxisomal fatty acid oxidation and decreased availability of non-esterified fatty acids could also contribute by decreasing the amounts of fatty acids, and hence decreasing the synthesis and the secretion of triglycerides (Rustan *et al.*, 1992). The hypotriglyceridemic effect of EPA could be an effect of low hepatic triglycerides production, as suggested by an increase in activity of carnitine palmitoyl transferase (fatty acids oxidation) and by a decrease in activity of phosphatidate phosphohydrolase (rate-limiting step of triglycerides synthesis) (Surette *et al.*, 1992), and / or by a decrease in the SREBP pathway (Le Jossic-Corcos *et al.*, 2005). The omega-3 PUFA affect the expression of genes via nuclear receptors, PPARs and SREBP. However, we did not find a decrease in the expression of SREBP-1c in the high-fructose diet fed rats. There are different affinities between omega-3 PUFA and PPAR for their different types. The EPA has a higher affinity for PPARα (Pawar and Jump, 2003), whereas omega-6 PUFA bind more easily to the PPARγ (Forman *et al.*, 1995). The EPA supplementation had no effect on the expression of PPARα in liver of the high-fat or the high-fructose diet fed rats, and no effect on the plasma triglycerides level was found. The gene expression of the PPARγ in the visceral adipose tissue was increased in the high-fructose diet fed rats. The omega-3 PUFA also act by inhibiting the maturation of SREBP (Kim *et al.*, 1999; Xu *et al.*, 1999b). We found that EPA supplementation increased the expression of SREBP-1c in the liver, but not in adipose tissue of the high-fat diet fed rats that could be due to high dietary fat content. The more comprehensive approach involving PPARα, PPARγ, and SREBP should be better studied to understand the hypotriglyceridemic effects of supplementation of EPA, and build

connections with possible therapeutic approaches, particularly in understanding the mechanisms of action of thiazolidinediones, which also act on PPARs.

Arachidonic acid, ARA (omega-6 PUFA) and EPA (omega-3 PUFA) compete for the formation of eicosanoids through cyclooxygenase and lipoxygenase enzymes. Pro-inflammatory eicosanoids are derived from ARA, whereas less inflammatory eicosanoids (such as prostaglandins) are derived from EPA (Simopoulos, 1999; Gallai *et al.*, 1995). The omega-6 PUFA to omega-3 PUFA ratio is considered an important factor to determine the effects of these PUFA on insulin sensitivity and dyslipidemia. A diet with n-6/n-3 ratio of 3.38 decreased plasma total cholesterol, increased plasma triglycerides, and decreased HDL, compared to a conventional diet with n-6/n-3 ratio of 51.1 in rats (Watanabe *et al.*, 2011), and Jeffery, *et al.* (1996) observed a decrease in plasma concentrations of total cholesterol, triglycerides and non-esterified fatty acids with a low n-6/n-3 ratio (0.33) compared to n-6/n-3 ratio (112.5). They used vegetable oil (α-linolenic acid and linoleic acid) as source of PUFA in their study. The α-linoleic acid is an effective substrate for β-oxidation than linoleic acid (Leyton *et al.*, 1987), so an increase intake of dietary omega-3 PUFA decrease non-esterified fatty acids as an effect of oxidation of fatty acids. This fact has been also reported by the use of fish oil in rats (Rustan *et al.*, 1992). Fukushima, *et al.* (2001) found a decrease in plasma total cholesterol in 24-week old rats fed with omega-3 PUFA (perilla oil) compared to omega-6 PUFA (borage oil) in 4-week duration; however the difference was not significant at 8 or 15 week of diet ingestion. Similar findings have been observed in another study with a decrease in plasma concentrations of total cholesterol and triglycerides in rats fed low n-6/n-3 ratio (1) compared to high ratio (16) at 6 months and 12 months of dietary intervention, but not on 18 months (Takeuchi *et al.*, 2009). These results suggest that the dietary n-6/n-3 ratio could affect plasma lipid profiles depending on the duration of feeding. Moreover, in another study, Jeffery, *et al.* (1997) found a decrease in total cholesterol with low n-6/n-3 ratio (1) than high ratio (100) and no change in plasma triglycerides and non-esterified fatty acids. The hypolipidemic ability could be related to reducetion in secretion of cholesterol and triglycerides from the liver (Zheng *et al.*, 2001). In addition, the type (EPA, DHA, ALA, LA) and the source (fish oil, vegetable oil) of omega-6 PUFA and omega-3 PUFA should also be considered in future studies of n-6/n-3 ratio.

Our results suggest that the effects of the supplementation with EPA were dependent on the diet fed to rats. In our study of 10 weeks, the supplementation with EPA had neither an effect on the body fat mass nor on the insulin sensitivity in rats. It decreased plasma triglycerides and non-esterified fatty acids in the high-fructose fed rats. Further studies are

required to delineate exact dose of EPA for its hypolipidemic and insulin sensitive effects. In addition, precise indications for the use of fish oil in different conditions of hyperlipidemia to differentiate the effects, if any, of pure EPA and fish oil have also been necessary.

5.3 Effects of supplementation of hesperidin and naringin in rats fed with a high-fat or a high-fructose diet:

The objective of this study was to determine the effects of supplementation with hesperidin and naringin (HN) for 8 weeks in the rats fed with the high-fat or the high-fructose diet, on obesity and insulin sensitivity. In this study, there were no group without the supplementation, hence the observed effects reflect the effects of hesperidin and naringin associated with the high-fat or the high-fructose diet.

In our study, body weight and body fat percentage were similar in all the groups at the end of experiment. In previous studies, contradictory results have been reported: some authors have reported no effect of hesperidin on body weight in rats (Wang *et al.*, 2011; Akiyama *et al.*, 2010; Ohtsuki *et al.*, 2003; Miyake *et al.*, 1998), and some have reported a decrease of perirenal adipose tissue weights in high-cholesterol diet fed rats (Wang *et al.*, 2011), and some have reported an increase in body weight in mice, in relation to plasma level of leptin (Jung *et al.*, 2004). A recent study of naringin have reported a decrease in body weight and body fat in mice (Pu *et al.*, 2012). Our results did not affect body weight and body fat on the supplementation of hesperidin and naringin in rats.

Basal insulinemia was not different among the groups at the end of the experiment. Previous studies have reported an increase in insulinemia in rats and in mice (Akiyama *et al.*, 2010; Jung *et al.*, 2004), which could be induced due to diabetic condition. The basal glycemia was not different among the groups at the end of the experiment. Previous studies have reported controversial results. A study reported hypoglycemic effect of hesperidin (10 g/kg of diet) (Akiyama *et al.*, 2010), an other study reported tendency to decrease glycemia in rats fed with cholesterol-rich diet supplemented with 0.08 % hesperidin (Wang *et al.*, 2011), and other studies using lemon flavonoids (O.2 % crude flavonoids, 0.2 % eriocitrin and 0.2 % hesperidin) or naringin alone (50 mg/kg) could not reduce hyperglycemia in rats (S and Pmo, 2011; Miyake *et al.*, 1998). The hypoglycemic effect has been explained by an increase in hepatic triglycerides (Jung *et al.*, 2004), an increase in expression of hepatic glucose kinase and a decrease in the expression of glucose-6-phosphatase (Akiyama *et al.*, 2010; Jung *et al.*, 2006), or a decrease in the expression of PEPCK and glucose-6-phosphatase in the liver (Pu *et al.*, 2012). The increase in glucose kinase and the decrease in glucose-6-phosphatase and PEPCK stimulate glycolysis and inhibit gluconeogenesis, which could improve hyperglycemia in diabetic animals.

Our results showed that the rats fed with the high-fat diet supplemented with HN, and high-fructose diet supplemented with HN had lower insulin sensitivity, compared to the control HN group at the end of the experiment. Previous studies showed that at the end of supplementation with hesperidin and naringin, the concentrations of plasma leptin (Jung *et al.*, 2004) and plasma adiponectin (Akiyama *et al.*, 2010) increased that could therefore, improve the insulin sensitivity. Moreover, aglycosides of the hesperidin and the naringin enhance the transcription of adiponectin and enhance the activity of PPARγ (Liu *et al.*, 2008), and therefore could improve the insulin sensitivity. Moreover, hesperidin and naringin inhibit the secretion of non-esterified fatty acids in response to TNFα in mouse adipocytes (Yoshida *et al.*, 2010), which could also improve the insulin sensitivity. The absence of the effect in our study could be explained by the fact that the high-fat or the high-fructose diet superimposed the effect of hesperidin and naringin on the insulin sensitivity.

The choice of using both hesperidine and naringine was based on the fact that supplementation of hesperidin or naringine alone had no effect on plasma lipid profile in humans (Demonty *et al.*, 2010). Plasma concentration of total cholesterol was similar among the groups at the end of the experiment. In previous studies, hesperidin and / or its metabolites have been reported to decrease plasma cholesterol (Wang *et al.*, 2011; Miceli *et al.*, 2007; Choi *et al.*, 2004; Kim *et al.*, 2003; Park *et al.*, 2001; Bok *et al.*, 1999; Monforte *et al.*, 1995), but in all these studies, rats were fed with a high-cholesterol diet. These flavanones are potent inhibitors of the activity of HMG-CoA reductase and ACAT *in vivo* (Choi *et al.*, 2004; Kim *et al.*, 2003; Park *et al.*, 2001; Bok *et al.*, 1999) or *in vitro* (Wilcox *et al.*, 2001), enzymes involved, individually or together, intestinal absorption of cholesterol, syn thesis and esterification of cholesterol, and hepatic VLDL-cholesterol secretion, and hence hesperidin and naringin could be effective as hypocholesterolemic agents by decreasing expression and / or activity of these enzymes in rats fed with a high-cholesterol diet. The absence of effect on plasma cholesterol level in our study was consistent with some authors (Wang *et al.*, 2011; Miyake *et al.*, 1998). We suggest that the hypocholesterolemic effect appears only in the case of dietary cholesterol intake or hypercholesterolemia. In addition, some authors have shown that these flavanones decrease plasma LDL, and increase plasma HDL (Miceli *et al.*, 2007; Monforte *et al.*, 1995), decrease plasma VLDL- and LDL-cholesterol and increase plasma HDL-cholesterol (Akiyama *et al.*, 2010), and hence could be effective to improve dyslipidemia. In our study, hesperidin and naringin resulted in higher VLDL-cholesterol and lower HDL-cholesterol in the high-fat or the high-fructose diet fed rats. In a study, a decrease in the LDL was associated with enhanced gene expression of LDL receptor, and reduced

expression and activity of microsomal triglyceride transfer protein *in vitro* (Wilcox *et al.*, 2001), which plays its role in the assembly of lipoproteins, mainly LDL and VLDL.

Plasma concentration of non-esterified fatty acids was not different among the groups at the end of the experiment that was consistent with a previous study (Ohtsuki *et al.*, 2003). However, the concentration of triglycerides was higher in the high-fat HN and the high-fructose HN groups compared to the control HN group at the end of the experiment. Variable results on the triglyceridemic level have been reported in rats. Previous studies have reported a decrease in the plasma triglycerides level (Miceli *et al.*, 2007; Choi *et al.*, 2004; Kim *et al.*, 2003; Kawaguchi *et al.*, 1997; Monforte *et al.*, 1995), a suppression of hypertriglyceridemia (Akiyama *et al.*, 2010), while some reported no affect (Ohtsuki *et al.*, 2003). The hypotriglyceridemic effect of flavanones could be an effect of repression of SREBP-1c in adipose tissue or liver by decreasing synthesis of triglycerides. In our study, the hypertriglyceridemia could be explained as a result of high amount of dietary fat or fructose in the diet, and could not be suppressed by the supplementation with hesperidin and naringin. These results are consistent with those in a previous study (Choi *et al.*, 1991) in rats fed with the high-fat diet.

In our study, the supplementation with the hesperidin and naringin, in the presence of the high-fat or the high-fructose diet for 8 weeks, decreased the insulin sensitivity in rats. Moreover, plasma concentration of triglycerides was increased, and no effect on plasma concentrations of total cholesterol and non-esterified fatty acids was observed. However, the absence of the high-fat or the high-fructose groups (without supplementation with hesperidin and naringin) hinders to determine and explain the effects of these flavanones on obesity, insulin sensitivity and dyslipidemia in our study.

- *CONCLUSION*

Obesity, a worldwide health issue, has been related to various metabolic disorders including insulin resistance, type 2 diabetes, cardiovascular problems, hypertension, dyslipidemia, renal problems, and cancer. The use of animal model has been a suitable choice to study obesity and its associated disorders. Rat has been used worldwide for this purpose; however longitudinal study of obesity and insulin resistance has not been studied in this animal model. The high-fat and the high-fructose diets have been used to induce obesity and insulin resistance in rats with variable results; however few studies have compared the effects of both these diets. In addition, the effects of nutritional supplementation to prevent obesity and its associated metabolic disorders were studied. Various nutritional and therapeutic agents have been studied for their anti-inflammatory and hypolipidemic effects.

Our study had two objectives; first was related to the characterization of rat model of obesity and low insulin sensitivity by studying the effects of a high-fat diet or a high-fructose diet by the longitudinal approach in rats. The changes in the expressions of genes involved for the development of obesity and insulin resistance in the hepatic and visceral adipose tissues were studied.

The second objective of this work was related to the nutritional supplementation in rats. The study of nutritional supplementation comprised of two parts. The first part was to study the effects of supplementation with the eicosapentaenoic acid (EPA) on obesity and insulin sensitivity by the longitudinal approach. The gene expression changes related to obesity and insulin sensitivity were studied in the hepatic and visceral adipose tissues. The second part was the supplementation with flavanones in the presence of the high-fat or the high-fructose diet in rats. Hesperidin and naringin were supplemented in the presence of the high-fat or the high-fructose diet in rats to study their effects on obesity and insulin sensitivity.

1 Characterization of an animal model

In the first study, we studied the effects of the high-fat diet and the high-fructose diet in rats. Male Wistar rats (12-week old) were fed with the high-fat (65 % energy by fat) diet or the high-fructose (65 % energy by fructose) diet for 10 weeks. We followed body weight, body fat mass (Fourier-transformed infrared spectroscopy), insulin sensitivity (euglycemic-

hyperinsulinemic clamp technique), plasma parameters and changes in the expression of genes in the hepatic and visceral adipose tissues that could be associated to the low insulin sensitivity and dyslipidemia at the start and at the end of the experiment in rats. The longitudinal approach allowed us to keep into account the basal values of the animals in our study that is not the case in previous studies related to obesity and insulin sensitivity in rats.

The high-fat diet for 10 weeks led to obesity (higher body fat mass) and low insulin sensitivity along with decreased expression of GLUT4 in the liver and visceral adipose tissue. The low insulin sensitivity has been associated with obesity and increased expression of leptin in the adipose tissue in rats. In our study, the high-fructose diet exhibited no change in insulin sensitivity and expression of insulin receptor and IRS-1 in the liver of rats; however GLUT4 expression in the liver and visceral adipose tissue was decreased. The high-fat diet induced 252 % increase in the body fat mass and the high-fructose diet induced 128 % increase that could confirm association of obesity and insulin resistance in the high-fat diet fed rats and not change in insulin sensitivity in the high-fructose diet fed rats (78 % increase in body fat mass in control diet fed rats).

The high-fat diet increased plasma total cholesterol (38 %) that could not reach significant that could be explained by absence of dietary cholesterol in the diet used in our study. We hypothesized that our result of no change in plasma concentrations of non-esterified fatty acids and triglycerides could be an effect of uptake of bulk amount of the non-esterified fatty acids and triglycerides by the liver. However, unchanged gene expressions of FAS, HSL and LPL in the liver did not support this hypothesis. Nevertheless, gene expression of TNF-α, FAS, ACC and perilipin were increased in visceral adipose tissue of the high-fat fed rats. It appears necessary to understand intra-individual variations in term of genetics and behaviour (feeding and physical activity). The study of feed intake and energy expenditure could have proved important tools to explain the balance between energy intake and energy expenditure. The high-fat diet had more deleterious response than the high-fructose diet to induce obesity and low IS in rats.

2 Supplementation with EPA

In this part of the study, male Wistar (12-week old) rats were supplemented with EPA (200-300 mg/kg/day, mixed in the cheese) along with the high-fat or high-fructose diet for 10 weeks to study the effects on low insulin sensitivity and dyslipidemia. Body weight, body fat

mass, insulin sensitivity, plasma parameters and the expression of genes in hepatic and visceral adipose tissues have been studied before and at the end of 10-week period. All the animals gained body weight and increased body fat mass as an effect of aging. In high-fat diet fed groups (with supplementation with EPA or not), the rats had increased body fat mass as an effect of lard in the high-fat diet. In the presence of EPA supplementation, the high-fat diet could not develop low insulin sensitivity that it developed without EPA supplementation. EPA supplementation avoided low insulin sensitivity in rats fed with the high-fat diet; however the gene expression study in the liver did not reveal any change in insulin signaling proteins. EPA increased gene expression of hepatic HSL in rats fed with the high-fat diet and the high-fructose diet suggesting the involvement of EPA in the process of lipolysis. EPA did not have any effect on plasma triglycerides, though it showed tendency to decrease plasma triglycerides and non-esterified fatty acids in the high-fructose diet fed rats that is consistent with an increase in the expressions of HSL and LPL in the liver. The EPA supplementation reduced the increase of plasma total cholesterol concentration from 36 % (without EPA supplementation) to 5 % (EPA supplementation) in the high-fat fed rats, though these values could not reach to the level of significance. EPA had no effect in hepatic PPARα expression in our study; however the gene expression of PPARγ in the visceral adipose tissue was increased in the high-fructose diet fed rats as an effect of EPA supplementation. The response of EPA supplementation was found to be variable in rats fed with various diets (high-fat and / or high-fructose diets). The hypotriglyceridemic effect of EPA supplementation was found in the high-fructose fed rats and hypocholesterolemic effect was observed non-significantly in the high-fat fed rats. Hence, the response of EPA has been dependent on the nature of the diet fed to the rats in our study.

3 Supplementation with hesperidin and naringin

In the last part of the thesis, the supplementation with hesperidin and naringin in the presence of the high-fat or the high-fructose diet for 8 weeks has been studied in rats. Body weight, body fat percentage, insulin sensitivity and plasma lipid profile have been followed at the end of 8-week period. The hypoglycemic response of these flavanones has been reported to be as an effect of decreased expression of glucose-6-phosphatase (gluconeogenesis) and increased expression of glucokinase (glycolysis). However, the supplementation with hesperidin and naringin could not counteract the low insulin sensitivity and glycemia that

could be the high-fat or high-fructose diet. We suggest that the short time course of the supplementation with hesperidin and naringin could not improve insulin sensitivity in rats. These flavonoids did not change plasma concentrations of total cholesterol and triglycerides. Controversial effect on the plasma triglycerides has been reported in rats, whereas beneficial effect to reduce plasma cholesterol has been reported in the high-cholesterol diet fed rats, and with similar dose in humans. In fact, these flavanones have been reported to potent inhibitors of the activities of the HMG-CoA reductase and the ACAT. Future studies of the age- and the dietary cholesterol-related response of hesperidin and naringin could help to elaborate the insulin sensitizing and hypolipidemic effects.

- *SYNTHESE*

1 Revue bibliographique

L'obésité est devenue ces dernières décennies un problème de santé publique majeur, souligné par l'Organisation Mondiale de la Santé. Sa prévalence mondiale a atteint des proportions épidémiques, avec plus d'un milliard de personnes en surpoids, dont 300 millions obèses (WHO, 2000). En 2009, 14,5 % de la population française était obèse et 31,9 % étaient en surpoids (Obépi, 2009). L'obésité est associée à de nombreux troubles cliniques, comme les maladies respiratoires, les troubles rénaux et cardiovasculaires, l'hypertension, l'insulinorésistance, le diabète de type 2, l'athérosclérose, la dyslipidémie ou encore les cancers.

L'obésité est une maladie chronique qui se définit comme une hypertrophie du tissu adipeux, conduisant à un poids élevé. Elle résulte d'un bilan positif entre l'ingestion et la dépense d'énergie. L'enèrgie en excès est stockée sous forme de graisses, menant au surpoids et ensuite à l'obésité. Le rôle du tissu adipeux dans l'installation d'affections comme le diabète de type 2 est particulièrement étudié. En effet une augmentation du tissu adipeux, et notamment du tissu adipeux viscéral, joue un rôle majeur dans l'établissement de l'insulinorésistance et le développement du diabète de type 2.

Les adipocytes accumulent les triglycérides dans des gouttelettes intracytoplasmiques. La régulation de la lipogenèse (synthèse des triglycérides) et de la lipolyse (dégradation des triglycérides fournissant des acides gras) dépend de l'équilibre entre les apports et les dépenses énergétiques. Cette régulation est à la fois nutritionnelle et hormonale.

1.1 Le tissu adipeux

1.1.1 Le tissu adipeux : lipogenèse et lipolyse

1.1.1.1 La lipogenèse

Le transport des lipides dans le plasma est assuré par les lipoprotéines. Pour les acides gras non-estérifiés le transport dans le plasma se fait par l'albumine. Les chylomicrons assurent le transport des triglycérides depuis l'intestin, suite à l'absorption des lipides ingérés. Les VLDL transportent, quant à eux, les triglycérides synthétisés par le foie.

La lipogenèse est la voie métabolique par laquelle l'adipocyte convertit les acides gras circulants en excès et le glucose (lipogenèse *de novo*) en triglycérides, forme de stockage au sein de la gouttelette lipidique.

Les acides gras destinés au stockage dans le tissu adipeux proviennent de l'hydrolyse des triglycérides des lipoprotéines par la lipoprotéine lipase (LPL) en acides gras et glycérol. L'entrée des acides gras dans l'adipocyte se fait par un transporteur 'Fatty Acid Transporter' (Abumrad *et al.*, 1993). Les acides gras sont alors amenés par la 'Fatty Acid Binding Protein' jusqu'à la gouttelette lipidique (Storch *et al.*, 2002), où ils se lient au glycérol phosphate pour former les triglycérides.

La voie de synthèse des acides gras à partir du glucose comporte deux étapes. A l'issue de la glycolyse, l'acétyl CoA est transformé en malonyl CoA par l'ACC (acétyl CoA carboxylase) qui est ensuite converti par la FAS (fatty acid synthase) en acide gras. Les acides gras non estérifiés se combinent avec le CoA pour former des thioesters, puis l'estérification des acides gras avec le glycérol conduit à la formation des triglycérides. Le glycérol, composant majeur des triglycérides, est activé par phosphorylation de son C-3 par la glycérol-kinase. La glycérol-3-phosphate acyltransférase lie un acide gras au glycérol-3-phosphate et génère un phosphate monoacylglycérol. Les acides gras incorporés dans les triglycérides sont liés à l'acyl-CoA par l'action de l'acyl-CoA synthétase. La liaison de deux molécules d'acyl-CoA au glycérol-3-phosphate donne le 1,2-diacylglycérol phosphate. Le phosphate est ensuite éliminé, par la phosphatidic acid phosphatase (PAP1), ce qui forme le 1,2-diacylglycérol, sur lequel peut se fixer un troisième acide gras. Le glucose est la source principale du glycérol phosphate servant à la synthèse des triglycérides. Il entre dans l'adipocyte par le transporteur GLUT4. Le glycérol phosphate provient de la glycolyse d'une part et de la glycéronéogènèse d'autre part. La glycéronéogenèse est la synthèse de glycérol par la PEPCK (phosphoénolpyruvate carboxykinase) à partir de substrats non glucidiques comme le pyruvate (Brito *et al.*, 1999). La lipogenèse dépend donc à la fois de l'apport des acides gras et de glucose.

- ***Régulation de la lipogenèse***

La lipogenèse est soumise à une régulation nutritionnelle (quantité de glucides et des acides gras circulants) et hormonale (concentrations en insuline et leptine). L'insuline favorise la lipogenèse, la leptine l'inhibe. La régulation de la lipogenèse par l'insuline et la leptine se fait par le biais de deux facteurs de transcription, le PPARγ (Vidal-Puig *et al.*, 1997) et le SREBP-1 (Kim *et al.*, 1998).

Le PPARγ est activé par les acides gras. Il se dimérise alors et se fixe sur une séquence PPRE (PPAR Response Element), au niveau du promoteur de certains gènes et en modifie l'expression. L'expression des gènes de la FABP, de la LPL, et de la PEPCK est activée par le PPARγ (Lapsys *et al.*, 2000; Martin *et al.*, 1997; Tontonoz *et al.*, 1995).

La principale isoforme du SREBP dans le tissu adipeux est le SREBP-1c. Il est activé par l'insuline et se fixe sur une séquence SRE (Sterol Regulatory Element) de l'ADN, ce qui stimule la synthèse d'enzymes de la lipogenèse. L'expression des gènes FAS et ACC est activée par le SREBP (Oh *et al.*, 2003; Horton *et al.*, 1998a; Shimano *et al.*, 1996).

1.1.1.2 La lipolyse

La lipolyse est le mécanisme de dégradation des triglycérides en acides gras et glycérol dans les adipocytes, libérés dans la circulation générale lorsque les dépenses énergétiques excèdent les apports ou en période interprandiale. Les triglycérides sont hydrolysés par la lipase hormone-sensible (HSL), la monoglycéride lipase et l'adipose triglycéride-lipase.

Les gouttelettes lipidiques sont stabilisées par une protéine, la périlipine. Pour que la HSL accède aux triglycérides, la périlipine doit auparavant quitter la gouttelette. La périlipine est soumise à la régulation hormonale par l'insuline et l'adrénaline. La fixation de l'adrénaline sur son récepteur β3 couplé à une protéine Gs déclenche une cascade de signalisation dont l'effecteur est l'adénylate cyclase, ce qui augmente le taux d'AMPc intracellulaire et active la PKA (Protéine Kinase A). La PKA activée phosphoryle la périlipine (Egan *et al.*, 1990) qui quitte ainsi la gouttelette et rend les triglycérides accessibles à la dégradation. La PKA phosphoryle aussi la HSL (Garton *et al.*, 1989) qui, ainsi activée, est transloquée du cytoplasme à la gouttelette. La HSL est l'enzyme de dégradation des triglycérides la plus importante du tissu adipeux, elle est aussi soumise à la régulation négative de l'insuline et positive de l'adrénaline (Frayn *et al.*, 1995). L'insuline exerce son action antilipolytique en diminuant la quantité de HSL et de triglycéride-lipase adipocytaire (Meilin *et al.*, 2011). L'activation du récepteur de l'insuline et des IRS (Insulin Receptor Substrate) conduit à l'activation de la PDE3 (phosphodiestérase 3) qui dégrade l'AMPc, inactivant ainsi la PKA et inhibant la lipolyse (Smith and Manganiello, 1989). L'adénosine peut aussi se fixer sur un récepteur α2 adrénergique couplé à une protéine Gi (inhibitrice), ce qui diminue le taux d'AMPc intracellulaire, et donc inhibe la PKA et la lipolyse (Schwabe *et al.*, 1975).

1.1.2 Le tissu adipeux : comme un organe endocrine

Le tissu adipeux est essentiellement composé d'adipocytes. Il constitue un organe de stockage des lipides mais aussi un organe endocrine. Sa localisation peut être sous-cutanée ou viscérale.

Il sécrète de manière régulée des molécules à action endocrine, paracrine et autocrine appelées adipocytokines ou adipokines, intervenant dans le contrôle du poids et du métabolisme énergétique. Ces molécules sont des hormones (leptine, résistine, adiponectine, visfatine) et des cytokines (TNFα, IL-6).

La leptine est la première adipokine découverte après l'identification de son gène déficient chez les souris *ob/ob* (Zhang *et al.*, 1994). Elle est sécrétée par les adipocytes. Elle intervient dans la régulation du poids du tissu adipeux, par une action centrale, en diminuant l'appétit et en augmentant les dépenses énergétiques, avec augmentation de la thermogenèse et de la lipolyse et une inhibition de la lipogenèse. Par ailleurs chez les souris *ob/ob*, l'administration de l'hormone améliore la sensibilité à l'insuline et réduit la néoglucogenèse (Pelleymounter *et al.*, 1995).

L'adiponectine a été caractérisée en 1995. L'expression du gène codant pour l'adiponectine est activée par l'insuline, qui stimule également sa sécrétion (Scherer *et al.*, 1995). La concentration d'adiponectine est diminuée chez le sujet obèse (Berg *et al.*, 2001) et l'hypoadiponectinémie est en relation étroite avec l'insulinorésistance (Kubota *et al.*, 2002). L'adiponectine pourrait améliorer la sensibilité à l'insuline en augmentant l'oxydation des lipides et en agissant directement sur la signalisation de l'insuline (récepteur, phosphorylation d'IRS-1 et protéine kinase B) (Yamauchi *et al.*, 2001). L'adiponectine est corrélée positivement avec la sensibilité à l'insuline et le taux plasmatique de HDL-cholestérol, et négativement avec la graisse viscérale et les concentrations en triglycérides et en apoB100 (Cnop *et al.*, 2003).

Le TNFα est un marqueur inflammatoire secrété par de nombreux tissu, dont le tissu adipeux, principalement par les macrophages du tissu adipeux (Weisberg *et al.*, 2003). Sa surexpression dans le tissu adipeux de sujets obèses et insulinorésistants (Hotamisligil *et al.*, 1993) a conduit à considérer l'obésité comme un état inflammatoire. Le TNFα induit

l'insulinorésistance en se fixant sur ces récepteurs présents sur les adipocytes, inhibe l'autophosphorylation du récepteur à l'insuline et la phosphorylation de l'IRS-1 (Stephens *et al.*, 1997; Hotamisligil *et al.*, 1996).

1.1.3 Insulinorésistance et obésité : rôle du tissu adipeux dans la pathogenèse

L'insuline régule le métabolisme lipidique et glucocidique de l'organisme et son rôle anabolique est indispensable à la mise en réserve (glycogenogenèse et lipogenèse) en utilisant des substrats glucidiques et lipidiques. Elle régule l'entrée du glucose dans les cellules de ses tissus cibles (foie, muscle, tissu adipeux), la synthèse de glycogène et la lipogenèse, elle inhibe la glycogénolyse, la néoglucogenèse hépatique, et la lipolyse intra-adipocytaire.

L'insulinorésistance est définie comme la diminution des effets biologiques de l'insuline, soit une diminution de la sensibilité à l'insuline de ces tissus cibles. Elle se caractérise par une altération des actions cellulaires de l'insuline, alors que l'insulinémie est normale voire augmentée par compensation. Ceci se traduit par l'incapacité de l'insuline à inhiber la production du glucose par le foie (Song *et al.*, 2001) et la captation du glucose par les muscles et le tissu adipeux, conduisant aux symptômes hyperglycémiques du diabète de type 2.

La signalisation insulinique commence par l'autophosphorylation du récepteur, ce qui active sa fonction tyrosine-kinase. Les substrats majeurs du récepteur à l'insuline sont les IRS, qui existent sous plusieurs formes, IRS-1 et IRS-2 étant les plus importants. Les IRS sont activés par phosphorylation des résidus tyrosines, tandis que la phosphorylation des résidus sérine-thréonine les inactive. Lorsque les IRS sont activés, ils activent à leur tour différentes voies : d'une part la voie de la MAP kinase (Mitogen Activating Protein Kinase) qui agit sur la prolifération, la différenciation et la croissance cellulaire, et d'autre part la voie de la PI3K, qui elle-même active la protéine kinase B, impliquée dans les effets métaboliques : translocation du transporteur GLUT4 vers la membrane, synthèse protéique, synthèse de glycogène, inhibition de lipolyse, inhibition de l'apoptose.

La prise et la perte de poids sont associées respectivement au développement de l'insulinorésistance et au rétablissement de la sensibilité à l'insuline. Il semble que la quantité de graisse viscérale soit un élément critique dans l'apparition de l'insulinorésistance.

Le premier mécanisme incriminé dans l'installation de l'insulinorésistance est l'augmentation de la concentration des acides gras non estérifiés dans le sang associée à l'expansion de la masse grasse. Dès les années 1960, Randle, *et al.* (1963) avancent qu'une compétition s'établit entre la disponibilité et l'oxydation des acides gras et le métabolisme du glucose dans les tissus musculaire et adipeux. Le tissu adipeux viscéral est particulièrement mis en cause du fait de son importante activité lipolytique, et par la libération des acides gras dans la veine porte qui sont directement déversés vers le foie (Jensen *et al.*, 1989). Dans le foie comme dans le muscle, les acides gras en excès sont stockés et l'accumulation des triglycérides produit un environnement qui interfère avec le métabolisme normal : le glucose est moins utilisé, la signalisation insulinique est altérée (phosphorylation des résidus sérine/threonine par diacylglycerol, inflammation, hyperlipidémie, hyperinsulinémie), ce qui conduit à une moindre captation du glucose et à une augmentation de la néoglucogenèse hépatique (Gan *et al.*, 2003).

Par ailleurs, l'augmentation de marqueurs circulants de l'inflammation (TNFα, IL-6, protéine C réactive ou CRP) et l'accumulation de macrophages dans le tissu adipeux de sujets obèses et insulinorésistants décrivent un état inflammatoire du statut obèse corrélé avec l'insulinorésistance (Pradhan *et al.*, 2001).

1.2 Intérêt du modèle animal dans l'étude de l'insulinorésistance

L'obésité associée à l'insulinorésistance fait l'objet de nombreuses études. Bien qu'il soit admis que de nombreux facteurs interviennent (prédispositions génétiques, réduction de l'activité), l'alimentation tient une place importante dans l'installation de l'insulinorésistance. La recherche de macronutriments en cause ou au contraire bénéfiques a fait l'objet d'études nutritionnelles dont l'intérêt est essentiellement à établir les liens entre l'alimentation et le développement de l'insulinorésistance, dans le but de proposer des préconisations de santé publique. Pour des raisons éthiques évidentes, ces études sont difficilement réalisables chez l'homme, et l'établissement de modèles animaux est indispensable. Le modèle animal a permis l'étude de l'obésité et de l'insulinorésistance induites par manipulation nutritionnelle : régime hyperlipidique, régime riche en saccharose, ou encore régime 'cafeteria' (Dourmashkin *et al.*, 2005; Woods *et al.*, 2003; Esteve *et al.*, 1994). Cette induction permet d'approcher au plus près les conditions d'instauration de l'obésité chez l'homme, mais des

manipulations génétiques (souris *ob/ob*) et des modèles spontanés (Zucker Fatty) (Srinivasan and Ramarao, 2007).

Le chien a été utilisé comme modèle animal dans notre laboratoire depuis quelques années pour l'étude de l''insulinorésistance et des dyslipidémies. Ce modèle nutritionnel d'obésité et d'insulinorésistance a été caractérisé, validé, et valorisé par des supplémentations nutritionnelles (acide nicotinique, thé vert, acides gras polyinsaturés oméga-3). Du fait du coût et de certaines limites de ce modèle, le développement d'un autre modèle animal a semblé intéressant. Nous avons choisi le rat comme modèle d'obésité nutritionnelle et d'insulinorésistance et utilisé ce modèle pour déterminer les effets des deux supplémentations nutritionnelles : acide eicosapentaénoïque et flavanones.

Le rat est un modèle animale bien documenté. Son génome a été séquencé et il existe de nombreuses ressources disponibles en biologie moléculaire. Sa durée de vie courte permet de réaliser des études rapides. Chez le rat, on peut induire l'obésité et l'insulinorésistance, en lui offrant un régime riche en graisse et / ou en fructose et ce modèle est aussi intéressant pour des études d'interventions nutritionnelles. Enfin, il reproduit la forme d'obésité viscérale principalement impliquée dans le syndrome d'insulinorésistance chez l'homme (Madsen *et al.*, 2010). Cependant, il a été rarement utilisé dans des études longitudinales.

1.3 Regimes : hyperlipidique ou riche en fructose

1.3.1 L'influence des lipides

Chez l'homme, comme chez le rat et le chien, un régime riche en lipides, et particulièrement en graisses saturées, induit une prise de poids, une insulinorésistance et une hyperlipidémie, contribuant au diabète de type 2 et au syndrome métabolique (Gayet *et al.*, 2004; Feskens *et al.*, 1995; Kromhout *et al.*, 1995; Hill *et al.*, 1992; Romieu *et al.*, 1988).

L'origine des troubles pourrait être liée à l'augmentation des acides gras non estérifiés plasmatiques résultant de l'excès de lipides dans l'alimentation, ainsi que de l'augmentation de tissu adipeux viscéral observée lors des régimes hyperlipidiques. Le mécanisme du développement de l'obésité et de l'insulinorésistance a été étudié, et notre étude pourrait être utile à cet égard en étudiant l'expression des ARNm des gènes cibles.

1.3.2 L'influence du fructose

La consommation de fructose a largement augmenté dans les dernières décennies par consommation de sodas très riches en fructose, ainsi que de préparations (desserts, céréales, sauces) sucrées avec du sirop de maïs riche en fructose.

Des études menées chez l'homme ont montré qu'une consommation importante de fructose induisait une augmentation des apports énergétiques et une prise de poids, une réduction de la sensibilité à l'insuline, et une dyslipidémie (Perez-Pozo *et al.*, 2010; Bantle *et al.*, 2000; Reiser *et al.*, 1989; Hallfrisch *et al.*, 1983).

Les mécanismes intervenant dans les anomalies métaboliques liées à la surconsommation de fructose s'exercent à différent niveaux. La sécrétion de leptine étant stimulée par l'insuline, et le fructose seul n'induisant pas de sécrétion d'insuline (Grant *et al.*, 1980), la sécrétion de leptine se trouve diminuée. Cette faible concentration de leptine augmente la prise alimentaire et le poids, du fait de sa faible action sur le système nerveux central (Sindelar *et al.*, 1999) et pourraient accroitre les désordres métaboliques. Par ailleurs un régime riche en fructose conduit une élévation des triglycérides plasmatiques plus importante qu'un régime riche en amidon (Castro *et al.*, 2011; Abraha *et al.*, 1998), ce qui participe à l'insulinorésistance et à l'hyperlipidémie (Jeppesen *et al.*, 1995) par la ré-estérification des acides gras non-estérifiés et lipogenèse *de novo*.

1.4 L'acide eicosapentaénoïque (EPA) et flavanones

1.4.1 L'acide eicosapentaénoïque (EPA)

Les acides gras polyinsaturés de type oméga-3 et -6 sont dits essentiels car l'organisme ne peut les synthétiser. Ils doivent être apportés par l'alimentation et participent à de nombreux processus : constitution des membranes cellulaires, croissance, développement cérébral, immunité et inflammation, etc. Parmi les acides gras oméga-3, les acides gras de omega-3 des longue-chaines, l'acide eicosapentaénoïque (EPA) et l'acide docosahexaénoïque (DHA) sont synthétisés en très faible quantité par l'organisme à partir de l'acide α-linolénique (ALA).

Les acides gras oméga-3 sont surtout reconnus pour leurs effets bénéfiques dans la réduction du risque de maladies cardiovasculaires (Einvik *et al.*, 2010; de Lorgeril *et al.*, 1994) par leurs effets antiarythmique (Leaf *et al.*, 2003), anti-inflammatoires (Calder, 2006), anti-athéroscléroses (von Schacky, 2003) et hypolipidémiants (Harris, 1997b). Par ailleurs, ils

interviennent dans l'amélioration du diabète de type 2 en favorisant la perte de poids chez le sujet obèse (Kunesova *et al.*, 2006). La composition en acides gras des phospholipides membranaires des cellules musculaires est corrélée avec les acides gras oméga-3 de l'alimentation (Andersson *et al.*, 2002), ainsi une alimentation riche en acides gras oméga-3 améliore-t-elle la fluidité membranaire et la sensibilité à l'insuline du muscle (Borkman *et al.*, 1993), et du corps entier (Mori *et al.*, 1997).

Les récentes modifications des habitudes alimentaires ont conduit à un déséquilibre du ratio oméga-6/oméga-3 en faveur des oméga-6, associé à l'insulinorésistance chez les individus avec le syndrome métabolique (Nigam *et al.*, 2009). On estime que le rapport oméga-6/oméga-3 dans l'alimentation occidentale est de 10/1 à 30/1 (NCEP, 1988), tandis qu'il devrait idéalement se situer entre 1/1 et 4/1 (Eaton and Konner, 1985). Cet excès d'oméga-6 ne permet pas l'utilisation optimale des oméga-3 (Brady *et al.*, 2004). En effet une compétition métabolique s'établit entre les acides gras oméga-3 et les acides gras oméga-6 puisque plusieurs enzymes leur sont communes, au détriment de l'exploitation des acides gras oméga-3. Le bénéfice des oméga-3 est donc estompé.

1.4.2 L'hespéridine et la naringine

Les flavonoïdes représentent la plus grande classe de composés polyphénoliques dérivés de plantes. Les flavanones (hespéridine, naringine) sont une sous-classe des flavonoïdes, ils sont le plus souvent liés à des glucosides et sont rarement libres (aglycones). L'hespéridine et la naringine sont des glycosides flavanones, composés d'une aglycone, l'hespérétine et la naringénine respectivement, lié à un disaccharide, le rutinose. L'hespéridine est l'hespérétine 7-O-β-rutinoside, et la naringine est la naringénine-7-rhamnoglucoside. L'hespéridine et la naringine ont été largement utilisées pour leurs propriétés anti-oxydantes, anti-inflammatoires et anticancéreuses. Celles-ci ont été récemment utilisées pour la prévention de l'obésité, l'insulinorésistance et la dyslipidémie (Akiyama *et al.*, 2010; Miceli *et al.*, 2007; Jung *et al.*, 2006).

Les flavanones pourraient avoir un effet hypoglycémiant (Akiyama *et al.*, 2009). Une supplémentation en hespéridine a diminué le glucose plasmatique chez des rats diabétiques (Akiyama *et al.*, 2010). L'hespéridine et la naringine ont augmenté les niveaux d'ARNm de la glucose kinase et diminué ceux de la glucose-6-phosphatase dans le foie de souris diabétiques (Jung *et al.*, 2006) ainsi que les activités des ces enzymes dans le foie de rats diabétiques

(Akiyama *et al.*, 2010). L'hespéridine augmente le niveau plasmatique d'adiponectine chez les rats diabétiques (Akiyama *et al.*, 2010) et associée à la naringénine, elle augmente l'expression de l'adiponectine et active le PPARγ d'une manière dose-dépendante dans les adipocytes (Liu *et al.*, 2008).

Les flavonoïdes des agrumes pourraient être efficaces contre l'hyperlipidémie. L'hespéridine et la naringine augmentent les HDL et diminuent le cholestérol plasmatique, les LDL et les triglycérides chez les rats (Miceli *et al.*, 2007; Monforte *et al.*, 1995). Bien que les flavonoïdes aient été étudiés depuis environ 50 ans, les mécanismes cellulaires impliqués dans leurs actions biologiques ne sont pas encore totalement connus. Ils pourraient réduire le risque de maladies cardiovasculaires eu piégeant des radicaux (effet antioxydant) et par leur action hypocholestérolémiante (Miceli *et al.*, 2007). L'hespéridine et la naringine inhibent les activités de l'HMG-CoA réductase et de l'acyl-CoA : cholestérol O-acyltransférase (ACAT), et ainsi abaissent le taux de cholestérol plasmatique chez les rats nourris avec un régime riche en cholestérol (Choi *et al.*, 2004; Kim *et al.*, 2003; Bok *et al.*, 1999). Par ailleurs, chez le rat sain, l'hespéridine a diminué les triglycérides plasmatiques seuls (Kawaguchi *et al.*, 1997), et ceci s'est s'accompagné d'une diminution du cholestérol plasmatique (Horcajada *et al.*, 2008). Dans une étude récente, chez les rats diabétiques, l'hespéridine a corrigé l'augmentation des triglycérides plasmatiques, du cholestérol total et du VLDL- et LDL-cholestérol ; et a augmenté le HDL-cholestérol (Akiyama *et al.*, 2010). L'hespéridine et la naringine ont augmenté l'expression du gène codant pour le récepteur LDL, qui améliore la captation et dégradation des LDL et réprimé l'activité et l'expression de la protéine microsomale de transfert des triglycérides (Wilcox *et al.*, 2001), ce qui inhibe la formation des VLDL.

2 OBJECTIF

Dans notre unité, le chien a été caractérisé comme modèle d'obésité et d'insulinorésistance, et utilisé pour mesurer les effets des acides gras polyinsaturés oméga-3, et des polyphénols de thé vert. Pour cette thèse, on a développé l'obésité et l'insulinorésistance chez le rat et étudié les effets de certains interventions nutritionnelles.

L'objectif de cette étude a été double. Notre premier objectif a été de caractériser un modèle « rat » de développement nutritionnel d'obésité et d'insulinorésistance, et dans un deuxième temps, d'étudier les effets de deux interventions nutritionnelles sur ce modèle. Nous avons mesuré les paramètres plasmatiques susceptibles d'être associés à une faible sensibilité à l'insuline, au début et à la fin du protocole. Une telle approche longitudinale nous a permis de caractériser le développement de la maladie de manière la plus satisfaisante possible. La plupart des perturbations plasmatiques résultent de modifications transcriptionnelles, touchant certains gènes impliqués dans le métabolisme glucido-lipidique. Afin de caractériser d'une manière complète le modèle « rat », nous avons mesuré l'expression des gènes dans le foie et le tissu adipeux viscéral, tissus cibles de l'insuline, semblant avoir un rôle clé dans le développement de l'obésité et de l'insulinorésistance. De nombreux agents nutritionnels et pharmacologiques, notamment les polyphénols, les statines, l'acide nicotinique, les fibrates, les flavonoïdes, les acides gras polyinsaturés oméga-3 (principalement sous la forme d'huile de poisson) ont été étudiés pour déterminer leurs effets anti-inflammatoires et hypolipidémiants. Il a été suggéré que l'huile de poisson diminue les triglycérides et a des effets controversés sur la sensibilité à l'insuline. Récemment, les études sont concentrées sur les effets particuliers des acides gras polyinsaturés d'huile de poisson. Les flavonoïdes sont largement étudiés pour leurs effets antioxydant et anticancéreux, et depuis quelques années, elles sont étudiées pour leurs effets hypolipidémiant et hypoglycémiant.

Pour caractériser le modèle, différents régimes ont été utilisés pour induire une obésité et/ou une insulinorésistance : un régime riche en lipides et un régime riche en fructose. Ces différents régimes doivent permettre de comparer les paramètres en jeu dans l'installation de l'obésité, dans le développement de l'insulinorésistance, dans les dyslipidémies et les modifications du profil lipoprotéinique, et enfin dans l'expression de gènes d'intérêt dans les tissus cibles de l'insuline (foie et tissu adipeux).

Notre second objectif était de mesurer l'effet de deux supplémentations nutritionnelles sur l'obésité et les troubles métaboliques associés : une supplémentation en acide eicosapentaénoïque (EPA) et une supplémentation en flavanones (hespéridine et naringine). On a donc étudié les paramètres mis en jeu dans la prévention de l'installation de l'obésité, des dyslipidémies et du profil lipoprotéinique et aussi dans l'amélioration de la sensibilité à l'insuline chez les rats ayant consommé les différents régimes (contrôle, riche en lipides, riche en fructose), avec ou sans supplémentation en EPA ou en hespéridine et naringine.

L'étude peut être divisée en trois parties.

I- La caractérisation de l'obésité et de l'insulinorésistance du modèle de rat soumis à un régime riche en matières grasses ou un régime riche en fructose.

II- Effets de la supplémentation en acide eicosapentaénoïque (EPA) chez ce modèle rat.

III- Effets de la supplémentation en hespéridine et naringine (HN) chez ce modèle rat, en présence d'un régime riche en matières grasses ou d'un régime riche en fructose.

3 Matériel et méthodes

3.1 Les régimes

3.1.1 Caractérisation du modèle rat d'obésité et d'insulinorésistance induites par un régime hyperlipidique ou riche en fructose

Dix huit rats Wistar adultes âgés de 12 semaines ont été aléatoirement divisés en trois groupes et ont été nourris pendant 10 semaines avec un régime témoin, un régime hyperlipidique, ou un régime riche en fructose. La composition des ces régimes figure dans le tableau 14.

3.1.2 Effets de la supplémentation en acide eicosapentaénoïque (EPA) chez le rat

Trente rats Wistar adultes âgés de 12 semaines ont été aléatoirement divisés en cinq groupes et ont été nourris pendant 10 semaines avec un régime témoin, un régime hyperlipidique (supplémenté en EPA ou non), ou un régime riche en fructose (supplémenté en EPA ou non). Les rats ayant consommé le régime témoin, le régime hyperlipidique, et le régime riche en fructose (sans supplémentation en EPA) sont les mêmes que dans la partie précédente. La composition des régimes est présentée dans le tableau 14. Les groupes supplémentés ont reçu 120 mg/rat/jour (200 - 300 mg/kg/jour) d'EPA (KD-Pharma Bexbach GmbH, Bexbach, Allemagne). L'EPA a été mélangé avec du fromage pour éviter son oxydation. Les autres groupes ont reçu le même fromage sans EPA.

3.1.3 Effets de la supplémentation en hespéridine et naringine (HN) chez le rat en présence d'un régime riche en matières grasses ou d'un régime riche en fructose

Dix huit rats Wistar adultes âgés de 31 semaines ont été répartis aléatoirement en trois groupes et ont été nourris pendant 8 semaines avec un régime témoin (supplémenté en hespéridine et naringine), un régime hyperlipidique (supplémenté en hespéridine et naringine), ou un régime riche en fructose (supplémenté en hespéridine et naringine). La

Tableau 14: Composition du régime témoin, du régime hyperlipidique et du régime riche en fructose.

a) Valeurs énergétiques du régime contrôle, du régime hyperlipidique, et du régime riche en fructose.

	Régime témoin	**Régime hyperlipidique**	**Régime riche en fructose**
Calories d'origine protéique	20	20	20
Calories d'origine lipidique	15	65	15
Calories d'origine glucidique	65	15	65
Valeurs énergétiques (kcal/kg)	**3488**	**4859**	**3488**

Les valeurs énergétiques sont présentées en % d'énergie (kcal %).

b) Composition de la matière sèche du régime contrôle, du régime hyperlipidique, et du régime riche en fructose.

	Régime témoin	**Régime hyperlipidique**	**Régime riche en fructose**
Caséine	20,5	28,6	20,5
Amidon de maïs	39,7	12,65	-
Dextrose / glucose	20	6,5	-
Fructose	-	-	59,7
Cellulose	6	6	6
Huile du tournesol	5,8	3,85	5,8
Saindoux	-	31,25	-
Minéraux[a]	7	9,75	7
Vitamines[b]	1	1,4	1
	100	**100**	**100**

Les composants alimentaires sont présentés en g par 100 g d'aliment.

[a] : Mineral mix SAFE ; [b] : Vitamin mix SAFE.

composition des régimes est présentée dans le tableau 14. Les rats ont reçu 8,75 mg/kg^{0,75} / jour d'hespéridine et la même quantité de naringine (Natural Orange Extract, et Citroflavonoides, Zoster, S. A., Beniel, l'Espagne, par Affinity Petcare, Barcelone, Espagne). L'hespéridine et la naringine ont été mélangées dans l'aliment.

Les paramètres mesurés ont été le poids, la composition corporelle par la méthode de dilution isotopique, la sensibilité à l'insuline, évaluée par la technique de clamp euglycémique-hyperinsulinémique, le profil lipidique plasmatique (triglycérides, cholestérol total et acides gras non-estérifiés), le profil lipoprotéinique plasmatique par FPLC, et les expressions ARNm des gènes impliqués dans l'obésité et la sensibilité à l'insuline dans les tissus adipeux et hépatique par PCR en temps réel. Les gènes étudiés ont été le récepteur à l'insuline et l'IRS-1 (pour la sensibilité à l'insuline), le GLUT4 (pour la capture du glucose), la leptine, le TNFα (pour l'inflammation), la FAS, l'ACC, la PEPCK et la LPL (pour la lipogenèse), la périlipine, la HSL et la lipase hépatique (pour la lipolyse), le SREBP-1c, le PPARα et le PPARγ, facteurs transcriptionnels clés dans le métabolisme glucido-lipidique.

4 Résultats

4.1 Caractérisation du modèle rat d'obésité et d'insulinorésistance induites par un régime hyperlipidique ou riche en fructose

- ***Poids et composition corporels***

Le poids et composition corporels sont montrés dans le tableau 15. Le gain de poids était similaire pour tous les groupes. A la fin du protocole, les rats ayant consommé le régime hyperlipidique avaient une masse grasse corporelle significativement plus élevée ($P < 0,05$) que les rats ayant consommé le régime témoin, à la fin du protocole.

- ***Sensibilité à l'insuline***

La glycémie, l'insulinémie et la sensibilité à l'insuline sont montrés dans le tableau 15. Aucune différence de glycémie ou d'insulinémie basale n'a été observée dans tous les groupes. Les rats ayant consommé le régime hyperlipidique ont présenté un plus faible ($P < 0,05$) taux de perfusion de glucose pendant le clamp euglycémique-hyperinsulinémique que les rats ayant consommé le régime témoin, à la fin du protocole. Les rats ayant consommé le régime riche en fructose n'avaient aucune modification de la sensibilité à l'insuline par rapport aux rats ayant consommé le régime témoin.

- ***Profils lipidiques plasmatiques***

Les profils lipidiques plasmatiques sont montrés dans le tableau 15. Les concentrations plasmatiques en cholestérol total, en triglycérides et en acides gras non-estérifiés n'ont pas été modifiées dans tous les groupes à la fin du protocole.

Les concentrations en cholestérol et en triglycérides dans les lipoprotéines plasmatiques n'ont pas été modifiées dans tous les groupes.

- ***L'expression ARNm des gènes dans le tissu hépatique***

Les expressions ARNm des gènes impliqués dans la signalisation de l'insuline (le récepteur à l'insuline et l'IRS-1) n'ont été modifiées dans le foie dans aucun groupe, à la fin du régime, par rapport au début du protocole. L'expression ARNm du gène du GLUT4 dans le foie est diminuée chez les rats ayant consommé le régime hyperlipidique et le régime riche en fructose, à la fin de l'expérimentation par rapport à la semaine 1. Il n'y a eu aucun changement dans les expressions ARNm des gènes de la FAS, de la LPL, de la PEPCK et de la HSL dans le foie à la fin de l'expérimentation, et celle de la lipase hépatique a été augmentée par rapport à la semaine 1 chez les rats ayant consommé le régime riche en fructose. L'expression ARNm du gène du SREBP-1c hépatique a été augmentée par rapport à la semaine 1, chez les rats ayant consommé le régime hyperlipidique. L'expression ARNm du PPARα hépatique a été augmentée à la semaine 11, par rapport à la semaine 1 chez les rats ayant consommé le régime riche en fructose.

- ***L'expression ARNm des gènes dans le tissu adipeux***

Les expressions ARNm des gènes impliqués dans la signalisation de l'insuline (le récepteur à l'insuline et l'IRS-1) dans le tissu adipeux viscéral n'ont été différentes à la semaine 11 dans aucun groupe. L'expression ARNm du GLUT4 dans le tissu adipeux viscéral a été plus faible chez les rats ayant consommé le régime hyperlipidique ou le régime riche en fructose par rapport aux rats ayant consommé le régime témoin, à la fin de l'expérimentation. Les expressions ARNm des gènes de la leptine et du TNFα dans le tissu adipeux ont été plus élevées chez des rats ayant consommé le régime hyperlipidique, par rapport aux rats ayant consommé le régime témoin. Les expressions ARNm de la FAS et de l'ACC dans le tissu adipeux ont été plus fortes chez des rats ayant consommé le régime hyperlipidique par rapport aux rats ayant consommé le régime témoin, et celle de la LPL à été similaire dans tous les groupes. L'expression ARNm de la périlipine dans le tissu adipeux viscéral a été plus élevée chez les rats ayant consommé le régime hyperlipidique par rapport aux rats ayant consommé le régime témoin, et celle de la HSL à été similaire dans tous les groupes. L'expression ARNm du gène du SREBP-1c dans le tissu adipeux viscéral n'a été différente dans aucun groupe. L'expression ARNm du gène du PPARγ dans le tissu adipeux viscéral a tendu à être plus faible ($P = 0,1023$) chez les rats ayant consommé le régime hyperlipidique par rapport aux rats ayant consommé le régime témoin.

Tableau 15: Poids, masse grasse corporelle, sensibilité à l'insuline, et profils lipidiques plasmatiques aux semaines 1 et 11 de l'expérimentation chez des rats ayant consommé le régime témoin, le régime hyperlipidique ou le régime riche en fructose.

	Témoins		**Hyperlipidique**		**Riche en fructose**				
	Semaine 1	**Semaine 11**	**Semaine 1**	**Semaine 11**	**Semaine 1**	**Semaine 11**	***Interaction***	***Temps***	***EPA-suppl.***
Poids corporel (g)	401±6	526±14	383±19	534±26	399±12	529±16	*NS*	♯♯	*NS*
Masse grasse corporelle (g)	35,6±3,3	63,3±9,5	25,6±4,7	90,0±12,2*	34,6±5,7	78,9±11,8	*	-	-
Glycémie basale (mmol/l)	5,51±0,11	4,93±0,25	5,60±0,15	5,31±0,19	5,35±0,19	5,52±0,31	*NS*	*NS*	*NS*
Insulinemia basale (pmol/l)	402±8,4	400±17,2	415±24,8	388±9,9	386±12,4	402±11,0	*NS*	*NS*	*NS*
GIR (mg/kg/min)	19,0±1,4	18,5±1,2	19,3±1,6	12,9±2,0*	18,2±1,5	15,3±1,4	*	-	-
Cholestérol total (mmol/l)	1,81±0,11	2,04±0,14	1,46±0,06	1,99±0,17	1,59±0,09	1,83±0,14	*NS*	♯	*NS*
Triglycérides (mmol/l)	1,89±0,15	1,64±0,10	1,86±0,12	1,76±0,12	1,78±0,11	2,00±0,30	*NS*	*NS*	*NS*
AGNE (mmol/l)	0,46±0,07	0,30±0,04	0,45±0,06	0,31±0,04	0,42±0,04	0,41±0,04	*NS*	♯	*NS*

NS, non significatif ; GIR, taux de perfusion de glucose (glucose infusion rate) dans le test de clamp euglycémique-hyperinsulinémique ; AGNE, acides gras non-estérifiés. * $P < 0,05$ pour les comparaisons entre les groupes pour l'interaction entre de temps et type du régime (Fixed effects model analysis a montré que les données des rats ayant consommé le régime hyperlipidique sont significativement différentes à la semaine 11, comparé aux rats témoins), ♯ $P < 0,05$ and ♯♯ $P < 0,0001$ pour les comparaisons entre les groupes pour l'effet du temps (significatif pour la semaine 11 vs. semaine 1) et - quand $P < 0,05$ pour les comparaisons entre les groupes pour l'interaction entre le temps et le type du régime, l'effet du temps et celui du type de régime n'ont pas été considérés. Les données sont présentées en moyennes avec les ESM (erreur standard de la moyenne) (n = 6).

4.2 Effets de la supplémentation en EPA chez le rat

4.2.1 Effets de la supplémentation en EPA chez des rats ayant consommé le régime hyperlipidique

- ***Poids et composition corporels***

Le poids et les compositions corporelles sont montrés dans le tableau 16. Le gain de poids était similaire pour tous les groupes. Les rats ayant consommé le régime hyperlipidique, supplémentés avec ou sans EPA, ont montré une augmentation de la masse grasse corporelle, par rapport à la semaine 1.

- ***Sensibilité à l'insuline***

La glycémie, l'insulinémie et la sensibilité à l'insuline sont montrés dans le tableau 16. Il n'y avait aucun changement de la glycémie basale et de l'insulinémie basale. Les rats ayant consommé le régime hyperlipidique sans EPA ont montré une diminution du taux de perfusion de glucose pendant le clamp euglycémique-hyperinsulinémique, par rapport au début du protocole, reflétant une diminution de la sensibilité à l'insuline.

- ***Profils lipidiques plasmatiques***

Les profils lipidiques plasmatiques sont montrés dans le tableau 16. Il n'y avait aucun changement des concentrations du cholestérol total, des triglycérides, et des acides gras non-estérifiés dans tous les groupes à la semaine 11, par rapport à la semaine 1.

La supplémentation en EPA a diminué le VLDL-cholestérol plasmatique chez les rats ayant consommé le régime hyperlipidique à la semaine 11, par rapport à la semaine 1.

- ***L'expression ARNm des gènes dans le tissu hépatique***

Les expressions ARNm du récepteur à l'insuline et d'IRS-1 dans le foie ont été inchangées, ainsi que celle du GLUT4 avec la supplémentation en EPA chez des rats ayant consommé le régime hyperlipidique. La supplémentation en EPA a tendu à diminuer (P =

Tableau 16: Poids, masse grasse corporelle, sensibilité à l'insuline, et profils lipidiques plasmatiques aux semaines 1 et 11 de l'expérimentation chez des rats ayant consommé le régime témoin, le régime hyperlipidique ou le régime hyperlipidique supplémenté en EPA.

	Témoins		**Hyperlipidique**		**Hyperlipidique EPA**				
	Semaine 1	**Semaine 11**	**Semaine 1**	**Semaine 11**	**Semaine 1**	**Semaine 11**	***Interaction***	***Temps***	***EPA-suppl.***
Poids corporel (g)	401±6	526±14	383±19	534±26	385±19	540±21	*NS*	‡	*NS*
Masse grasse corporelle (g)	35,6±3,3	63,3±9,5	25,6±4,7	90,0±12,2*	33,2±3,8	99,0±10,3*	*	-	-
GIR (mg/kg/min)	19,0±1,4	18,5±1,2	19,3±1,6	12,9±2,0*	19,4±2,4	16,0±0,7	*	-	-
Cholestérol total (mmol/l)	1,81±0,11	2,04±0,14	1,46±0,06	1,99±0,17	1,62±0,12	1,71±0,08	*NS*	‡	*NS*
Triglycérides (mmol/l)	1,89±0,15	1,64±0,10	1,86±0,12	1,76±0,12	1,76±0,17	1,60±0,18	*NS*	‡	*NS*
AGNE (mmol/l)	0,46±0,07	0,30±0,04	0,45±0,06	0,31±0,04	0,48±0,07	0,44±0,06	*NS*	‡	*NS*

EPA, acide eicosapentaénoïque ; EPA-suppl., supplémentation en EPA ; NS, non significatif ; GIR, taux de perfusion de glucose (glucose infusion rate) dans le test de clamp euglycémique-hyperinsulinémique ; AGNE, acides gras non-estérifiés. * $P < 0,05$, pour les comparaisons entre les groupes pour l'interaction entre de temps et la supplémentation en EPA (Fixed effects model analysis a montré que les données sont significativement différentes à la semaine 11, comparé aux rats témoins), ‡ $P < 0,05$ pour les comparaisons entre les groupes pour l'effet du temps (significatif pour la semaine 11 vs. Semaine 1) et - quand $P < 0,05$ pour les comparaisons entre les groupes pour l'interaction entre le temps et la supplémentation en EPA, l'effet du temps et celui de la supplémentation en EPA n'ont pas été considérés. Les données sont présentées en moyennes avec les ESMs (n = 6).

0,0639) l'expression ARNm de la LPL dans le foie chez des rats ayant consommé le régime hyperlipidique par rapport à la semaine 1, et n'a pas changé les expressions des gènes de la FAS et de la PEPCK dans le foie. L'expression ARNm de la HSL dans le foie a été augmentée chez les rats ayant consommé le régime hyperlipidique supplémenté en EPA par rapport à la semaine 1, et celle de la lipase hépatique a été inchangée. L'expression ARNm du SREBP-1c hépatique a été augmentée chez les rats ayant consommé le régime hyperlipidique supplémentés en EPA ou non, par rapport à la semaine 1, et celle du PPARα hépatique a été inchangée.

- ***L'expression ARNm des gènes dans le tissu adipeux viscéral***

Les expressions ARNm des gènes d'IRS-1 et du GLUT4 ont été plus élevées dans le tissu adipeux chez des rats ayant consommé le régime hyperlipidique supplémenté en EPA par rapport aux rats ayant consommé le régime hyperlipidique sans la supplémentation. Les niveaux d'ARNm du récepteur à l'insuline, de la leptine, du TNFα, des enzymes de la lipogenèses (la FAS, l'ACC et la LPL), des enzymes de la lipolyse (la HSL et la périlipine), et des facteurs transcriptionnels (le SREBP-1c et le PPARγ) ont été similaires à la fin de la supplémentation en EPA chez des rats ayant consommé le régime hyperlipidique par rapport aux rats ayant consommé le régime hyperlipidique sans la supplémentation.

4.2.2 Effets de la supplémentation en EPA chez des rats ayant consommé le régime riche en fructose

- ***Poids et composition corporels***

Le poids et composition corporels sont montrés dans le tableau 17. Le poids corporel des rats ayant consommé le régime riche en fructose supplémenté en EPA a été plus élevé à la semaine 11, par rapport aux rats témoins. Il n'y avait aucun changement de la masse grasse corporelle.

- ***Sensibilité à l'insuline***

La glycémie, l'insulinémie et la sensibilité à l'insuline sont montrés dans le tableau 17. Il n'y a eu aucun changement de la glycémie basale, de l'insulinémie basale et du taux de perfusion de glucose pendant le clamp euglycémique-hyperinsulinémique chez les rats ayant consommé le régime riche en fructose supplémenté ou non en EPA.

- ***Profils lipidiques plasmatiques***

Les profils lipidiques plasmatiques sont montrés dans le tableau 17. Il n'y a eu aucun changement de concentration plasmatique du cholestérol total. Chez les rats ayant consommé le régime riche en fructose supplémenté en EPA, la concentration en triglycérides a tendu à diminuer ($P = 0,086$) ainsi que la concentration plasmatique des acides gras non-estérifiés ($P = 0,05$).

Il n'y a eu aucun changement des triglycérides lipoprotéinique plasmatique, et le VLDL-cholestérol plasmatique a été augmenté à la semaine 11 chez les rats ayant consommé le régime riche en fructose supplémenté en EPA par rapport à la semaine 1.

- ***L'expression ARNm des gènes dans le tissu hépatique***

Les expressions ARNm des gènes majeurs impliqués dans la signalisation de l'insuline (le récepteur à l'insuline et l'IRS-1) et l'absorption du glucose (le transporteur GLUT4) dans le foie ont été inchangées chez des rats ayant consommé le régime riche en fructose supplémenté en EPA par rapport au début du protocole. L'expression ARNm de la LPL dans le foie a été

augmentée chez les rats ayant consommé le régime riche en fructose supplémenté en EPA par rapport au début du protocole, et celles de la FAS et de la PEPCK ont été inchangées. L'expression ARNm de la HSL dans le foie a été augmentée chez les rats ayant consommé le régime riche en fructose supplémenté en EPA par rapport au début du protocole, et celle de la lipase hépatique a été similaire. La supplémentation en EPA n'a pas changé les expressions ARNm du SREBP-1c et du PPARα chez des rats ayant consommé le régime riche en fructose par rapport au début du protocole.

- ***L'expression ARNm des gènes dans le tissu adipeux viscéral***

Les expressions ARNm de gènes majeurs impliqués dans la signalisation de l'insuline (le récepteur à l'insuline et l'IRS-1) et l'absorption du glucose (le transporteur GLUT4) dans le tissu adipeux viscéral ont été similaires, ainsi que celles de la leptine et du TNFα à la fin de la supplémentation en EPA chez des rats ayant consommé le régime riche en fructose et des rats ayant consommé le régime riche en fructose sans la supplémentation. L'expression ARNm de la FAS dans le tissu adipeux viscéral a été plus forte chez des rats ayant consommé le régime riche en fructose supplémenté en EPA, mais celles d'ACC et de la LPL ont été similaires par rapport aux rats ayant consommé le régime riche en fructose sans la supplémentation. L'expression génique de la périlipine et de la HSL dans le tissu adipeux viscéral n'a pas été différente à la fin de la supplémentation en EPA chez des rats ayant consommé le régime riche en fructose. L'expression ARNm du PPARγ dans le tissu adipeux viscéral a été plus élevée chez des rats ayant consommé le régime riche en fructose supplémenté en EPA, et celle du SREBP-1c a été similaire par rapport aux rats ayant consommé le régime riche en fructose sans la supplémentation.

Tableau 17: Poids, masse grasse corporelle, sensibilité à l'insuline, et profils lipidiques plasmatiques aux semaines 1 et 11 de l'expérimentation chez des rats ayant consommé le régime témoin, le régime riche en fructose ou le régime riche en fructose supplémenté en EPA.

	Témoin		**Riche en fructose**		**Riche en fructose EPA**				
	Semaine 1	**Semaine 11**	**Semaine 1**	**Semaine 11**	**Semaine 1**	**Semaine 11**	***Interaction***	***Temps***	***EPA-suppl.***
Poids corporel (g)	401±6	526±14	399±12	529±16	395±18	553±17*	*	-	-
Masse grasse corporelle (g)	35,6±3,3	63,3±9,5	34,6±5,7	78,9±11,8	30,8±3,6	79,5±11,7	*NS*	ǂ	*NS*
GIR (mg/kg/min)	19,0±1,4	18,5±1,2	18,2±1,5	15,3±1,4	20,4±0,9	16,8±0,7	*NS*	*NS*	*NS*
Cholestérol total (mmol/l)	1,81±0,11	2,04±0,14	1,59±0,09	1,83±0,14	1,59±0,08	1,83±0,24	*NS*	ǂ	*NS*
Triglycérides (mmol/l)	1,89±0,15	1,64±0,10	1,78±0,11	2,00±0,30	2,49±0,19	1,73±0,11x	*	-	-
AGNE (mmol/l)	0,46±0,07	0,30±0,04	0,42±0,04	0,41±0,04	0,62±0,02	0,30±0,03y	*	-	-

EPA, acide eicosapentaénoïque ; EPA-suppl., supplémentation en EPA ; NS, non significatif ; GIR, taux de perfusion de glucose (glucose infusion rate) dans le test de clamp euglycémique-hyperinsulinémique ; AGNE, acides gras non-estérifiés. * $P < 0,05$, pour les comparaisons entre les groupes pour l'interaction entre de temps et la supplémentation en EPA (Fixed effects model analysis a montré que les données des rats ayant consommé le régime riche en fructose supplémenté en EPA sont significativement différentes à la semaine 11, comparé aux rats témoins), x $P = 0.086$ et y $P = 0.05$ présent une tendance à diminuer à la semaine 11, comparé aux rats témoins, ǂ $P < 0,05$ pour les comparaisons entre les groupes pour l'effet du temps (significatif pour la semaine 11 vs. Semaine 1) et - quand $P < 0,05$ pour les comparaisons entre les groupes pour l'interaction entre le temps et la supplémentation en EPA, l'effet du temps et celui de la supplémentation en EPA n'ont pas été considérés. Les données sont présentées en moyennes avec les ESM (erreur standard de la moyenne) (n = 6).

4.3 Effets de la supplémentation en hespéridine et naringine chez le rat en présence d'un régime hyperlipidique ou d'un régime riche en fructose

- ***Poids et composition corporels***

Le poids et composition corporels sont montrés dans le tableau 18. Le poids corporel et le pourcentage de masse grasse corporelle ont été similaires dans tous les groupes à la fin de l'expérimentation.

- ***Sensibilité à l'insuline***

La glycémie, l'insulinémie et la sensibilité à l'insuline sont montrés dans le tableau 18. Il y avait un effet significatif de la supplémentation en HN chez les rats ayant consommé le régime hyperlipidique ou le régime riche en fructose sur la glycémie basale à la fin de l'expérimentation. L'insulinémie basale a été similaire entre les groupes à la fin de l'expérimentation. Les rats ayant consommé le régime hyperlipidique supplémenté en HN ou le régime riche en fructose supplémenté en HN ont eu un plus faible taux de perfusion de glucose pendant le clamp euglycémique-hyperinsulinémique, que les rats ayant consommé le régime témoins supplémenté en HN, à la fin de l'expérimentation.

- ***Profils lipidiques plasmatiques***

Le profils lipidiques plasmatiques sont montrés dans le tableau 18. La concentration plasmatique du cholestérol total a été similaire dans tous les groupes à la fin de l'expérimentation. La concentration plasmatique des triglycérides a été plus élevée chez des rats ayant consommé le régime hyperlipidique supplémenté en HN ou le régime riche en fructose supplémenté en HN par rapport aux rats ayant consommé le régime témoins supplémenté en HN, à la fin de l'expérimentation. La concentration plasmatique des acides gras non-estérifiés n'est différente dans aucun groupe, à la fin de l'expérimentation.

Tableau 18: Poids, pourcentage de masse grasse corporelle, sensibilité à l'insuline, et profils lipidiques plasmatiques à la fin de l'expérimentation chez des rats ayant consommé le régime témoin supplémenté en HN, le régime hyperlipidique supplémenté en HN ou le régime riche en fructose supplémenté en HN.

	Rats nourris régime témoin HN	**Rats nourris régime hyperlipidique HN**	**Rats nourris régime riche en fructose HN**	***ANOVA***
Poids corporel (g)	537 ± 38	571 ± 31	628 ± 21	*NS*
Masse grasse (g)	109,5 ± 14,5	133,2 ± 16,1	137,0 ± 18,1	*NS*
Glycémie basale (g/l)	0,88 ± 0,05	0,77 ± 0,04	0,81 ± 0,05	*
Insulinémie basale (ng/ml)	2,04 ± 0,34	1,75 ± 0,22	3,14 ± 0,57	*NS*
GIR (mg/kg/min)	18,9 ± 1,4	10,7 ± 0,9*	12,6 ± 1,3*	*
Cholestérol total (mmol/l)	2,11 ± 0,17	2,09 ± 0,12	2,19 ± 0,13	*NS*
Triglycérides (mmol/l)	1,50 ± 0,38	2,68 ± 0,28*	2,63 ± 0,18*	*
AGNE (mmol/l)	0,37 ± 0,08	0,33 ± 0,05	0,51 ± 0,05	*NS*

HN, hespéridine et naringine; NS, non significatif; GIR, taux de perfusion de glucose (glucose infusion rate) dans le test de clamp euglycémique-hyperinsulinémique; AGNE, acides gras non-estérifiés. * $P < 0,05$ comparé aux rats témoins à la fin du protocole. Les données sont présentées en moyennes avec les ESM (erreur standard de la moyenne) (n = 6).

5 Discussion

5.1 Caractérisation du modèle rat d'obésité et d'insulinorésistance induites par un régime hyperlipidique ou riche en fructose

L'élaboration et la caractérisation de modèles animaux utilisés pour l'étude de l'obésité et de la sensibilité à l'insuline sont importantes. L'objectif de cette partie d'étude était le développement de l'obésité et de l'insulinorésistance induits par l'alimentation chez le rat, ou plus précisément de caractériser le rat comme un modèle d'obésité et d'insulinorésistance induites par un régime hyperlipidique ou un régime riche en fructose par une approche longitudinale.

Nous avons composé les groupes de rats de manière aléatoire et utilisé une approche longitudinale, ce qui suppose la détermination des valeurs basales des paramètres biochimiques afin d'exclure les différences préexistantes. Ce faisant, nous avons mesuré des différences de concentration plasmatique en cholestérol total entre les groupes au début de l'expérimentation (semaine 1) alors que les autres paramètres (poids corporel, masse grasse corporelle, glycémie basale et insulinémie basale, sensibilité à l'insuline, les concentrations plasmatiques des triglycérides et des acides gras non-estérifiés) étaient similaires. De telles variations de concentration en lipides ont été reportées chez des rats, la concentration du cholestérol total plasmatique peut ainsi varier de 1,04 mmol/l à 3,38 mmol/l (Wesche, 2009), tout en étant considérée comme normale. La concentration en cholestérol total chez les rats adultes variait entre 1,249 et 2,109 mmol/l à la semaine 1 dans notre étude, ce qui suggère une variation entre les rats.

La randomisation avait pour but d'éliminer les différences entre les groupes mais la détermination des valeurs basales est restée une nécessité. Elles ont été prises en compte dans l'analyse statistique, ce qui a contribué à annuler les différences préexistantes.

L'induction de l'obésité et l'étude de la résistance à l'insuline ont été possibles en utilisant des régimes de composition précise, semi-purifiés ou purifiés, des régimes standard (croquettes) enrichis en lipides ou en fructose ou des régimes 'cafeteria'. Cependant tous ces régimes ne sont pas satisfaisants : les régimes « cafeteria » sont considérés impropres (Moore, 1987), les régimes standards peuvent contenir des contaminants tels que les phytochimiques,

dérivés de plantes, et les régimes standards enrichis en lipides ou fructose peuvent être déséquilibrés et conduire à une malnutrition. Des phytoestrogènes ont par exemple été décelés dans l'aliment standard des rongeurs et pourraient affecter les voies endocriniennes (Kanno *et al.*, 2002). Nous avons donc choisi d'utiliser un aliment semi-purifié pour éliminer ou réduire les effets d'une déficience ou un déséquilibre en macro- et micronutriments en ajustant leur quantité.

Plusieurs caractéristiques du régime hyperlipidique choisi pour induire l'obésité et le développement de l'insulinorésistance doivent être renseignées : type, quantité et source des lipides. En effet, le pourcentage de lipides présents dans un aliment influe sur le développement et le degré de l'obésité, de la dyslipidémie et de l'insulinorésistance. Ghibaudi, *et al.* (2002) ont montré que le poids et la masse grasse corporelle augmentaient chez le rat proportionnellement à la quantité de lipides. La source de matières grasses dans l'alimentation doit aussi être prise en compte. Buettner, *et al.* (2006) ont ainsi montré qu'une alimentation riche en acides gras saturés avait un effet plus délétère sur le développement de l'insulinorésistance et de la stéatose hépatique qu'un régime riche en acides gras polyinsaturés (huile de poisson). Pour notre étude, nous avons donc choisi le saindoux riche en acides gras saturés comme source principale de lipides.

Outre les régimes hyperlipidiques, des régimes riches en fructose avec 60 % à 65 % d'énergie sous forme de fructose ont été largement utilisés (Mohamed Salih *et al.*, 2009; Sanchez-Lozada *et al.*, 2007; Nakagawa *et al.*, 2006). Nous avons de ce fait décidé d'étudier le développement de l'obésité et de l'insulinorésistance en utilisant un régime hyperlipidique (65 % d'énergie lipidique) ou riche en fructose (65 % d'énergie), par une approche longitudinale pendant 10 semaines.

Dans notre étude, les rats ont tous eu le même gain du poids, pendant la période de 10 semaines. Indépendement de l'augmentation du poids corporel, l'augmentation de la masse grasse corporelle peut présenter un risque dans le développement de l'insulinorésistance et les pertubations métaboliques associées. Dans notre étude, l'augmentation de la masse grasse chez des rats ayant consommé le régime hyperlipidique reflète la propriété obésogène du saindoux, riche en acides gras saturés, utilisé comme source de lipides. Lors de l'installation de l'obésité, les adipocytes augmentent en taille et en nombre. Le tissu adipeux sécrète des adipokines, notamment la leptine qui agit sur le système nerveux central et régule la prise alimentaire et la dépense énergétique. La leptine agit sur ses récepteurs au niveau de l'hypothalamus et inhibe l'appétit ; l'absence de la leptine, ou de ses récepteurs, ou la

résistance à la leptine conduit à une prise alimentaire incontrôlée et à l'obésité. Dans notre étude, l'expression génique de la leptine dans le tissu adipeux viscéral a été plus élevée chez les rats ayant consommé le régime hyperlipidique par rapport aux rats témoins, ce qui est cohérent avec l'augmentation de la masse grasse corporelle. Li, *et al.* (2002) ont observé une augmentation de l'expression de la leptine chez les rats consommant un régime hyperlipidique, liée à une expansion du tissu adipeux. Nos résultats concernant le poids et la masse grasse corporels chez les rats ayant consommé le régime riche en fructose montrent que la forte teneur en fructose n'a pas conduit à l'obésité. Dans l'étude de Chicco, *et al.* (2003) un gain de poids significatif n'a été obtenu qu'après 15 ou 30 semaines d'un régime riche en saccharose, ce qui suggère la nécessité d'une période prolongée pour obtenir un gain de poids. En outre, les niveaux d'ARNm de la leptine dans le tissu adipeux n'ont pas été modifiés chez les rats ayant consommé le régime riche en fructose. Ceci nous a amené à émettre l'hypothèse selon laquelle le développement de l'obésité chez les rats pourrait être leptine-dépendant : le régime hyperlipidique pourrait stimuler l'expression de la leptine dans le tissu adipeux, cette hyperleptinémie pourrait développer une résistance à la leptine, d'où un apport énergétique très élevé et, finalement, l'obésité.

Chez nos rats ayant consommé le régime hyperlipidique, les résultats concernant la faible sensibilité à l'insuline et la masse grasse corporelle élevée ont été cohérents, avec ce qui a été décrit chez des rats ayant consommé un régime riche en lipides où une faible sensibilité à l'insuline était associée à une augmentation de tissu adipeux péri-épididymaire (Sridhar *et al.*, 2008; Vinayagamoorthi *et al.*, 2008). Dans notre étude, chez les rats nourris avec le régime riche en fructose, aucun changement de sensibilité à l'insuline et de glycémie basale n'a été observé. Dans une étude précédente, ce régime riche en fructose a augmenté le glycogène hépatique (Yadav *et al.*, 2009), par inhibition de la glycogénolyse plutôt que par synthèse de glycogène. En effet, le fructose 1-phosphate, un intermédiaire du métabolisme hépatique du fructose, inhibe l'activité de la glycogène-phosphorylase en épuisant le phosphate inorganique (Kaufmann and Froesch, 1973), et donc inhibe la glycogénolyse. L'augmentation de la quantité hépatique de glycogène pourrait être un mécanisme permettant d'empêcher le développement de l'hyperglycémie.

La production accrue des acides gras non-estérifiés par la lipogenèse *de novo* pourrait jouer un rôle important dans le développement de la résistance à l'insuline et de la dyslipidémie. La conversion du fructose en triose-phosphates (glycéraldéhyde-3-phosphate et dihydroxyacétone phosphate) peut fournir du glycérol nécessaire à la lipogenèse hépatique,

conduisant à une augmentation de la production des triglycérides. De plus, le fructose inhibe l'oxydation lipidique et favorise la synthèse des VLDL hépatiques (Topping and Mayes, 1972).

L'augmentation des concentrations en triglycérides et en acides gras non-estérifiés pourrait conduire à une augmentation de la phosphorylation sérine / thréonine de l'IRS-1, et ainsi réduire la signalisation de l'insuline. En effet, l'accumulation intramyocytaire des lipides a été associée avec une augmentation de la phosphorylation de la sérine et une diminution de l'action d'insuline (Morino *et al.*, 2005). Dans notre étude, les rats ayant consommé le régime riche en fructose n'ont pas développé de dyslipidémie et aucun changement dans les niveaux en ARNm du récepteur à l'insuline et de l'IRS-1 dans le foie n'a été mis en évidence. Il faut noter qu'un régime riche en saccharose induit une baisse de la sensibilité à l'insuline qui touche le foie avant de toucher les muscles (Pagliassotti *et al.*, 1996). Nous avons évalué la sensibilité à l'insuline par la technique du clamp euglycémique-hyperinsulinémique, qui est la méthode standard d'évaluation directe de la sensibilité à l'insuline du corps entier (DeFronzo *et al.*, 1979), par mesure de la captation du glucose induite par l'insuline dans des conditions hyperinsulinémiques. Il est possible que dans notre étude, le régime riche en fructose ait pu induire une insulinorésistance hépatique, ce qu'a suggéré la diminution des ARNm du transporteur GLUT4 dans ce tissu, sans diminuer la sensibilité à l'insuline du corps entier, et les niveaux d'ARNm du récepteur à l'insuline et de l'IRS-1 étant d'ailleurs inchangés dans le tissu adipeux viscéral. Il faut noter que la diminution de l'absorption du glucose dans l'insulinorésistance n'est pas nécessairement corrélée avec l'expression du GLUT4 (Pedersen *et al.*, 1990) et que cette absorption dépend aussi de l'activité intrinsèque du GLUT4 (Konrad *et al.*, 2002). La fluidité de la membrane joue également un rôle majeur dans la translocation du GLUT4 à la membrane et dans l'absorption du glucose (Sandra *et al.*, 1984). L'altération de la cascade de signalisation de l'insuline observée dans l'insulinorésistance réduit l'efficacité de l'insuline, malgré des concentrations en insuline normales voire élevées. Le récepteur à l'insuline est le premier effecteur de cette signalisation. L'expression de ce récepteur dans le foie et le tissu adipeux viscéral n'a pas été différente entre nos groupes, tout comme l'expression du gène d'IRS-1, indiquant que la signalisation de l'insuline semble peu ou pas affectée dans le foie et les tissus adipeux. Nos résultats suggèrent que le régime riche en fructose n'a pu que faiblement affecter l'efficacité de l'insuline. De plus, la durée de notre expérimentation pourrait ne pas avoir été suffisante pour permettre le développement de l'insulinorésistance du corps entier. Nous avons observé l'augmentation d'expression ARNm

d'ACC associée avec l'insulinorésistance chez les rats ayant consommé le régime hyperlipidique. Une diminution d'expression d'ACC est en corrélation positive avec la sensibilité à l'insuline (Rosa *et al.*, 2003). L'activation d'ACC pourrait inhiber l'oxydation des acides gras en augmentant les malonyl-CoA et augmenter les triglycérides intramyocytaires et conduire l'insulinorésistance. Il a par ailleurs été suggéré que le PPARγ pourrait jouer un rôle dans la sensibilité à l'insuline du tissu adipeux en stimulant l'expression de certains gènes : adiponectine (Iwaki *et al.*, 2003), résistine (Ghosh *et al.*, 2003; Patel *et al.*, 2003) et GLUT4 (Giron *et al.*, 1999), et pourrait améliorer la sensibilité à l'insuline. Les faibles expressions de PPARγ et de GLUT4 que nous avons mesurées dans le tissu adipeux viscéral des rats ayant consommé le régime hyperlipidique confirme le lien existant entre ces gènes.

L'association de l'inflammation et du développement du diabète de type 2 (Hotamisligil *et al.*, 1993), a conduit à considérer l'obésité comme un état inflammatoire chronique et a amené à explorer les conséquences de l'inflammation dans le développement de l'insensibilité à l'insuline. Le tissu adipeux, particulièrement le tissu adipeux viscéral, produit des cytokines pro-inflammatoires, notamment TNFα, et cette production augmente avec l'expansion du tissu adipeux (Hotamisligil *et al.*, 1993). Dans notre étude, les niveaux d'ARNm du TNFα dans le tissu adipeux viscéral ont été augmentés chez les rats ayant consommé le régime hyperlipidique. Plusieurs études chez le rat ont étudié l'effet d'une induction nutritionnelle de l'obésité et de l'insulinorésistance sur la production du TNFα et ont rapporté des résultats contradictoires. Morin, *et al.* (1997) ont observé une augmentation de l'expression du TNFα dans le tissu adipeux viscéral chez des rats nourris avec un régime hyperlipidique, non corrélée avec le statut insulino-résistant, tandis que Li, *et al.* (2002) n'ont constaté aucun changement de TNFα. Nos résultats suggèrent une relation positive entre l'inflammation (expression accrue de TNFα) et l'obésité, associée à une faible sensibilité à l'insuline chez les rats ayant consommé le régime hyperlipidique.

Concernant les lipides plasmatiques, aucune modification de la concentration en cholestérol total ou de la concentration en cholestérol dans les lipoprotéines n'a été mesurée chez les rats ayant consommé le régime hyperlipidique ou le régime riche en fructose. Ceci pourrait s'expliquer par l'absence de cholestérol dans l'aliment. Ce résultat est cohérent avec ceux de Sinitskaya, *et al.* (2007) qui n'ont montré aucune variation du taux de cholestérol dans les lipoprotéines plasmatiques à la fin d'un régime hyperlipidique, mais sont contradictoires avec ceux de Mohamed Salih, et al. (2009) qui ont montré une augmentation

du VLDL-cholestérol et du LDL-cholestérol et une diminution du HDL-cholestérol chez les rats ayant consommé un régime riche en fructose. La plupart des souches de rats sont résistantes au développement de l'athérosclérose (Moghadasian, 2002) et possèdent la capacité de maintenir un faible taux de cholestérol total et de LDL-cholestérol et un taux élevé du HDL-cholestérol. De ce fait, il est généralement nécessaire d'inclure du cholestérol et de l'acide cholique dans l'aliment pour induire l'athérosclérose (Andrus *et al.*, 1956), comme l'acide cholique favorise l'absorption intestinale des matières grasses et du cholestérol (Wang *et al.*, 1999a).

Nous n'avons observé aucun changement dans les concentrations en acides gras non-estérifiés plasmatiques et en triglycérides quelque soit le régime. Dans la littérature, des résultats contradictoires ont été décrits. Certains auteurs ont montré une augmentation de la concentration des acides gras non-estérifiés plasmatiques (Posey *et al.*, 2009; Sinitskaya *et al.*, 2007) et des triglycérides (Sridhar *et al.*, 2008; Vinayagamoorthi *et al.*, 2008) chez des rats ayant consommé un régime hyperlipidique tandis que d'autres n'ont montré aucune variation des acides gras non-estérifiés (Morens *et al.*, 2006) et des triglycérides (Matveyenko *et al.*, 2009; Sinitskaya *et al.*, 2007; Morens *et al.*, 2006). Nos résultats concernant les concentrations en acides gras non-estérifiés et en triglycérides chez les rats ayant consommé le régime riche en fructose diffèrent de ceux précédemment décrits (Mohamed Salih *et al.*, 2009; Shih *et al.*, 2009). Il est possible que le foie ait pu capturer les acides gras non-estérifiés et augmenter leur oxydation, comme l'a suggéré l'augmentation de l'expression hépatique du gène de PPARα chez les rats ayant consommé le régime riche en fructose. En effet une augmentation du PPARα favorise la captation et le catabolisme des acides gras en stimulant l'expression des gènes impliqués dans le transport et l'oxydation des acides gras. Enfin, il est possible que d'éventuelles perturbations des lipides plasmatiques puissent n'être décelables qu'en postprandial, et non chez des rats à jeun depuis 12h, comme cela a été décrit par les études précédentes (Koo *et al.*, 2008; Lee *et al.*, 2006b).

Il est évident que l'augmentation de la masse grasse a été liée à la lipogenèse adipocytaire. Dans le tissu adipeux viscéral, les niveaux d'ARNm de la FAS et de l'ACC ont été plus importants chez les rats ayant consommé le régime hyperlipidique que chez les rats témoins, ce qui a suggéré une de lipogenèse *de novo* accrue, tandis que, dans le foie, cette expression inchangée pourrait indiquer une lipogenèse hépatique elle aussi inchangée. L'augmentation de l'expression hépatique du SREBP-1c chez les rats ayant consommé le régime hyperlipidique a pu refléter une synthèse accrue des triglycérides, mais la

triglycéridémie normale chez ces rats a toutefois suggéré une accumulation des triglycérides dans le foie. En outre, l'expression inchangée du SREBP-1c dans le tissu adipeux viscéral chez les rats ayant consommé le régime riche en fructose a été cohérent avec la triglycéridémie normale. Une forte lipogenèse *de novo* dans le foie et le tissu adipeux des rats ayant consommé le régime riche en fructose a été exclue, car aucune changement de l'expression des gènes de la FAS, et la captation et l'utilisation des acides gras non-estérifiés plasmatiques n'a été observé. Une relation positive entre la sensibilité à l'insuline et la LPL plasmatique (Hanyu *et al.*, 2004), et l'activité de la LPL musculaire (Pollare *et al.*, 1991) a été établie, mais dans notre étude, l'expression ARNm hépatique de la LPL chez les rats ayant consommé le régime hyperlipidique ou riche en fructose n'a pas été modifiée. Le régime riche en fructose n'a pas modifié la lipogenèse hépatique et adipocytaire, ce qui est cohérent avec l'absence du changement du profil lipidique plasmatique des triglycérides et des acides gras non-estérifiés et la stabilité de la sensibilité à l'insuline.

La relation entre diminution du PPARγ et faible sensibilité à l'insuline a été établie (Kubota *et al.*, 1999). Dans notre étude, les plus faibles niveaux d'ARNm du PPARγ dans le tissu adipeux viscéral chez les rats ayant consommé le régime hyperlipidique par rapport aux rats témoins est à lier à l'insulinorésistance mesurée chez ces rats. L'activation du PPARγ augmente l'expression du gène de la LPL dans le tissu adipeux chez le rat (Schoonjans *et al.*, 1996a). Nos résultats concernant l'expression de la LPL dans le tissu adipeux sont incohérents avec cette donnée.

La HSL est une enzyme clé de la lipolyse, soumise à une régulation négative de l'insuline (Stralfors and Honnor, 1989). L'expression ARNm de la HSL est augmentée lors de la perte de poids, en lien avec l'augmentation de la lipolyse (Sztalryd and Kraemer, 1994), et les souris knock-out pour ce gène ont une lipolyse accrue (Martinez-Botas *et al.*, 2000). Il existe, dans l'obésité, une augmentation paradoxale de la lipolyse, principalement due à la résistance du tissu adipeux et à l'action antilipolytique de l'insuline. Dans l'adipocyte, la périlipine stabilise la gouttelette lipidique et empêche l'action de la HSL. Son expression est accrue dans le tissu adipeux de sujets obèses (Kern *et al.*, 2004), et diminue après une perte de poids chez le chien (Leray *et al.*, 2008). Dans notre étude, le niveau d'ARNm de la HSL a été inchangé et celui de la périlipine augmenté dans les tissus hépatique et adipeux de rats ayant consommé un régime hyperlipidique par rapport aux rats témoins. Cette augmentation de l'expression de la périlipine a pu inhiber la lipolyse, en empêchant l'action de la HSL.

En conclusion, le régime hyperlipidique a rendu les rats adultes de notre étude obèses, avec une importante masse grasse corporelle et a diminué leur sensibilité à l'insuline. La faible expression de GLUT4 dans le foie et le tissu adipeux des rats ayant consommé le régime hyperlipidique ou le régime riche en fructose pourrait conduire à un défaut de la captation de glucose dans les tissus cibles. Le régime hyperlipidique a augmenté l'expression du TNFα et de la leptine dans le tissu adipeux, du SREBP-1c dans le foie, de la périlipine, de la FAS et de l'ACC dans le tissu adipeux et a diminué l'expression du PPARγ. Le régime riche en fructose a, quant à lui, augmenté l'expression de la FAS et de l'ACC dans le tissu adipeux viscéral. En bref, les 10 semaines de consommation d'aliment lipidique ont conduit à l'obésité et l'insulinorésistance, tandis que des rats nourris avec le régime riche en fructose n'ont montré aucun changement dans la sensibilité à l'insuline et la lipidémie. Le régime hyperlipidique a eu une réponse plus délétère que le régime riche en fructose, et semble plus efficace pour induire l'obésité et l'insulinorésistance chez le rat.

5.2 Effets de la supplémentation en EPA chez le rat

L'objectif de cette partie était d'observer les effets d'une supplémentation en EPA dans le régime hyperlipidique ou le régime riche en fructose, sur la sensibilité à l'insuline et le profil lipidique plasmatique chez les rats. En outre, notre objectif était d'étudier le rôle d'EPA dans le métabolisme des lipides et des glucides.

Dans notre étude, tous les rats ont pris du poids d'une manière similaire. En fait, ce gain du poids a pu être un effet de la croissance. Les rats ayant consommé le régime hyperlipidique (supplémenté en EPA ou non) ont vu augmenter leur masse grasse, par rapport à la semaine 1, et l'EPA n'a réduit ni le poids corporel, ni la masse grasse. La nature des matières graisses alimentaires intervient dans le développement d'obésité et d'insulinorésistance. L'importante masse grasse corporelle chez les rats ayant consommé le régime hyperlipidique pourrait s'expliquer par l'utilisation de saindoux comme source de lipides dans l'aliment. Il est possible qu'un tel régime entraîne le stockage des graisses à partir des calories supplémentaires, menant à l'obésité, mais nous n'avons pas mesuré la prise alimentaire. Des études précédentes ont montré que la substitution de 6 g/jour de graisses par de l'huile de poisson (EPA et DHA) entrainait une diminution de la masse grasse chez des individus sains (Couet *et al.*, 1997). En outre, les acides gras polyinsaturés oméga-3 semblent protéger contre l'installation de l'obésité chez les rats sains et réduire la masse grasse des rats obèses (Shirouchi *et al.*, 2007; Minami *et al.*, 2002; Raclot *et al.*, 1997). Dans notre étude, nous n'avons pas mesuré la prise alimentaire, mais l'accès libre des animaux à leur aliment pourrait avoir conduit à une augmentation des calories ingérées, aboutissant à une augmentation de la masse grasse corporelle. Reseland, *et al.* (2001a) ont mesuré une diminution des taux circulants de leptine à la fin d'une supplémentation en acides gras polyinsaturés oméga 3, liée à une diminution de son expression (Reseland *et al.*, 2001b; Raclot *et al.*, 1997). Nous n'avons trouvé aucun changement d'expression de la leptine, du poids corporel et de la masse grasse à la fin de la supplémentation en EPA.

Dans notre étude, l'insulinémie basale et la glycémie basale n'ont été modifiées dans aucun groupe. Chez les rats ayant consommé le régime riche en fructose (avec ou sans supplémentation en EPA) la sensibilité à l'insuline est inchangée par rapport aux rats témoins. Chez les rats ayant consommé le régime hyperlipidique non supplémenté en EPA, la sensibilité à l'insuline a diminué, et la supplémentation en EPA a empêché cette diminution de la sensibilité à l'insuline. Des études antérieures ont montré un rôle bénéfique des acides

gras oméga-3 sur la sensibilité à l'insuline de rats ayant consommé un régime hyperlipidique (Andersen *et al.*, 2008; Lombardo *et al.*, 2007; Storlien *et al.*, 1987), mais Gillam, *et al.* (2009) n'ont pas obtenu d'amélioration de la sensibilité à l'insuline chez les rats Zucker *fa/fa*. Chez des rats ayant consommé le régime hyperlipidique, l'augmentation des niveaux d'ARNm de l'IRS-1 et du GLUT4 dans le tissu adipeux suggère que l'EPA pourrait améliorer la signalisation de l'insuline et l'absorption du glucose, sans toutefois modifier ces paramètres dans le foie. Il a été décrit qu'une supplémentation en EPA facilitait l'absorption du glucose et améliorait la sensibilité à l'insuline en augmentant l'expression de GLUT4, dans le muscle squelettique (Mori *et al.*, 1997). L'étude de l'expression des gènes impliqués dans la signalisation de l'insuline et l'absorption du glucose dans le muscle pourrait permettre de mieux comprendre l'effet bénéfique d'une supplémentation en EPA sur de la sensibilité à l'insuline. Notre expérimentation a duré 10 semaines et nous avons choisi une supplémentation en EPA pur chez des rats Wistar, tandis que l'étude de Mori, et al. (1997) durait 17 à 18 semaines, utilisait une supplémentation en huile de poisson, et était menée chez des rats Otsuka Long-Evans Tokushima Fatty. L'amélioration de la captation du glucose par une supplémentation en EPA pourrait exiger plus de temps dans le foie et le muscle que dans le tissu adipeux. Chez la souris, les acides gras polyinsaturés oméga-3 ont empêché la détérioration de la sensibilité à l'insuline hépatique induite par un régime hyperlipidique et ont diminué le contenu en diglycérides et triglycérides du foie, par une voie impliquant le PPARα, (Neschen *et al.*, 2007). L'amélioration de la sensibilité à l'insuline par une supplémentation en huile de poisson chez les rats ayant consommé un régime riche en saccharose s'est accompagnée d'une augmentation du niveau plasmatique d'adiponectine (Rossi *et al.*, 2005). Il est possible qu'un tel mécanisme ait eu lieu dans notre étude de la supplémentation en EPA chez les rats ayant consommé le régime hyperlipidique ou riche en fructose, mais l'amélioration de la sensibilité à l'insuline n'a pas atteint le niveau significatif. Le régime riche en fructose n'ayant pas induit de détérioration de la sensibilité à l'insuline, un éventuel effet de la supplémentation en EPA est difficile à mettre en évidence.

Les acides gras oméga-3 semblent efficaces pour améliorer la dyslipidémie et les profils lipoprotéiniques. Raclot, *et al.* (1997) ont comparé l'efficacité du DHA et de l'EPA, et ont constaté que le DHA a un effet anti-adipogénique supérieur à l'EPA. Dans notre étude, la quantité d'EPA supplémenté est relativement faible par rapport à la quantité de matières grasses contenue dans le régime hyperlipidique. Le taux plasmatique du cholestérol total n'a pas été modifié au terme du régime hyperlipidique ou riche en fructose. Le cholestérol

contenu dans l'aliment semble jouer un rôle dans l'éventuel l'effet hypocholestérolémiant des acides gras polyinsaturés oméga-3, et, en absence de cholestérol dans l'aliment, ils pourraient ne pas exercer d'effet hypocholestérolémiant. Ainsi, une supplémentation en EPA (300 mg/kg/jour) pendant 5 semaines a-t-elle réduit le cholestérol total plasmatique de rats ayant consommé un régime riche en cholestérol alimentaire (1 % de cholestérol et 1 % d'acide cholique), mais pas de rats ayant consommé un régime témoin (Ku *et al.*, 1999). Mizuguchi, *et al.* (1992) ont mesuré l'effet de l'EPA chez des rats ayant consommé un régime témoin pendant 7 jours et un régime riche en cholestérol (1 % de cholestérol et 1 % d'acide cholique) pendant 4 semaines. L'EPA a réduit le cholestérol total plasmatique, aux doses de 100, 300 et 1000 mg/kg d'aliment chez les rats hypercholestérolémiques, et aux doses de 300 et 1000 mg/kg d'aliment chez les rats ayant consommé un régime témoin. Dans une autre étude, une supplémentations en EPA (1 g/kg de poids corporel) ou en huile de poisson (3 g/kg de poids corporel) a réduit le cholestérol total plasmatique par rapport au groupe témoin (Cummings *et al.*, 2010), ce qui suggère que l'EPA pourrait avoir un effet hypocholestérolémiant lorsqu'il est utilisé à fortes doses. Dans notre étude, il est possible que la faible dose d'EPA (200 - 300 mg/kg de poids corporel/jour) n'ait pas pu affecter la concentration du cholestérol total plasmatique des rats ayant consommé le régime hyperlipidique ou le régime riche en fructose. L'administration d'EPA chez le rat a réduit le cholestérol total plasmatique au bout de 2 semaines (Spady, 1993), mais pas de 17 à 18 semaines (Mori *et al.*, 1997), ce qui suggère que l'administration d'EPA à long terme pourrait ne pas être bénéfique sur la cholestérolémie.

Dans notre étude, les concentrations plasmatiques en triglycérides et en acides gras non-estérifiés n'ont pas été changées au terme du régime hyperlipidique ou riche en fructose par rapport aux rats témoins. La supplémentation en EPA a tendu à diminuer les concentrations plasmatiques en triglycérides et en acides gras non-estérifiés chez les rats ayant consommé le régime riche en fructose. L'effet bénéfique de l'EPA et du DHA sur la sensibilité à l'insuline pourrait passer par une accumulation des triglycérides dans le muscle et le tissu adipeux (Storlien *et al.*, 1987) et par une modification de la composition en acides gras des phospholipides membranaires (Luo *et al.*, 1996). Les acides gras oméga-3 à longue chaîne et leurs dérivés sont également des ligands de certains facteurs de transcription tels que le PPAR et peuvent donc influencer la régulation des gènes impliqués dans l'adipogenèse, la sensibilité à l'insuline et le métabolisme des lipides (Banga *et al.*, 2009; Itoh *et al.*, 2008). Une administration d'EPA pendant 5 jours ou 2 semaines a diminué le niveau plasmatique des triglycérides (Spady, 1993; Aarsland *et al.*, 1990), mais une administration de 0.3 à 1.0

g/kg/jour pendant 4 ou 17 semaines n'a pas eu d'incidence sur des triglycérides plasmatiques (Naba *et al.*, 2006; Mori *et al.*, 1997). Une étude intéressante a montré quant à elle, qu'une supplémentation en huile de poisson (3 g/kg de poids corporel) réduisait les triglycérides plasmatiques à la fin de 3 mois et 4 mois de traitement, que cet effet n'est pas visible avant 1 mois ou 2 mois, et après 5 mois, 6 mois ou 7 mois (Cummings *et al.*, 2010). Dans notre étude, la supplémentation en EPA n'a eu aucun effet sur les niveaux plasmatiques des triglycérides et des acides gras non-estérifiés chez les rats ayant consommé le régime hyperlipidique, mais a diminué ceux des rats ayant consommé le régime riche en fructose. Ceci est en accord avec une étude chez des rats ayant consommé un régime riche en glucose ou fructose (Herzberg and Rogerson, 1988). Les acides gras oméga-3 répriment les gènes de la lipogenèse comme celui de FAS dans le foie (Blake and Clarke, 1990). Une étude a montré une diminution d'activité de la FAS dans le foie de rats ayant consommé un régime riche en saccharose (Peyron-Caso *et al.*, 2003), ce qui pourrait diminuer les triglycérides plasmatiques et les acides gras non-estérifiés. Dans notre étude, la diminution de l'expression de la FAS dans le foie n'a pas été significative chez les rats ayant consommé le régime riche en fructose avec la supplémentation en EPA. Dans notre étude, l'augmentation de l'expression de la FAS dans le tissu adipeux chez des rats ayant consommé le régime riche en fructose suggère une augmentation de la lipogenèse *de novo* dans les adipocytes. Raclot, *et al.* (1997) a montré que les acides gras polyinsaturés oméga 3 diminuaient l'expression de la HSL. L'administration des acides gras polyinsaturés a stimulé l'expression ARNm de la HSL dans le foie et le tissu adipeux (Sun *et al.*, 2011), et dans notre étude, nous avons également trouvé une augmentation des niveaux d'ARNm de la HSL dans le foie des rats ayant consommé le régime hyperlipidique ou le régime riche en fructose, ce qui suggère une simulation de la lipolyse hépatique par l'EPA, mais pas dans le tissu adipeux. Une stimulation de l'expression de la PEPCK par les acides gras a été montrée (Antras-Ferry *et al.*, 1994), ce qui favorise la lipogenèse. Nous n'avons trouvé aucun changement dans l'expression du gène de la PEPCK dans le foie. Il existe des différences inter-tissulaires quant à la modulation de l'expression de ces gènes (FAS, HSL, PEPCK), et des différences inter-régionales entre graisse sous-cutanée et la graisse viscérale (Raclot *et al.*, 1997). L'huile de poisson pourrait avoir un plus grand effet sur les triglycérides que d'autres acides gras polyinsaturés (huile de tournesol et huile de lin), car l'huile de poisson a diminué les triglycérides par l'inhibition de l'activité d'acyl-CoA : diacylglycérol transferase (Rustan *et al.*, 1992). L'augmentation de l'oxydation peroxysomale des acides gras et leur moins grande disponibilité des acides gras pourraient diminuer la quantité en acides gras et donc diminuer la synthèse et la sécrétion des triglycérides (Rustan *et*

al., 1992). L'effet hypotriglycéridémiant de l'EPA pourrait s'expliquer par la diminution de la production hépatique des triglycérides, ce qui suggère l'augmentation de l'activité de carnitine palmitoyl transferase (oxydation des acides gras) et la diminution de l'activité de la phosphatidate phosphohydrolase (étape limitante de la synthèse des triglycérides) (Surette *et al.*, 1992), et / ou par une diminution de la voie de SREBP (Le Jossic-Corcos *et al.*, 2005). Les acides gras oméga-3 affectent l'expression des gènes via les récepteurs nucléaires, PPARs et SREBP. Cependant, nous n'avons pas trouvé une diminution de l'expression hépatique du SREBP-1c chez des rats ayant consommé le régime riche en fructose. Ils se lient aux PPARs avec une affinité différente selon le type. Ainsi, l'EPA a une affinité supérieure pour PPARα (Pawar and Jump, 2003), alors que les oméga-6 se lient plus facilement au PPARγ (Forman *et al.*, 1995). La supplémentation en EPA n'a eu aucun effet sur l'expression du gène du PPARα dans le foie des rats ayant consommé le régime hyperlipidique ou riche en fructose, et aucun effet sur le taux plasmatique des triglycérides n'a été trouvé. L'expression du gène du PPARγ dans le tissu adipeux viscéral a été augmentée chez les rats ayant consommé le régime riche en fructose. Les acides gras oméga 3 agissent aussi en inhibant la maturation des SREBP (Kim *et al.*, 1999; Xu *et al.*, 1999b). Nous avons trouvé que la supplémentation en EPA a augmenté l'expression du gène de SREBP-1c dans le foie, mais pas dans le tissu adipeux, chez les rats ayant consommé le régime hyperlipidique qui pourrait être induite par la forte teneur en lipides. L'étude plus approfondie des voies impliquant le PPARα, le PPARγ, et le SREBP-1c devrait permettre de comprendre les effets hypotriglycéridémiant de la supplémentation en EPA et faire des liens avec des approches thérapeutiques, en particulier dans la compréhension des mécanismes d'action des thiazolidinediones, qui agissent également sur les PPARs.

L'acide arachidonique, ARA (acides gras polyinsaturé oméga-6) et l'EPA (acides gras polyinsaturé oméga-3) entrent en compétition pour former des eicosanoïdes par la cyclo-oxygénase et la lipoxygenase. Les eicosanoïdes pro-inflammatoires dérivent de l'ARA, tandis que les eicosanoïdes anti-inflammatoires (tels que les prostaglandines) dérivent de l'EPA (Simopoulos, 1999; Gallai *et al.*, 1995). Le ratio « acides gras polyinsaturés n-6 sur acides gras polyinsaturés n-3 » est considéré comme un facteur important pour déterminer les effets de ces acides gras polyinsaturés sur la sensibilité à l'insuline et les dyslipidémies. Une alimentation apportant un ratio n-6/n-3 de 3,38 a diminué le cholestérol total, augmenté les triglycérides et diminué le HDL par rapport à une alimentation apportant un ratio n-6/n-3 de 51,1 chez le rat (Watanabe *et al.*, 2011), et Jeffery, *et al.* (1996) ont observé une diminution

des niveaux plasmatique du cholestérol total, des triglycérides et des acides gras non-estérifiés pour le plus faible ratio n-6/n-3 (0,33) par rapport au ratio n-6/n-3 (112,5). Ils ont utilisé de l'huile végétale (l'acide α-linolénique et l'acide linoléique) comme source des acides gras polyinsaturés. L'acide α-linolénique (oméga-3) est un meilleur substrat pour la β-oxydation- que l'acide linoléique (oméga-6) (Leyton *et al.*, 1987), et une ingestion élevée en acides gras polyinsaturés oméga-3 pourrait réduire les concentrations en acides gras non-estérifiés plasmatiques en stimulant l'oxydation des acides gras, ainsi que cela a été montré chez le rat (Rustan *et al.*, 1992). Fukushima, *et al.* (2001) ont trouvé une plus faible concentration en cholestérol total chez des rats, âgés de 24 semaines, ayant consommé des acides gras polyinsaturés oméga-3 (huile de périlla) que chez des rats ayant consommé des cides gras polyinsaturés oméga-6 (huile de bourrache) pendant 4 semaines, puis cette différence disparaissait au bout de 8 et 15 semaines de régime. Une diminution des niveaux de cholestérol plasmatique total et des triglycérides a également été décrite chez les rats ayant consommé un faible ratio n-6/n-3 (1) par rapport à un ratio élevé (16) au bout de 6 mois et 12 mois de régime, mais pas au bout de 18 mois (Takeuchi *et al.*, 2009). Ces résultats suggèrent que le ratio n-6/n-3 pourrait affecter le profil lipidique plasmatique en fonction de la durée d'alimentation. Par ailleurs, Jeffery, *et al.* (1997) ont mesuré une diminution du cholestérol total pour les rats ayant ingéré un ratio n-6/n-3 (1) par rapport à un ratio élevé (100), sans modification des triglycérides et des acides gras non-estérifiés. Cet effet hypolipémiant pourrait s'expliquer par une moindre sécrétion de cholestérol et des triglycérides par le foie (Zheng *et al.*, 2001). En outre, le type (EPA, DHA, ALA, LA) et l'origine (l'huile de poisson, l'huile végétale) des acides gras polyinsaturés oméga-6 et acides gras polyinsaturés oméga-3 devraient également être prise en compte dans les études futures sur le ratio n-6/n-3.

Nos résultats suggèrent que les effets de la supplémentation en EPA dépendent de l'aliment que reçoivent les rats. Dans notre étude de 10 semaines, la supplémentation en EPA n'a eu aucun effet sur la masse grasse, ni sur la sensibilité à l'insuline chez les rats. Elle a diminué les triglycérides plasmatiques et les acides gras non-estérifiés chez les rats ayant consommé le régime riche en fructose. Des études complémentaires sont nécessaires pour déterminer la dose d'EPA ayant un effet hypolipidémiant et insulinosensibilisant, pour préciser leur effet dans différentes conditions d'hyperlipidémie, et pour distinguer les effets de l'EPA pur et de l'huile de poisson.

5.3 Effets de la supplémentation en hespéridine et naringine chez le rat en présence d'un régime hyperlipidique ou d'un régime riche en fructose

L'objectif de cette étude était de déterminer les effets d'une supplémentation en hespéridine et naringine pendant 8 semaines chez des rats recevant un régime hyperlipidique ou un régime riche en fructose, sur l'obésité et la sensibilité à l'insuline. Dans cette étude, il n'y avait pas de groupe de rats sans supplémentation, les effets observés reflètent donc les effets de l'hespéridine et la naringine associées à un le régime hyperlipidique ou un régime riche en fructose.

Dans notre étude, le poids corporel et le pourcentage de la masse grasse ont été similaire dans tous les groupes à la fin de l'expérimentation. Les études précédentes ont rapporté des résultats contradictoires : certains auteurs n'ont mis en évidence aucun effet de l'hespéridine sur le poids corporel chez le rat (Wang *et al.*, 2011; Akiyama *et al.*, 2010; Ohtsuki *et al.*, 2003; Miyake *et al.*, 1998), d'autres une diminution de la masse de tissu adipeux périrénal chez des rats consommant un régime riche en cholestérol (Wang *et al.*, 2011), et d'autres enfin une prise de poids chez la souris, en relation avec le niveau plasmatique de leptine (Jung *et al.*, 2004). Une étude récente utilisant la naringine a rapporté une diminution du poids et de la masse grasse viscérale chez la souris (Pu *et al.*, 2012). Nos résultats n'ont montré aucun effet de la supplémentation en hespéridine et naringine sur le poids et la masse grasse chez le rat.

L'insulinémie basale n'a pas varié entre les groupes à la fin de l'expérimentation dans notre étude. Les études précédentes ont montré une augmentation de l'insulinémie chez le rat et la souris (Akiyama *et al.*, 2010; Jung *et al.*, 2004), qui pourrait être induite à cause de la condition diabétique. Dans notre étude, la glycémie basale n'a pas varié entre les groupes à la fin de l'expérimentation. La littérature rapporte à ce sujet des résultats contradictoires. Ainsi, une étude a montré un effet hypoglycémiant de l'hespéridine (10 g/kg d'aliment) (Akiyama *et al.*, 2010), une autre une tendance à la diminution de la glycémie chez des rats consommant un régime riche en cholestérol supplémenté avec 0,08 % d'hespéridine (Wang *et al.*, 2011), d'autres, utilisant les flavonoïdes de citron (0,2 % d'eriocitrin et 0,2 % d'hespéridine) ou la naringine seule (50 mg/kg) n'ont observé aucun effet sur l'hyperglycémie de rats diabétiques (S and Pmo, 2011; Miyake *et al.*, 1998). L'effet hypoglycémiant s'explique par une augmentation du glycogène hépatique (Jung *et al.*, 2004), une augmentation de l'expression de la glucose-kinase hépatique et une diminution de l'expression de la glucose-6-phosphatase

(Akiyama *et al.*, 2010; Jung *et al.*, 2006), ou encore une diminution de l'expression de la PEPCK et de la glucose-6-phosphatase dans le foie (Pu *et al.*, 2012). L'augmentation de la glucose-kinase et la diminution de la glucose-6-phosphatase et de la PEPCK stimulent la glycolyse et inhibent la gluconéogenèse, ce qui pourrait améliorer l''hyperglycémie d'animaux diabétiques.

Nos résultats ont montré que les rats ayant consommé le régime hyperlipidique supplémenté en hespéridine et naringine et le régime riche en fructose supplémenté en hespéridine et naringine ont une plus faible sensibilité à l'insuline que les rats témoins supplémentés. Des précédentes études ont montré qu'au terme d'une supplémentation en hespéridine et naringine, les concentrations plasmatiques en leptine (Jung *et al.*, 2004) et en adiponectine (Akiyama *et al.*, 2010) étaient augmentées, ce qui donc pourrait améliorer la sensibilité à l'insuline. De plus, les aglycosides de l'hespéridine et de la naringine stimulent la transcription de l'adiponectine, et augmentent l'activité du PPARγ (Liu *et al.*, 2008), et ainsi améliorent la sensibilité à l'insuline. Par ailleurs, l'hespéridine et la naringine inhibent la sécrétion des acides gras non-estérifiés en réponse au TNFα par les adipocytes de souris (Yoshida *et al.*, 2010), ce qui pourrait également améliorer la sensibilité à l'insuline. L'absence d'effet dans notre étude pourrait s'expliquer par le fait que les régimes hyperlipidique ou riche en fructose aient masqué l'effet de l'hespéridine et de la naringine sur la sensibilité à l'insuline.

Le choix de l'utilisation du mélange d'hespéridine et de naringine est lié au fait qu'aucun effet d'une supplémentation en hespéridine seule ou la naringine seule n'a été observé sur le profil lipidique chez l'homme (Demonty *et al.*, 2010). La concentration plasmatique du cholestérol total a été similaire entre les groupes à la fin de l'expérimentation. Dans des études précédentes, l'hespéridine et/ou ses métabolites ont diminué le cholestérol plasmatique (Wang *et al.*, 2011; Miceli *et al.*, 2007; Choi *et al.*, 2004; Kim *et al.*, 2003; Park *et al.*, 2001; Bok *et al.*, 1999; Monforte *et al.*, 1995), mais dans toutes ces études, les rats ont été nourris avec un régime riche en cholestérol. Ces flavanones sont de puissants inhibiteurs de l'activité de la HMG-CoA réductase et l'ACAT *in vivo* (Choi *et al.*, 2004; Kim *et al.*, 2003; Park *et al.*, 2001; Bok *et al.*, 1999) et *in vitro* (Wilcox *et al.*, 2001), enzymes impliqués dans l'absorption intestinale et l'estérification du cholestérol pour l'ACAT, et dans la synthèse du cholestérol et la sécrétion du VLDL-cholestérol pour la HMG-CoA réducatse, ce qui explique leur effet hypocholestérolémiant chez des animaux consommant un régime riche en cholestérol. L'absence d'effet, dans notre étude, sur le cholestérol total plasmatique était

compatible avec les travaux précédentes (Wang *et al.*, 2011; Miyake *et al.*, 1998). De fait, cet effet hypocholestérolémiant pourrait n'apparaitre qu'en cas d'apport de cholestérol par l'alimentation. Par ailleurs, certains auteurs ont montré que ces flavanones pourraient diminuer les LDL et augmenter les HDL plasmatiques (Miceli *et al.*, 2007; Monforte *et al.*, 1995), diminuer le cholestérol dans les VLDL et LDL et augmenter le HDL-cholestérol (Akiyama *et al.*, 2010), et ainsi, pourraient corriger la dyslipidémie. Dans notre étude, nous avons observé que l'hespéridine et la naringine ont augmenté le VLDL-cholestérol et ont diminué le HDL-cholestérol chez des rats ayant consommé le régime hyperlipidique ou riche en fructose. Une étude a montré que la diminution des LDL était associée à une surexpression du gène du récepteur LDL, et à une diminution de l'expression et de l'activité de la protéine microsomale de transfert des triglycérides (MTP) *in vitro* (Wilcox *et al.*, 2001), qui joue son rôle dans l'assemblage des lipoprotéines, principalement triglycérides et VLDL.

La concentration des acides gras non-estérifiés plasmatiques n'a pas été différente entre les groupes à la fin de l'expérimentation. Ceci était cohérent avec une étude précédente (Ohtsuki *et al.*, 2003). En revanche, à la fin de l'expérimentation, les concentrations en triglycérides étaient plus élevées chez les rats ayant consommé le régime hyperlipidique supplémenté en hespéridine et naringine ou le régime riche en fructose supplémenté en hespéridine et naringine que chez les rats ayant consommé le régime témoin supplémenté en hespéridine et naringine. Des études précédentes chez le rat ont rapporté une diminution des triglycérides plasmatiques (Miceli *et al.*, 2007; Choi *et al.*, 2004; Kim *et al.*, 2003; Kawaguchi *et al.*, 1997; Monforte *et al.*, 1995), une correction de l'hypertriglycéridémie (Akiyama *et al.*, 2010), tandis que d'autres n'ont mesuré aucun effet (Ohtsuki *et al.*, 2003). L'effet hypotriglycéridémiant des flavanones pourrait être un effet de répression de SREBP-1c dans le tissu adipeux et le foie en diminuant la synthèse des triglycérides. Dans notre étude, l'hypertriglycéridémie a pu s'expliquer par la forte teneur en matières grasses ou en fructose dans les régimes et n'a pu être corrigée par la supplémentation avec l'hespéridine et la naringine. Ces résultats sont cohérents avec ceux d'une étude précédente (Choi *et al.*, 1991) chez des rats recevant un régime hyperlipidique.

Dans notre étude, la supplémentation en hespéridine et naringine, en présence d'un régime hyperlipidique ou d'un régime riche en fructose pendant 8 semaines, a diminué la sensibilité à l'insuline chez les rats par rapport aux rats témoins. Par ailleurs, la concentration plasmatique des triglycérides a été augmentée, et aucun effet sur les concentrations du cholestérol total plasmatique et des acides gras non-estérifiés n'a été observé. Cependant,

l'absence des groupes des rats ayant consommé un régime hyperlipidique et riche en fructose (sans supplémentation en hespéridine et naringine) empêche de déterminer et d'expliquer les effets de ces flavanones sur l'obésité, la dyslipidémie et la sensibilité à l'insuline.

6 Conclusion

Nous avons donc, dans cette étude, caractérisé le modèle de rat d'étude de l'obésité et de la sensibilité à l'insuline. Le régime hyperlipidique a induit une obésité et diminué la sensibilité à l'insuline, ce qui est cohérent avec la diminution de l'expression de GLUT4 dans le foie et le tissu adipeux. Au contraire, le régime riche en fructose n'a induit aucun changement de la sensibilité à l'insuline et de la lipidémie. Le régime hyperlipidique a eu une réponse plus délétère que le régime riche en fructose pour induire l'obésité et diminuer la sensibilité à l'insuline.

La supplémentation en EPA a limité la baisse de la sensibilité à l'insuline chez les rats ayant consommé le régime hyperlipidique, sans modifier l'expression de gènes impliqués dans la signalisation de l'insuline dans le foie. Bien que la supplémentation en EPA a augmenté l'expression de la HSL hépatique chez les rats ayant consommé le régime hyperlipidique, elle n'a eu aucun effet sur les triglycérides plasmatiques. La supplémentation en EPA a tendu à diminuer les triglycérides et les acides gras non-estérifiés chez les rats ayant consommé le régime riche en fructose, avec une diminution de l'expression de la FAS et l'augmentation des expressions géniques de la LPL et de la HSL dans le foie. La réponse d'EPA a été différente selon le régime donné aux rats. La supplémentation en EPA était dépendante de la nature du régime (régime riche en fructose) pour diminuer les triglycérides plasmatiques.

La supplémentation en hespéridine et naringine n'a pas pu empêcher la baisse de la sensibilité à l'insuline, peut être en raison de la nature (hyperlipidique ou riche en fructose) du régime ou de la courte durée de la supplémentation. Cette supplémentation n'a pas modifié le cholestérol total et la triglycéridémie, alors que des effets bénéfiques ont été rapportés chez les rats nourris avec un régime riche en cholestérol. Dans l'avenir, des études liées au cholestérol alimentaire pourraient aider à comprendre les effets de l'hespéridine et de la naringine sur la lipidémie, particulièrement la cholestérolémie.

- *BIBLIOGRAPHY*

Aarsland, A., M. Lundquist, B. Borretsen and R. K. Berge (1990). On the effect of peroxisomal beta-oxidation and carnitine palmitoyltransferase activity by eicosapentaenoic acid in liver and heart from rats. *Lipids* **25**(9): 546-8.

Abel, E. D., O. Peroni, J. K. Kim, Y. B. Kim, O. Boss, E. Hadro, T. Minnemann, G. I. Shulman and B. B. Kahn (2001). Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* **409**(6821): 729-33.

Abraha, A., S. M. Humphreys, M. L. Clark, D. R. Matthews and K. N. Frayn (1998). Acute effect of fructose on postprandial lipaemia in diabetic and non-diabetic subjects. *Br J Nutr* **80**(2): 169-75.

Abumrad, N. A., M. R. el-Maghrabi, E. Z. Amri, E. Lopez and P. A. Grimaldi (1993). Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J Biol Chem* **268**(24): 17665-8.

Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs and M. Krieger (1996). Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* **271**(5248): 518-20.

Afonso, R. A., W. W. Lautt, R. T. Ribeiro, D. J. Legare and M. P. Macedo (2007). Insulin resistance in two animal models of obesity: A comparison of HISS-dependent and HISS-independent insulin action in high-fat diet-fed and Zucker rats. *Proc West Pharmacol Soc* **50**: 110-4.

Ahima, R. S. and J. S. Flier (2000). Leptin. *Annu Rev Physiol* **62**: 413-37.

Ahren, B., T. Gudbjartsson, A. N. Al-Amin, H. Martensson, U. Myrsen-Axcrona, S. Karlsson, H. Mulder and F. Sundler (1999). Islet perturbations in rats fed a high-fat diet. *Pancreas* **18**(1): 75-83.

Akiyama, S., S. Katsumata, K. Suzuki, Y. Ishimi, J. Wu and M. Uehara (2010). Dietary hesperidin exerts hypoglycemic and hypolipidemic effects in streptozotocin-induced marginal type 1 diabetic rats. *J Clin Biochem Nutr* **46**(1): 87-92.

Akiyama, S., S. Katsumata, K. Suzuki, Y. Nakaya, Y. Ishimi and M. Uehara (2009). Hypoglycemic and hypolipidemic effects of hesperidin and cyclodextrin-clathrated hesperetin in Goto-Kakizaki rats with type 2 diabetes. *Biosci Biotechnol Biochem* **73**(12): 2779-82.

Akiyama, T., I. Tachibana, H. Shirohara, N. Watanabe and M. Otsuki (1996). High-fat hypercaloric diet induces obesity, glucose intolerance and hyperlipidemia in normal adult male Wistar rat. *Diabetes Res Clin Pract* **31**(1-3): 27-35.

Al-Hasani, H. and H. G. Joost (2005). Nutrition-/diet-induced changes in gene expression in white adipose tissue. *Best Pract Res Clin Endocrinol Metab* **19**(4): 589-603.

Alaupovic, P., W. J. Mack, C. Knight-Gibson and H. N. Hodis (1997). The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler Thromb Vasc Biol* **17**(4): 715-22.

Alberti, K. G. and P. Z. Zimmet (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* **15**(7): 539-53.

Altshuler, D., J. N. Hirschhorn, M. Klannemark, C. M. Lindgren, M. C. Vohl, J. Nemesh, C. R. Lane, S. F. Schaffner, S. Bolk, C. Brewer, T. Tuomi, D. Gaudet, T. J. Hudson, M. Daly, L. Groop and E. S. Lander (2000). The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* **26**(1): 76-80.

Anai, M., M. Funaki, T. Ogihara, A. Kanda, Y. Onishi, H. Sakoda, K. Inukai, M. Nawano, Y. Fukushima, Y. Yazaki, M. Kikuchi, Y. Oka and T. Asano (1999). Enhanced insulin-stimulated activation of phosphatidylinositol 3-kinase in the liver of high-fat-fed rats. *Diabetes* **48**(1): 158-69.

Anderson, B. M. and D. W. Ma (2009). Are all n-3 polyunsaturated fatty acids created equal? *Lipids Health Dis* **8**: 33.

Andersen, G., K. Harnack, H. F. Erbersdobler and V. Somoza (2008). Dietary eicosapentaenoic acid and docosahexaenoic acid are more effective than alpha-linolenic acid in improving insulin sensitivity in rats. *Ann Nutr Metab* **52**(3): 250-6.

Andersson, A., C. Nalsen, S. Tengblad and B. Vessby (2002). Fatty acid composition of skeletal muscle reflects dietary fat composition in humans. *Am J Clin Nutr* **76**(6): 1222-9.

Andrus, S. B., L. C. Fillios, G. V. Mann and F. J. Stare (1956). Experimental production of gross atherosclerosis in the rat. *J Exp Med* **104**(4): 539-54.

Annuzzi, G., A. Rivellese, B. Capaldo, L. Di Marino, C. Iovine, G. Marotta and G. Riccardi (1991). A controlled study on the effects of n-3 fatty acids on lipid and glucose metabolism in non-insulin-dependent diabetic patients. *Atherosclerosis* **87**(1): 65-73.

Antras-Ferry, J., G. Le Bigot, P. Robin, D. Robin and C. Forest (1994). Stimulation of phosphoenolpyruvate carboxykinase gene expression by fatty acids. *Biochem Biophys Res Commun* **203**(1): 385-91.

Aoyama, T., J. M. Peters, N. Iritani, T. Nakajima, K. Furihata, T. Hashimoto and F. J. Gonzalez (1998). Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *J Biol Chem* **273**(10): 5678-84.

Arita, Y., S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi and Y. Matsuzawa (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* **257**(1): 79-83.

Aubert, J., O. Champigny, P. Saint-Marc, R. Negrel, S. Collins, D. Ricquier and G. Ailhaud (1997). Up-regulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells. *Biochem Biophys Res Commun* **238**(2): 606-11.

Auboeuf, D., J. Rieusset, L. Fajas, P. Vallier, V. Frering, J. P. Riou, B. Staels, J. Auwerx, M. Laville and H. Vidal (1997). Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes* **46**(8): 1319-27.

Avruch, J. (2007). MAP kinase pathways: the first twenty years. *Biochim Biophys Acta* **1773**(8): 1150-60.

Bachorik, P. S., F. A. Franklin, D. G. Virgil and P. O. Kwiterovich, Jr. (1982). High-affinity uptake and degradation of apolipoprotein E free high-density lipoprotein and low-density lipoprotein in cultured porcine hepatocytes. *Biochemistry* **21**(22): 5675-84.

Bado, A., S. Levasseur, S. Attoub, S. Kermorgant, J. P. Laigneau, M. N. Bortoluzzi, L. Moizo, T. Lehy, M. Guerre-Millo, Y. Le Marchand-Brustel and M. J. Lewin (1998). The stomach is a source of leptin. *Nature* **394**(6695): 790-3.

Bai, Y., S. Zhang, K. S. Kim, J. K. Lee and K. H. Kim (1996). Obese gene expression alters the ability of 30A5 preadipocytes to respond to lipogenic hormones. *J Biol Chem* **271**(24): 13939-42.

Baker, P. W. and G. F. Gibbons (2000). Effect of dietary fish oil on the sensitivity of hepatic lipid metabolism to regulation by insulin. *J Lipid Res* **41**(5): 719-26.

Balasse, E. O. and H. A. Ooms (1973). Role of plasma free fatty acids in the control of insulin secretion in man. *Diabetologia* **9**(2): 145-51.

Balk, E. M., A. H. Lichtenstein, M. Chung, B. Kupelnick, P. Chew and J. Lau (2006). Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis* **189**(1): 19-30.

Banerji, M. A., R. L. Chaiken, D. Gordon, J. G. Kral and H. E. Lebovitz (1995). Does intra-abdominal adipose tissue in black men determine whether NIDDM is insulin-resistant or insulin-sensitive? *Diabetes* **44**(2): 141-6.

Banga, A., R. Unal, P. Tripathi, I. Pokrovskaya, R. J. Owens, P. A. Kern and G. Ranganathan (2009). Adiponectin translation is increased by the PPARgamma agonists pioglitazone and omega-3 fatty acids. *Am J Physiol Endocrinol Metab* **296**(3): E480-9.

Banks, W. A. (2008). The blood-brain barrier as a cause of obesity. *Curr Pharm Des* **14**(16): 1606-14.

Banks, W. A. and C. L. Farrell (2003). Impaired transport of leptin across the blood-brain barrier in obesity is acquired and reversible. *Am J Physiol Endocrinol Metab* **285**(1): E10-5.

Bantle, J. P., S. K. Raatz, W. Thomas and A. Georgopoulos (2000). Effects of dietary fructose on plasma lipids in healthy subjects. *Am J Clin Nutr* **72**(5): 1128-34.

Barak, Y., D. Liao, W. He, E. S. Ong, M. C. Nelson, J. M. Olefsky, R. Boland and R. M. Evans (2002). Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer. *Proc Natl Acad Sci U S A* **99**(1): 303-8.

Baron, A. D., G. Brechtel, P. Wallace and S. V. Edelman (1988). Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol* **255**(6 Pt 1): E769-74.

Barrett, P. H. and G. F. Watts (2003). Kinetic studies of lipoprotein metabolism in the metabolic syndrome including effects of nutritional interventions. *Curr Opin Lipidol* **14**(1): 61-8.

Basciano, H., L. Federico and K. Adeli (2005). Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond)* **2**(1): 5.

Baynes, C., A. D. Henderson, V. Anyaoku, W. Richmond, C. L. Hughes, D. G. Johnston and R. S. Elkeles (1991). The role of insulin insensitivity and hepatic lipase in the dyslipidaemia of type 2 diabetes. *Diabet Med* **8**(6): 560-6.

Bell, K. S., C. Schmitz-Peiffer, M. Lim-Fraser, T. J. Biden, G. J. Cooney and E. W. Kraegen (2000). Acute reversal of lipid-induced muscle insulin resistance is associated with rapid alteration in PKC-theta localization. *Am J Physiol Endocrinol Metab* **279**(5): E1196-201.

Bell, G. I., T. Kayano, J. B. Buse, C. F. Burant, J. Takeda, D. Lin, H. Fukumoto and S. Seino (1990). Molecular biology of mammalian glucose transporters. *Diabetes Care* **13**(3): 198-208.

Benavente-Garcia, O. and J. Castillo (2008). Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. *J Agric Food Chem* **56**(15): 6185-205.

Berenguer, M., L. Martinez, S. Giorgetti-Peraldi, Y. Le Marchand-Brustel and R. Govers (2010). A serum factor induces insulin-independent translocation of GLUT4 to the cell surface which is maintained in insulin resistance. *PLoS One* **5**(12): e15560.

Berg, A. H. and P. E. Scherer (2005). Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* **96**(9): 939-49.

Berg, A. H., T. P. Combs, X. Du, M. Brownlee and P. E. Scherer (2001). The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* **7**(8): 947-53.

Berge, R. K., L. Madsen, H. Vaagenes, K. J. Tronstad, M. Gottlicher and A. C. Rustan (1999). In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. *Biochem J* **343 Pt 1**: 191-7.

Berger, J., C. Biswas, P. P. Vicario, H. V. Strout, R. Saperstein and P. F. Pilch (1989). Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting. *Nature* **340**(6228): 70-2.

Bergman, R. N., S. P. Kim, I. R. Hsu, K. J. Catalano, J. D. Chiu, M. Kabir, J. M. Richey and M. Ader (2007). Abdominal obesity: role in the pathophysiology of metabolic disease and cardiovascular risk. *Am J Med* **120**(2 Suppl 1): S3-8; discussion S29-32.

Bergman, R. N., S. P. Kim, K. J. Catalano, I. R. Hsu, J. D. Chiu, M. Kabir, K. Hucking and M. Ader (2006). Why visceral fat is bad: mechanisms of the metabolic syndrome. *Obesity (Silver Spring)* **14 Suppl 1**: 16S-19S.

Berthou, L., N. Duverger, F. Emmanuel, S. Langouet, J. Auwerx, A. Guillouzo, J. C. Fruchart, E. Rubin, P. Denefle, B. Staels and D. Branellec (1996). Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J Clin Invest* **97**(11): 2408-16.

Bessesen, D. H. (2001). The role of carbohydrates in insulin resistance. *J Nutr* **131**(10): 2782S-2786S.

Beutler, B., I. W. Milsark and A. C. Cerami (1985). Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* **229**(4716): 869-71.

Bezard, J., J. P. Blond, A. Bernard and P. Clouet (1994). The metabolism and availability of essential fatty acids in animal and human tissues. *Reprod Nutr Dev* **34**(6): 539-68.

Birnbaum, M. J. (1989). Identification of a novel gene encoding an insulin-responsive glucose transporter protein. *Cell* **57**(2): 305-15.

Blake, W. L. and S. D. Clarke (1990). Suppression of rat hepatic fatty acid synthase and S14 gene transcription by dietary polyunsaturated fat. *J Nutr* **120**(12): 1727-9.

Blanquart, C., O. Barbier, J. C. Fruchart, B. Staels and C. Glineur (2002). Peroxisome proliferator-activated receptor alpha (PPARalpha) turnover by the ubiquitin-proteasome system controls the ligand-induced expression level of its target genes. *J Biol Chem* **277**(40): 37254-9.

Bluher, S. and C. S. Mantzoros (2009). Leptin in humans: lessons from translational research. *Am J Clin Nutr* **89**(3): 991S-997S.

Boden, G. (2003). Effects of free fatty acids (FFA) on glucose metabolism: significance for insulin resistance and type 2 diabetes. *Exp Clin Endocrinol Diabetes* **111**(3): 121-4.

Boden, G., X. Chen and N. Iqbal (1998). Acute lowering of plasma fatty acids lowers basal insulin secretion in diabetic and nondiabetic subjects. *Diabetes* **47**(10): 1609-12.

Boden, G., X. Chen, J. Ruiz, J. V. White and L. Rossetti (1994). Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* **93**(6): 2438-46.

Bok, S. H., S. H. Lee, Y. B. Park, K. H. Bae, K. H. Son, T. S. Jeong and M. S. Choi (1999). Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. *J Nutr* **129**(6): 1182-5.

Bolaffi, J. L., G. G. Rodd, J. Wang and G. M. Grodsky (1994). Interrelationship of changes in islet nicotine adeninedinucleotide, insulin secretion, and cell viability induced by interleukin-1 beta. *Endocrinology* **134**(2): 537-42.

Borkman, M., L. H. Storlien, D. A. Pan, A. B. Jenkins, D. J. Chisholm and L. V. Campbell (1993). The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N Engl J Med* **328**(4): 238-44.

Bouchard, C. (1991). Current understanding of the etiology of obesity: genetic and nongenetic factors. *Am J Clin Nutr* **53**(6 Suppl): 1561S-1565S.

Brady, L. M., S. S. Lovegrove, S. V. Lesauvage, B. A. Gower, A. M. Minihane, C. M. Williams and J. A. Lovegrove (2004). Increased n-6 polyunsaturated fatty acids do not attenuate the effects of long-chain n-3 polyunsaturated fatty acids on insulin sensitivity or triacylglycerol reduction in Indian Asians. *Am J Clin Nutr* **79**(6): 983-91.

Braissant, O., F. Foufelle, C. Scotto, M. Dauca and W. Wahli (1996). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* **137**(1): 354-66.

Brand, K., K. A. Dugi, J. D. Brunzell, D. N. Nevin and S. Santamarina-Fojo (1996). A novel A-->G mutation in intron I of the hepatic lipase gene leads to alternative splicing resulting in enzyme deficiency. *J Lipid Res* **37**(6): 1213-23.

Brasaemle, D. L., B. Rubin, I. A. Harten, J. Gruia-Gray, A. R. Kimmel and C. Londos (2000). Perilipin A increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis. *J Biol Chem* **275**(49): 38486-93.

Bray, G. A. and D. A. York (1979). Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol Rev* **59**(3): 719-809.

Breckenridge, W. C., J. A. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren and G. Gardiner (1982). Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis* **45**(2): 161-79.

Brito, M. N., N. A. Brito, S. R. Brito, M. A. Moura, N. H. Kawashita, I. C. Kettelhut and R. H. Migliorini (1999). Brown adipose tissue triacylglycerol synthesis in rats adapted to a high-protein, carbohydrate-free diet. *Am J Physiol* **276**(4 Pt 2): R1003-9.

Brown, K. K., B. R. Henke, S. G. Blanchard, J. E. Cobb, R. Mook, I. Kaldor, S. A. Kliewer, J. M. Lehmann, J. M. Lenhard, W. W. Harrington, P. J. Novak, W. Faison, J. G. Binz, M. A. Hashim, W. O. Oliver, H. R. Brown, D. J. Parks, K. D. Plunket, W. Q. Tong, J. A. Menius, K. Adkison, S. A. Noble and T. M. Willson (1999). A novel N-aryl tyrosine activator of peroxisome proliferator-activated receptor-gamma reverses the diabetic phenotype of the Zucker diabetic fatty rat. *Diabetes* **48**(7): 1415-24.

Brown, W. V. and M. L. Baginsky (1972). Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem Biophys Res Commun* **46**(2): 375-82.

Browning, L. M., J. D. Krebs, C. S. Moore, G. D. Mishra, M. A. O'Connell and S. A. Jebb (2007). The impact of long chain n-3 polyunsaturated fatty acid supplementation on inflammation, insulin sensitivity and CVD risk in a group of overweight women with an inflammatory phenotype. *Diabetes Obes Metab* **9**(1): 70-80.

Bruynseels, K., N. Bergans, N. Gillis, F. van Dorpen, P. Van Hecke, W. Stalmans and F. Vanstapel (1999). On the inhibition of hepatic glycogenolysis by fructose. A 31P-NMR study in perfused rat liver using the fructose analogue 2,5-anhydro-D-mannitol. *NMR Biomed* **12**(3): 145-56.

Bryant, N. J., R. Govers and D. E. James (2002). Regulated transport of the glucose transporter GLUT4. *Nat Rev Mol Cell Biol* **3**(4): 267-77.

Buettner, R., J. Scholmerich and L. C. Bollheimer (2007). High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring)* **15**(4): 798-808.

Buettner, R., K. G. Parhofer, M. Woenckhaus, C. E. Wrede, L. A. Kunz-Schughart, J. Scholmerich and L. C. Bollheimer (2006). Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J Mol Endocrinol* **36**(3): 485-501.

Calder, P. C. (2006). n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* **83**(6 Suppl): 1505S-1519S.

Calderhead, D. M., K. Kitagawa, G. E. Lienhard and G. W. Gould (1990). Translocation of the brain-type glucose transporter largely accounts for insulin stimulation of glucose transport in BC3H-1 myocytes. *Biochem J* **269**(3): 597-601.

Cambuli, V. M., M. C. Musiu, M. Incani, M. Paderi, R. Serpe, V. Marras, E. Cossu, M. G. Cavallo, S. Mariotti, S. Loche and M. G. Baroni (2008). Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. *J Clin Endocrinol Metab* **93**(8): 3051-7.

Camps, L., M. Reina, M. Llobera, G. Bengtsson-Olivecrona, T. Olivecrona and S. Vilaro (1991). Lipoprotein lipase in lungs, spleen, and liver: synthesis and distribution. *J Lipid Res* **32**(12): 1877-88.

Camps, L., M. Reina, M. Llobera, S. Vilaro and T. Olivecrona (1990). Lipoprotein lipase: cellular origin and functional distribution. *Am J Physiol* **258**(4 Pt 1): C673-81.

Carlson, L. A., L. Holmquist and P. Nilsson-Ehle (1986). Deficiency of hepatic lipase activity in post-heparin plasma in familial hyper-alpha-triglyceridemia. *Acta Med Scand* **219**(5): 435-47.

Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore and B. Williamson (1975). An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* **72**(9): 3666-70.

Carvalho, E., K. Kotani, O. D. Peroni and B. B. Kahn (2005). Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. *Am J Physiol Endocrinol Metab* **289**(4): E551-61.

Castro, M. C., M. L. Massa, H. Del Zotto, J. J. Gagliardino and F. Francini (2011). Rat liver uncoupling protein 2: changes induced by a fructose-rich diet. *Life Sci* **89**(17-18): 609-14.

Catapano, A. L. (1982). Apolipoprotein C-II and lipoprotein lipase activity. *Ric Clin Lab* **12**(1): 35-40.

Cazarolli, L. H., L. Zanatta, E. H. Alberton, M. S. Figueiredo, P. Folador, R. G. Damazio, M. G. Pizzolatti and F. R. Silva (2008). Flavonoids: cellular and molecular mechanism of action in glucose homeostasis. *Mini Rev Med Chem* **8**(10): 1032-8.

Cefalu, W. T., Z. Q. Wang, S. Werbel, A. Bell-Farrow, J. R. Crouse, 3rd, W. H. Hinson, J. G. Terry and R. Anderson (1995). Contribution of visceral fat mass to the insulin resistance of aging. *Metabolism* **44**(7): 954-9.

Chandrasekar, B. and G. Fernandes (1994). Decreased pro-inflammatory cytokines and increased antioxidant enzyme gene expression by omega-3 lipids in murine lupus nephritis. *Biochem Biophys Res Commun* **200**(2): 893-8.

Chang, L., S. H. Chiang and A. R. Saltiel (2004). Insulin signaling and the regulation of glucose transport. *Mol Med* **10**(7-12): 65-71.

Chawla, A., W. A. Boisvert, C. H. Lee, B. A. Laffitte, Y. Barak, S. B. Joseph, D. Liao, L. Nagy, P. A. Edwards, L. K. Curtiss, R. M. Evans and P. Tontonoz (2001). A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol Cell* **7**(1): 161-71.

Cheatham, B. and C. R. Kahn (1995). Insulin action and the insulin signaling network. *Endocr Rev* **16**(2): 117-42.

Chen, S. H., G. Habib, C. Y. Yang, Z. W. Gu, B. R. Lee, S. A. Weng, S. R. Silberman, S. J. Cai, J. P. Deslypere, M. Rosseneu and et al. (1987). Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. *Science* **238**(4825): 363-6.

Chessler, S. D., W. Y. Fujimoto, J. B. Shofer, E. J. Boyko and D. S. Weigle (1998). Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. *Diabetes* **47**(2): 239-43.

Cheung, A. T., D. Ree, J. K. Kolls, J. Fuselier, D. H. Coy and M. Bryer-Ash (1998). An in vivo model for elucidation of the mechanism of tumor necrosis factor-alpha (TNF-alpha)-induced insulin resistance: evidence for differential regulation of insulin signaling by TNF-alpha. *Endocrinology* **139**(12): 4928-35.

Chicco, A., M. E. D'Alessandro, L. Karabatas, C. Pastorale, J. C. Basabe and Y. B. Lombardo (2003). Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. *J Nutr* **133**(1): 127-33.

Chinetti, G., S. Lestavel, V. Bocher, A. T. Remaley, B. Neve, I. P. Torra, E. Teissier, A. Minnich, M. Jaye, N. Duverger, H. B. Brewer, J. C. Fruchart, V. Clavey and B. Staels (2001). PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med* **7**(1): 53-8.

Choi, K. C., S. Y. Lee, H. J. Yoo, O. H. Ryu, K. W. Lee, S. M. Kim, S. H. Baik and K. M. Choi (2007). Effect of PPAR-delta agonist on the expression of visfatin, adiponectin, and resistin in rat adipose tissue and 3T3-L1 adipocytes. *Biochem Biophys Res Commun* **357**(1): 62-7.

Choi, G. S., S. Lee, T. S. Jeong, M. K. Lee, J. S. Lee, U. J. Jung, H. J. Kim, Y. B. Park, S. H. Bok and M. S. Choi (2004). Evaluation of hesperetin 7-O-lauryl ether as lipid-lowering agent in high-cholesterol-fed rats. *Bioorg Med Chem* **12**(13): 3599-605.

Choi, S. Y., M. C. Komaromy, J. Chen, L. G. Fong and A. D. Cooper (1994). Acceleration of uptake of LDL but not chylomicrons or chylomicron remnants by cells that secrete apoE and hepatic lipase. *J Lipid Res* **35**(5): 848-59.

Choi, J. S., T. Yokozawa and H. Oura (1991). Improvement of hyperglycemia and hyperlipemia in streptozotocin-diabetic rats by a methanolic extract of Prunus davidiana stems and its main component, prunin. *Planta Med* **57**(3): 208-11.

Choudhury, J. and A. J. Sanyal (2005). Insulin resistance in NASH. *Front Biosci* **10**: 1520-33.

Chu, A. J., M. A. Walton, J. K. Prasad and A. Seto (1999). Blockade by polyunsaturated n-3 fatty acids of endotoxin-induced monocytic tissue factor activation is mediated by the depressed receptor expression in THP-1 cells. *J Surg Res* **87**(2): 217-24.

Chua, S. C., Jr., I. K. Koutras, L. Han, S. M. Liu, J. Kay, S. J. Young, W. K. Chung and R. L. Leibel (1997). Fine structure of the murine leptin receptor gene: splice site suppression is required to form two alternatively spliced transcripts. *Genomics* **45**(2): 264-70.

Chung, B. H., J. P. Segrest and F. Franklin (1998). In vitro production of beta-very low density lipoproteins and small, dense low density lipoproteins in mildly hypertriglyceridemic plasma: role of activities of lecithin:cholester acyltransferase, cholesterylester transfer proteins and lipoprotein lipase. *Atherosclerosis* **141**(2): 209-25.

Clarke, S. D. (2001). Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *J Nutr* **131**(4): 1129-32.

Clavey, V., S. Lestavel-Delattre, C. Copin, J. M. Bard and J. C. Fruchart (1995). Modulation of lipoprotein B binding to the LDL receptor by exogenous lipids and apolipoproteins CI, CII, CIII, and E. *Arterioscler Thromb Vasc Biol* **15**(7): 963-71.

Clee, S. M., H. Zhang, N. Bissada, L. Miao, E. Ehrenborg, P. Benlian, G. X. Shen, A. Angel, R. C. LeBoeuf and M. R. Hayden (1997). Relationship between lipoprotein lipase and high density lipoprotein cholesterol in mice: modulation by cholesteryl ester transfer protein and dietary status. *J Lipid Res* **38**(10): 2079-89.

Clement, K., C. Vaisse, N. Lahlou, S. Cabrol, V. Pelloux, D. Cassuto, M. Gourmelen, C. Dina, J. Chambaz, J. M. Lacorte, A. Basdevant, P. Bougneres, Y. Lebouc, P. Froguel and B. Guy-Grand (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**(6674): 398-401.

Cnop, M., P. J. Havel, K. M. Utzschneider, D. B. Carr, M. K. Sinha, E. J. Boyko, B. M. Retzlaff, R. H. Knopp, J. D. Brunzell and S. E. Kahn (2003). Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* **46**(4): 459-69.

Cohen, B., D. Novick and M. Rubinstein (1996). Modulation of insulin activities by leptin. *Science* **274**(5290): 1185-8.

Coleman, D. L. (1978). Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* **14**(3): 141-8.

Combs, T. P., A. H. Berg, S. Obici, P. E. Scherer and L. Rossetti (2001). Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* **108**(12): 1875-81.

Comuzzie, A. G., T. Funahashi, G. Sonnenberg, L. J. Martin, H. J. Jacob, A. E. Black, D. Maas, M. Takahashi, S. Kihara, S. Tanaka, Y. Matsuzawa, J. Blangero, D. Cohen and A. Kissebah (2001). The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab* **86**(9): 4321-5.

Connelly, M. A. and D. L. Williams (2004). Scavenger receptor BI: a scavenger receptor with a mission to transport high density lipoprotein lipids. *Curr Opin Lipidol* **15**(3): 287-95.

Connelly, P. W. (1999). The role of hepatic lipase in lipoprotein metabolism. *Clin Chim Acta* **286**(1-2): 243-55.

Cortez-Pinto, H., J. Chatham, V. P. Chacko, C. Arnold, A. Rashid and A. M. Diehl (1999). Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA* **282**(17): 1659-64.

Costa, R. R., N. R. Villela, M. G. Souza, B. C. Boa, F. Z. Cyrino, S. V. Silva, P. C. Lisboa, E. G. Moura, T. C. Barja-Fidalgo and E. Bouskela (2011). High fat diet induces central obesity, insulin resistance and microvascular dysfunction in hamsters. *Microvasc Res* **82**(3): 416-22.

Cottrell, D. A., B. J. Marshall and J. M. Falko (2003). Therapeutic approaches to dyslipidemia in diabetes mellitus and metabolic syndrome. *Curr Opin Cardiol* **18**(4): 301-8.

Couet, C., J. Delarue, P. Ritz, J. M. Antoine and F. Lamisse (1997). Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int J Obes Relat Metab Disord* **21**(8): 637-43.

Cryer, A. (1981). Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int J Biochem* **13**(5): 525-41.

Cumin, F., H. P. Baum and N. Levens (1996). Leptin is cleared from the circulation primarily by the kidney. *Int J Obes Relat Metab Disord* **20**(12): 1120-6.

Cummings, B. P., K. L. Stanhope, J. L. Graham, S. C. Griffen and P. J. Havel (2010). Supplementation with EPA or fish oil for 11 months lowers circulating lipids, but does not delay the onset of diabetes in UC Davis-type 2 diabetes mellitus rats. *Br J Nutr* **104**(11): 1628-34.

Cusin, I., K. E. Zakrzewska, O. Boss, P. Muzzin, J. P. Giacobino, D. Ricquier, B. Jeanrenaud and F. Rohner-Jeanrenaud (1998). Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. *Diabetes* **47**(7): 1014-9.

Cusin, I., F. Rohner-Jeanrenaud, A. Stricker-Krongrad and B. Jeanrenaud (1996). The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats. Reduced sensitivity compared with lean animals. *Diabetes* **45**(10): 1446-50.

Czarnecka, H. and S. Yokoyama (1996). Regulation of cellular cholesterol efflux by lecithin:cholesterol acyltransferase reaction through nonspecific lipid exchange. *J Biol Chem* **271**(4): 2023-8.

Dallinga-Thie, G. M., M. Groenendijk, R. N. Blom, T. W. De Bruin and E. De Kant (2001). Genetic heterogeneity in the apolipoprotein C-III promoter and effects of insulin. *J Lipid Res* **42**(9): 1450-6.

Das, U. N. (2005a). A defect in the activity of Delta6 and Delta5 desaturases may be a factor predisposing to the development of insulin resistance syndrome. *Prostaglandins Leukot Essent Fatty Acids* **72**(5): 343-50.

Das, U. N. (2005b). Pathophysiology of metabolic syndrome X and its links to the perinatal period. *Nutrition* **21**(6): 762-73.

Dashti, N., M. Gandhi, X. Liu, X. Lin and J. P. Segrest (2002). The N-terminal 1000 residues of apolipoprotein B associate with microsomal triglyceride transfer protein to create a lipid transfer pocket required for lipoprotein assembly. *Biochemistry* **41**(22): 6978-87.

de Lorgeril, M., S. Renaud, N. Mamelle, P. Salen, J. L. Martin, I. Monjaud, J. Guidollet, P. Touboul and J. Delaye (1994). Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* **343**(8911): 1454-9.

De Vos, P., A. M. Lefebvre, S. G. Miller, M. Guerre-Millo, K. Wong, R. Saladin, L. G. Hamann, B. Staels, M. R. Briggs and J. Auwerx (1996). Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. *J Clin Invest* **98**(4): 1004-9.

Deeb, S. S., L. Fajas, M. Nemoto, J. Pihlajamaki, L. Mykkanen, J. Kuusisto, M. Laakso, W. Fujimoto and J. Auwerx (1998). A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* **20**(3): 284-7.

DeFronzo, R. A., E. Jacot, E. Jequier, E. Maeder, J. Wahren and J. P. Felber (1981). The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* **30**(12): 1000-7.

DeFronzo, R. A., J. D. Tobin and R. Andres (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* **237**(3): E214-23.

Delbosc, S., E. Paizanis, R. Magous, C. Araiz, T. Dimo, J. P. Cristol, G. Cros and J. Azay (2005). Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat. *Atherosclerosis* **179**(1): 43-9.

Demonty, I., Y. Lin, Y. E. Zebregs, M. A. Vermeer, H. C. van der Knaap, M. Jakel and E. A. Trautwein (2010). The citrus flavonoids hesperidin and naringin do not affect serum cholesterol in moderately hypercholesterolemic men and women. *J Nutr* **140**(9): 1615-20.

Denke, M. A. (2002). Dietary prescriptions to control dyslipidemias. *Circulation* **105**(2): 132-5.

Diard, P., M. I. Malewiak, D. Lagrange and S. Griglio (1994). Hepatic lipase may act as a ligand in the uptake of artificial chylomicron remnant-like particles by isolated rat hepatocytes. *Biochem J* **299 (Pt 3)**: 889-94.

Donahue, R. P., R. D. Abbott, E. Bloom, D. M. Reed and K. Yano (1987). Central obesity and coronary heart disease in men. *Lancet* **1**(8537): 821-4.

Douen, A. G., T. Ramlal, G. D. Cartee and A. Klip (1990). Exercise modulates the insulin-induced translocation of glucose transporters in rat skeletal muscle. *FEBS Lett* **261**(2): 256-60.

Dourmashkin, J. T., G. Q. Chang, E. C. Gayles, J. O. Hill, S. K. Fried, C. Julien and S. F. Leibowitz (2005). Different forms of obesity as a function of diet composition. *Int J Obes (Lond)* **29**(11): 1368-78.

Ducimetiere, P., J. Richard and F. Cambien (1986). The pattern of subcutaneous fat distribution in middle-aged men and the risk of coronary heart disease: the Paris Prospective Study. *Int J Obes* **10**(3): 229-40.

Dugani, C. B. and A. Klip (2005). Glucose transporter 4: cycling, compartments and controversies. *EMBO Rep* **6**(12): 1137-42.

Dullaart, R. P., F. Perton, M. M. van der Klauw, H. L. Hillege and W. J. Sluiter (2009). High plasma lecithin:cholesterol acyltransferase activity does not predict low incidence of cardiovascular events: possible attenuation of cardioprotection associated with high HDL cholesterol. *Atherosclerosis* **208**(2): 537-42.

Dunbar, J. C., Y. Hu and H. Lu (1997). Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* **46**(12): 2040-3.

Eaton, S. B. and M. Konner (1985). Paleolithic nutrition. A consideration of its nature and current implications. *N Engl J Med* **312**(5): 283-9.

Eckel, R. H. (1989). Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* **320**(16): 1060-8.

Ecker, J., T. Langmann, C. Moehle and G. Schmitz (2007). Isomer specific effects of Conjugated Linoleic Acid on macrophage ABCG1 transcription by a SREBP-1c dependent mechanism. *Biochem Biophys Res Commun* **352**(3): 805-11.

Egan, J. J., A. S. Greenberg, M. K. Chang, S. A. Wek, M. C. Moos, Jr. and C. Londos (1992). Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. *Proc Natl Acad Sci U S A* **89**(18): 8537-41.

Egan, J. J., A. S. Greenberg, M. K. Chang and C. Londos (1990). Control of endogenous phosphorylation of the major cAMP-dependent protein kinase substrate in adipocytes by insulin and beta-adrenergic stimulation. *J Biol Chem* **265**(31): 18769-75.

Einvik, G., T. O. Klemsdal, L. Sandvik and E. M. Hjerkinn (2010). A randomized clinical trial on n-3 polyunsaturated fatty acids supplementation and all-cause mortality in elderly men at high cardiovascular risk. *Eur J Cardiovasc Prev Rehabil* **17**(5): 588-92.

Emilsson, V., Y. L. Liu, M. A. Cawthorne, N. M. Morton and M. Davenport (1997). Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* **46**(2): 313-6.

Eriksson, J., L. Koranyi, R. Bourey, C. Schalin-Jantti, E. Widen, M. Mueckler, A. M. Permutt and L. C. Groop (1992). Insulin resistance in type 2 (non-insulin-dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT-4) gene in human skeletal muscle. *Diabetologia* **35**(2): 143-7.

Esteve, M., I. Rafecas, J. A. Fernandez-Lopez, X. Remesar and M. Alemany (1994). Effect of a cafeteria diet on energy intake and balance in Wistar rats. *Physiol Behav* **56**(1): 65-71.

Evans, J. L., I. D. Goldfine, B. A. Maddux and G. M. Grodsky (2002). Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* **23**(5): 599-622.

Ezaki, O., E. Tsuji, K. Momomura, M. Kasuga and H. Itakura (1992). Effects of fish and safflower oil feeding on subcellular glucose transporter distributions in rat adipocytes. *Am J Physiol* **263**(1 Pt 1): E94-101.

Farooqi, I. S., S. A. Jebb, G. Langmack, E. Lawrence, C. H. Cheetham, A. M. Prentice, I. A. Hughes, M. A. McCamish and S. O'Rahilly (1999). Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* **341**(12): 879-84.

Fernandez-Galaz, C., T. Fernandez-Agullo, C. Perez, S. Peralta, C. Arribas, A. Andres, J. M. Carrascosa and M. Ros (2002). Long-term food restriction prevents ageing-associated central leptin resistance in wistar rats. *Diabetologia* **45**(7): 997-1003.

Feskens, E. J., S. M. Virtanen, L. Rasanen, J. Tuomilehto, J. Stengard, J. Pekkanen, A. Nissinen and D. Kromhout (1995). Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care* **18**(8): 1104-12.

Flachs, P., V. Mohamed-Ali, O. Horakova, M. Rossmeisl, M. J. Hosseinzadeh-Attar, M. Hensler, J. Ruzickova and J. Kopecky (2006). Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia* **49**(2): 394-7.

Flegal, K. M., M. D. Carroll, C. L. Ogden and L. R. Curtin (2010). Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* **303**(3): 235-41.

Florez, H., A. Mendez, P. Casanova-Romero, C. Larreal-Urdaneta, S. Castillo-Florez, D. Lee and R. Goldberg (2006). Increased apolipoprotein C-III levels associated with insulin resistance contribute to dyslipidemia in normoglycemic and diabetic subjects from a triethnic population. *Atherosclerosis* **188**(1): 134-41.

Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman and R. M. Evans (1995). 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* **83**(5): 803-12.

Frank, P. G. and Y. L. Marcel (2000). Apolipoprotein A-I: structure-function relationships. *J Lipid Res* **41**(6): 853-72.

Frayn, K. N., S. W. Coppack, B. A. Fielding and S. M. Humphreys (1995). Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. *Adv Enzyme Regul* **35**: 163-78.

Frederiksen, K. S., E. M. Wulf, K. Wassermann, P. Sauerberg and J. Fleckner (2003). Identification of hepatic transcriptional changes in insulin-resistant rats treated with peroxisome proliferator activated receptor-alpha agonists. *J Mol Endocrinol* **30**(3): 317-29.

Fried, S. K., C. D. Russell, N. L. Grauso and R. E. Brolin (1993). Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *J Clin Invest* **92**(5): 2191-8.

Friedberg, C. E., M. J. Janssen, R. J. Heine and D. E. Grobbee (1998). Fish oil and glycemic control in diabetes. A meta-analysis. *Diabetes Care* **21**(4): 494-500.

Friedman, J. E., W. M. Sherman, M. J. Reed, C. W. Elton and G. L. Dohm (1990). Exercise training increases glucose transporter protein GLUT-4 in skeletal muscle of obese Zucker (fa/fa) rats. *FEBS Lett* **268**(1): 13-6.

Friedman, J. M. and J. L. Halaas (1998). Leptin and the regulation of body weight in mammals. *Nature* **395**(6704): 763-70.

Fruebis, J., T. S. Tsao, S. Javorschi, D. Ebbets-Reed, M. R. Erickson, F. T. Yen, B. E. Bihain and H. F. Lodish (2001). Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* **98**(4): 2005-10.

Fukuhara, A., M. Matsuda, M. Nishizawa, K. Segawa, M. Tanaka, K. Kishimoto, Y. Matsuki, M. Murakami, T. Ichisaka, H. Murakami, E. Watanabe, T. Takagi, M. Akiyoshi, T. Ohtsubo, S. Kihara, S. Yamashita, M. Makishima, T. Funahashi, S. Yamanaka, R. Hiramatsu, Y. Matsuzawa and I. Shimomura (2005). Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* **307**(5708): 426-30.

Fukushima, M., T. Ohhashi, S. Ohno, H. Saitoh, K. Sonoyama, K. Shimada, M. Sekikawa and M. Nakano (2001). Effects of diets enriched in n-6 or n-3 fatty acids on cholesterol metabolism in older rats chronically fed a cholesterol-enriched diet. *Lipids* **36**(3): 261-6.

Galati, G. and P. J. O'Brien (2004). Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radic Biol Med* **37**(3): 287-303.

Galic, S., J. S. Oakhill and G. R. Steinberg (2010). Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* **316**(2): 129-39.

Gallai, V., P. Sarchielli, A. Trequattrini, M. Franceschini, A. Floridi, C. Firenze, A. Alberti, D. Di Benedetto and E. Stragliotto (1995). Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. *J Neuroimmunol* **56**(2): 143-53.

Gan, S. K., A. D. Kriketos, A. M. Poynten, S. M. Furler, C. H. Thompson, E. W. Kraegen, L. V. Campbell and D. J. Chisholm (2003). Insulin action, regional fat, and myocyte lipid: altered relationships with increased adiposity. *Obes Res* **11**(11): 1295-305.

Ganda, O. P. (2009). Refining lipoprotein assessment in diabetes: apolipoprotein B makes sense. *Endocr Pract* **15**(4): 370-6.

Garcia-Pelayo, M. C., E. Garcia-Peregrin and M. Martinez-Cayuela (2004). Differential translational effects of myristic acid and eicosapentaenoic acid on 3-hydroxy-3-methylglutaryl-CoA reductase from Reuber H35 hepatoma cells. *Exp Biol Med (Maywood)* **229**(8): 781-6.

Garton, A. J., D. G. Campbell, D. Carling, D. G. Hardie, R. J. Colbran and S. J. Yeaman (1989). Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. *Eur J Biochem* **179**(1): 249-54.

Garvey, W. T., T. P. Huecksteadt, R. Monzon and S. Marshall (1989). Dexamethasone regulates the glucose transport system in primary cultured adipocytes: different mechanisms of insulin resistance after acute and chronic exposure. *Endocrinology* **124**(5): 2063-73.

Garvey, W. T., L. Maianu, J. A. Hancock, A. M. Golichowski and A. Baron (1992). Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. *Diabetes* **41**(4): 465-75.

Gautier, T., D. Masson, J. P. de Barros, A. Athias, P. Gambert, D. Aunis, M. H. Metz-Boutigue and L. Lagrost (2000). Human apolipoprotein C-I accounts for the ability of plasma high density lipoproteins to inhibit the cholesteryl ester transfer protein activity. *J Biol Chem* **275**(48): 37504-9.

Gavrilova, O., B. Marcus-Samuels, D. Graham, J. K. Kim, G. I. Shulman, A. L. Castle, C. Vinson, M. Eckhaus and M. L. Reitman (2000). Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* **105**(3): 271-8.

Gayet, C., E. Bailhache, H. Dumon, L. Martin, B. Siliart and P. Nguyen (2004). Insulin resistance and changes in plasma concentration of TNFalpha, IGF1, and NEFA in dogs during weight gain and obesity. *J Anim Physiol Anim Nutr (Berl)* **88**(3-4): 157-65.

Gbaguidi, F. G., G. Chinetti, D. Milosavljevic, E. Teissier, J. Chapman, G. Olivecrona, J. C. Fruchart, S. Griglio, J. Fruchart-Najib and B. Staels (2002). Peroxisome proliferator-activated receptor (PPAR) agonists decrease lipoprotein lipase secretion and glycated LDL uptake by human macrophages. *FEBS Lett* **512**(1-3): 85-90.

Gebhardt, D. O., A. Beintema, F. C. Reman and C. M. van Gent (1979). The lipoprotein composition of amniotic fluid. *Clin Chim Acta* **94**(1): 93-100.

Gerrits, P. M., A. L. Olson and J. E. Pessin (1993). Regulation of the GLUT4/muscle-fat glucose transporter mRNA in adipose tissue of insulin-deficient diabetic rats. *J Biol Chem* **268**(1): 640-4.

Ghibaudi, L., J. Cook, C. Farley, M. van Heek and J. J. Hwa (2002). Fat intake affects adiposity, comorbidity factors, and energy metabolism of sprague-dawley rats. *Obes Res* **10**(9): 956-63.

Ghosh, S., A. K. Singh, B. Aruna, S. Mukhopadhyay and N. Z. Ehtesham (2003). The genomic organization of mouse resistin reveals major differences from the human resistin: functional implications. *Gene* **305**(1): 27-34.

Gillam, M., A. Noto, P. Zahradka and C. G. Taylor (2009). Improved n-3 fatty acid status does not modulate insulin resistance in fa/fa Zucker rats. *Prostaglandins Leukot Essent Fatty Acids* **81**(5-6): 331-9.

Girard, J. (2000). [Fatty acids and beta cells]. *Diabetes Metab* **26 Suppl 3**: 6-9.

Girard, J. (1995). [Role of free fatty acids in the insulin resistance of non-insulin-dependent diabetes]. *Diabete Metab* **21**(2): 79-88.

Giron, M. D., R. Salto, P. Hortelano, J. L. Periago, A. M. Vargas and M. D. Suarez (1999). Increased diaphragm expression of GLUT4 in control and streptozotocin-diabetic rats by fish oil-supplemented diets. *Lipids* **34**(8): 801-7.

Glomset, J. A. (1973). The metabolic role of lecithin: cholesterol acyltransferase: perspectives from pathology. *Adv Lipid Res* **11**(0): 1-65.

Goldberg, I. J. and M. Merkel (2001). Lipoprotein lipase: physiology, biochemistry, and molecular biology. *Front Biosci* **6**: D388-405.

Gong, Y., R. Ishida-Takahashi, E. C. Villanueva, D. C. Fingar, H. Munzberg and M. G. Myers, Jr. (2007). The long form of the leptin receptor regulates STAT5 and ribosomal protein S6 via alternate mechanisms. *J Biol Chem* **282**(42): 31019-27.

Gonzalez-Periz, A., R. Horrillo, N. Ferre, K. Gronert, B. Dong, E. Moran-Salvador, E. Titos, M. Martinez-Clemente, M. Lopez-Parra, V. Arroyo and J. Claria (2009). Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. *FASEB J* **23**(6): 1946-57.

Grant, A. M., M. R. Christie and S. J. Ashcroft (1980). Insulin release from human pancreatic islets in vitro. *Diabetologia* **19**(2): 114-7.

Greenwood, M. R. (1985). The relationship of enzyme activity to feeding behavior in rats: lipoprotein lipase as the metabolic gatekeeper. *Int J Obes* **9 Suppl 1**: 67-70.

Griffin, M. E., M. J. Marcucci, G. W. Cline, K. Bell, N. Barucci, D. Lee, L. J. Goodyear, E. W. Kraegen, M. F. White and G. I. Shulman (1999). Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* **48**(6): 1270-4.

Grinspoon, S. K., H. Askari, M. L. Landt, D. M. Nathan, D. A. Schoenfeld, D. L. Hayden, M. Laposata, J. Hubbard and A. Klibanski (1997). Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. *Am J Clin Nutr* **66**(6): 1352-6.

Gross, B. and B. Staels (2007). PPAR agonists: multimodal drugs for the treatment of type-2 diabetes. *Best Pract Res Clin Endocrinol Metab* **21**(4): 687-710.

Grunfeld, C., C. Zhao, J. Fuller, A. Pollack, A. Moser, J. Friedman and K. R. Feingold (1996). Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. *J Clin Invest* **97**(9): 2152-7.

Guerin, M., W. Le Goff, T. S. Lassel, A. Van Tol, G. Steiner and M. J. Chapman (2001). Atherogenic role of elevated CE transfer from HDL to VLDL(1) and dense LDL in type 2 diabetes : impact of the degree of triglyceridemia. *Arterioscler Thromb Vasc Biol* **21**(2): 282-8.

Guerre-Millo, M., C. Rouault, P. Poulain, J. Andre, V. Poitout, J. M. Peters, F. J. Gonzalez, J. C. Fruchart, G. Reach and B. Staels (2001). PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. *Diabetes* **50**(12): 2809-14.

Guerre-Millo, M., P. Gervois, E. Raspe, L. Madsen, P. Poulain, B. Derudas, J. M. Herbert, D. A. Winegar, T. M. Willson, J. C. Fruchart, R. K. Berge and B. Staels (2000). Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* **275**(22): 16638-42.

Guo, D. and D. B. Donner (1996). Tumor necrosis factor promotes phosphorylation and binding of insulin receptor substrate 1 to phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. *J Biol Chem* **271**(2): 615-8.

Gustafson, A., P. Alaupovic and R. H. Furman (1966). Studies of the composition and structure of serum lipoproteins. Separation and characterization of phospholipid-protein residues obtained by partial delipidization of very low density lipoproteins of human serum. *Biochemistry* **5**(2): 632-40.

Haffner, S. M., M. P. Stern, B. D. Mitchell, H. P. Hazuda and J. K. Patterson (1990). Incidence of type II diabetes in Mexican Americans predicted by fasting insulin and glucose levels, obesity, and body-fat distribution. *Diabetes* **39**(3): 283-8.

Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley and J. M. Friedman (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269**(5223): 543-6.

Halle, M., A. Berg, H. Northoff and J. Keul (1998). Importance of TNF-alpha and leptin in obesity and insulin resistance: a hypothesis on the impact of physical exercise. *Exerc Immunol Rev* **4**: 77-94.

Halleux, C. M., M. Takahashi, M. L. Delporte, R. Detry, T. Funahashi, Y. Matsuzawa and S. M. Brichard (2001). Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochem Biophys Res Commun* **288**(5): 1102-7.

Hallfrisch, J., K. C. Ellwood, O. E. t. Michaelis, S. Reiser, T. M. O'Dorisio and E. S. Prather (1983). Effects of dietary fructose on plasma glucose and hormone responses in normal and hyperinsulinemic men. *J Nutr* **113**(9): 1819-26.

Haluzik, M., O. Gavrilova and D. LeRoith (2004). Peroxisome proliferator-activated receptor-alpha deficiency does not alter insulin sensitivity in mice maintained on regular or high-fat diet: hyperinsulinemic-euglycemic clamp studies. *Endocrinology* **145**(4): 1662-7.

Hanyu, O., T. Miida, K. Obayashi, T. Ikarashi, S. Soda, S. Kaneko, S. Hirayama, K. Suzuki, Y. Nakamura, K. Yamatani and Y. Aizawa (2004). Lipoprotein lipase (LPL) mass in preheparin serum reflects insulin sensitivity. *Atherosclerosis* **174**(2): 385-90.

Haque, M. S., Y. Minokoshi, M. Hamai, M. Iwai, M. Horiuchi and T. Shimazu (1999). Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. *Diabetes* **48**(9): 1706-12.

Harbourne, J. B. (1993). *The flavonoids: advances in research since 1986.*, London: Chapman & Hall.

Harris, W. S. (1997a). n-3 fatty acids and serum lipoproteins: animal studies. *Am J Clin Nutr* **65**(5 Suppl): 1611S-1616S.

Harris, W. S. (1997b). n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* **65**(5 Suppl): 1645S-1654S.

Havel, R. J. (1986). Functional activities of hepatic lipoprotein receptors. *Annu Rev Physiol* **48**: 119-34.

Haynes, W. G., W. I. Sivitz, D. A. Morgan, S. A. Walsh and A. L. Mark (1997). Sympathetic and cardiorenal actions of leptin. *Hypertension* **30**(3 Pt 2): 619-23.

He, B., S. Zhao, W. Zhang, Y. Li, Y. Lu and P. Han (2010). Salicylate prevents hepatic oxidative stress activation caused by short-term elevation of free fatty acids in vivo. *Diabetes Res Clin Pract* **89**(2): 150-6.

Hegele, R. A., J. A. Little, C. Vezina, G. F. Maguire, L. Tu, T. S. Wolever, D. J. Jenkins and P. W. Connelly (1993). Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. *Arterioscler Thromb* **13**(5): 720-8.

Henquin, J. C., M. Gembal, P. Detimary, Z. Y. Gao, C. Warnotte and P. Gilon (1994). Multisite control of insulin release by glucose. *Diabete Metab* **20**(2): 132-7.

Hertog, M. G., P. C. Hollman, M. B. Katan and D. Kromhout (1993). Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr Cancer* **20**(1): 21-9.

Herzberg, G. R. and M. Rogerson (1988). Hepatic fatty acid synthesis and triglyceride secretion in rats fed fructose- or glucose-based diets containing corn oil, tallow or marine oil. *J Nutr* **118**(9): 1061-7.

Hickman, D. L. and M. Swan (2010). Use of a body condition score technique to assess health status in a rat model of polycystic kidney disease. *J Am Assoc Lab Anim Sci* **49**(2): 155-9.

Hileman, S. M., J. Tornoe, J. S. Flier and C. Bjorbaek (2000). Transcellular transport of leptin by the short leptin receptor isoform ObRa in Madin-Darby Canine Kidney cells. *Endocrinology* **141**(6): 1955-61.

Hill, J. O., D. Lin, F. Yakubu and J. C. Peters (1992). Development of dietary obesity in rats: influence of amount and composition of dietary fat. *Int J Obes Relat Metab Disord* **16**(5): 321-33.

Hodis, H. N. (1999). Triglyceride-rich lipoprotein remnant particles and risk of atherosclerosis. *Circulation* **99**(22): 2852-4.

Holleboom, A. G., J. A. Kuivenhoven, M. Vergeer, G. K. Hovingh, J. N. van Miert, N. J. Wareham, J. J. Kastelein, K. T. Khaw and S. M. Boekholdt (2009). Plasma levels of lecithin:cholesterol acyltransferase and risk of future coronary artery disease in apparently healthy men and women: a prospective case-control analysis nested in the EPIC-Norfolk population study. *J Lipid Res* **51**(2): 416-21.

Holman, G. D., I. J. Kozka, A. E. Clark, C. J. Flower, J. Saltis, A. D. Habberfield, I. A. Simpson and S. W. Cushman (1990). Cell surface labeling of glucose transporter isoform GLUT4 by bis-mannose photolabel. Correlation with stimulation of glucose transport in rat adipose cells by insulin and phorbol ester. *J Biol Chem* **265**(30): 18172-9.

Homma, H., H. Kurachi, Y. Nishio, T. Takeda, T. Yamamoto, K. Adachi, K. Morishige, M. Ohmichi, Y. Matsuzawa and Y. Murata (2000). Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. *J Biol Chem* **275**(15): 11404-11.

Horcajada, M. N., V. Habauzit, A. Trzeciakiewicz, C. Morand, A. Gil-Izquierdo, J. Mardon, P. Lebecque, M. J. Davicco, W. S. Chee, V. Coxam and E. Offord (2008). Hesperidin inhibits ovariectomized-induced osteopenia and shows differential effects on bone mass and strength in young and adult intact rats. *J Appl Physiol* **104**(3): 648-54.

Horton, J. D., N. A. Shah, J. A. Warrington, N. N. Anderson, S. W. Park, M. S. Brown and J. L. Goldstein (2003). Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc Natl Acad Sci U S A* **100**(21): 12027-32.

Horton, J. D., J. L. Goldstein and M. S. Brown (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* **109**(9): 1125-31.

Horton, J. D., Y. Bashmakov, I. Shimomura and H. Shimano (1998a). Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci U S A* **95**(11): 5987-92.

Horton, J. D., I. Shimomura, M. S. Brown, R. E. Hammer, J. L. Goldstein and H. Shimano (1998b). Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J Clin Invest* **101**(11): 2331-9.

Hotamisligil, G. S. and E. Erbay (2008). Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol* **8**(12): 923-34.

Hotamisligil, G. S., P. Arner, R. L. Atkinson and B. M. Spiegelman (1997). Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance. *Diabetes* **46**(3): 451-5.

Hotamisligil, G. S., P. Peraldi, A. Budavari, R. Ellis, M. F. White and B. M. Spiegelman (1996). IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* **271**(5249): 665-8.

Hotamisligil, G. S., P. Arner, J. F. Caro, R. L. Atkinson and B. M. Spiegelman (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* **95**(5): 2409-15.

Hotamisligil, G. S., A. Budavari, D. Murray and B. M. Spiegelman (1994a). Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J Clin Invest* **94**(4): 1543-9.

Hotamisligil, G. S., D. L. Murray, L. N. Choy and B. M. Spiegelman (1994b). Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A* **91**(11): 4854-8.

Hotamisligil, G. S., N. S. Shargill and B. M. Spiegelman (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **259**(5091): 87-91.

Hotta, K., T. Funahashi, N. L. Bodkin, H. K. Ortmeyer, Y. Arita, B. C. Hansen and Y. Matsuzawa (2001). Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* **50**(5): 1126-33.

Houseknecht, K. L., C. S. Mantzoros, R. Kuliawat, E. Hadro, J. S. Flier and B. B. Kahn (1996). Evidence for leptin binding to proteins in serum of rodents and humans: modulation with obesity. *Diabetes* **45**(11): 1638-43.

Hu, E., J. B. Kim, P. Sarraf and B. M. Spiegelman (1996). Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. *Science* **274**(5295): 2100-3.

Hua, X., C. Yokoyama, J. Wu, M. R. Briggs, M. S. Brown, J. L. Goldstein and X. Wang (1993). SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. *Proc Natl Acad Sci U S A* **90**(24): 11603-7.

Huang, S. and M. P. Czech (2007). The GLUT4 glucose transporter. *Cell Metab* **5**(4): 237-52.

Hummel, K. P., M. M. Dickie and D. L. Coleman (1966). Diabetes, a new mutation in the mouse. *Science* **153**(740): 1127-8.

Hwa, J. J., A. B. Fawzi, M. P. Graziano, L. Ghibaudi, P. Williams, M. Van Heek, H. Davis, M. Rudinski, E. Sybertz and C. D. Strader (1997). Leptin increases energy expenditure and selectively promotes fat metabolism in ob/ob mice. *Am J Physiol* **272**(4 Pt 2): R1204-9.

IDF (2011). The global burden. IDF Diabetes Atlas. *International Diabetes Federation***:** http://www.idf.org/diabetesatlas/5e/the-global-burden.

IDF (2006). The IDF consensus worldwide definition of the METABOLIC SYNDROME. *International Diabetes Federation***:** 1-24 http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf.

Ihm, J., J. L. Ellsworth, B. Chataing and J. A. Harmony (1982). Plasma protein-facilitated coupled exchange of phosphatidylcholine and cholesteryl ester in the absence of cholesterol esterification. *J Biol Chem* **257**(9): 4818-27.

Ikemoto, S., M. Takahashi, N. Tsunoda, K. Maruyama, H. Itakura and O. Ezaki (1996). High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. *Metabolism* **45**(12): 1539-46.

Isganaitis, E. and R. H. Lustig (2005). Fast food, central nervous system insulin resistance, and obesity. *Arterioscler Thromb Vasc Biol* **25**(12): 2451-62.

Itoh, T., L. Fairall, K. Amin, Y. Inaba, A. Szanto, B. L. Balint, L. Nagy, K. Yamamoto and J. W. Schwabe (2008). Structural basis for the activation of PPARgamma by oxidized fatty acids. *Nat Struct Mol Biol* **15**(9): 924-31.

Iwaki, M., M. Matsuda, N. Maeda, T. Funahashi, Y. Matsuzawa, M. Makishima and I. Shimomura (2003). Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* **52**(7): 1655-63.

Jain, S., M. Gaiha, J. Bhattacharjee and S. Anuradha (2002). Effects of low-dose omega-3 fatty acid substitution in type-2 diabetes mellitus with special reference to oxidative stress--a prospective preliminary study. *J Assoc Physicians India* **50**: 1028-33.

Jalovaara, K., M. Santaniemi, M. Timonen, J. Jokelainen, Y. A. Kesaniemi, O. Ukkola, S. Keinanen-Kiukaanniemi and U. Rajala (2008). Low serum adiponectin level as a predictor of impaired glucose regulation and type 2 diabetes mellitus in a middle-aged Finnish population. *Metabolism* **57**(8): 1130-4.

James, D. E., J. Hiken and J. C. Lawrence, Jr. (1989). Isoproterenol stimulates phosphorylation of the insulin-regulatable glucose transporter in rat adipocytes. *Proc Natl Acad Sci U S A* **86**(21): 8368-72.

Jansen, H. (2004). Hepatic lipase: friend or foe and under what circumstances? *Curr Atheroscler Rep* **6**(5): 343-7.

Jansen, H., A. J. Verhoeven, L. Weeks, J. J. Kastelein, D. J. Halley, A. van den Ouweland, J. W. Jukema, J. C. Seidell and J. C. Birkenhager (1997). Common C-to-T substitution at position -480 of the hepatic lipase promoter associated with a lowered lipase activity in coronary artery disease patients. *Arterioscler Thromb Vasc Biol* **17**(11): 2837-42.

Jaworski, K., E. Sarkadi-Nagy, R. E. Duncan, M. Ahmadian and H. S. Sul (2007). Regulation of triglyceride metabolism. IV. Hormonal regulation of lipolysis in adipose tissue. *Am J Physiol Gastrointest Liver Physiol* **293**(1): G1-4.

Jeffery, N. M., E. A. Newsholme and P. C. Calder (1997). Level of polyunsaturated fatty acids and the n-6 to n-3 polyunsaturated fatty acid ratio in the rat diet alter serum lipid levels and lymphocyte functions. *Prostaglandins Leukot Essent Fatty Acids* **57**(2): 149-60.

Jeffery, N. M., P. Sanderson, E. J. Sherrington, E. A. Newsholme and P. C. Calder (1996). The ratio of n-6 to n-3 polyunsaturated fatty acids in the rat diet alters serum lipid levels and lymphocyte functions. *Lipids* **31**(7): 737-45.

Jellema, A., J. Plat and R. P. Mensink (2004). Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. *Eur J Clin Invest* **34**(11): 766-73.

Jensen, M. D., M. W. Haymond, R. A. Rizza, P. E. Cryer and J. M. Miles (1989). Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest* **83**(4): 1168-73.

Jeppesen, J., Y. I. Chen, M. Y. Zhou, P. Schaaf, A. Coulston and G. M. Reaven (1995). Postprandial triglyceride and retinyl ester responses to oral fat: effects of fructose. *Am J Clin Nutr* **61**(4): 787-91.

Ji, Y., B. Jian, N. Wang, Y. Sun, M. L. Moya, M. C. Phillips, G. H. Rothblat, J. B. Swaney and A. R. Tall (1997). Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. *J Biol Chem* **272**(34): 20982-5.

Ji, Z. S., S. J. Lauer, S. Fazio, A. Bensadoun, J. M. Taylor and R. W. Mahley (1994). Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. *J Biol Chem* **269**(18): 13429-36.

Jiao, K., H. Liu, J. Chen, D. Tian, J. Hou and A. D. Kaye (2008). Roles of plasma interleukin-6 and tumor necrosis factor-alpha and FFA and TG in the development of insulin resistance induced by high-fat diet. *Cytokine* **42**(2): 161-9.

Jump, D. B. (2002). The biochemistry of n-3 polyunsaturated fatty acids. *J Biol Chem* **277**(11): 8755-8.

Jump, D. B. and S. D. Clarke (1999). Regulation of gene expression by dietary fat. *Annu Rev Nutr* **19**: 63-90.

Jump, D. B., S. D. Clarke, A. Thelen and M. Liimatta (1994). Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J Lipid Res* **35**(6): 1076-84.

Jung, U. J., M. K. Lee, Y. B. Park, M. A. Kang and M. S. Choi (2006). Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. *Int J Biochem Cell Biol* **38**(7): 1134-45.

Jung, U. J., M. K. Lee, K. S. Jeong and M. S. Choi (2004). The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. *J Nutr* **134**(10): 2499-503.

Kadowaki, T. and T. Yamauchi (2005). Adiponectin and adiponectin receptors. *Endocr Rev* **26**(3): 439-51.

Kahn, B. B., M. J. Charron, H. F. Lodish, S. W. Cushman and J. S. Flier (1989). Differential regulation of two glucose transporters in adipose cells from diabetic and insulin-treated diabetic rats. *J Clin Invest* **84**(2): 404-11.

Kahn, B. B., E. S. Horton and S. W. Cushman (1987). Mechanism for enhanced glucose transport response to insulin in adipose cells from chronically hyperinsulinemic rats. Increased translocation of glucose transporters from an enlarged intracellular pool. *J Clin Invest* **79**(3): 853-8.

Kamohara, S., R. Burcelin, J. L. Halaas, J. M. Friedman and M. J. Charron (1997). Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* **389**(6649): 374-7.

Kampmann, U., B. Christensen, T. S. Nielsen, S. B. Pedersen, L. Orskov, S. Lund, N. Moller and N. Jessen (2011). GLUT4 and UBC9 Protein Expression Is Reduced in Muscle from Type 2 Diabetic Patients with Severe Insulin Resistance. *PLoS One* **6**(11): e27854.

Kannappan, S., N. Palanisamy and C. V. Anuradha (2010). Suppression of hepatic oxidative events and regulation of eNOS expression in the liver by naringenin in fructose-administered rats. *Eur J Pharmacol* **645**(1-3): 177-84.

Kanno, J., H. Kato, T. Iwata and T. Inoue (2002). Phytoestrogen-low diet for endocrine disruptor studies. *J Agric Food Chem* **50**(13): 3883-5.

Karpe, F. and E. E. Ehrenborg (2009). PPARdelta in humans: genetic and pharmacological evidence for a significant metabolic function. *Curr Opin Lipidol* **20**(4): 333-6.

Kaufmann, U. and E. R. Froesch (1973). Inhibition of phosphorylase-a by fructose-1-phosphate, alpha-glycerophosphate and fructose-1,6-diphosphate: explanation for fructose-induced hypoglycaemia in hereditary fructose intolerance and fructose-1,6-diphosphatase deficiency. *Eur J Clin Invest* **3**(5): 407-13.

Kavanagh, K., K. L. Jones, J. Sawyer, K. Kelley, J. J. Carr, J. D. Wagner and L. L. Rudel (2007). Trans fat diet induces abdominal obesity and changes in insulin sensitivity in monkeys. *Obesity (Silver Spring)* **15**(7): 1675-84.

Kawaguchi, K., T. Mizuno, K. Aida and K. Uchino (1997). Hesperidin as an inhibitor of lipases from porcine pancreas and Pseudomonas. *Biosci Biotechnol Biochem* **61**(1): 102-4.

Kawakami, M. and A. Cerami (1981). Studies of endotoxin-induced decrease in lipoprotein lipase activity. *J Exp Med* **154**(3): 631-9.

Keim, N. L., J. S. Stern and P. J. Havel (1998). Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *Am J Clin Nutr* **68**(4): 794-801.

Kelada, A. S., S. L. Macaulay and J. Proietto (1992). Cyclic AMP acutely stimulates translocation of the major insulin-regulatable glucose transporter GLUT4. *J Biol Chem* **267**(10): 7021-5.

Kern, P. A., G. Di Gregorio, T. Lu, N. Rassouli and G. Ranganathan (2004). Perilipin expression in human adipose tissue is elevated with obesity. *J Clin Endocrinol Metab* **89**(3): 1352-8.

Kern, P. A., M. Saghizadeh, J. M. Ong, R. J. Bosch, R. Deem and R. B. Simsolo (1995). The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* **95**(5): 2111-9.

Kershaw, E. E. and J. S. Flier (2004). Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* **89**(6): 2548-56.

Kersten, S. (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep* **2**(4): 282-6.

Kersten, S., S. Mandard, P. Escher, F. J. Gonzalez, S. Tafuri, B. Desvergne and W. Wahli (2001). The peroxisome proliferator-activated receptor alpha regulates amino acid metabolism. *FASEB J* **15**(11): 1971-8.

Kersten, S., J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne and W. Wahli (1999). Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* **103**(11): 1489-98.

Kido, Y., J. Nakae and D. Accili (2001). Clinical review 125: The insulin receptor and its cellular targets. *J Clin Endocrinol Metab* **86**(3): 972-9.

Kieffer, T. J., R. S. Heller, C. A. Leech, G. G. Holz and J. F. Habener (1997). Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes* **46**(6): 1087-93.

Kim, H. K., T. S. Jeong, M. K. Lee, Y. B. Park and M. S. Choi (2003). Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. *Clin Chim Acta* **327**(1-2): 129-37.

Kim, H. I., J. W. Kim, S. H. Kim, J. Y. Cha, K. S. Kim and Y. H. Ahn (2000a). Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. *Diabetes* **49**(9): 1517-24.

Kim, J. K., O. Gavrilova, Y. Chen, M. L. Reitman and G. I. Shulman (2000b). Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J Biol Chem* **275**(12): 8456-60.

Kim, H. J., M. Takahashi and O. Ezaki (1999). Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. *J Biol Chem* **274**(36): 25892-8.

Kim, J. B., P. Sarraf, M. Wright, K. M. Yao, E. Mueller, G. Solanes, B. B. Lowell and B. M. Spiegelman (1998). Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest* **101**(1): 1-9.

Kinnunen, P. K., R. L. Jackson, L. C. Smith, A. M. Gotto, Jr. and J. T. Sparrow (1977). Activation of lipoprotein lipase by native and synthetic fragments of human plasma apolipoprotein C-II. *Proc Natl Acad Sci U S A* **74**(11): 4848-51.

Kinsella, J. E. (1991). Alpha-linolenic acid: functions and effects on linoleic acid metabolism and eicosanoid-mediated reactions. *Adv Food Nutr Res* **35**: 1-184.

Klein-Platat, C., J. Drai, M. Oujaa, J. L. Schlienger and C. Simon (2005). Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. *Am J Clin Nutr* **82**(6): 1178-84.

Kletzien, R. F., S. D. Clarke and R. G. Ulrich (1992). Enhancement of adipocyte differentiation by an insulin-sensitizing agent. *Mol Pharmacol* **41**(2): 393-8.

Knudsen, P., M. Antikainen, S. Ehnholm, M. Uusi-Oukari, H. Tenkanen, S. Lahdenpera, J. Kahri, M. Tilly-Kiesi, A. Bensadoun, M. R. Taskinen and C. Ehnholm (1996). A compound heterozygote for hepatic lipase gene mutations Leu334-->Phe and Thr383-->Met: correlation between hepatic lipase activity and phenotypic expression. *J Lipid Res* **37**(4): 825-34.

Knudsen, P., J. Eriksson, S. Lahdenpera, J. Kahri, L. Groop and M. R. Taskinen (1995). Changes of lipolytic enzymes cluster with insulin resistance syndrome. Botnia Study Group. *Diabetologia* **38**(3): 344-50.

Komaromy, M., S. Azhar and A. D. Cooper (1996). Chinese hamster ovary cells expressing a cell surface-anchored form of hepatic lipase. Characterization of low density lipoprotein and chylomicron remnant uptake and selective uptake of high density lipoprotein-cholesteryl ester. *J Biol Chem* **271**(28): 16906-14.

Konrad, D., P. J. Bilan, Z. Nawaz, G. Sweeney, W. Niu, Z. Liu, C. N. Antonescu, A. Rudich and A. Klip (2002). Need for GLUT4 activation to reach maximum effect of insulin-mediated glucose uptake in brown adipocytes isolated from GLUT4myc-expressing mice. *Diabetes* **51**(9): 2719-26.

Koo, H. Y., M. A. Wallig, B. H. Chung, T. Y. Nara, B. H. Cho and M. T. Nakamura (2008). Dietary fructose induces a wide range of genes with distinct shift in carbohydrate and lipid metabolism in fed and fasted rat liver. *Biochim Biophys Acta* **1782**(5): 341-8.

Koranyi, L., D. James, M. Mueckler and M. A. Permutt (1990). Glucose transporter levels in spontaneously obese (db/db) insulin-resistant mice. *J Clin Invest* **85**(3): 962-7.

Koranyi, L. I., R. E. Bourey, H. Vuorinen-Markkola, V. A. Koivisto, M. Mueckler, M. A. Permutt and H. Yki-Jarvinen (1991). Level of skeletal muscle glucose transporter protein correlates with insulin-stimulated whole body glucose disposal in man. *Diabetologia* **34**(10): 763-5.

Kosaki, A., K. Yamada and H. Kuzuya (1996). Reduced expression of the leptin gene (ob) by catecholamine through a G(S) protein-coupled pathway in 3T3-L1 adipocytes. *Diabetes* **45**(12): 1744-9.

Kozarsky, K. F., M. H. Donahee, A. Rigotti, S. N. Iqbal, E. R. Edelman and M. Krieger (1997). Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature* **387**(6631): 414-7.

Kraegen, E. W., D. E. James, L. H. Storlien, K. M. Burleigh and D. J. Chisholm (1986). In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration. *Diabetologia* **29**(3): 192-8.

Kramer, D. K., L. Al-Khalili, B. Guigas, Y. Leng, P. M. Garcia-Roves and A. Krook (2007). Role of AMP kinase and PPARdelta in the regulation of lipid and glucose metabolism in human skeletal muscle. *J Biol Chem* **282**(27): 19313-20.

Krapp, A., S. Ahle, S. Kersting, Y. Hua, K. Kneser, M. Nielsen, J. Gliemann and U. Beisiegel (1996). Hepatic lipase mediates the uptake of chylomicrons and beta-VLDL into cells via the LDL receptor-related protein (LRP). *J Lipid Res* **37**(5): 926-36.

Krebs, C. and G. Singleton (1993). Indexes of Condition for Small Mammals. *Australian Journal of Zoology* **41**(4): 317-323.

Krieger, M. (2001). Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems. *J Clin Invest* **108**(6): 793-7.

Kriegler, M., C. Perez, K. DeFay, I. Albert and S. D. Lu (1988). A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* **53**(1): 45-53.

Kromhout, D., A. Menotti, B. Bloemberg, C. Aravanis, H. Blackburn, R. Buzina, A. S. Dontas, F. Fidanza, S. Giampaoli, A. Jansen and et al. (1995). Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: the Seven Countries Study. *Prev Med* **24**(3): 308-15.

Ku, K., H. Oku, T. Kaneda, M. Onoe and Z. Zhang (1999). Beneficial effects of omega-3 fatty acid treatment on the recovery of cardiac function after cold storage of hyperlipidemic rats. *Metabolism* **48**(10): 1203-9.

Kubota, N., W. Yano, T. Kubota, T. Yamauchi, S. Itoh, H. Kumagai, H. Kozono, I. Takamoto, S. Okamoto, T. Shiuchi, R. Suzuki, H. Satoh, A. Tsuchida, M. Moroi, K. Sugi, T. Noda, H. Ebinuma, Y. Ueta, T. Kondo, E. Araki, O. Ezaki, R. Nagai, K. Tobe, Y. Terauchi, K. Ueki, Y. Minokoshi and T. Kadowaki (2007). Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab* **6**(1): 55-68.

Kubota, N., Y. Terauchi, T. Yamauchi, T. Kubota, M. Moroi, J. Matsui, K. Eto, T. Yamashita, J. Kamon, H. Satoh, W. Yano, P. Froguel, R. Nagai, S. Kimura, T. Kadowaki and T. Noda (2002). Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* **277**(29): 25863-6.

Kubota, N., Y. Terauchi, H. Miki, H. Tamemoto, T. Yamauchi, K. Komeda, S. Satoh, R. Nakano, C. Ishii, T. Sugiyama, K. Eto, Y. Tsubamoto, A. Okuno, K. Murakami, H. Sekihara, G. Hasegawa, M. Naito, Y. Toyoshima, S. Tanaka, K. Shiota, T. Kitamura, T. Fujita, O. Ezaki, S. Aizawa, T. Kadowaki and et al. (1999). PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* **4**(4): 597-609.

Kuhnau, J. (1976). The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* **24**: 117-91.

Kunesova, M., R. Braunerova, P. Hlavaty, E. Tvrzicka, B. Stankova, J. Skrha, J. Hilgertova, M. Hill, J. Kopecky, M. Wagenknecht, V. Hainer, M. Matoulek, J. Parizkova, A. Zak and S. Svacina (2006). The influence of n-3 polyunsaturated fatty acids and very low calorie diet

during a short-term weight reducing regimen on weight loss and serum fatty acid composition in severely obese women. *Physiol Res* **55**(1): 63-72.

Kunitake, S. T., C. M. Mendel and L. K. Hennessy (1992). Interconversion between apolipoprotein A-I-containing lipoproteins of pre-beta and alpha electrophoretic mobilities. *J Lipid Res* **33**(12): 1807-16.

Kusunoki, M., K. Tsutsumi, M. Nakayama, T. Kurokawa, T. Nakamura, H. Ogawa, Y. Fukuzawa, M. Morishita, T. Koide and T. Miyata (2007). Relationship between serum concentrations of saturated fatty acids and unsaturated fatty acids and the homeostasis model insulin resistance index in Japanese patients with type 2 diabetes mellitus. *J Med Invest* **54**(3-4): 243-7.

Lagrost, L., C. Dengremont, A. Athias, C. de Geitere, J. C. Fruchart, C. Lallemant, P. Gambert and G. Castro (1995). Modulation of cholesterol efflux from Fu5AH hepatoma cells by the apolipoprotein content of high density lipoprotein particles. Particles containing various proportions of apolipoproteins A-I and A-II. *J Biol Chem* **270**(22): 13004-9.

Lambert, G., M. J. Amar, P. Martin, J. Fruchart-Najib, B. Foger, R. D. Shamburek, H. B. Brewer, Jr. and S. Santamarina-Fojo (2000). Hepatic lipase deficiency decreases the selective uptake of HDL-cholesteryl esters in vivo. *J Lipid Res* **41**(5): 667-72.

Lambert, G., M. B. Chase, K. Dugi, A. Bensadoun, H. B. Brewer, Jr. and S. Santamarina-Fojo (1999). Hepatic lipase promotes the selective uptake of high density lipoprotein-cholesteryl esters via the scavenger receptor B1. *J Lipid Res* **40**(7): 1294-303.

Lang, C. H., C. Dobrescu and G. J. Bagby (1992). Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. *Endocrinology* **130**(1): 43-52.

Lapsys, N. M., A. D. Kriketos, M. Lim-Fraser, A. M. Poynten, A. Lowy, S. M. Furler, D. J. Chisholm and G. J. Cooney (2000). Expression of genes involved in lipid metabolism correlate with peroxisome proliferator-activated receptor gamma expression in human skeletal muscle. *J Clin Endocrinol Metab* **85**(11): 4293-7.

Lawrence, J. C., Jr., R. C. Piper, L. J. Robinson and D. E. James (1992). GLUT4 facilitates insulin stimulation and cAMP-mediated inhibition of glucose transport. *Proc Natl Acad Sci U S A* **89**(8): 3493-7.

Lawton, C. L., H. J. Delargy, J. Brockman, F. C. Smith and J. E. Blundell (2000). The degree of saturation of fatty acids influences post-ingestive satiety. *Br J Nutr* **83**(5): 473-82.

Le Jossic-Corcos, C., C. Gonthier, I. Zaghini, E. Logette, I. Shechter and P. Bournot (2005). Hepatic farnesyl diphosphate synthase expression is suppressed by polyunsaturated fatty acids. *Biochem J* **385**(Pt 3): 787-94.

Le Marchand-Brustel, Y., C. Olichon-Berthe, T. Gremeaux, J. F. Tanti, N. Rochet and E. Van Obberghen (1990). Glucose transporter in insulin sensitive tissues of lean and obese mice. Effect of the thermogenic agent BRL 26830A. *Endocrinology* **127**(6): 2687-95.

Le Roith, D. and Y. Zick (2001). Recent advances in our understanding of insulin action and insulin resistance. *Diabetes Care* **24**(3): 588-97.

Leaf, A., J. X. Kang, Y. F. Xiao and G. E. Billman (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation* **107**(21): 2646-52.

Lee, C. H., P. Olson, A. Hevener, I. Mehl, L. W. Chong, J. M. Olefsky, F. J. Gonzalez, J. Ham, H. Kang, J. M. Peters and R. M. Evans (2006a). PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc Natl Acad Sci U S A* **103**(9): 3444-9.

Lee, Y. C., Y. H. Ko, Y. P. Hsu and L. T. Ho (2006b). Plasma leptin response to oral glucose tolerance and fasting/re-feeding tests in rats with fructose-induced metabolic derangements. *Life Sci* **78**(11): 1155-62.

Lee, S., C. Andrieu, F. Saltel, O. Destaing, J. Auclair, V. Pouchkine, J. Michelon, B. Salaun, R. Kobayashi, P. Jurdic, E. D. Kieff and B. S. Sylla (2004). IkappaB kinase beta phosphorylates Dok1 serines in response to TNF, IL-1, or gamma radiation. *Proc Natl Acad Sci U S A* **101**(50): 17416-21.

Lee, S. J., L. A. Moye, H. Campos, G. H. Williams and F. M. Sacks (2003). Hypertriglyceridemia but not diabetes status is associated with VLDL containing apolipoprotein CIII in patients with coronary heart disease. *Atherosclerosis* **167**(2): 293-302.

Lefevre, M., C. H. Sloop and P. S. Roheim (1988). Characterization of dog prenodal peripheral lymph lipoproteins. Evidence for the peripheral formation of lipoprotein-unassociated apoA-I with slow pre-beta electrophoretic mobility. *J Lipid Res* **29**(9): 1139-48.

Lemberger, T., O. Braissant, C. Juge-Aubry, H. Keller, R. Saladin, B. Staels, J. Auwerx, A. G. Burger, C. A. Meier and W. Wahli (1996). PPAR tissue distribution and interactions with other hormone-signaling pathways. *Ann N Y Acad Sci* **804**: 231-51.

Lemieux, S. and J. P. Despres (1994). Metabolic complications of visceral obesity: contribution to the aetiology of type 2 diabetes and implications for prevention and treatment. *Diabete Metab* **20**(4): 375-93.

Leone, T. C., C. J. Weinheimer and D. P. Kelly (1999). A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. *Proc Natl Acad Sci U S A* **96**(13): 7473-8.

Leray, V., S. Serisier, S. Khosniat, L. Martin, H. Dumon and P. Nguyen (2008). Adipose tissue gene expression in obese dogs after weight loss. *J Anim Physiol Anim Nutr (Berl)* **92**(3): 390-8.

Leth, T. and U. Justesen (1998). *Analysis of flavonoids in fruits, vegetables and beverages by HPCL-UV and LC-MS and estimation of the total daily flavonoid intake in Denmark. In Polyphenols in Food*, Luxembourg: Off. Official Publications Eur. Commun.

Levin, B. E., A. A. Dunn-Meynell and W. A. Banks (2004). Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling before obesity onset. *Am J Physiol Regul Integr Comp Physiol* **286**(1): R143-50.

Leyton, J., P. J. Drury and M. A. Crawford (1987). Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Br J Nutr* **57**(3): 383-93.

Li, F. Y., K. K. Cheng, K. S. Lam, P. M. Vanhoutte and A. Xu (2011). Cross-talk between adipose tissue and vasculature: role of adiponectin. *Acta Physiol (Oxf)* **203**(1): 167-80.

Li, J. J., C. J. Huang and D. Xie (2008). Anti-obesity effects of conjugated linoleic acid, docosahexaenoic acid, and eicosapentaenoic acid. *Mol Nutr Food Res* **52**(6): 631-45.

Lin, M. H., S. C. Lu, P. C. Huang, Y. C. Liu and S. Y. Liu (2005). A high-cholesterol, n-3 polyunsaturated fatty acid diet causes different responses in rats and hamsters. *Ann Nutr Metab* **49**(6): 386-91.

Li, J., X. Yu, W. Pan and R. H. Unger (2002). Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. *Am J Physiol Endocrinol Metab* **282**(6): E1334-41.

Li, W. W., M. M. Dammerman, J. D. Smith, S. Metzger, J. L. Breslow and T. Leff (1995). Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. *J Clin Invest* **96**(6): 2601-5.

Lindroos, A. K., L. Lissner, B. Carlsson, L. M. Carlsson, J. Torgerson, C. Karlsson, K. Stenlof and L. Sjostrom (1998). Familial predisposition for obesity may modify the predictive value of serum leptin concentrations for long-term weight change in obese women. *Am J Clin Nutr* **67**(6): 1119-23.

Liu, L., S. Shan, K. Zhang, Z. Q. Ning, X. P. Lu and Y. Y. Cheng (2008). Naringenin and hesperetin, two flavonoids derived from Citrus aurantium up-regulate transcription of adiponectin. *Phytother Res* **22**(10): 1400-3.

Lombardo, Y. B., G. Hein and A. Chicco (2007). Metabolic syndrome: effects of n-3 PUFAs on a model of dyslipidemia, insulin resistance and adiposity. *Lipids* **42**(5): 427-37.

Lorente-Cebrian, S., P. Perez-Matute, J. A. Martinez, A. Marti and M. J. Moreno-Aliaga (2006). Effects of eicosapentaenoic acid (EPA) on adiponectin gene expression and secretion in primary cultured rat adipocytes. *J Physiol Biochem* **62**(2): 61-9.

Lund, S., H. Vestergaard, P. H. Andersen, O. Schmitz, L. B. Gotzsche and O. Pedersen (1993). GLUT-4 content in plasma membrane of muscle from patients with non-insulin-dependent diabetes mellitus. *Am J Physiol* **265**(6 Pt 1): E889-97.

Lundasen, T., W. Liao, B. Angelin and M. Rudling (2003). Leptin induces the hepatic high density lipoprotein receptor scavenger receptor B type I (SR-BI) but not cholesterol 7alpha-hydroxylase (Cyp7a1) in leptin-deficient (ob/ob) mice. *J Biol Chem* **278**(44): 43224-8.

Luo, J., S. W. Rizkalla, J. Boillot, C. Alamowitch, H. Chaib, F. Bruzzo, N. Desplanque, A. M. Dalix, G. Durand and G. Slama (1996). Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids. *J Nutr* **126**(8): 1951-8.

Madsen, A. N., G. Hansen, S. J. Paulsen, K. Lykkegaard, M. Tang-Christensen, H. S. Hansen, B. E. Levin, P. J. Larsen, L. B. Knudsen, K. Fosgerau and N. Vrang (2010). Long-term characterization of the diet-induced obese and diet-resistant rat model: a polygenetic rat model mimicking the human obesity syndrome. *J Endocrinol* **206**(3): 287-96.

Maeda, N., M. Takahashi, T. Funahashi, S. Kihara, H. Nishizawa, K. Kishida, H. Nagaretani, M. Matsuda, R. Komuro, N. Ouchi, H. Kuriyama, K. Hotta, T. Nakamura, I. Shimomura and Y. Matsuzawa (2001). PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* **50**(9): 2094-9.

Maffei, M., H. Fei, G. H. Lee, C. Dani, P. Leroy, Y. Zhang, R. Proenca, R. Negrel, G. Ailhaud and J. M. Friedman (1995). Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. *Proc Natl Acad Sci U S A* **92**(15): 6957-60.

Malik, S. (2003). Transcriptional regulation of the apolipoprotein AI gene. *Front Biosci* **8**: d360-8.

Mamputu, J. C., L. Levesque and G. Renier (2000). Proliferative effect of lipoprotein lipase on human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* **20**(10): 2212-9.

Mangelsdorf, D. J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon and R. M. Evans (1995). The nuclear receptor superfamily: the second decade. *Cell* **83**(6): 835-9.

Marcel, Y. L., C. Vezina, B. Teng and A. Sniderman (1980). Transfer of cholesterol esters between human high density lipoproteins and triglyceride-rich lipoproteins controlled by a plasma protein factor. *Atherosclerosis* **35**(2): 127-33.

Marette, A., J. M. Richardson, T. Ramlal, T. W. Balon, M. Vranic, J. E. Pessin and A. Klip (1992). Abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. *Am J Physiol* **263**(2 Pt 1): C443-52.

Marin, P., I. Hogh-Kristiansen, S. Jansson, M. Krotkiewski, G. Holm and P. Bjorntorp (1992). Uptake of glucose carbon in muscle glycogen and adipose tissue triglycerides in vivo in humans. *Am J Physiol* **263**(3 Pt 1): E473-80.

Marques-Vidal, P., C. Azema, X. Collet, C. Vieu, H. Chap and B. Perret (1994). Hepatic lipase promotes the uptake of HDL esterified cholesterol by the perfused rat liver: a study using reconstituted HDL particles of defined phospholipid composition. *J Lipid Res* **35**(3): 373-84.

Marsh, J. B., D. L. Topping and P. J. Nestel (1987). Comparative effects of dietary fish oil and carbohydrate on plasma lipids and hepatic activities of phosphatidate phosphohydrolase, diacylglycerol acyltransferase and neutral lipase activities in the rat. *Biochim Biophys Acta* **922**(2): 239-43.

Martin, G., K. Schoonjans, A. M. Lefebvre, B. Staels and J. Auwerx (1997). Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. *J Biol Chem* **272**(45): 28210-7.

Martinez-Botas, J., J. B. Anderson, D. Tessier, A. Lapillonne, B. H. Chang, M. J. Quast, D. Gorenstein, K. H. Chen and L. Chan (2000). Absence of perilipin results in leanness and reverses obesity in Lepr(db/db) mice. *Nat Genet* **26**(4): 474-9.

Masuzaki, H., Y. Ogawa, K. Hosoda, T. Kawada, T. Fushiki and K. Nakao (1995a). Augmented expression of the obese gene in the adipose tissue from rats fed high-fat diet. *Biochem Biophys Res Commun* **216**(1): 355-8.

Masuzaki, H., Y. Ogawa, N. Isse, N. Satoh, T. Okazaki, M. Shigemoto, K. Mori, N. Tamura, K. Hosoda, Y. Yoshimasa and et al. (1995b). Human obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. *Diabetes* **44**(7): 855-8.

Masuzaki, H., Y. Ogawa, N. Sagawa, K. Hosoda, T. Matsumoto, H. Mise, H. Nishimura, Y. Yoshimasa, I. Tanaka, T. Mori and K. Nakao (1997). Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med* **3**(9): 1029-33.

Matsui, H., K. Okumura, K. Kawakami, M. Hibino, Y. Toki and T. Ito (1997). Improved insulin sensitivity by bezafibrate in rats: relationship to fatty acid composition of skeletal-muscle triglycerides. *Diabetes* **46**(3): 348-53.

Matsuura, F., N. Wang, W. Chen, X. C. Jiang and A. R. Tall (2006). HDL from CETP-deficient subjects shows enhanced ability to promote cholesterol efflux from macrophages in an apoE- and ABCG1-dependent pathway. *J Clin Invest* **116**(5): 1435-42.

Matthews, D. R., A. S. Rudenski, M. A. Burnett, P. Darling and R. C. Turner (1985). The half-life of endogenous insulin and C-peptide in man assessed by somatostatin suppression. *Clin Endocrinol (Oxf)* **23**(1): 71-9.

Matveyenko, A. V., T. Gurlo, M. Daval, A. E. Butler and P. C. Butler (2009). Successful versus failed adaptation to high-fat diet-induced insulin resistance: the role of IAPP-induced beta-cell endoplasmic reticulum stress. *Diabetes* **58**(4): 906-16.

McTernan, C. L., P. G. McTernan, A. L. Harte, P. L. Levick, A. H. Barnett and S. Kumar (2002). Resistin, central obesity, and type 2 diabetes. *Lancet* **359**(9300): 46-7.

Meier, U. and A. M. Gressner (2004). Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem* **50**(9): 1511-25.

Meilin, E., M. Aviram and T. Hayek (2011). Insulin increases macrophage triglyceride accumulation under diabetic conditions through the down regulation of hormone sensitive lipase and adipose triglyceride lipase. *Biofactors* **37**(2): 95-103.

Miceli, N., M. R. Mondello, M. T. Monforte, V. Sdrafkakis, P. Dugo, M. L. Crupi, M. F. Taviano, R. De Pasquale and A. Trovato (2007). Hypolipidemic effects of Citrus bergamia Risso et Poiteau juice in rats fed a hypercholesterolemic diet. *J Agric Food Chem* **55**(26): 10671-7.

Middleton, E., Jr., C. Kandaswami and T. C. Theoharides (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev* **52**(4): 673-751.

Mildner, A. M. and S. D. Clarke (1991). Porcine fatty acid synthase: cloning of a complementary DNA, tissue distribution of its mRNA and suppression of expression by somatotropin and dietary protein. *J Nutr* **121**(6): 900-7.

Miles, P. D., O. M. Romeo, K. Higo, A. Cohen, K. Rafaat and J. M. Olefsky (1997). TNF-alpha-induced insulin resistance in vivo and its prevention by troglitazone. *Diabetes* **46**(11): 1678-83.

Miller, A. L. and L. C. Smith (1973). Activation of lipoprotein lipase by apolipoprotein glutamic acid. Formation of a stable surface film. *J Biol Chem* **248**(9): 3359-62.

Minami, A., N. Ishimura, S. Sakamoto, E. Takishita, K. Mawatari, K. Okada and Y. Nakaya (2002). Effect of eicosapentaenoic acid ethyl ester v. oleic acid-rich safflower oil on insulin resistance in type 2 diabetic model rats with hypertriacylglycerolaemia. *Br J Nutr* **87**(2): 157-62.

Mittendorfer, B., M. G. Jeschke, S. E. Wolf and L. S. Sidossis (1998). Nutritional hepatic steatosis and mortality after burn injury in rats. *Clin Nutr* **17**(6): 293-9.

Miyake, Y., K. Yamamoto, N. Tsujihara and T. Osawa (1998). Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids* **33**(7): 689-95.

Mizuguchi, K., T. Yano, M. Kojima, Y. Tanaka, M. Ishibashi, A. Masada, M. Sato, M. Mizota, K. Fukutake and Y. Saito (1992). Hypolipidemic effect of ethyl all-cis-5,8,11,14,17-eicosapentaenoate (EPA-E) in rats. *Jpn J Pharmacol* **59**(3): 307-12.

Moghadasian, M. H. (2002). Experimental atherosclerosis: a historical overview. *Life Sci* **70**(8): 855-65.

Mohamed Salih, S., P. Nallasamy, P. Muniyandi, V. Periyasami and A. Carani Venkatraman (2009). Genistein improves liver function and attenuates non-alcoholic fatty liver disease in a rat model of insulin resistance. *J Diabetes* **1**(4): 278-87.

Moller, D. E. and K. D. Kaufman (2005). Metabolic syndrome: a clinical and molecular perspective. *Annu Rev Med* **56**: 45-62.

Monforte, M. T., A. Trovato, S. Kirjavainen, A. M. Forestieri, E. M. Galati and R. B. Lo Curto (1995). Biological effects of hesperidin, a Citrus flavonoid. (note II): hypolipidemic activity on experimental hypercholesterolemia in rat. *Farmaco* **50**(9): 595-9.

Montague, C. T., J. B. Prins, L. Sanders, J. E. Digby and S. O'Rahilly (1997). Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes* **46**(3): 342-7.

Monzillo, L. U., O. Hamdy, E. S. Horton, S. Ledbury, C. Mullooly, C. Jarema, S. Porter, K. Ovalle, A. Moussa and C. S. Mantzoros (2003). Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. *Obes Res* **11**(9): 1048-54.

Moore, B. J. (1987). The cafeteria diet--an inappropriate tool for studies of thermogenesis. *J Nutr* **117**(2): 227-31.

Moore, G. B., H. Chapman, J. C. Holder, C. A. Lister, V. Piercy, S. A. Smith and J. C. Clapham (2001). Differential regulation of adipocytokine mRNAs by rosiglitazone in db/db mice. *Biochem Biophys Res Commun* **286**(4): 735-41.

Morens, C., V. Sirot, A. J. Scheurink and G. van Dijk (2006). Low-carbohydrate diets affect energy balance and fuel homeostasis differentially in lean and obese rats. *Am J Physiol Regul Integr Comp Physiol* **291**(6): R1622-9.

Mori, T. A., R. J. Woodman, V. Burke, I. B. Puddey, K. D. Croft and L. J. Beilin (2003). Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. *Free Radic Biol Med* **35**(7): 772-81.

Mori, Y., Y. Murakawa, J. Yokoyama, N. Tajima, Y. Ikeda, H. Nobukata, T. Ishikawa and Y. Shibutani (1999). Effect of highly purified eicosapentaenoic acid ethyl ester on insulin resistance and hypertension in Dahl salt-sensitive rats. *Metabolism* **48**(9): 1089-95.

Mori, Y., Y. Murakawa, S. Katoh, S. Hata, J. Yokoyama, N. Tajima, Y. Ikeda, H. Nobukata, T. Ishikawa and Y. Shibutani (1997). Influence of highly purified eicosapentaenoic acid ethyl ester on insulin resistance in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus. *Metabolism* **46**(12): 1458-64.

Morin, C. L., R. H. Eckel, T. Marcel and M. J. Pagliassotti (1997). High fat diets elevate adipose tissue-derived tumor necrosis factor-alpha activity. *Endocrinology* **138**(11): 4665-71.

Morino, K., K. F. Petersen, S. Dufour, D. Befroy, J. Frattini, N. Shatzkes, S. Neschen, M. F. White, S. Bilz, S. Sono, M. Pypaert and G. I. Shulman (2005). Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *J Clin Invest* **115**(12): 3587-93.

Morris, D. L. and L. Rui (2009). Recent advances in understanding leptin signaling and leptin resistance. *Am J Physiol Endocrinol Metab* **297**(6): E1247-59.

Mu, J., J. T. Brozinick, Jr., O. Valladares, M. Bucan and M. J. Birnbaum (2001). A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol Cell* **7**(5): 1085-94.

Mueckler, M. (1990). Family of glucose-transporter genes. Implications for glucose homeostasis and diabetes. *Diabetes* **39**(1): 6-11.

Muoio, D. M., G. L. Dohm, F. T. Fiedorek, Jr., E. B. Tapscott and R. A. Coleman (1997). Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* **46**(8): 1360-3.

Muoio, D. M., J. M. Way, C. J. Tanner, D. A. Winegar, S. A. Kliewer, J. A. Houmard, W. E. Kraus and G. L. Dohm (2002). Peroxisome proliferator-activated receptor-alpha regulates fatty acid utilization in primary human skeletal muscle cells. *Diabetes* **51**(4): 901-9.

Murakami, T., S. Michelagnoli, R. Longhi, G. Gianfranceschi, F. Pazzucconi, L. Calabresi, C. R. Sirtori and G. Franceschini (1995). Triglycerides are major determinants of cholesterol esterification/transfer and HDL remodeling in human plasma. *Arterioscler Thromb Vasc Biol* **15**(11): 1819-28.

Mustad, V. A., S. Demichele, Y. S. Huang, A. Mika, N. Lubbers, N. Berthiaume, J. Polakowski and B. Zinker (2006). Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse. *Metabolism* **55**(10): 1365-74.

Myers, M. G., Jr., R. L. Leibel, R. J. Seeley and M. W. Schwartz (2010). Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab* **21**(11): 643-51.

Naba, H., C. Kakinuma, S. Ohnishi and T. Ogihara (2006). Improving effect of ethyl eicosapentanoate on statin-induced rhabdomyolysis in Eisai hyperbilirubinemic rats. *Biochem Biophys Res Commun* **340**(1): 215-20.

Naderali, E. K. and G. Williams (2003). Prolonged endothelial-dependent and -independent arterial dysfunction induced in the rat by short-term feeding with a high-fat, high-sucrose diet. *Atherosclerosis* **166**(2): 253-9.

Nagy, L., P. Tontonoz, J. G. Alvarez, H. Chen and R. M. Evans (1998). Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. *Cell* **93**(2): 229-40.

Nakagawa, T., H. Hu, S. Zharikov, K. R. Tuttle, R. A. Short, O. Glushakova, X. Ouyang, D. I. Feig, E. R. Block, J. Herrera-Acosta, J. M. Patel and R. J. Johnson (2006). A causal role for uric acid in fructose-induced metabolic syndrome. *Am J Physiol Renal Physiol* **290**(3): F625-31.

Nakamura, N., T. Hamazaki, M. Kobayashi, M. Ohta and K. Okuda (1998). Effects of eicosapentaenoic acids on remnant-like particles, cholesterol concentrations and plasma fatty acid composition in patients with diabetes mellitus. *In Vivo* **12**(3): 311-4.

Nakamura, M. T. (1995). Another obese gene function. *Nature* **374**(6518): 124.

Nakano, Y., T. Tobe, N. H. Choi-Miura, T. Mazda and M. Tomita (1996). Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem* **120**(4): 803-12.

NCEP (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* **285**(19): 2486-97.

NCEP (1988). Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel. *Arch Intern Med* **148**(1): 36-69.

Neschen, S., K. Morino, J. Dong, Y. Wang-Fischer, G. W. Cline, A. J. Romanelli, J. C. Rossbacher, I. K. Moore, W. Regittnig, D. S. Munoz, J. H. Kim and G. I. Shulman (2007). n-3 Fatty acids preserve insulin sensitivity in vivo in a peroxisome proliferator-activated receptor-alpha-dependent manner. *Diabetes* **56**(4): 1034-41.

Neschen, S., I. Moore, W. Regittnig, C. L. Yu, Y. Wang, M. Pypaert, K. F. Petersen and G. I. Shulman (2002). Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. *Am J Physiol Endocrinol Metab* **282**(2): E395-401.

Neuringer, M., G. J. Anderson and W. E. Connor (1988). The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Annu Rev Nutr* **8**: 517-41.

Nieto-Vazquez, I., S. Fernandez-Veledo, D. K. Kramer, R. Vila-Bedmar, L. Garcia-Guerra and M. Lorenzo (2008). Insulin resistance associated to obesity: the link TNF-alpha. *Arch Physiol Biochem* **114**(3): 183-94.

Nigam, A., N. Frasure-Smith, F. Lesperance and P. Julien (2009). Relationship between n-3 and n-6 plasma fatty acid levels and insulin resistance in coronary patients with and without metabolic syndrome. *Nutr Metab Cardiovasc Dis* **19**(4): 264-70.

Nikkila, E. A., J. K. Huttunen and C. Ehnholm (1977). Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. Relationship to plasma triglyceride metabolism. *Diabetes* **26**(1): 11-21.

Nobukata, H., T. Ishikawa, M. Obata and Y. Shibutani (2000). Long-term administration of highly purified eicosapentaenoic acid ethyl ester prevents diabetes and abnormalities of blood coagulation in male WBN/Kob rats. *Metabolism* **49**(7): 912-9.

Nomura, S., S. Kanazawa and S. Fukuhara (2003). Effects of eicosapentaenoic acid on platelet activation markers and cell adhesion molecules in hyperlipidemic patients with Type 2 diabetes mellitus. *J Diabetes Complications* **17**(3): 153-9.

Nyby, M. D., K. Matsumoto, K. Yamamoto, K. Abedi, P. Eslami, G. Hernandez, V. Smutko, M. E. Berger and M. L. Tuck (2005). Dietary fish oil prevents vascular dysfunction and oxidative stress in hyperinsulinemic rats. *Am J Hypertens* **18**(2 Pt 1): 213-9.

O'Rahilly, S. (1998). Life without leptin. *Nature* **392**(6674): 330-1.

Obépi (2009). Enquête épidémiologique nationale sur le surpoids et l'obésité. Une enquête INSERM / TNS HEALTHCARE (KANTARHEALTH) / ROCHE. *ObEpi***:** 1-53 http://www.roche.fr/gear/newcontents/servlet/staticfilesServlet?type=data&communityId=re719001&id=static/attachedfile/re7300002/re72700003/AttachedFile_10101.pdf.

Ofei, F., S. Hurel, J. Newkirk, M. Sopwith and R. Taylor (1996). Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* **45**(7): 881-5.

Ogawa, S., K. Takeuchi, K. Sugimura, M. Fukuda, R. Lee, S. Ito and T. Sato (2000). Bezafibrate reduces blood glucose in type 2 diabetes mellitus. *Metabolism* **49**(3): 331-4.

Oh, S. Y., S. K. Park, J. W. Kim, Y. H. Ahn, S. W. Park and K. S. Kim (2003). Acetyl-CoA carboxylase beta gene is regulated by sterol regulatory element-binding protein-1 in liver. *J Biol Chem* **278**(31): 28410-7.

Ohtsuki, K., A. Abe, H. Mitsuzumi, M. Kondo, K. Uemura, Y. Iwasaki and Y. Kondo (2003). Glucosyl hesperidin improves serum cholesterol composition and inhibits hypertrophy in vasculature. *J Nutr Sci Vitaminol (Tokyo)* **49**(6): 447-50.

Oka, Y., T. Asano, Y. Shibasaki, M. Kasuga, Y. Kanazawa and F. Takaku (1988). Studies with antipeptide antibody suggest the presence of at least two types of glucose transporter in rat brain and adipocyte. *J Biol Chem* **263**(26): 13432-9.

Okada, Y., T. Yoshino, A. Takeuchi, M. Endoh, H. Ohta, Y. Jinno, T. Yokoyama, T. Izawa and E. Kobayshi (2000). Effects of the K+ channel opener KRN4884 on the cardiovascular metabolic syndrome model in rats. *J Cardiovasc Pharmacol* **35**(2): 287-93.

Okamoto, Y., H. Ishida, T. Taminato, K. Tsuji, T. Kurose, Y. Tsuura, S. Kato, H. Imura and Y. Seino (1992). Role of cytosolic Ca2+ in impaired sensitivity to glucose of rat pancreatic islets exposed to high glucose in vitro. *Diabetes* **41**(12): 1555-61.

Olatunji, L. A. and A. O. Soladoye (2007). Increased magnesium intake prevents hyperlipidemia and insulin resistance and reduces lipid peroxidation in fructose-fed rats. *Pathophysiology* **14**(1): 11-5.

Ong, J. M., T. G. Kirchgessner, M. C. Schotz and P. A. Kern (1988). Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated rat adipocytes. *J Biol Chem* **263**(26): 12933-8.

Ooi, E. M., P. H. Barrett, D. C. Chan and G. F. Watts (2008). Apolipoprotein C-III: understanding an emerging cardiovascular risk factor. *Clin Sci (Lond)* **114**(10): 611-24.

Ookuma, M., K. Ookuma and D. A. York (1998). Effects of leptin on insulin secretion from isolated rat pancreatic islets. *Diabetes* **47**(2): 219-23.

Osborne, T. F. (2000). Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J Biol Chem* **275**(42): 32379-82.

Ouchi, N., S. Kihara, Y. Arita, Y. Okamoto, K. Maeda, H. Kuriyama, K. Hotta, M. Nishida, M. Takahashi, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Funahashi and Y. Matsuzawa (2000). Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* **102**(11): 1296-301.

Pagliassotti, M. J., P. A. Prach, T. A. Koppenhafer and D. A. Pan (1996). Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. *Am J Physiol* **271**(5 Pt 2): R1319-26.

Park, Y. B., K. M. Do, S. H. Bok, M. K. Lee, T. S. Jeong and M. S. Choi (2001). Interactive effect of hesperidin and vitamin E supplements on cholesterol metabolism in high cholesterol-fed rats. *Int J Vitam Nutr Res* **71**(1): 36-44.

Park, K. S., T. P. Ciaraldi, L. Abrams-Carter, S. Mudaliar, S. E. Nikoulina and R. R. Henry (1997). PPAR-gamma gene expression is elevated in skeletal muscle of obese and type II diabetic subjects. *Diabetes* **46**(7): 1230-4.

Patel, L., A. C. Buckels, I. J. Kinghorn, P. R. Murdock, J. D. Holbrook, C. Plumpton, C. H. Macphee and S. A. Smith (2003). Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* **300**(2): 472-6.

Pawar, A. and D. B. Jump (2003). Unsaturated fatty acid regulation of peroxisome proliferator-activated receptor alpha activity in rat primary hepatocytes. *J Biol Chem* **278**(38): 35931-9.

Peck, G. R., J. A. Chavez, W. G. Roach, B. A. Budnik, W. S. Lane, H. K. Karlsson, J. R. Zierath and G. E. Lienhard (2009). Insulin-stimulated phosphorylation of the Rab GTPase-activating protein TBC1D1 regulates GLUT4 translocation. *J Biol Chem* **284**(44): 30016-23.

Pedersen, H., M. Petersen, A. Major-Pedersen, T. Jensen, N. S. Nielsen, S. T. Lauridsen and P. Marckmann (2003). Influence of fish oil supplementation on in vivo and in vitro oxidation resistance of low-density lipoprotein in type 2 diabetes. *Eur J Clin Nutr* **57**(5): 713-20.

Pedersen, O., C. R. Kahn and B. B. Kahn (1992). Divergent regulation of the Glut 1 and Glut 4 glucose transporters in isolated adipocytes from Zucker rats. *J Clin Invest* **89**(6): 1964-73.

Pedersen, O., C. R. Kahn, J. S. Flier and B. B. Kahn (1991). High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (Glut 4) in fat cells of rats. *Endocrinology* **129**(2): 771-7.

Pedersen, O., J. F. Bak, P. H. Andersen, S. Lund, D. E. Moller, J. S. Flier and B. B. Kahn (1990). Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. *Diabetes* **39**(7): 865-70.

Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone and F. Collins (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* **269**(5223): 540-3.

Perez-Matute, P., N. Perez-Echarri, J. A. Martinez, A. Marti and M. J. Moreno-Aliaga (2007). Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: role of apoptosis, adiponectin and tumour necrosis factor-alpha. *Br J Nutr* **97**(2): 389-98.

Perez-Pozo, S. E., J. Schold, T. Nakagawa, L. G. Sanchez-Lozada, R. J. Johnson and J. L. Lillo (2010). Excessive fructose intake induces the features of metabolic syndrome in healthy adult men: role of uric acid in the hypertensive response. *Int J Obes (Lond)* **34**(3): 454-61.

Peschon, J. J., D. S. Torrance, K. L. Stocking, M. B. Glaccum, C. Otten, C. R. Willis, K. Charrier, P. J. Morrissey, C. B. Ware and K. M. Mohler (1998). TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. *J Immunol* **160**(2): 943-52.

Peters, J. M., R. C. Cattley and F. J. Gonzalez (1997). Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. *Carcinogenesis* **18**(11): 2029-33.

Petersen, K. F. and G. I. Shulman (2006). Etiology of insulin resistance. *Am J Med* **119**(5 Suppl 1): S10-6.

Peyron-Caso, E., A. Quignard-Boulange, M. Laromiguiere, S. Feing-Kwong-Chan, A. Veronese, B. Ardouin, G. Slama and S. W. Rizkalla (2003). Dietary fish oil increases lipid mobilization but does not decrease lipid storage-related enzyme activities in adipose tissue of insulin-resistant, sucrose-fed rats. *J Nutr* **133**(7): 2239-43.

Peyron-Caso, E., S. Fluteau-Nadler, M. Kabir, M. Guerre-Millo, A. Quignard-Boulange, G. Slama and S. W. Rizkalla (2002). Regulation of glucose transport and transporter 4 (GLUT-4) in muscle and adipocytes of sucrose-fed rats: effects of N-3 poly- and monounsaturated fatty acids. *Horm Metab Res* **34**(7): 360-6.

Pighin, D., L. Karabatas, A. Rossi, A. Chicco, J. C. Basabe and Y. B. Lombardo (2003). Fish oil affects pancreatic fat storage, pyruvate dehydrogenase complex activity and insulin secretion in rats fed a sucrose-rich diet. *J Nutr* **133**(12): 4095-101.

Plum, L., X. Ma, B. Hampel, N. Balthasar, R. Coppari, H. Munzberg, M. Shanabrough, D. Burdakov, E. Rother, R. Janoschek, J. Alber, B. F. Belgardt, L. Koch, J. Seibler, F. Schwenk, C. Fekete, A. Suzuki, T. W. Mak, W. Krone, T. L. Horvath, F. M. Ashcroft and J. C. Bruning (2006). Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet-sensitive obesity. *J Clin Invest* **116**(7): 1886-901.

Pocai, A., K. Morgan, C. Buettner, R. Gutierrez-Juarez, S. Obici and L. Rossetti (2005). Central leptin acutely reverses diet-induced hepatic insulin resistance. *Diabetes* **54**(11): 3182-9.

Pollare, T., B. Vessby and H. Lithell (1991). Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. *Arterioscler Thromb* **11**(5): 1192-203.

Pomp, D. (1997). Genetic dissection of obesity in polygenic animal models. *Behav Genet* **27**(4): 285-306.

Posey, K. A., D. J. Clegg, R. L. Printz, J. Byun, G. J. Morton, A. Vivekanandan-Giri, S. Pennathur, D. G. Baskin, J. W. Heinecke, S. C. Woods, M. W. Schwartz and K. D. Niswender (2009). Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *Am J Physiol Endocrinol Metab* **296**(5): E1003-12.

Pouliot, M. C., J. P. Despres, A. Nadeau, S. Moorjani, D. Prud'Homme, P. J. Lupien, A. Tremblay and C. Bouchard (1992). Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. *Diabetes* **41**(7): 826-34.

Powell, L. M., S. C. Wallis, R. J. Pease, Y. H. Edwards, T. J. Knott and J. Scott (1987). A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. *Cell* **50**(6): 831-40.

Pradhan, A. D., J. E. Manson, N. Rifai, J. E. Buring and P. M. Ridker (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* **286**(3): 327-34.

Pruneta, V., D. Autran, G. Ponsin, C. Marcais, L. Duvillard, B. Verges, F. Berthezene and P. Moulin (2001). Ex vivo measurement of lipoprotein lipase-dependent very low density lipoprotein (VLDL)-triglyceride hydrolysis in human VLDL: an alternative to the postheparin assay of lipoprotein lipase activity? *J Clin Endocrinol Metab* **86**(2): 797-803.

Pu, P., D. M. Gao, S. Mohamed, J. Chen, J. Zhang, X. Y. Zhou, N. J. Zhou, J. Xie and H. Jiang (2012). Naringin ameliorates metabolic syndrome by activating AMP-activated protein kinase in mice fed a high-fat diet. *Arch Biochem Biophys* **518**(1): 61-70.

Pykalisto, O. J., P. H. Smith and J. D. Brunzell (1975). Determinants of human adipose tissue lipoprotein lipase. Effect of diabetes and obesity on basal- and diet-induced activity. *J Clin Invest* **56**(5): 1108-17.

Qian, H., M. J. Azain, M. M. Compton, D. L. Hartzell, G. J. Hausman and C. A. Baile (1998). Brain administration of leptin causes deletion of adipocytes by apoptosis. *Endocrinology* **139**(2): 791-4.

Qiu, W., C. Taghibiglou, R. K. Avramoglu, S. C. Van Iderstine, M. Naples, H. Ashrafpour, S. Mhapsekar, R. Sato and K. Adeli (2005). Oleate-mediated stimulation of microsomal triglyceride transfer protein (MTP) gene promoter: implications for hepatic MTP overexpression in insulin resistance. *Biochemistry* **44**(8): 3041-9.

Quarfordt, S. H., G. Michalopoulos and B. Schirmer (1982). The effect of human C apolipoproteins on the in vitro hepatic metabolism of triglyceride emulsions in the rat. *J Biol Chem* **257**(24): 14642-7.

Querfeld, U., M. M. Hoffmann, G. Klaus, F. Eifinger, M. Ackerschott, D. Michalk and P. A. Kern (1999). Antagonistic effects of vitamin D and parathyroid hormone on lipoprotein lipase in cultured adipocytes. *J Am Soc Nephrol* **10**(10): 2158-64.

Raclot, T., R. Groscolas, D. Langin and P. Ferre (1997). Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues. *J Lipid Res* **38**(10): 1963-72.

Rajala, M. W., S. Obici, P. E. Scherer and L. Rossetti (2003). Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. *J Clin Invest* **111**(2): 225-30.

Randle, P. J., A. L. Kerbey and J. Espinal (1988). Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones. *Diabetes Metab Rev* **4**(7): 623-38.

Randle, P. J., P. B. Garland, C. N. Hales and E. A. Newsholme (1963). The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1**(7285): 785-9.

Ravussin, E., R. E. Pratley, M. Maffei, H. Wang, J. M. Friedman, P. H. Bennett and C. Bogardus (1997). Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nat Med* **3**(2): 238-40.

Reaven, G. M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* **37**(12): 1595-607.

Reichl, D., T. M. Forte, J. L. Hong, D. N. Rudra and J. Pflug (1985). Human lymphedema fluid lipoproteins: particle size, cholesterol and apolipoprotein distributions, and electron microscopic structure. *J Lipid Res* **26**(12): 1399-411.

Reilly, S. M. and C. H. Lee (2008). PPAR delta as a therapeutic target in metabolic disease. *FEBS Lett* **582**(1): 26-31.

Reiser, S., A. S. Powell, D. J. Scholfield, P. Panda, M. Fields and J. J. Canary (1989). Day-long glucose, insulin, and fructose responses of hyperinsulinemic and nonhyperinsulinemic men adapted to diets containing either fructose or high-amylose cornstarch. *Am J Clin Nutr* **50**(5): 1008-14.

Renier, G., E. Skamene, J. B. DeSanctis and D. Radzioch (1994). Induction of tumor necrosis factor alpha gene expression by lipoprotein lipase. *J Lipid Res* **35**(2): 271-8.

Renier, G., E. Skamene, J. DeSanctis and D. Radzioch (1993). Dietary n-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice. Modulation of macrophage secretory activities. *Arterioscler Thromb* **13**(10): 1515-24.

Reseland, J. E., S. A. Anderssen, K. Solvoll, I. Hjermann, P. Urdal, I. Holme and C. A. Drevon (2001a). Effect of long-term changes in diet and exercise on plasma leptin concentrations. *Am J Clin Nutr* **73**(2): 240-5.

Reseland, J. E., F. Haugen, K. Hollung, K. Solvoll, B. Halvorsen, I. R. Brude, M. S. Nenseter, E. N. Christiansen and C. A. Drevon (2001b). Reduction of leptin gene expression by dietary polyunsaturated fatty acids. *J Lipid Res* **42**(5): 743-50.

Riemens, S. C., A. Van Tol, W. J. Sluiter and R. P. Dullaart (1999). Acute and chronic effects of a 24-hour intravenous triglyceride emulsion challenge on plasma lecithin: cholesterol acyltransferase, phospholipid transfer protein, and cholesteryl ester transfer protein activities. *J Lipid Res* **40**(8): 1459-66.

Riemens, S., A. van Tol, W. Sluiter and R. Dullaart (1998). Elevated plasma cholesteryl ester transfer in NIDDM: relationships with apolipoprotein B-containing lipoproteins and phospholipid transfer protein. *Atherosclerosis* **140**(1): 71-9.

Rigotti, A., B. L. Trigatti, M. Penman, H. Rayburn, J. Herz and M. Krieger (1997). A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci U S A* **94**(23): 12610-5.

Rinninger, F. and R. C. Pittman (1988). Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters by human fibroblasts and Hep G2 hepatoma cells. *J Lipid Res* **29**(9): 1179-94.

Rivellese, A. A., A. Maffettone, C. Iovine, L. Di Marino, G. Annuzzi, M. Mancini and G. Riccardi (1996). Long-term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. *Diabetes Care* **19**(11): 1207-13.

Roberts, C. K., K. Liang, R. J. Barnard, C. H. Kim and N. D. Vaziri (2004). HMG-CoA reductase, cholesterol 7alpha-hydroxylase, LDL receptor, SR-B1, and ACAT in diet-induced syndrome X. *Kidney Int* **66**(4): 1503-11.

Robinson, D. R., M. Urakaze, R. Huang, H. Taki, E. Sugiyama, C. T. Knoell, L. Xu, E. T. Yeh and P. E. Auron (1996). Dietary marine lipids suppress continuous expression of interleukin-1 beta gene transcription. *Lipids* **31 Suppl**: S23-31.

Roden, M., T. B. Price, G. Perseghin, K. F. Petersen, D. L. Rothman, G. W. Cline and G. I. Shulman (1996). Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* **97**(12): 2859-65.

Romieu, I., W. C. Willett, M. J. Stampfer, G. A. Colditz, L. Sampson, B. Rosner, C. H. Hennekens and F. E. Speizer (1988). Energy intake and other determinants of relative weight. *Am J Clin Nutr* **47**(3): 406-12.

Rosa, G., M. Manco, N. Vega, A. V. Greco, M. Castagneto, H. Vidal and G. Mingrone (2003). Decreased muscle acetyl-coenzyme A carboxylase 2 mRNA and insulin resistance in formerly obese subjects. *Obes Res* **11**(11): 1306-12.

Rosen, E. D., P. Sarraf, A. E. Troy, G. Bradwin, K. Moore, D. S. Milstone, B. M. Spiegelman and R. M. Mortensen (1999). PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell* **4**(4): 611-7.

Rosenvinge, A., R. Krogh-Madsen, B. Baslund and B. K. Pedersen (2007). Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNFalpha therapy. *Scand J Rheumatol* **36**(2): 91-6.

Ross, J. A. and C. M. Kasum (2002). Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr* **22**: 19-34.

Rossi, A. S., Y. B. Lombardo, J. M. Lacorte, A. G. Chicco, C. Rouault, G. Slama and S. W. Rizkalla (2005). Dietary fish oil positively regulates plasma leptin and adiponectin levels in sucrose-fed, insulin-resistant rats. *Am J Physiol Regul Integr Comp Physiol* **289**(2): R486-R494.

Rousseau, V., D. J. Becker, L. N. Ongemba, J. Rahier, J. C. Henquin and S. M. Brichard (1997). Developmental and nutritional changes of ob and PPAR gamma 2 gene expression in rat white adipose tissue. *Biochem J* **321 (Pt 2)**: 451-6.

Ruan, H. and H. F. Lodish (2003). Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. *Cytokine Growth Factor Rev* **14**(5): 447-55.

Rustan, A. C., E. N. Christiansen and C. A. Drevon (1992). Serum lipids, hepatic glycerolipid metabolism and peroxisomal fatty acid oxidation in rats fed omega-3 and omega-6 fatty acids. *Biochem J* **283 (Pt 2)**: 333-9.

Rusznyák, S. T. and A. Szent-Györgyi (1936). Vitamin P: Flavonols as Vitamins. *Nature*(138): 27-27.

S, X. and O. Pmo (2011). Naringin Ameliorates Atherogenic Dyslipidemia but Not Hyperglycemia in Rats with Type 1 Diabetes. *J Cardiovasc Pharmacol*.

Sainsbury, A., I. Cusin, F. Rohner-Jeanrenaud and B. Jeanrenaud (1997). Adrenalectomy prevents the obesity syndrome produced by chronic central neuropeptide Y infusion in normal rats. *Diabetes* **46**(2): 209-14.

Sakurai, H., H. Chiba, H. Miyoshi, T. Sugita and W. Toriumi (1999). IkappaB kinases phosphorylate NF-kappaB p65 subunit on serine 536 in the transactivation domain. *J Biol Chem* **274**(43): 30353-6.

Sanchez-Lozada, L. G., E. Tapia, A. Jimenez, P. Bautista, M. Cristobal, T. Nepomuceno, V. Soto, C. Avila-Casado, T. Nakagawa, R. J. Johnson, J. Herrera-Acosta and M. Franco (2007). Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. *Am J Physiol Renal Physiol* **292**(1): F423-9.

Sandra, A., D. J. Fyler and S. J. Marshall (1984). Effects of lipids on the transport activity of the reconstituted glucose transport system from rat adipocyte. *Biochim Biophys Acta* **778**(3): 511-5.

Sartippour, M. R. and G. Renier (2000). Differential regulation of macrophage peroxisome proliferator-activated receptor expression by glucose : role of peroxisome proliferator-activated receptors in lipoprotein lipase gene expression. *Arterioscler Thromb Vasc Biol* **20**(1): 104-10.

Satoh, H., M. T. Nguyen, P. D. Miles, T. Imamura, I. Usui and J. M. Olefsky (2004). Adenovirus-mediated chronic "hyper-resistinemia" leads to in vivo insulin resistance in normal rats. *J Clin Invest* **114**(2): 224-31.

Scarpace, P. J., M. Matheny, B. H. Pollock and N. Tumer (1997). Leptin increases uncoupling protein expression and energy expenditure. *Am J Physiol* **273**(1 Pt 1): E226-30.

Scherer, P. E., S. Williams, M. Fogliano, G. Baldini and H. F. Lodish (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* **270**(45): 26746-9.

Schmitz, G. and J. Ecker (2008). The opposing effects of n-3 and n-6 fatty acids. *Prog Lipid Res* **47**(2): 147-55.

Schoonjans, K., J. Peinado-Onsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels and J. Auwerx (1996a). PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* **15**(19): 5336-48.

Schoonjans, K., B. Staels and J. Auwerx (1996b). The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* **1302**(2): 93-109.

Schulze, M. B., I. Shai, E. B. Rimm, T. Li, N. Rifai and F. B. Hu (2005). Adiponectin and future coronary heart disease events among men with type 2 diabetes. *Diabetes* **54**(2): 534-9.

Schultz, J. R., J. G. Verstuyft, E. L. Gong, A. V. Nichols and E. M. Rubin (1993). Protein composition determines the anti-atherogenic properties of HDL in transgenic mice. *Nature* **365**(6448): 762-4.

Schwabe, U., R. Ebert and H. C. Erbler (1975). Adenosine release from fat cells: effect on cyclic AMP levels and hormone actions. *Adv Cyclic Nucleotide Res* **5**: 569-84.

Schwartz, M. W., D. G. Baskin, K. J. Kaiyala and S. C. Woods (1999). Model for the regulation of energy balance and adiposity by the central nervous system. *Am J Clin Nutr* **69**(4): 584-96.

Schwartz, M. W., D. G. Baskin, T. R. Bukowski, J. L. Kuijper, D. Foster, G. Lasser, D. E. Prunkard, D. Porte, Jr., S. C. Woods, R. J. Seeley and D. S. Weigle (1996). Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. *Diabetes* **45**(4): 531-5.

Segal, K. R., M. Landt and S. Klein (1996). Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. *Diabetes* **45**(7): 988-91.

Segrest, J. P., M. K. Jones, H. De Loof and N. Dashti (2001). Structure of apolipoprotein B-100 in low density lipoproteins. *J Lipid Res* **42**(9): 1346-67.

Settasatian, N., M. Duong, L. K. Curtiss, C. Ehnholm, M. Jauhiainen, J. Huuskonen and K. A. Rye (2001). The mechanism of the remodeling of high density lipoproteins by phospholipid transfer protein. *J Biol Chem* **276**(29): 26898-905.

Seufert, J., T. J. Kieffer and J. F. Habener (1999). Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. *Proc Natl Acad Sci U S A* **96**(2): 674-9.

Sevilla, L., A. Guma, G. Enrique-Tarancon, S. Mora, P. Munoz, M. Palacin, X. Testar and A. Zorzano (1997). Chronic high-fat feeding and middle-aging reduce in an additive fashion Glut4 expression in skeletal muscle and adipose tissue. *Biochem Biophys Res Commun* **235**(1): 89-93.

Shafi, S., S. E. Brady, A. Bensadoun and R. J. Havel (1994). Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J Lipid Res* **35**(4): 709-20.

Shan, W. F., B. Q. Chen, S. J. Zhu, L. Jiang and Y. F. Zhou (2011). Effects of GLUT4 expression on insulin resistance in patients with advanced liver cirrhosis. *J Zhejiang Univ Sci B* **12**(8): 677-82.

Sheng, T. and K. Yang (2008). Adiponectin and its association with insulin resistance and type 2 diabetes. *J Genet Genomics* **35**(6): 321-6.

Shibata, M., F. Hakuno, D. Yamanaka, H. Okajima, T. Fukushima, T. Hasegawa, T. Ogata, Y. Toyoshima, K. Chida, K. Kimura, H. Sakoda, A. Takenaka, T. Asano and S. Takahashi (2010). Paraquat-induced oxidative stress represses phosphatidylinositol 3-kinase activities leading to impaired glucose uptake in 3T3-L1 adipocytes. *J Biol Chem* **285**(27): 20915-25.

Shih, C. C., C. H. Lin, W. L. Lin and J. B. Wu (2009). Momordica charantia extract on insulin resistance and the skeletal muscle GLUT4 protein in fructose-fed rats. *J Ethnopharmacol* **123**(1): 82-90.

Shimabukuro, M., Y. T. Zhou, Y. Lee and R. H. Unger (1998). Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. *J Biol Chem* **273**(6): 3547-50.

Shimamoto, K. and N. Ura (2006). Mechanisms of insulin resistance in hypertensive rats. *Clin Exp Hypertens* **28**(6): 543-52.

Shimano, H., N. Yahagi, M. Amemiya-Kudo, A. H. Hasty, J. Osuga, Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, T. Gotoda, S. Ishibashi and N. Yamada (1999). Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J Biol Chem* **274**(50): 35832-9.

Shimano, H., J. D. Horton, R. E. Hammer, I. Shimomura, M. S. Brown and J. L. Goldstein (1996). Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest* **98**(7): 1575-84.

Shimomura, I., H. Shimano, B. S. Korn, Y. Bashmakov and J. D. Horton (1998). Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J Biol Chem* **273**(52): 35299-306.

Shirouchi, B., K. Nagao, N. Inoue, T. Ohkubo, H. Hibino and T. Yanagita (2007). Effect of dietary omega 3 phosphatidylcholine on obesity-related disorders in obese Otsuka Long-Evans Tokushima fatty rats. *J Agric Food Chem* **55**(17): 7170-6.

Shoelson, S. E., J. Lee and A. B. Goldfine (2006). Inflammation and insulin resistance. *J Clin Invest* **116**(7): 1793-801.

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. *J Clin Invest* **106**(2): 171-6.

Siddle, K. (2011). Signalling by insulin and IGF receptors: supporting acts and new players. *J Mol Endocrinol* **47**(1): R1-10.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* **56**(8): 365-79.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *Am J Clin Nutr* **70**(3 Suppl): 560S-569S.

Simpson, I. A. and S. W. Cushman (1986). Hormonal regulation of mammalian glucose transport. *Annu Rev Biochem* **55**: 1059-89.

Simsolo, R. B., J. M. Ong, B. Saffari and P. A. Kern (1992). Effect of improved diabetes control on the expression of lipoprotein lipase in human adipose tissue. *J Lipid Res* **33**(1): 89-95.

Sindelar, D. K., P. J. Havel, R. J. Seeley, C. W. Wilkinson, S. C. Woods and M. W. Schwartz (1999). Low plasma leptin levels contribute to diabetic hyperphagia in rats. *Diabetes* **48**(6): 1275-80.

Sinitskaya, N., S. Gourmelen, C. Schuster-Klein, B. Guardiola-Lemaitre, P. Pevet and E. Challet (2007). Increasing the fat-to-carbohydrate ratio in a high-fat diet prevents the development of obesity but not a prediabetic state in rats. *Clin Sci (Lond)* **113**(10): 417-25.

Sivitz, W. I., S. L. DeSautel, T. Kayano, G. I. Bell and J. E. Pessin (1989). Regulation of glucose transporter messenger RNA in insulin-deficient states. *Nature* **340**(6228): 72-4.

Sloop, C. H., L. Dory and P. S. Roheim (1987). Interstitial fluid lipoproteins. *J Lipid Res* **28**(3): 225-37.

Smith, U. (2002). Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance--is insulin resistance initiated in the adipose tissue? *Int J Obes Relat Metab Disord* **26**(7): 897-904.

Smith, C. J. and V. C. Manganiello (1989). Role of hormone-sensitive low Km cAMP phosphodiesterase in regulation of cAMP-dependent protein kinase and lipolysis in rat adipocytes. *Mol Pharmacol* **35**(3): 381-6.

Song, S., S. Andrikopoulos, C. Filippis, A. W. Thorburn, D. Khan and J. Proietto (2001). Mechanism of fat-induced hepatic gluconeogenesis: effect of metformin. *Am J Physiol Endocrinol Metab* **281**(2): E275-82.

Soria, A., A. Chicco, M. Eugenia D'Alessandro, A. Rossi and Y. B. Lombardo (2002). Dietary fish oil reverse epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipemic sucrose fed rat model small star, filled. *J Nutr Biochem* **13**(4): 209-218.

Soukas, A., P. Cohen, N. D. Socci and J. M. Friedman (2000). Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* **14**(8): 963-80.

Soutar, A. K., C. W. Garner, H. N. Baker, J. T. Sparrow, R. L. Jackson, A. M. Gotto and L. C. Smith (1975). Effect of the human plasma apolipoproteins and phosphatidylcholine acyl donor on the activity of lecithin: cholesterol acyltransferase. *Biochemistry* **14**(14): 3057-64.

Spady, D. K. (1993). Regulatory effects of individual n-6 and n-3 polyunsaturated fatty acids on LDL transport in the rat. *J Lipid Res* **34**(8): 1337-46.

Sridhar, M. G., R. Vinayagamoorthi, V. Arul Suyambunathan, Z. Bobby and N. Selvaraj (2008). Bitter gourd (Momordica charantia) improves insulin sensitivity by increasing skeletal muscle insulin-stimulated IRS-1 tyrosine phosphorylation in high-fat-fed rats. *Br J Nutr* **99**(4): 806-12.

Srinivasan, K. and P. Ramarao (2007). Animal models in type 2 diabetes research: an overview. *Indian J Med Res* **125**(3): 451-72.

Stanley Mainzen Prince, P. and N. Kamalakkannan (2006). Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. *J Biochem Mol Toxicol* **20**(2): 96-102.

Stefan, N., B. Vozarova, T. Funahashi, Y. Matsuzawa, C. Weyer, R. S. Lindsay, J. F. Youngren, P. J. Havel, R. E. Pratley, C. Bogardus and P. A. Tataranni (2002). Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. *Diabetes* **51**(6): 1884-8.

Stehno-Bittel, L. (2008). Intricacies of fat. *Phys Ther* **88**(11): 1265-78.

Stephens, J. M., J. Lee and P. F. Pilch (1997). Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *J Biol Chem* **272**(2): 971-6.

Stephens, J. M. and P. H. Pekala (1991). Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. *J Biol Chem* **266**(32): 21839-45.

Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima and M. A. Lazar (2001). The hormone resistin links obesity to diabetes. *Nature* **409**(6818): 307-12.

Storch, J., J. H. Veerkamp and K. T. Hsu (2002). Similar mechanisms of fatty acid transfer from human anal rodent fatty acid-binding proteins to membranes: liver, intestine, heart muscle, and adipose tissue FABPs. *Mol Cell Biochem* **239**(1-2): 25-33.

Storlien, L. H., E. W. Kraegen, D. J. Chisholm, G. L. Ford, D. G. Bruce and W. S. Pascoe (1987). Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* **237**(4817): 885-8.

Storlien, L. H., D. E. James, K. M. Burleigh, D. J. Chisholm and E. W. Kraegen (1986). Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *Am J Physiol* **251**(5 Pt 1): E576-83.

Stralfors, P. and R. C. Honnor (1989). Insulin-induced dephosphorylation of hormone-sensitive lipase. Correlation with lipolysis and cAMP-dependent protein kinase activity. *Eur J Biochem* **182**(2): 379-85.

Stuart, C. A., G. Wen, W. C. Gustafson and E. A. Thompson (2000). Comparison of GLUT1, GLUT3, and GLUT4 mRNA and the subcellular distribution of their proteins in normal human muscle. *Metabolism* **49**(12): 1604-9.

Stuart, C. A., D. Yin, M. E. Howell, R. J. Dykes, J. J. Laffan and A. A. Ferrando (2006). Hexose transporter mRNAs for GLUT4, GLUT5, and GLUT12 predominate in human muscle. *Am J Physiol Endocrinol Metab* **291**(5): E1067-73.

Sul, H. S., M. J. Latasa, Y. Moon and K. H. Kim (2000). Regulation of the fatty acid synthase promoter by insulin. *J Nutr* **130**(2S Suppl): 315S-320S.

Sun, C., Z. W. Wei and Y. Li (2011). DHA regulates lipogenesis and lipolysis genes in mice adipose and liver. *Mol Biol Rep* **38**(2): 731-7.

Sun, Q., L. Li, R. Li, M. Yang, H. Liu, M. J. Nowicki, H. Zong, J. Xu and G. Yang (2009). Overexpression of visfatin/PBEF/Nampt alters whole-body insulin sensitivity and lipid profile in rats. *Ann Med* **41**(4): 311-20.

Suresh, Y. and U. N. Das (2003). Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus. Effect of omega-3 fatty acids. *Nutrition* **19**(3): 213-28.

Surette, M. E., J. Whelan, K. S. Broughton and J. E. Kinsella (1992). Evidence for mechanisms of the hypotriglyceridemic effect of n-3 polyunsaturated fatty acids. *Biochim Biophys Acta* **1126**(2): 199-205.

Szapary, P. O. and D. J. Rader (2001). Pharmacological management of high triglycerides and low high-density lipoprotein cholesterol. *Curr Opin Pharmacol* **1**(2): 113-20.

Sztalryd, C. and F. B. Kraemer (1994). Regulation of hormone-sensitive lipase during fasting. *Am J Physiol* **266**(2 Pt 1): E179-85.

Takahashi, M., N. Tsuboyama-Kasaoka, T. Nakatani, M. Ishii, S. Tsutsumi, H. Aburatani and O. Ezaki (2002). Fish oil feeding alters liver gene expressions to defend against PPARalpha activation and ROS production. *Am J Physiol Gastrointest Liver Physiol* **282**(2): G338-48.

Takahashi, Y. and T. Ide (2000). Dietary n-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. *Br J Nutr* **84**(2): 175-84.

Takeuchi, H., S. Sekine, O. Noguchi, Y. Murano, T. Aoyama and T. Matsuo (2009). Effect of life-long dietary n-6/n-3 fatty acid ratio on life span, serum lipids and serum glucose in Wistar rats. *J Nutr Sci Vitaminol (Tokyo)* **55**(5): 394-9.

Tall, A. R., E. Abreu and J. Shuman (1983). Separation of a plasma phospholipid transfer protein from cholesterol ester/phospholipid exchange protein. *J Biol Chem* **258**(4): 2174-80.

Tan, K. C., M. B. Cooper, K. L. Ling, B. A. Griffin, D. J. Freeman, C. J. Packard, J. Shepherd, C. N. Hales and D. J. Betteridge (1995). Fasting and postprandial determinants for the occurrence of small dense LDL species in non-insulin-dependent diabetic patients with and without hypertriglyceridaemia: the involvement of insulin, insulin precursor species and insulin resistance. *Atherosclerosis* **113**(2): 273-87.

Tanaka, T., J. Yamamoto, S. Iwasaki, H. Asaba, H. Hamura, Y. Ikeda, M. Watanabe, K. Magoori, R. X. Ioka, K. Tachibana, Y. Watanabe, Y. Uchiyama, K. Sumi, H. Iguchi, S. Ito, T. Doi, T. Hamakubo, M. Naito, J. Auwerx, M. Yanagisawa, T. Kodama and J. Sakai (2003).

Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci U S A* **100**(26): 15924-9.

Taouis, M., C. Dagou, C. Ster, G. Durand, M. Pinault and J. Delarue (2002). N-3 polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. *Am J Physiol Endocrinol Metab* **282**(3): E664-71.

Tappy, L. and K. A. Lê (2010). Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev* **90**(1): 23-46.

Tartaglia, L. A. (1997). The leptin receptor. *J Biol Chem* **272**(10): 6093-6.

Taskinen, M. R. (1987). Lipoprotein lipase in diabetes. *Diabetes Metab Rev* **3**(2): 551-70.

Tato, F., G. L. Vega and S. M. Grundy (1997). Determinants of plasma HDL-cholesterol in hypertriglyceridemic patients. Role of cholesterol-ester transfer protein and lecithin cholesteryl acyl transferase. *Arterioscler Thromb Vasc Biol* **17**(1): 56-63.

Taylor, K. G., D. J. Galton and G. Holdsworth (1979). Insulin-independent diabetes: a defect in the activity of lipoprotein lipase in adipose tissue. *Diabetologia* **16**(5): 313-7.

Thorens, B., M. J. Charron and H. F. Lodish (1990). Molecular physiology of glucose transporters. *Diabetes Care* **13**(3): 209-18.

Tontonoz, P., E. Hu, J. Devine, E. G. Beale and B. M. Spiegelman (1995). PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol* **15**(1): 351-7.

Tontonoz, P., E. Hu and B. M. Spiegelman (1994). Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* **79**(7): 1147-56.

Topping, D. L. and P. A. Mayes (1972). The immediate effects of insulin and fructose on the metabolism of the perfused liver. Changes in lipoprotein secretion, fatty acid oxidation and esterification, lipogenesis and carbohydrate metabolism. *Biochem J* **126**(2): 295-311.

Tordjman, K. M., K. A. Leingang, D. E. James and M. M. Mueckler (1989). Differential regulation of two distinct glucose transporter species expressed in 3T3-L1 adipocytes: effect of chronic insulin and tolbutamide treatment. *Proc Natl Acad Sci U S A* **86**(20): 7761-5.

Tornvall, P., G. Olivecrona, F. Karpe, A. Hamsten and T. Olivecrona (1995). Lipoprotein lipase mass and activity in plasma and their increase after heparin are separate parameters with different relations to plasma lipoproteins. *Arterioscler Thromb Vasc Biol* **15**(8): 1086-93.

Torti, F. M., B. Dieckmann, B. Beutler, A. Cerami and G. M. Ringold (1985). A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia. *Science* **229**(4716): 867-9.

Tracey, K. J., S. F. Lowry, T. J. Fahey, 3rd, J. D. Albert, Y. Fong, D. Hesse, B. Beutler, K. R. Manogue, S. Calvano, H. Wei and et al. (1987). Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. *Surg Gynecol Obstet* **164**(5): 415-22.

Travers, M. E. and M. I. McCarthy (2011). Type 2 diabetes and obesity: genomics and the clinic. *Hum Genet* **130**(1): 41-58.

Tulipano, G., A. V. Vergoni, D. Soldi, E. E. Muller and D. Cocchi (2004). Characterization of the resistance to the anorectic and endocrine effects of leptin in obesity-prone and obesity-resistant rats fed a high-fat diet. *J Endocrinol* **183**(2): 289-98.

Unger, R. H., Y. T. Zhou and L. Orci (1999). Regulation of fatty acid homeostasis in cells: novel role of leptin. *Proc Natl Acad Sci U S A* **96**(5): 2327-32.

Utzschneider, K. M., D. B. Carr, J. Tong, T. M. Wallace, R. L. Hull, S. Zraika, Q. Xiao, J. S. Mistry, B. M. Retzlaff, R. H. Knopp and S. E. Kahn (2005). Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. *Diabetologia* **48**(11): 2330-3.

Uysal, K. T., S. M. Wiesbrock, M. W. Marino and G. S. Hotamisligil (1997). Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* **389**(6651): 610-4.

Vague, J. (1947). La différenciation sexuelle, facteur déterminant des formes de l'obésité. *Presse Méd* **55**(30): 339-340.

van den Hoek, A. M., B. Teusink, P. J. Voshol, L. M. Havekes, J. A. Romijn and H. Pijl (2008). Leptin deficiency per se dictates body composition and insulin action in ob/ob mice. *J Neuroendocrinol* **20**(1): 120-7.

van der Velde, A. E. (2010). Reverse cholesterol transport: from classical view to new insights. *World J Gastroenterol* **16**(47): 5908-15.

Van Harmelen, V., S. Reynisdottir, K. Cianflone, E. Degerman, J. Hoffstedt, K. Nilsell, A. Sniderman and P. Arner (1999). Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. *J Biol Chem* **274**(26): 18243-51.

van Zwieten, P. A. (1999). Diabetes and hypertension: experimental models for pharmacological studies. *Clin Exp Hypertens* **21**(1-2): 1-16.

Vazquez-Vela, M. E., N. Torres and A. R. Tovar (2008). White adipose tissue as endocrine organ and its role in obesity. *Arch Med Res* **39**(8): 715-28.

Vidal-Puig, A. J., R. V. Considine, M. Jimenez-Linan, A. Werman, W. J. Pories, J. F. Caro and J. S. Flier (1997). Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* **99**(10): 2416-22.

Villanueva, E. C. and M. G. Myers, Jr. (2008). Leptin receptor signaling and the regulation of mammalian physiology. *Int J Obes (Lond)* **32 Suppl 7**: S8-12.

Vinayagamoorthi, R., Z. Bobby and M. G. Sridhar (2008). Antioxidants preserve redox balance and inhibit c-Jun-N-terminal kinase pathway while improving insulin signaling in fat-fed rats: evidence for the role of oxidative stress on IRS-1 serine phosphorylation and insulin resistance. *J Endocrinol* **197**(2): 287-96.

Von Diemen, V., E. N. Trindade and M. R. Trindade (2006). [Experimental model to induce obesity in rats]. *Acta Cir Bras* **21**(6): 425-9.

von Schacky, C. (2003). The role of omega-3 fatty acids in cardiovascular disease. *Curr Atheroscler Rep* **5**(2): 139-45.

Vu-Dac, N., K. Schoonjans, V. Kosykh, J. Dallongeville, J. C. Fruchart, B. Staels and J. Auwerx (1995). Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. *J Clin Invest* **96**(2): 741-50.

Vu-Dac, N., K. Schoonjans, B. Laine, J. C. Fruchart, J. Auwerx and B. Staels (1994). Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. *J Biol Chem* **269**(49): 31012-8.

Wabitsch, M., P. B. Jensen, W. F. Blum, C. T. Christoffersen, P. Englaro, E. Heinze, W. Rascher, W. Teller, H. Tornqvist and H. Hauner (1996). Insulin and cortisol promote leptin production in cultured human fat cells. *Diabetes* **45**(10): 1435-8.

Wallach, D., E. E. Varfolomeev, N. L. Malinin, Y. V. Goltsev, A. V. Kovalenko and M. P. Boldin (1999). Tumor necrosis factor receptor and Fas signaling mechanisms. *Annu Rev Immunol* **17**: 331-67.

Wang, C. Y. and J. K. Liao (2012). A mouse model of diet-induced obesity and insulin resistance. *Methods Mol Biol* **821**: 421-33.

Wang, X., J. Hasegawa, Y. Kitamura, Z. Wang, A. Matsuda, W. Shinoda, N. Miura and K. Kimura (2011). Effects of Hesperidin on the Progression of Hypercholesterolemia and Fatty Liver Induced by High-Cholesterol Diet in Rats. *J Pharmacol Sci*.

Wang, D. Q., F. Lammert, D. E. Cohen, B. Paigen and M. C. Carey (1999a). Cholic acid aids absorption, biliary secretion, and phase transitions of cholesterol in murine cholelithogenesis. *Am J Physiol* **276**(3 Pt 1): G751-60.

Wang, M. Y., Y. Lee and R. H. Unger (1999b). Novel form of lipolysis induced by leptin. *J Biol Chem* **274**(25): 17541-4.

Wang, C. S., W. J. McConathy, H. U. Kloer and P. Alaupovic (1985). Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J Clin Invest* **75**(2): 384-90.

Warden, C. H., C. C. Hedrick, J. H. Qiao, L. W. Castellani and A. J. Lusis (1993). Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. *Science* **261**(5120): 469-72.

Watanabe, N., K. Onuma, K. Fujimoto, S. Miyake and T. Nakamura (2011). Long-term effect of an enteral diet with a different n-6/n-3 ratio on fatty acid composition and blood parameters in rats. *J Oleo Sci* **60**(3): 109-15.

Way, J. M., W. W. Harrington, K. K. Brown, W. K. Gottschalk, S. S. Sundseth, T. A. Mansfield, R. K. Ramachandran, T. M. Willson and S. A. Kliewer (2001). Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene expression in multiple insulin-sensitive tissues. *Endocrinology* **142**(3): 1269-77.

Webber, J. (2003). Energy balance in obesity. *Proc Nutr Soc* **62**(2): 539-43.

Weinstock, P. H., S. Levak-Frank, L. C. Hudgins, H. Radner, J. M. Friedman, R. Zechner and J. L. Breslow (1997). Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. *Proc Natl Acad Sci U S A* **94**(19): 10261-6.

Weisberg, S. P., D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel and A. W. Ferrante, Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **112**(12): 1796-808.

Welsh, G. I., I. Hers, D. C. Berwick, G. Dell, M. Wherlock, R. Birkin, S. Leney and J. M. Tavare (2005). Role of protein kinase B in insulin-regulated glucose uptake. *Biochem Soc Trans* **33**(Pt 2): 346-9.

Wesche, P. (2009). Rodents: clinical pathology. *BSAVA manual of rodents and ferrets*. E. Keeble and A. Meredith. Gloucester, England, BSAVA British small animal veterinary association**:** 43.

Westerveld, H. T., J. C. de Graaf, H. H. van Breugel, J. W. Akkerman, J. J. Sixma, D. W. Erkelens and J. D. Banga (1993). Effects of low-dose EPA-E on glycemic control, lipid profile, lipoprotein(a), platelet aggregation, viscosity, and platelet and vessel wall interaction in NIDDM. *Diabetes Care* **16**(5): 683-8.

Weyer, C., T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R. E. Pratley and P. A. Tataranni (2001). Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**(5): 1930-5.

White, M. F. (2003). Insulin signaling in health and disease. *Science* **302**(5651): 1710-1.

White, M. F. (2002). IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* **283**(3): E413-22.

Whitlock, G., S. Lewington, P. Sherliker, R. Clarke, J. Emberson, J. Halsey, N. Qizilbash, R. Collins and R. Peto (2009). Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* **373**(9669): 1083-96.

WHO (2004). Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. **363:** 157-63.

WHO (2000). Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser*. **894:** i-xii, 1-253.

WHO (1995). Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser*. **854:** 1-452.

Wilcox, G. (2005). Insulin and insulin resistance. *Clin Biochem Rev* **26**(2): 19-39.

Wilcox, L. J., N. M. Borradaile, L. E. de Dreu and M. W. Huff (2001). Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *J Lipid Res* **42**(5): 725-34.

Windler, E. and R. J. Havel (1985). Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. *J Lipid Res* **26**(5): 556-65.

Wong, S. H. and J. B. Marsh (1988). Inhibition of apolipoprotein secretion and phosphatidate phosphohydrolase activity by eicosapentaenoic and docosahexaenoic acids in the perfused rat liver. *Metabolism* **37**(12): 1177-81.

Wood, I. S., L. Hunter and P. Trayhurn (2003). Expression of Class III facilitative glucose transporter genes (GLUT-10 and GLUT-12) in mouse and human adipose tissues. *Biochem Biophys Res Commun* **308**(1): 43-9.

Wood, I. S. and P. Trayhurn (2003). Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr* **89**(1): 3-9.

Woods, S. C., R. J. Seeley, P. A. Rushing, D. D'Alessio and P. Tso (2003). A controlled high-fat diet induces an obese syndrome in rats. *J Nutr* **133**(4): 1081-7.

Woods, S. C., R. J. Seeley, D. Porte, Jr. and M. W. Schwartz (1998). Signals that regulate food intake and energy homeostasis. *Science* **280**(5368): 1378-83.

Wu, P., J. M. Peters and R. A. Harris (2001). Adaptive increase in pyruvate dehydrogenase kinase 4 during starvation is mediated by peroxisome proliferator-activated receptor alpha. *Biochem Biophys Res Commun* **287**(2): 391-6.

Xu, G. F., F. Zhu, C. X. Liu, H. Li and M. Shi (2005). [Study on serum leptin level of urban resident and the relationship between body mass index, blood lipid, insulin and leptin level]. *Wei Sheng Yan Jiu* **34**(2): 205-7.

Xu, H. E., M. H. Lambert, V. G. Montana, D. J. Parks, S. G. Blanchard, P. J. Brown, D. D. Sternbach, J. M. Lehmann, G. B. Wisely, T. M. Willson, S. A. Kliewer and M. V. Milburn (1999a). Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol Cell* **3**(3): 397-403.

Xu, J., M. T. Nakamura, H. P. Cho and S. D. Clarke (1999b). Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J Biol Chem* **274**(33): 23577-83.

Yadav, H., S. Jain, M. Yadav, P. R. Sinha, G. B. Prasad and F. Marotta (2009). Epigenomic derangement of hepatic glucose metabolism by feeding of high fructose diet and its prevention by Rosiglitazone in rats. *Dig Liver Dis* **41**(7): 500-8.

Yamamoto, T., H. Fukumoto, G. Koh, H. Yano, K. Yasuda, K. Masuda, H. Ikeda, H. Imura and Y. Seino (1991). Liver and muscle-fat type glucose transporter gene expression in obese and diabetic rats. *Biochem Biophys Res Commun* **175**(3): 995-1002.

Yamamoto, K., F. Kamo-Yamada, S. Cho and M. Sugano (1980). Some observations on the cholesterol esterifying and cholesterol ester hydrolyzing activities in dog plasma. *J Biochem* **87**(5): 1271-8.

Yamauchi, T., Y. Nio, T. Maki, M. Kobayashi, T. Takazawa, M. Iwabu, M. Okada-Iwabu, S. Kawamoto, N. Kubota, T. Kubota, Y. Ito, J. Kamon, A. Tsuchida, K. Kumagai, H. Kozono, Y. Hada, H. Ogata, K. Tokuyama, M. Tsunoda, T. Ide, K. Murakami, M. Awazawa, I. Takamoto, P. Froguel, K. Hara, K. Tobe, R. Nagai, K. Ueki and T. Kadowaki (2007). Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* **13**(3): 332-9.

Yamauchi, T., J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N. H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai and T. Kadowaki (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* **423**(6941): 762-9.

Yamauchi, T., J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B. B. Kahn and T. Kadowaki (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* **8**(11): 1288-95.

Yamauchi, T., J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M. L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel and T. Kadowaki (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* **7**(8): 941-6.

Yancey, P. G., A. E. Bortnick, G. Kellner-Weibel, M. de la Llera-Moya, M. C. Phillips and G. H. Rothblat (2003). Importance of different pathways of cellular cholesterol efflux. *Arterioscler Thromb Vasc Biol* **23**(5): 712-9.

Yaqoob, P., E. J. Sherrington, N. M. Jeffery, P. Sanderson, D. J. Harvey, E. A. Newsholme and P. C. Calder (1995). Comparison of the effects of a range of dietary lipids upon serum and tissue lipid composition in the rat. *Int J Biochem Cell Biol* **27**(3): 297-310.

Yaspelkis, B. B., 3rd, J. R. Davis, M. Saberi, T. L. Smith, R. Jazayeri, M. Singh, V. Fernandez, B. Trevino, N. Chinookoswong, J. Wang, Z. Q. Shi and N. Levin (2001). Leptin administration improves skeletal muscle insulin responsiveness in diet-induced insulin-resistant rats. *Am J Physiol Endocrinol Metab* **280**(1): E130-42.

Yatagai, T., S. Nagasaka, A. Taniguchi, M. Fukushima, T. Nakamura, A. Kuroe, Y. Nakai and S. Ishibashi (2003). Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. *Metabolism* **52**(10): 1274-8.

Yokota, T., K. Oritani, I. Takahashi, J. Ishikawa, A. Matsuyama, N. Ouchi, S. Kihara, T. Funahashi, A. J. Tenner, Y. Tomiyama and Y. Matsuzawa (2000). Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* **96**(5): 1723-32.

Yoshida, H., N. Takamura, T. Shuto, K. Ogata, J. Tokunaga, K. Kawai and H. Kai (2010). The citrus flavonoids hesperetin and naringenin block the lipolytic actions of TNF-alpha in mouse adipocytes. *Biochem Biophys Res Commun* **394**(3): 728-32.

Young, P. W., M. A. Cawthorne, P. J. Coyle, J. C. Holder, G. D. Holman, I. J. Kozka, D. M. Kirkham, C. A. Lister and S. A. Smith (1995). Repeat treatment of obese mice with BRL 49653, a new potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. *Diabetes* **44**(9): 1087-92.

Zakrzewska, K. E., I. Cusin, A. Stricker-Krongrad, O. Boss, D. Ricquier, B. Jeanrenaud and F. Rohner-Jeanrenaud (1999). Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat. *Diabetes* **48**(2): 365-70.

Zakrzewska, K. E., I. Cusin, A. Sainsbury, F. Rohner-Jeanrenaud and B. Jeanrenaud (1997). Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. *Diabetes* **46**(4): 717-9.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold and J. M. Friedman (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**(6505): 425-32.

Zhao, S., Y. Chu, C. Zhang, Y. Lin, K. Xu, P. Yang, J. Fan and E. Liu (2008). Diet-induced central obesity and insulin resistance in rabbits. *J Anim Physiol Anim Nutr (Berl)* **92**(1): 105-11.

Zhao, Y., S. Joshi-Barve, S. Barve and L. H. Chen (2004). Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. *J Am Coll Nutr* **23**(1): 71-8.

Zheng, X., M. Avella and K. M. Botham (2001). Comparison of the effects of dietary n-3 and n-6 polyunsaturated fatty acids on very-low-density lipoprotein secretion when delivered to hepatocytes in chylomicron remnants. *Biochem J* **357**(Pt 2): 481-7.

Zhong, S., I. J. Goldberg, C. Bruce, E. Rubin, J. L. Breslow and A. Tall (1994). Human ApoA-II inhibits the hydrolysis of HDL triglyceride and the decrease of HDL size induced by hypertriglyceridemia and cholesteryl ester transfer protein in transgenic mice. *J Clin Invest* **94**(6): 2457-67.

Zhou, Q. L., J. G. Park, Z. Y. Jiang, J. J. Holik, P. Mitra, S. Semiz, A. Guilherme, A. M. Powelka, X. Tang, J. Virbasius and M. P. Czech (2004). Analysis of insulin signalling by RNAi-based gene silencing. *Biochem Soc Trans* **32**(Pt 5): 817-21.

Ziemke, F. and C. S. Mantzoros (2010). Adiponectin in insulin resistance: lessons from translational research. *Am J Clin Nutr* **91**(1): 258S-261S.

Zierath, J. R., E. U. Frevert, J. W. Ryder, P. O. Berggren and B. B. Kahn (1998). Evidence against a direct effect of leptin on glucose transport in skeletal muscle and adipocytes. *Diabetes* **47**(1): 1-4.

Zierler, K. L. (1976). Fatty acids as substrates for heart and skeletal muscle. *Circ Res* **38**(6): 459-63.

Zisman, A., O. D. Peroni, E. D. Abel, M. D. Michael, F. Mauvais-Jarvis, B. B. Lowell, J. F. Wojtaszewski, M. F. Hirshman, A. Virkamaki, L. J. Goodyear, C. R. Kahn and B. B. Kahn (2000). Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med* **6**(8): 924-8.

Zorzano, A., W. Wilkinson, N. Kotliar, G. Thoidis, B. E. Wadzinkski, A. E. Ruoho and P. F. Pilch (1989). Insulin-regulated glucose uptake in rat adipocytes is mediated by two transporter isoforms present in at least two vesicle populations. *J Biol Chem* **264**(21): 12358-63.

Obesity and insulin resistance in rats fed with high-fat or high-fructose diet. Nutritional interventions with EPA or flavanones.

High prevalence of obesity and related disorders has made the use of animal models essential. We aimed to study obesity and insulin sensitivity (IS) in rats fed with either a control, a high-fat (HF), or a high-fructose (HFr) diet. After 10 weeks, HF led to obesity, higher plasma cholesterol (TC) level and lower IS and higher expression of fatty acid synthase, TNFα, leptin and perilipin in adipose tissue; whereas HFr resulted in no change in IS and lipidemia, but enhanced PPARα expression. HF had more deleterious effects than HFr to induce obesity and lower IS in rats.

Nutritional interventions have been used to lower obesity and improve IS. We studied the effects of EPA supplementation (EPA-suppl.) for 10 weeks. EPA-suppl. avoided the drop in IS in HF fed rats, associated with higher expression of IRS-1 and GLUT4 in adipose tissue; however hepatic gene expression did not support any insulin-sensitizing effect. EPA-suppl. tended to lower plasma triglycerides (TG) and non-esterified fatty acids and enhanced HSL and LPL expression in the liver of HFr fed rats. The response to EPA-suppl. was therefore dependent on the nature of the diet.

Flavonoids have been studied for their potential hypolipidemic effect. We studied the effects of hesperidin and naringin supplementation (HN-suppl.). HN-suppl. did not change plasma TC and TG, though hypocholesterolemic effect has been reported in rats fed with cholesterol-enriched diet. HN-suppl. did not counteract the drop in IS that could be due to the nature of the diet and the short duration of the study (8 weeks). The lack of insulin-sensitizing effect could be due to use of too old rats, indeed other studies used younger rats.

Key words: rats, obesity, insulin sensitivity, high-fat diet, high-fructose diet, eicosapentaenoic acid, hesperidin, naringin.

Obésité et insulinorésistance chez des rats consommant des régimes enrichis en matières grasses ou en fructose. Intervention nutritionnelle avec de l'EPA ou des flavanones.

La forte prévalence de l'obésité et des troubles associés a rendu nécessaire le recours à des modèles animaux. Nous avons étudié l'obésité et la sensibilité à l'insuline (SI) chez des rats consommant un régime témoin, hyperlipidique (HF), ou riche en fructose (HFr). Après 10 semaines, les rats HF sont devenus obèses, leur cholestérolémie (TC) a été augmentée et leur SI diminuée, et l'expression de FAS, de TNFα et de leptine a été augmentée dans le tissu adipeux. Au contraire, HFr n'a induit de changement ni dans la SI ni dans le profil lipidique, mais une plus forte expression de PPARα. HF a donc eu des effets nettement plus délétères que HFr en ce qui concerne l'induction de l'obésité et la baisse de SI.

Nous avons étudié l'effet d'une supplémentation en EPA (EPA-suppl.) pendant 10 semaines. Elle a empêché la baisse de SI chez les rats HF, lié une expression plus élevée d'IRS-1 et de GLUT4 dans le tissu adipeux, alors que l'expression hépatique des gènes n'a pas permis de confirmer une sensibilisation à l'insuline. EPA-suppl. a tendu à diminuer les triglycérides (TG) et les acides gras non estérifiés chez les rats HFr chez lesquels l'expression hépatique de LPL et d'HSL a été augmentée. La réponse à EPA-suppl. a donc été dépendante du régime.

Nous avons étudié l'effet d'une supplémentation en hespéridine et naringine (HN-suppl.). Celle-ci n'a modifié ni la TC ni la TG. HN-suppl. n'a pas évité à la chute de SI, ce qui pourrait être lié à la nature du régime et à la courte durée de l'étude (8 semaines). L'absence d'effet sur la SI, pourrait aussi s'expliquer par l'utilisation de rats trop âgés, alors que les précédentes études ont été réalisées sur des rats plus jeunes.

Mots clés : rats, obésité, sensibilité à l'insuline, régime hyperlipidique, régime riche en fructose, acide eicosapentaénoïque, hespéridine, naringine.