
# DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN

**Esther Guiu Jurado**

# Deregulation of fatty acid metabolism in the adipose tissue of obese women

*Doctoral Thesis*

*Esther Guiu Jurado*
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UNIVERSITAT ROVIRA i VIRGILI
*Departament de Medicina i Cirurgia*

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*Doctoral Thesis*

Directed by Prof. Cristóbal Manuel Richart Jurado
and
Dra. Maria Teresa Auguet Quintillà

Department of Medicine and Surgery

UNIVERSITAT ROVIRA I VIRGILI

Tarragona 2016

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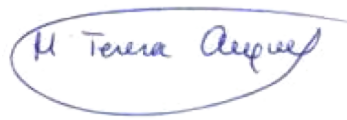
FEM CONSTAR que aquest treball titulat "**Deregulation of fatty acid metabolism in the adipose tissue of obese women**", que presenta Esther Guiu Jurado per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció al Departament de Medicina i Cirurgia d'aquesta universitat i que compleix els requeriments per poder optar a menció internacional.

Tarragona, 7 de Gener de 2016

El director de la tesi doctoral

Prof. Cristóbal Manuel Richart Jurado

La codirectora de la tesi doctoral



Dra. Maria Teresa Auguet Quintillà

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Als meus pares

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"The important thing is not to stop questioning."

**Albert Einstein**

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TABLE OF CONTENT

TABLE OF CONTENT

# I. LIST OF ABBREVIATIONS

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

| | | |
|---|---|---|
| **A** | **AACE** | American Association of Clinical Endocrinologists |
| | **AC** | Adenylate cyclase |
| | **ACC1** | Acetyl-CoA carboxylase 1 |
| | **ACLY** | ATP citrate lyase |
| | **ACS** | Acyl-CoA synthetase |
| | **ADA** | American Diabetes Association |
| | **ADD1** | Adipocyte determination and differentiation-dependent factor 1 |
| | **AGB** | Adjustable gastric band |
| | **AGPAT** | 1-Acylglycerol-3-phosphate O -acyltransferase |
| | **AHA** | American Heart Association |
| | **5'-AMP** | 5'-adenosine monophosphate |
| | **AMPK** | AMP-activated protein kinase |
| | **ANP** | Atrial natriuretic peptide |
| | **α2-AR** | Alpha 2-adrenergic receptor |
| | **AT** | Adipose tissue |
| | **Atg7** | Autophagy-related 7gene |
| | **ATGL** | Adipose triglyceride lipase |
| | **ATP** | Adenosine triphosphate |
| **B** | **BAT** | Brown adipose tissue |
| | **β–AR** | Beta-adrenergic receptor |
| | **BMI** | Body mass index |
| | **BNP** | Brain natriuretic peptide |
| | **BP** | Blood pressure |

## I. LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| | **BPD-DS** | Biliopancreatic diversion with duodenal switch |
| **C** | | |
| | **cAMP** | Cyclic adenosine monophosphate |
| | **CD36** | Fatty acid translocase |
| | **C/EBP** | CCAAT/enhancer-binding protein |
| | **cGMP** | Cyclic guanosine monophosphate |
| | **ChREBP** | Carbohydrate-responsive element-binding protein |
| | **CM** | Chylomicrons |
| | **CoA** | Coenzyme A |
| | **CPT1** | Carnitine palmitoyltransferase I |
| | **CPT2** | Carnitine palmitoyltransferase I |
| | **CREB** | cAMP responsive element binding protein |
| | **CRP** | C-reactive protein |
| | **Csf1** | Colony stimulating factor-1 |
| **D** | | |
| | **DAG** | Diacylglycerol |
| | **DBP** | Diastolic blood pressure |
| | **DGAT** | Diacylglycerol acyltransferase |
| | **DHAP** | Dihydroxyacetone phosphate |
| | **DM2** | Diabetes mellitus type 2 |
| **E** | | |
| | **EGIR** | European Group for the Study of Insulin Resistance |
| | **ER** | Endoplasmic reticulum |
| **F** | | |
| | **FA** | Fatty acid |
| | **FABP4** | Fatty acid binding protein 4 |

| | | |
|---|---|---|
| | **FABPpm** | Fatty acid binding protein plasma membrane |
| | **F1,6BP** | Fructose 1,6 bisphosphate |
| | **FAS** | Fatty acid synthase |
| | **FATP** | Fatty acid transport protein |
| | **FDA** | Food and Drug Administration |
| | **FFA** | Free fatty acid |
| **G** | **GABA** | γ-aminobutyric acid |
| | **GADH** | Glyceraldehyde 3-phosphate |
| | **GC** | Guanylate cyclase |
| | **GAPDH** | Glyceraldehyde-3-phosphate |
| | **GLUT4** | Glucose transporter 4 |
| | **G3P** | Glycerol-3-phosphate |
| | **G6P** | Glucose-6-phosphate |
| | **GPAT** | Glycerol-3-phosphate acyltransferase |
| | **GPDH** | Glycerol-3-phosphate dehydrogenase |
| **H** | **HbA1c** | Glycated haemoglobin |
| | **HDL-C** | High-density lipoprotein cholesterol |
| | **HOMA2-IR** | Homeostatic Model Assessment Method Insulin Resistance |
| | **HSL** | Hormone sensitive lipase |
| **I** | **IDF** | International Diabetes Federation |
| | **IFG** | Impaired fasting glucose |
| | **IGT** | Impaired glucose tolerance |

## I. LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| | **IL1** | Interleukin 1 |
| | **IL6** | Interleukin 6 |
| | **IR** | Insulin resistance |
| **L** | **LDL-C** | Low-density lipoprotein cholesterol |
| | **LPA** | Lysophosphatidic acid |
| | **LPL** | Lipoprotein lipase |
| | **LxRα** | Liver X receptor alpha |
| **M** | **MAG** | Monoacylglycerol |
| | **mALB** | Microalbuminuria |
| | **MCP1** | Monocyte Chemoattractant Protein 1 |
| | **MetS** | Metabolic syndrome |
| | **MGL** | Monoacylglycerol lipase |
| | **MO** | Morbidly obese |
| **N** | **NAFLD** | Non-alcoholic fatty liver disease |
| | **NASH** | Non-alcoholic steatohepatitis |
| | **NCEP ATP III** | National Cholesterol Education Program Adult Treatment Panel III |
| | **NEFA** | Non-esterified fatty acids |
| | **NHLBI** | National Heart, Lung, and Blood Institute |
| **P** | **PA** | Phosphatidic acid |
| | **PAP** | Phosphatidic acid phosphatase |
| | **PDE3B** | Phosphodiesterase 3B |

| | | |
|---|---|---|
| | **PI3K** | Phosphatidylinositol 3-kinase |
| | **PKA** | cAMP-dependent protein kinase |
| | **PKG** | cGMP-dependent proteinkinase |
| | **PLIN** | Perilipin |
| | **PPARα** | Peroxisome proliferator-activated receptor alpha |
| | **PPARδ** | Peroxisome proliferator-activated receptor delta |
| | **PPARγ** | Peroxisome proliferator-activated receptor gamma |
| | **PREF-1** | Preadipocyte factor 1 |
| **R** | **RBP4** | Retinol binding protein 4 |
| | **ROS** | Reactive oxygen species |
| | **RXRs** | Retinoid X receptors |
| | **RYGB** | Roux-en-Y gastric bypass |
| **S** | **SAT** | Subcutaneous adipose tissue |
| | **SBP** | Systolic blood pressure |
| | **SG** | Sleeve gastrectomy |
| | **SOX9** | SRY(sex determining region Y)-box 9 |
| | **SREBP1c** | Sterol regulatory element binding protein 1c |
| | **SVF** | Stromal vascular fractions |
| **T** | **TCA cycle** | Tricarboxylic acid cycle |
| | **TG** | Triglyceride |
| | **TGFβ** | Transforming growth factor beta |
| | **TNFα** | Tumor necrosis factor alpha |
| | **TNFR I** | Tumor necrosis factor receptor I |

## I. LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| | **TNFRII** | Tumor necrosis factor receptor II |
| **V** | | |
| | **VAT** | Visceral adipose tissue |
| | **VLDL** | Very low-density lipoprotein |
| **W** | | |
| | **WAT** | White adipose tissue |
| | **WC** | Waist circumference |
| | **WHO** | World Health Organization |
| | **WHR** | Waist-hip ratio |

# II. INTRODUCTION

UNIVERSITAT ROVIRA I VIRGILI
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# 1. OBESITY: THE EPIDEMIC OF THE 21st CENTURY

**Obesity** is a worldwide epidemic and is considered one of the greatest public health challenges of the 21st century. Its prevalence has tripled in both developed and developing countries since the 1980s, and the number of those affected continues to rise at an alarming rate [1].

Obesity is typically defined as the state of having excess of body weight relative to height that results from an imbalance between caloric intake and energy expenditure. However, this simple definition misrepresents an etiologically complex phenotype primarily associated with excess adiposity that can manifest metabolically and not just as a function of body size [2,3]. Obesity increases the risk of chronic disease morbidity, including type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease, hypertension, dyslipidemia, and certain cancers, and consequently, it is associated with an increase in mortality [4,5].

## 1.1. Epidemiology

The prevalence of obesity is increasing at an alarming rate in many parts of the world. According to the World Health Organization (WHO), in 2014, more than 1.9 billion adults were overweight. Of these, over 600 million were obese. Globally, approximately 39% of the total population was overweight, and 13% were obese [1]. Gallus *et al.*, using data from a pan-European survey, showed that 34.8% of the European adult population was overweight (40.5% of men and 29.3% of women), and 12.8% (14.0% of men and 11.5% of women) were obese [6]. **Figure 1** shows the percent prevalence of overweight and obesity in 16 European countries. Specifically, in Spain, 39.2% of the adult population was overweight and 14.0% was obese. Furthermore, Rodríguez-Rodríguez *et al.* found that the prevalence of overweight was higher in men than in women in Spain. In contrast, the prevalence of obesity was similar between both sexes. Moreover, 47.8% of the population had excess body weight and 70.2% had

excess body fat. These parameters were higher in men than in women and increased with age [7]. In addition, a number of behavioral and environmental factors have contributed to the long-term rise in overweight and obesity rates in industrialized countries. The economic crisis is also likely to have contributed to a further growth in obesity because many families have been forced to cut their food expenditures and switch to lower-priced and less healthy foods [8,9].

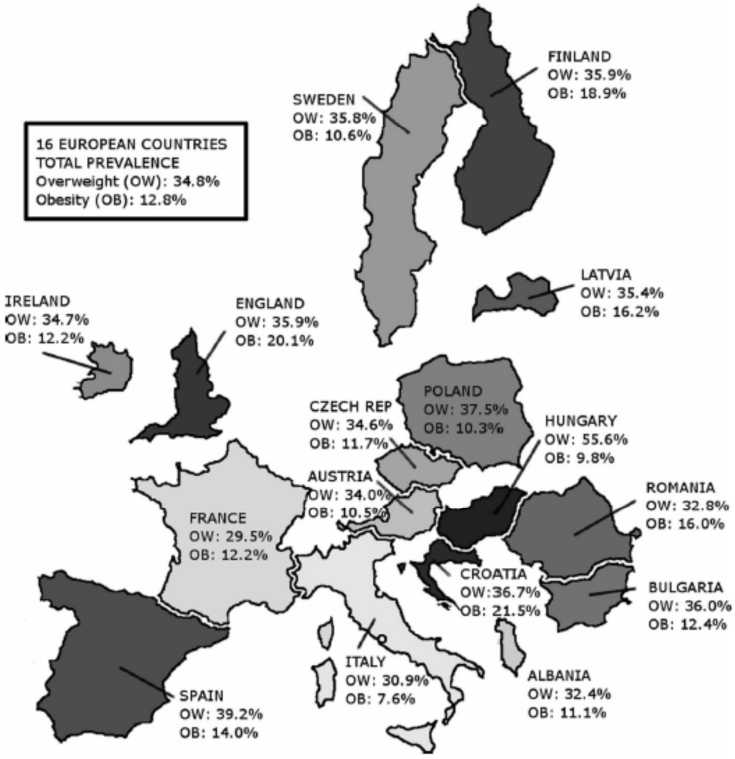


**Figure 1. Percent of overweight (25 ≤ BMI <30 kg/m$^2$) and obesity (BMI ≥ 30 kg/m$^2$) among adults from 16 European countries.** Countries are colored according to their prevalence of overweight/obesity (light, relatively low prevalence; dark, relatively high prevalence). Prevalence estimates for the overall population were computed by weighting each country in proportion to that country's adult population [6].

Although overweight and obesity are considered a problem of high-income countries, their prevalence is increasing in low- and middle-income countries as well, particularly in urban settings. In these developing countries with emerging economies, the rate of increase of this health problem is more than 30% higher than in developed countries [1].

## 1.2. Clinical diagnosis and measurement of fat distribution

The most commonly used method for classifying an individual as overweight or obese is based on the **body mass index** (BMI), a value that is determined by dividing body weight (in kilograms) by the square of height (in meters).

The classification of overweight and obesity, according to BMI, is shown in **Table 1**. This classification is based primarily on the association between BMI and increased health risks. In adults, overweight is classified as a BMI ≥ 25.0 kg/m$^2$, and obesity is defined as a BMI ≥ 30.0 kg/m$^2$ [10].

**Table 1. Classification of overweight and obesity in adults based on increasing health risk.** Adapted from the World Health Organization (WHO) report, Nº894 [10].

| Classification | BMI (kg/m$^2$) | Associated Health Risks |
|---|---|---|
| Underweight | <18.5 | Low (but the risk of other clinical problems increases) |
| Normal | 18.5-24.9 | Average |
| Overweight: | ≥25 | |
| Preobese | 25.0-29.9 | Increased |
| Obese class I | 30.0-34.9 | Moderately increased |
| Obese class II | 35.0-39.9 | Severely increased |
| Obese class III | ≥40 | Very severely increased |

The major limitation of using BMI is that it does not differentiate between the weight of fat and the weight of muscle and, therefore, may lead to the misclassification of very muscular individuals as overweight [11]. Therefore, other methods, in addition to the measurement of BMI, would be valuable in identifying individuals that are at an increased risk for obesity-related illness.

**Waist circumference** (WC) is another clinical measurement that may be used to assess weight-related health risks. The WHO has described sex-specific WC values that are related to an increased or substantially increased risk of metabolic complications related to obesity (**Table 2**). WC correlates closely with BMI (*r*= 0.84-0.88) [12] and provides an estimate of intra-abdominal fat which is more strongly associated with health risks than fat stored in other regions of the body [13,14].

**Table 2. Sex-specific waist circumference and risk of metabolic complications associated with obesity in Caucasians.** Adapted from World Health Organization (WHO) report, Nº894 [10].

| Sex | WC (cm) | Risk of metabolic complications |
|---|---|---|
| Men | ≥94 | Increased |
| | ≥102 | Substantially increased |
| Women | ≥80 | Increased |
| | ≥88 | Substantially increased |

Although BMI and WC are the recommended and most clinically feasible means of identifying patients who are overweight or obese in clinical practice, numerous body composition assessment techniques are available. These include bioelectrical impedance, dual-energy X-ray absorptiometry, body density, and total body water estimates [15,16]. Collectively, these techniques allow for the measurement of fat, fat-free mass, bone mineral content, total body water, extracellular water, total adipose tissue, adipose subdepots, skeletal muscle, select organs, and ectopic fat depots.

## 1.3. Etiology

Obesity is the result of genetic, behavioral, environmental, physiological, social, and cultural factors that result in an imbalance in energy homeostasis and promote excessive fat deposition [17,18]. The relative contribution of each of these factors has been studied extensively, and although genes play an important role in the regulation of body weight, the World Health Organization Consultation on Obesity concluded that behavioral and environmental factors, such as sedentary lifestyles combined with excess energy intake, are primarily responsible for the dramatic increase in obesity during the last 2 decades [10]. All of these factors create an **obesogenic environment**. The obesogenicity of an environment has been defined as the sum of the influences, opportunities, and conditions of life that facilitate overweight and obesity through several intersecting mechanisms [19].

In past decades, environmental changes linked with globalization and modernization have promoted overeating and have reduced physical activity because there is a growing availability of abundant, inexpensive, calorie-dense, highly palatable foods and sugar-sweetened beverages as well as a decrease in energy expended in leisure-time physical activities. In addition, the frequent disruption of sleep and circadian rhythms and a variety of other cultural and economic factors also predispose individuals to weight gain. Finally, hereditary factors, such as genetics, family history and racial/ethnic differences, also lead to the development of obesity [3,19–21]. A summary of the major risk factors and determinants of obesity are shown in **Figure 2**.

In summary, obesity is influenced by a complex interaction between genetic, metabolic, behavioral and environmental factors and also seems to be related to numerous social and economic changes inherent to modern society. Ultimately, personal behavior in response to these conditions continues to play a dominant role in preventing obesity. It is important to note that, apart from genetics, every risk factor discussed above is modifiable.

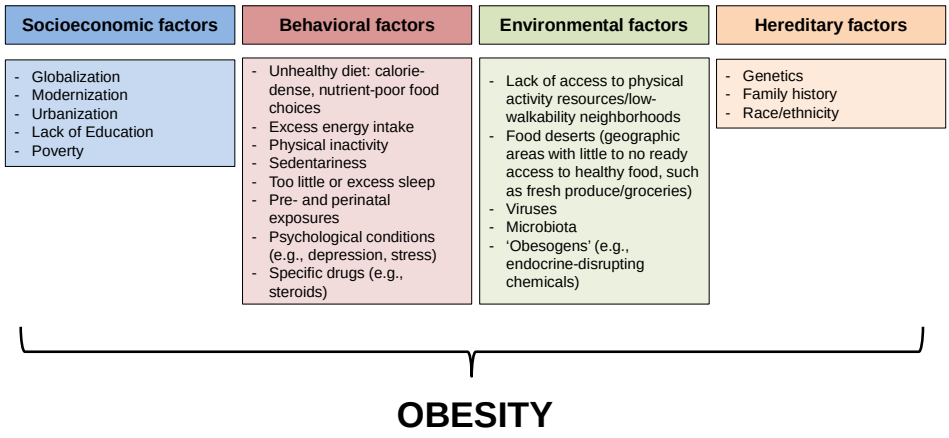


**Figure 2. Major risk factors of obesity.** Adapted from [3].

## 1.4. Physiopathology

**Energy homeostasis** is the balance between energy intake and energy expenditure. When intake exceeds expenditure, energy is stored almost exclusively within white adipose tissue (WAT) in the form of triglycerides (TGs), whereas when expenditure exceeds intake, these energy stores are drawn upon to provide energy to support the ongoing metabolic needs of the organism.

The complex process of energy homeostasis is tightly regulated by the cross talk of central and peripheral signaling systems and depends on constant signal integration [22–25]. Key peripheral tissues and organs implicated in this process are the adipose tissue and the gastrointestinal tract along with its associated digestive organs, such as the liver and the pancreas.

Dietary fat is absorbed through the gastrointestinal tract in the form of circulating chylomicrons (CM) and very low-density lipoprotein (VLDL), part of which is metabolized to provide energy and the rest of which is stored in the adipose tissue and liver [26]. As a consequence of energy storage, adipose tissue releases several adipokines, such as leptin, which regulate energy homeostasis by signaling to the brain and peripheral tissues.

Adipose fat accumulation is achieved by the *de* novo synthesis of fatty acids as well as by fatty uptake, while adipose fat mobilization is accomplished during lipolysis to provide energy to oxidative tissues, such as skeletal muscles and the heart. Moreover, the liver, apart from its role in short-term energy storage, is also an important site for energy conversion. It changes energy sources from one form to another, such as glycogen to glucose, fatty acids to TGs and saturated fatty acids to unsaturated fatty acids (**Figure 3**). In addition, insulin is secreted by the pancreas in response to meals and circulating nutrients to act as a peripheral signal to the brain to control energy intake and metabolism. Like insulin and leptin levels, the endocannabinoid system has also been suggested to regulate food intake and energy expenditure [27,28].

Obesity develops as a result of a period of chronic **energy imbalance** that is characterized by a maintained increase in energy intake. Fat mass accumulation during the development of obesity is characterized by adipocyte hyperplasia and hypertrophy and is associated with increased angiogenesis, macrophage infiltration, extracellular matrix component production and, endothelial cell activation and by the production and release of several inflammatory mediators [29]. The dysregulation of the functions of pro- and anti-inflammatory cytokines/adipokines and their production in obese individuals leads to a state of chronic low-grade inflammation [30,31]. Furthermore, obesity also promotes WAT macrophage accumulation [32], which can also contribute to inflammation. This inflammation induces lipolysis and the release of free fatty acids (FFAs) in adipose tissue. Consequently, there is ectopic fat accumulation and a proinflammatory environment that develops insulin resistance [33]. Obesity also induces hepatic proinflammatory cytokine production, which can result in insulin resistance and steatosis in the liver [34,35]. Moreover, in obese patients, the accumulation of lipids and proinflammatory macrophages in skeletal muscle inhibits insulin signaling [36]. In addition, obesity is associated with macrophage infiltration, IL-1β secretion and decreased insulin secretion by the pancreas [37]. In the digestive system, obesity decreases the numbers of eosinophils and innate lymphoid cells in the gut and increases intestinal

permeability and metabolic endotoxemia [38,39]. Finally, most of the genes associated with obesity are expressed in the brain and affect food intake [40–42]. However, a better understanding of the complex neural systems that regulate energy homeostasis during the development of obesity is needed (**Figure 4**).

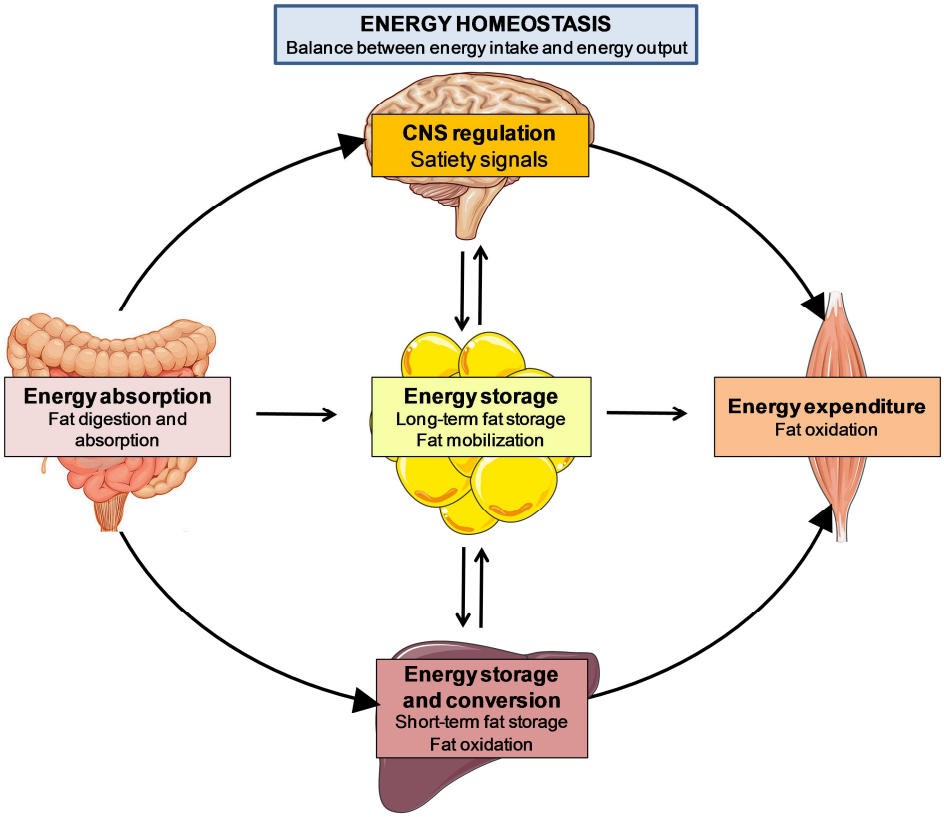


**Figure 3. Adipose tissue is at the crossroad of energy homeostasis.** Adapted from [43]. CNS, central nervous system

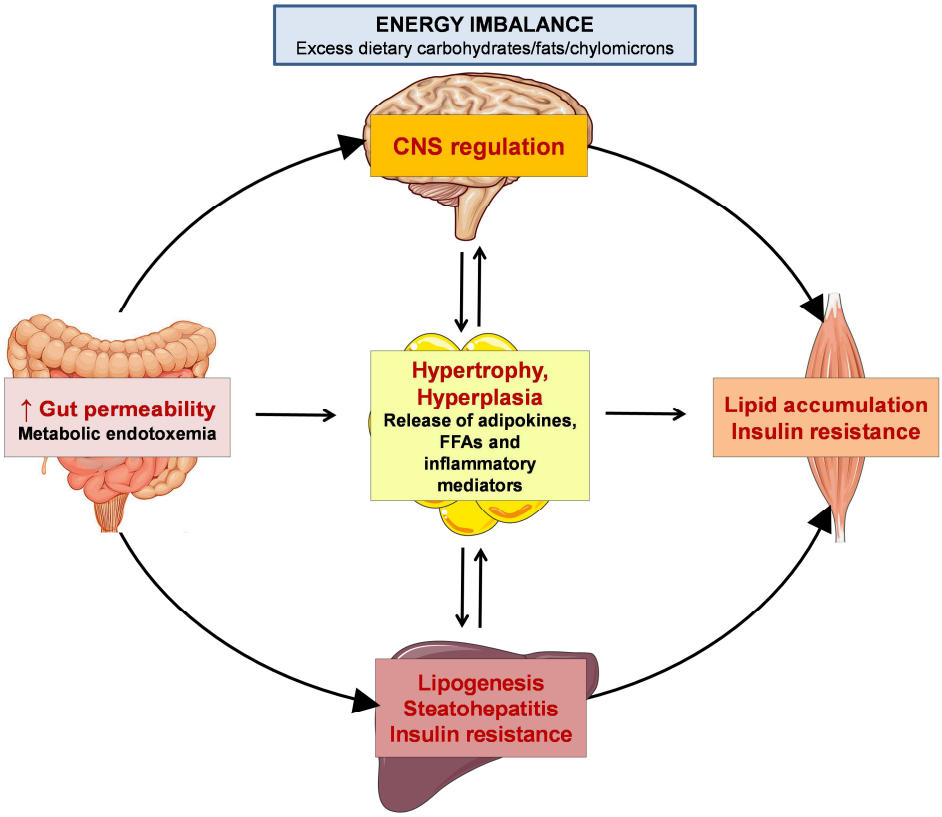


**Figure 4. Adipose tissue is at the crossroad of energy imbalance.** CNS, central nervous system; FFAs, free fatty acids.

## 1.5. Obesity comorbidities

Obesity significantly increases the risk and worsens the prognosis of many diseases and complications, including diabetes mellitus type 2 (DM2), dyslipidemia, hypertension, metabolic syndrome, non-alcoholic fatty live disease (NAFLD), cardiovascular disease and several types of cancer (**Table 3**) [5,44,45].

An analysis from the Framingham Heart Study showed that both overweight and obesity increased the development of **cardiovascular risk** factors, such as hypertension, hypercholesterolemia, and diabetes [46]. It is well known that this increased risk is characterized by metabolic changes

that alter lipid profiles and increase the potential for atherosclerosis due to inflammation.

Obesity clearly increases the risk of developing **DM2**. Large population studies have confirmed the links between excess weight and the development of insulin resistance (IR) and diabetes, which suggests that patients with excessive weight are at substantial risk for developing diabetes [5,44].

Obesity also predisposes individuals to gastrointestinal and hepatic complications. Non-alcoholic fatty liver disease (**NAFLD**), or hepatic steatosis, is becoming an increasingly important health issue because it is the most common cause of chronic liver disease in the Western world, and its incidence is increasing rapidly [47]. The increasing rates of NAFLD are linked to the obesity epidemic because the most important risk factors for hepatic steatosis are obesity, insulin resistance, and hyperlipidemia [48].

However, obesity itself does not necessarily lead to these comorbidities [49–51]. A group of obese individuals has been identified that appears to be protected against obesity-related metabolic disturbances [52–54]. These individuals are considered to be "**metabolically healthy obese**". Despite having excessive body fat, they display a favorable metabolic profile characterized by high insulin sensitivity and favorable lipid and inflammation profiles [55,52]. Because of this, obesity could be considered a heterogeneous disorder with a variable risk profile.

However, because of the substantial risks associated with excess body fat, the obesity epidemic represents a critical public health issue that has the potential to incur major healthcare costs.

It is well known that obesity is associated with an increased risk of death. Recent estimates have shown that approximately 2.8 million deaths per year in the European Union result from overweight- and obesity-related causes [56].

**Table 3. Obesity-related comorbidities and complications.** Adapted from Tsigos *et al*. [57].

| Obesity-related health risks and complications | |
|---|---|
| **Metabolic complications** | Diabetes, insulin resistance, dyslipidemia, metabolic syndrome, hyperuricemia, gout, low-grade inflammation |
| **Cardiovascular disorders** | Hypertension, coronary heart disease, congestive heart failure, stroke, venous thromboembolism |
| **Respiratory disease** | Asthma, hypoxemia, sleep apnea syndrome, obesity hypoventilation syndrome |
| **Cancers** | Esophagus, small intestine, colon, rectum, liver, gallbladder, pancreas, kidney, leukemia, multiple myeloma, and lymphoma<br>In women: endometrial, cervix uteri, ovary, breast<br>In men: prostate |
| **Osteoarthritis** (knee) and an increase in pain in the weight bearing joints | |
| **Gastrointestinal** | Gallbladder disease, NAFLD, non-alcoholic steatohepatitis (NASH), gastro-esophageal reflux, hernia |
| **Urinary incontinence** | |
| **Reproductive health** | Menstrual irregularity, infertility, hirsutism, polycystic ovaries, miscarriage, gestational diabetes, preeclampsia, macrosomia, fetal distress, malformation, dystocia, primary caesarean section |
| **Miscellaneous** | Idiopathic intracranial hypertension, proteinuria, skin infection, nephrotic syndrome, lymphedema, complications from anesthesia, periodontal disease |
| **Psychological and social consequences** | Low self-esteem, anxiety, depression, stigmatization, discrimination in hiring, college acceptance and wages |

## 1.6. Metabolic syndrome

**Metabolic syndrome** (MetS, also called syndrome X or insulin resistance syndrome) is a constellation of metabolic abnormalities that

appear to directly promote the development of cardiovascular disease and DM2 [58,59].

The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) recognized elevated waist circumference, or abdominal obesity, as an independent component of MetS. Elevated triglyceride concentrations, low high-density lipoprotein cholesterol (HDL-C) levels, elevated blood pressure, and high fasting glucose concentrations are also recognized as components of MetS (**Table 4**) [58]. Subjects with ≥3 factors are classified as having MetS [58]. The American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) agree with this definition but lowered the threshold for elevated fasting glucose concentrations from 110 mg/dL to 100 mg/dL because of the importance of this factor in assessing diabetic risk [59].

**Table 4. Clinical components of metabolic syndrome.** Adapted from the third report of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) [58].

| Risk Factor | Defining level |
|---|---|
| **Abdominal obesity (WC)** | |
| Men | > 102 cm |
| Women | > 88 cm |
| **Triglycerides** | ≥ 150 mg/dL |
| **HDL-C** | |
| Men | < 40 mg/dL |
| Women | < 50 mg/dL |
| **Blood pressure** | ≥ 130/ ≥ 85 mm Hg |
| **Fasting glucose** | ≥ 110 mg/dL |

HLD-C, high-density lipoprotein cholesterol; WC, waist circumference

Additional diagnostic criteria of MetS have also been proposed by other institutions, such as the WHO or the International Diabetes Federation (IDF) (**Table 5**).

**Table 5. Different criteria proposed for a clinical diagnosis of MetS in humans.** Subjects with altered metabolic parameters in three of the six categories are classified as having MetS. Adapted from [60].

| Metabolic parameters | WHO (1998) | EGIR (1999) | ATP III (2001) | AACE (2001) | ATP III (2004) | IDF (2005) | AHA/NHLBI (2005) |
|---|---|---|---|---|---|---|---|
| **IR** | IGT, IFG, DM2 or lowered insulin sensitivity **(a)** plus any 2 of the following: | Plasma insulin >75$^{th}$ percentile plus any 2 of the following: | None, but any 3 of the following 5 features: | IGT or IFG plus any of the following based on clinical judgment: | None, but any 3 of the following 5 features: | None | None, but any 3 of the following 5 features: |
| **Body weight** | Waist-to-hip ratio > 0.90 and/or BMI> 30 kg/m$^2$ | WC ≥ 94 cm | WC ≥ 102 cm | BMI ≥ 25 kg/m$^2$ | WC ≥ 102 cm | Increased WC > 94 cm plus any 2 of the following: | WC ≥ 102 cm |
| **Lipids** | TG ≥ 1.7mmol/L and/or HDL-C < 0.91 mmol/L | TG ≥ 2.0 mmol/L and/or HDL-C < 1.01 mmol/L or treated for dyslipidemia | TG ≥ 1.69 mmol/L, HDL-C < 1.03 mmol/L | TG ≥ 1.69 mmol/L, HDL-C < 1.03 mmol/L | TG ≥ 1.69 mmol/L, HDL-C < 1.03 mmol/L | TG ≥ 1.7 mmol/L /or on TG Rx, HDL-C < 1.03 mmol/L or on HDL-C Rx | TG ≥ 1.69 mmol/L or on TG Rx, HDL-C < 1.03 mmol/L or on HDL-C Rx |
| **Blood pressure** | ≥160/90 mm Hg | ≥140/90 mm Hg or on hypertension Rx | ≥130/85 mm Hg | ≥130/85 mm Hg | ≥130/85 mm Hg | ≥130/85 mm Hg or on hypertension Rx | ≥130/85 mm Hg or on hypertension Rx |
| **Glucose** | IGT, IFG or DM2 | IGT or IFG (but not DM2) | > 6.11 mmol/L (includes DM2) | IGT or IFG (but not DM2) | > 5.6 mmol/L (includes DM2) | ≥ 5.6 mmol/L (includes DM2) | ≥ 5.6 mmol/L or on hypoglycemic |
| **Other** | mALB | | | Other features of IR **(b)** | | | |

The diagnostic criteria proposed by the World Health Organization (WHO) (1998); European Group for the Study of Insulin Resistance (EGIR) (1999); Adult Treatment Panel III (ATP III) (2001); American Association of Clinical Endocrinologists (AACE) (2003); ATP III (2004); International Diabetes Federation (IDF) (2005); and American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) (2005). BMI, body mass index; DM2, diabetes mellitus type 2; HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IR, insulin resistance; mALB, microalbuminuria; TG, triglycerides; WC, waist circumference. **(a)** Insulin sensitivity measured under hyperinsulinemic euglycemic conditions; glucose uptake below lowest quartile for the background population under investigation. **(b)** Includes family history of type 2 diabetes mellitus, sedentary lifestyle, advanced age, and ethnic groups susceptible to type 2 diabetes mellitus.

## 1.7. Obesity management

Considering obesity as a chronic medical disease state helps to frame the concept of using a three-stepped intensification of care approach to weight management (**Figure 5**). First, all patients should be counseled on evidence-based **lifestyle modifications** that include diet, physical activity and behavioral change therapies. Second, if lifestyle modifications are not effective, pharmacotherapy should then be considered to provide an additional benefit. Third, if these approaches fail, consideration should be given to **bariatric surgery**. Although they are invasive, surgical procedures have been demonstrated to be the most effective, long-term treatment for individuals with severe or moderate obesity that is complicated by comorbid conditions and that is not responsive to non-surgical approaches [61].

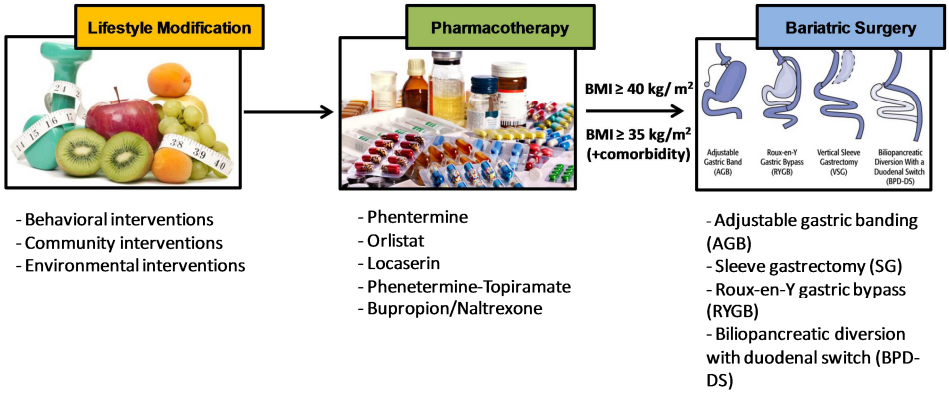


**Figure 5. Three-stepped intensification of care approach to weight management [61].**

### 1.7.1. Lifestyle modifications

Lifestyle modifications are recommended as the primary intervention for overweight and obese individuals. Behavioral modification uses strategies focusing on behavioral changes that are targeted at reducing overeating and sedentary activities to achieve and maintain weight loss. Furthermore,

they are the most accessible and economical approaches to care because of their non-invasive nature and their weight-independent benefits [62]. Lifestyle modifications can be divided into three broad categories: behavioral, community and environmental interventions [63].

Behavioral interventions have formed the cornerstone of obesity prevention and treatment. They are focused on behavior-related aspects and specifically focus on increasing energy expenditure and reducing energy intake to achieve weight loss.

Community interventions are implemented in a combination of local settings, such as neighborhoods, schools, communal sites, social care facilities and cultural centers. They combine behavioral measures and local environmental changes to address the supply and demand for food and/or physical activity.

Environmental interventions modify a target population's environment and are often outside the healthcare sector. Therefore, they have the potential to reach large numbers of individuals simultaneously and may have a more lasting effect on behavioral changes as they become incorporated into structures, systems, policies and sociocultural norms. These interventions consist of the imposition of taxes and/or subsidies to promote healthy eating, the adoption of mandatory food labelling schemes, the implementation of educational mass media campaigns to increase health information and knowledge, and the regulation of food advertisement to children.

If the patient is not able to achieve their weight and health goals by lifestyle changes alone and meets the indications for drug therapy, then addition of adjunctive pharmacotherapy should be considered.

### 1.7.2. Pharmacotherapy

According to current Food and Drug Administration (FDA) guidelines, pharmacotherapy is approved for patients with a BMI ≥ 30 kg/m$^2$ or ≥ 27 kg/m$^2$ when also complicated by an obesity-related comorbidity [61].

Historically, pharmacotherapy for obesity has witnessed the rise and fall of several promising drug candidates that eventually had to be withdrawn due to unacceptable safety concerns [64].

Currently, there are two groups of approved drugs that can be used: **1)** medications approved for obesity management *per se* (appetite suppressants or satiety enhancers and gastrointestinal blockers) (**Table 6**) and **2)** medications used for the treatment of obesity-related comorbidities that affect body weight (**Table 7**) [61,65,66].

Among the currently approved anti-obesity drugs, four noradrenergic agents (phentermine, benzphetamine, diethylpropion, phendimetrazine) were approved as adjuncts in the management of obesity in 1960. Phentermine remains the most often prescribed drug for short-term use for weight loss [61,67]. Orlistat was approved by the FDA in 1999 as the first lipase inhibitor for obesity management [64,67]. Subsequently, after a gap of more than a decade, two new therapies, lorcaserin and phentermine/topiramate were approved in 2012. In 2014, the FDA finally approved the combination of bupropion/naltrexone as a treatment option for the management of obesity [61,64,67].

Medications that are FDA-approved for other conditions and have been found to result in weight loss have also been tested as potential obesity treatments. For example, metformin is an antihyperglycemic drug approved for the treatment of DM2 that has been demonstrated to reduce both energy intake and body weight [68–73].

In summary, the ideal anti-obesity drug should selectively reduce body fat stores, especially visceral fat, by ameliorating the regulatory or metabolic disturbances involved in the pathogenesis of obesity. Furthermore, it should exhibit minor side effects, be preferentially administered orally for long-term

use, and be widely accessible [66]. Further studies are needed to find the ideal anti-obesity drug.

**Table 6. Weight loss medications approved by the FDA for obesity treatment.**

| Medications | Year of FDA approval | Mechanism |
|---|---|---|
| Noradrenergic activation | | |
| Phentermine<br>Diethylpropion<br>Phendimetrazine<br>Benzphetamine | 1959 | Enhances satiety by inhibiting the reuptake of noradrenaline<br>Increases hypothalamic noradrenaline levels |
| Gastrointestinal lipase inhibiton | | |
| Orlistat | 1999 | Reduces body weight by binding and inhibiting lipases produced by the pancreas and the stomach<br>Reduces the absorption of ingested dietary fat by approximately 30% |
| Serotonin receptor activation | | |
| Lorcaserin | 2012 | Selective activation of serotonin 2C ($5HT_{2C}$) receptors in anorexigenic pro-opiomelanocortin neurons in the hypothalamus resulting in the release of α-melanocortin stimulating hormone, which acts on melanocortin receptors to decrease food intake and enhance satiety |
| Combined therapy | | |
| Phentermine/Topiramate extended release | 2012 | Phentermine reduces appetite through an increased noradrenaline in the hypothalamus. However, the precise mechanism of action of topiramate on reducing appetite is not thoroughly understood. It is thought that it has some effect on γ-aminobutyric acid (GABA) receptors |
| Bupropion/Naltrexone extended release | 2014 | Bupropion reduces food intake by acting on adrenergic and dopaminergic receptors in the hypothalamus. Naltrexone is an opioid receptor antagonist which inhibits food intake |

**Table 7. Medications approved by the FDA for other conditions that have been tested as obesity treatments.**

| Condition | Medications | Mechanism |
|---|---|---|
| **DM2** | Biguanide | |
| | Metformin | Improves insulin sensitivity and produces small, sustained weight loss of approximately 2% |
| | Glucagon-like peptide-1 | |
| | Exenatide<br>Liraglutide | Reduces fasting and post-prandial glucose levels, slows gastric emptying and decreases food intake by 19% |
| **Neurobehavioral disorders** | Antidepressant | |
| | Bupropion | Reduces food intake by acting on adrenergic and dopaminergic receptors in the hypothalamus |
| | Anticonvulsant | |
| | Topiramate | Induces appetite suppression and satiety via GABA receptor-mediated inhibitory activity |

### 1.7.3. Bariatric surgery

According to the National Institutes of Health Consensus Development Conference on bariatric surgery [74], patients with a BMI ≥ 40 kg/ $m^2$ or those with a BMI ≥ 35 kg/$m^2$ who also have associated high-risk comorbid conditions can be considered as surgical candidates. Bariatric surgery procedures promote weight loss and improvement in comorbidities through multiple mechanisms [75].

Weight loss surgeries have traditionally been classified into three categories based on anatomical changes: restrictive, restrictive and malabsorptive, and malabsorptive. Restrictive approaches limit the amount of food consumed by reducing the size of the stomach, whereas malabsorptive approaches limit the absorption of nutrients by bypassing

portions of the intestine [76]. However, more recently, the clinical benefits of bariatric surgery in achieving weight loss and improving metabolic comorbidities have largely been attributed to changes in the physiological responses of gut hormones and changes in adipose tissue metabolism [77,78].

There are four types of bariatric surgery, which are usually performed laparoscopically: adjustable gastric band (AGB), sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB) and biliopancreatic diversion with duodenal switch (BPD-DS) (**Figure 6**) [75]. Each of these surgical procedures is described in detail below.

The choice of which procedure to use depends on many factors, including local expertise and experience with the different bariatric surgical procedures and the complexity and reversibility of the procedure. In addition, the patient's general health, the presence of risk factors associated with high perioperative morbidity and mortality, and the nature of any obesity-associated comorbidities might influence the risk-benefit ratio and the choice to use a given procedure. A patient's preference and compliance and the effects of a specific procedure on comorbidities must also be considered.

In conclusion, bariatric surgery has increased in popularity because of its greater ability to induce long-term weight loss than medical or pharmacological treatments. Furthermore, bariatric surgery is safe and beneficial in severely obese patients as it induces long-term metabolic benefits. Because of the low risk of surgery and the well-supported sustained benefits of surgically induced weight loss, it is likely that bariatric surgery will continue to evolve and to have an expanding role in obesity treatment [79].

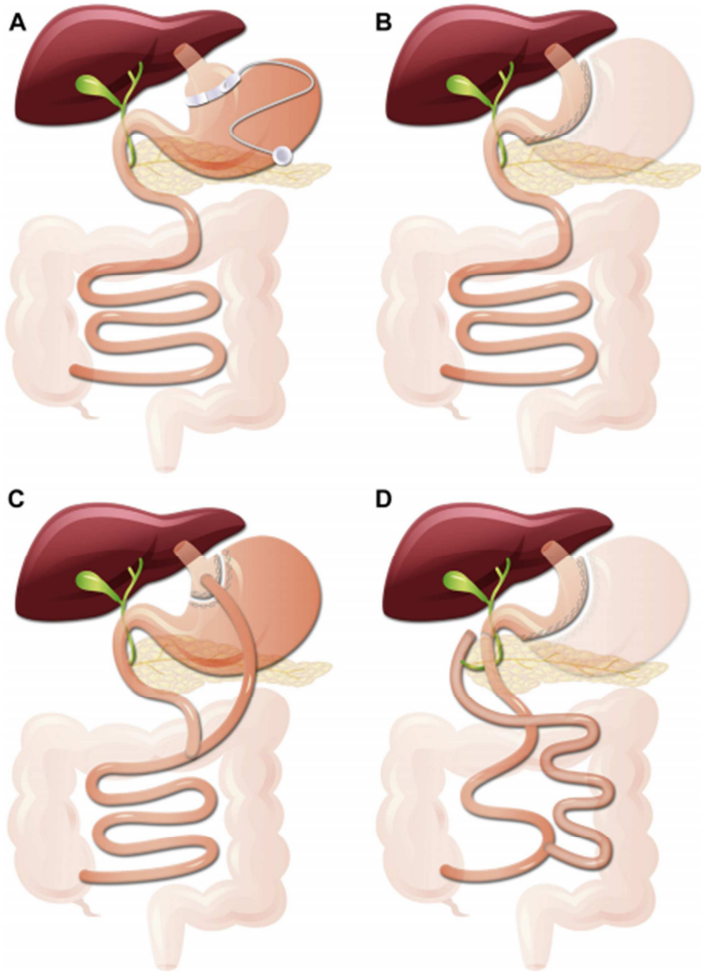


**Figure 6. Types of bariatric surgical procedures.** **(A)** Adjustable gastric band. **(B)** Sleeve gastrectomy. **(C)** Roux-en-Y gastric bypass. **(D)** Biliopancreatic diversion with duodenal switch (BPD-DS). Adapted from [75].

**Adjustable gastric band (AGB)** surgery uses an implanted, inflatable-band device that is placed at the topmost part of the stomach and is connected to a reservoir port placed just under the skin. Band adjustment results in the "adjustment" of the upper stomach pouch outlet. The pouch fills with food quickly and the band slows the passage of food from the pouch to the lower part of the stomach, which allows the patient to achieve appetite control and satiety with less food.

**Sleeve gastrectomy (SG)** is a longitudinal resection of the stomach, which preserves its vagal innervation, starting from the antrum 5-6 cm from the pylorus

and finishing at the fundus close to the cardia. Approximately 75% to 80% of the stomach is resected and the remaining gastric sleeve is calibrated with a French bougie. The ideal remaining stomach volume after the procedure is approximately 150 mL.

**Roux-en-Y bypass (RYGB)** consists of a reduction in the volume of the stomach to a small, 15-mL pouch by stapling off a section of it and connecting it to the small intestine further down in the digestive system. The length of the alimentary loop can be modified, but most of the time it is standardized at 150 cm to ensure that the RYGB has a greater restrictive component than malabsorptive component.

**Biliopancreatic diversion with duodenal switch (BPD-DS)** involves a gastric restriction with an SG where malabsorption results from a bypass in the small intestine. The duodenum is transected approximately 4 cm distal to the pylorus and anastomosed to a 250 cm alimentary limb of ileum. The biliopancreatic limb, which consists of the distal duodenum, jejunum, and proximal ileum, contains the biliopancreatic secretions and is attached to the alimentary limb approximately 100 cm from the end of the ileum or ileocecal valve area. The BPD-DS surgery has a more significant malabsorptive component than that of RYGB surgery.

## 1.8. Dysfunction of adipose tissue in obesity

Adipose tissue (AT) dysfunction is one of the early abnormalities that occurs in the development of obesity. It appears to be an important mechanism determining the individual's risk of developing obesity-related comorbidities [80–84]. AT dysfunction may develop under conditions of continuous positive energy balance in patients with an impaired ability to expand of subcutaneous AT stores [85,86]. The inability to store excess calories in "healthy" subcutaneous fat depots may represent a critical node in ectopic fat accumulation [51,81,84]. Consequently, several mechanisms are activated leading to AT dysfunction, such as adipocyte hypertrophy, AT hypoxia, other AT stresses, autophagy and inflammation (**Figure 7**). AT dysfunction is characterized by ectopic fat accumulation, an increased number of AT-infiltrating immune cells, enlarged adipocytes, and increased autophagy and apoptosis, as well as changes in AT mRNA and protein expression patterns [87]. It is important to note that, with the development of AT dysfunction, adipokine secretion is significantly altered and shifts to a proinflammatory, atherogenic and diabetogenic pattern [82].

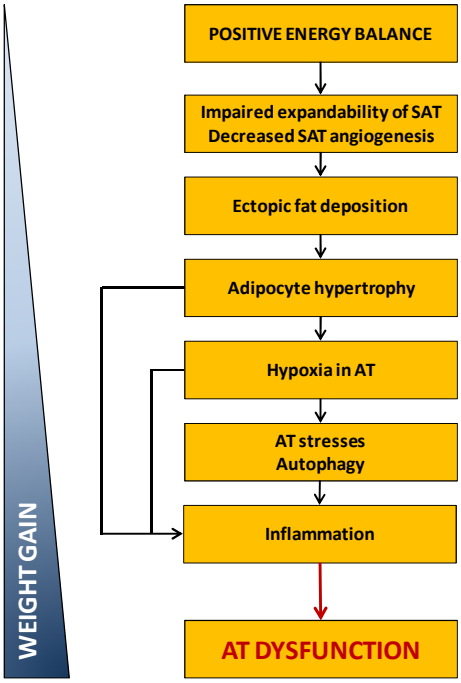


**Figure 7. Development of adipose tissue dysfunction.**
A positive energy balance causes expanding fat mass by increasing the average fat cell volume and the number of adipocytes. Potential mechanisms for the development of adipose tissue dysfunction include impaired expandability of SAT, ectopic fat accumulation, genetic factors, inflammatory processes in AT, hypoxia, and other stresses such as endoplasmic reticulum, oxidative and metabolic stress. AT, adipose tissue; SAT, subcutaneous adipose tissue. Adapted from [87,81].

### 1.8.1. Impaired subcutaneous adipose tissue expandability and vascularization

Impaired AT expandability leads to ectopic accumulation of lipids in the liver and muscle, insulin resistance, and metabolic disease. AT dysfunction and obesity-related comorbidities may be the consequence of an impaired capacity to store fat in healthy fat depots [85,88]. Some authors suggest that excessive energy intake primarily promotes fat accumulation in visceral fat depots and, subsequently, contributes to hepatic and peripheral insulin resistance [89,90]. In this sense, subcutaneous AT plays a "buffering" role by protecting other organs from ectopic fat deposition [85]. In several obese subjects, subcutaneous AT expandability has been shown to be impaired and the excess lipids transported toward other tissues [85]. However, unimpaired subcutaneous AT expandability may underline the insulin sensitivity of the healthy obese phenotype [50,51,91].

Brakenhielm *et al.* have shown that the expansion of fat masses is dependent on angiogenesis [92]. Under conditions of impaired vascularization and angiogenesis, adipose tissue accumulation is inhibited [93]. However, the specific role of angiogenesis in the development of human obesity has not yet been elucidated.

### 1.8.2. Ectopic fat deposition

Despite having a positive energy balance, not all obese subjects have the same risk of developing obesity-related comorbidities. Subjects with peripheral obesity (distributed subcutaneously) are at little or no risk of developing complications associated with obesity, whereas individuals with central obesity (fat accumulation in visceral depots) are much more prone to these complications [94].

There is strong clinical and epidemiologic evidence for the adverse metabolic and cardiovascular effects of ectopic fat deposition [95–97]. The biology of visceral fat is different from that of subcutaneous adipose tissue.

Visceral adipose tissue has decreased insulin sensitivity, increased lipolytic activity, lower angiogenic potential, different cellular composition and different expression levels of key genes related to adipocyte biology [98]. Moreover, the anatomical site of visceral adipose tissue could contribute to an increased cardiometabolic risk because visceral fat depots drain into the portal vein making the liver a target of metabolites, cytokines and adipokines released from visceral fat [80].

### 1.8.3. Hypertrophy and hyperplasia

Increases in fat mass manifest as increases in both intracellular lipids and greater adipocyte size (**hypertrophy**) and as an increase in the number of adipocytes (**hyperplasia**). Adipose hypertrophy and hyperplasia are associated with intracellular abnormalities of adipocyte function, particularly endoplasmic reticulum (ER) and mitochondrial stress. These processes lead to the increased release of adipokines, free fatty acids, and inflammatory mediators that cause adipocyte dysfunction and induce adverse effects in the liver, pancreatic β-cells, and skeletal muscle, as well as in the heart and vascular beds [29].

Adipocyte hypertrophy plays an important role in obesity-related disorders and has been shown to be the major determinant of obesity development due to increased triglyceride storage [99]. In morbidly obese women, large fat cells in the visceral region are linked to dyslipidemia, whereas large subcutaneous adipocytes correlate with impaired glucose metabolism, hyperinsulinemia and IR. Overall, hyperplasia in AT is less deleterious than hypertrophy with regard to the metabolic complications of obesity. Women with combined hyperplasia have been shown to have a more benign glucose, insulin and lipid metabolic profile than those with hypertrophy. Moreover, none of the women with general adipose hyperplasia had diabetes or dyslipidemia [100]. Studies in subjects with either insulin-sensitive or insulin-resistant healthy obesity have shown that a higher average and maximal adipocyte volume is associated with

significantly impaired whole-body insulin sensitivity, increased circulating indicators of inflammation and oxidative stress, and increased numbers of macrophages within AT [50].

### 1.8.4. Hypoxia

It has been suggested that the deregulation of AT is a specific response to relative hypoxia in clusters of adipocytes that become too distant from the vasculature as the adipose tissue mass expands [101–103]. The concept that the development of hypoxia reinforces the initiation and progression of the inflammatory response in adipose tissue in obesity is primarily related to the direct effects of a hypoxic state in obesity [103]. Hypoxia may induce both oxidative and ER stress [101,102]. Evidence of hypoxia in adipose tissue has been demonstrated in several obese mouse models and in obese patients [102,103]. In humans, short-term, whole-body hypoxia decreases insulin sensitivity [104], and short-term, whole-body hyperoxygenation increases insulin sensitivity [105]. Furthermore, in mouse models, obesity is associated with lower oxygen partial pressures in subcutaneous and visceral AT [106,107]. *In vitro* studies on adipocytes have shown that experimental hypoxia stimulates the release of inflammation-related adipokines [102].

Hypoxia in expanding AT in obesity may be a pathogenic factor that causes stress and an inflammatory response within adipose tissue and, subsequently, leads to its dysfunction. However, further studies are needed to determine how AT hypoxia leads to impaired AT function.

### 1.8.5. Adipose tissue stresses

Impaired subcutaneous expandability, ectopic fat accumulation and adipocyte hypertrophy may induce several forms of stress in adipose tissue. Obesity causes metabolic, inflammatory, oxidative and ER stress in adipose tissue [108]. Adipose tissue responds to these stresses with the activation of stress-sensing pathways, which may lead to cellular malfunction and

contribute to obesity-related comorbidities [108–111]. It has been shown that stresses are associated with increased immune cell infiltration into AT [112].

In addition to AT, macrophages may mediate the link between the stress response in adipose tissue and the risk of obesity-induced metabolic diseases [113]. Moreover, several studies have demonstrated that FFAs induce ER stress in different cells, including adipocytes [114].

Taken together, when a positive energy balance exists, adipose tissue is exposed to several stresses that may induce a proinflammatory state and adipose tissue dysfunction in obesity.

### 1.8.6. Autophagy

Recently, the involvement of autophagy in the pathogenesis of human diseases that include alterations in lipid metabolism and adipose tissue biology has been increasingly studied [115–118]. Autophagy is a process by which intracellular components are targeted for lysosomal degradation by a highly regulated process of vesicle formation and fusion [117]. It is induced in response to conditions of nutrient starvation to increase the release of amino acids, FA, and monosaccharides for use as an energy supply [119]. Defects in autophagy cause an inability of the cell to synthesize proteins that are required for survival. This process might be a compensatory mechanism in response to stress. Autophagy is important for cellular housekeeping because it eliminates unnecessary, damaged and/or harmful cellular products and organelles [119].

Autophagy also contributes to carbohydrate and protein degradation and may be involved in the regulation of lipid metabolism [117]. Furthermore, it has been shown that autophagy is also implicated in the physiopathology of obesity and its comorbidities [116]. Studies in animals, which have a targeted deletion of the autophagy-related 7 gene (atg7) in adipose tissue, have shown that autophagy contributes to the regulation of fat mass and the balance between white and brown adipocytes. Interestingly, these mutant mice were resistant to high-fat-diet-induced obesity [120]. In humans,

autophagy is upregulated in the AT of obese and/or DM2 patients, predominantly in visceral adipose tissue, and correlates with the degree of obesity, visceral fat distribution, and adipocyte hypertrophy [115–117].

In summary, the activation of autophagy may occur together with the development of insulin resistance and could precede the development of obesity-associated morbidities. Moreover, autophagy could represent a previously unrecognized protective mechanism against obesity-associated adipose tissue dysfunction, or it could be a symptom of impaired AT function.

### 1.8.7. Immune cell infiltration

Mechanisms including adipocyte hypertrophy, nutritional surplus, hypoxia and AT stresses that cause proinflammatory adipokine secretion may lead to the attraction of proinflammatory immune cells to adipose tissue and cause chronic, low-grade inflammation (**Figure 8**). Obesity and inflammation are highly integrated processes in the pathogenesis of insulin resistance, diabetes, atherosclerosis, and NAFLD [37]. In fact, in the majority of obese patients, adipose tissue expansion is associated with the increased infiltration of proinflammatory immune cells into adipose tissue causing chronic low-grade inflammation [121].

Macrophage infiltration into adipose tissue increases proportionally with increased BMI, body fat mass and adipocyte hypertrophy and represents a reversible process in obese patients who are losing weight [122,123].

It was found that macrophages could be recruited from the circulation by chemoattractant proteins, including monocyte chemoattractant protein 1 (MCP1), chemerin, progranulin and colony stimulating factor-1 (Csf1), in response to the death of hypertrophied adipocytes [32].

## II. INTRODUCTION

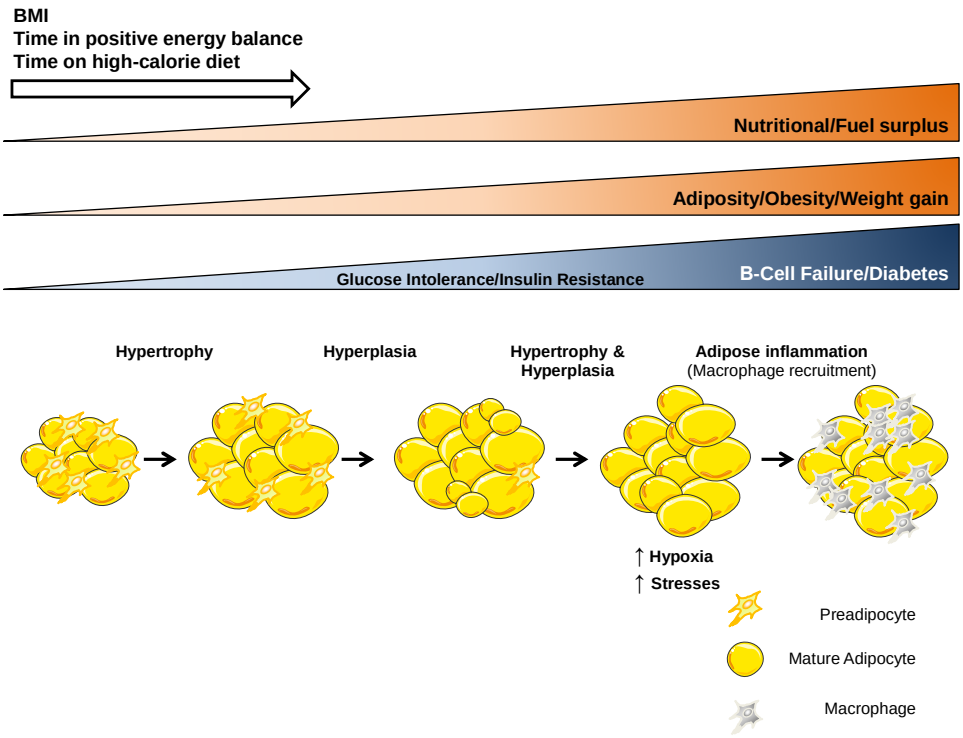


**Figure 8. Macrophage infiltration into adipose tissue during the development of obesity.** With a positive energy balance, adipocyte hypertrophy develops. Furthermore, during the process of fat accumulation, several mechanisms (hypoxia, stress, and the secretion of leptin, MCP1 and progranulin) cause the activation of circulating monocytes, which subsequently transmigrate into adipose tissue. Monocytes differentiate into macrophages and interact with adipocyte and endothelial cells resulting in an increased secretion of proinflammatory cytokines, adipokines and angiogenic factors.

Studying morbidly obese patients with or without insulin resistance, Klöting *et al.* found that increased macrophage infiltration into visceral adipose tissue was a strong predictor of the insulin-resistant obese phenotype (**Figure 9**) independent of BMI and total body fat mass [50]. Therefore, macrophage infiltration into adipose tissue could represent the link between adipose tissue dysfunction and whole-body insulin resistance.

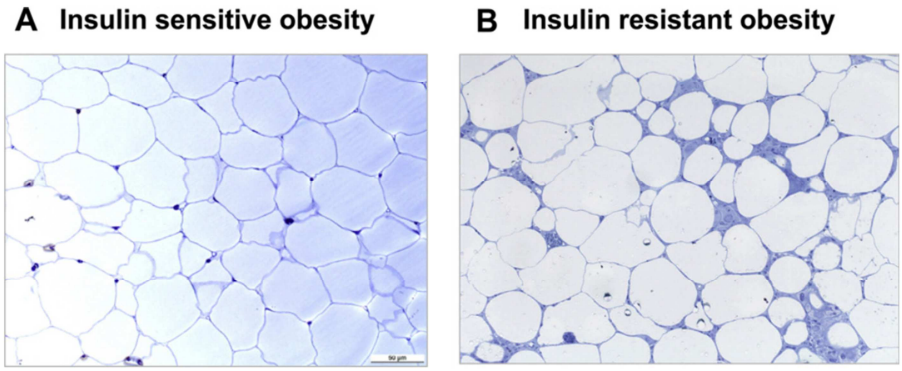


**Figure 9. Inflammation of visceral adipose tissue in insulin-resistant obesity.** Hematoxylin and eosin staining of omental adipose tissue sections from **(A)** a 52 year-old male patient with a BMI of 44.8 kg/m$^2$ with insulin-sensitive healthy obesity and **(B)** an age, sex, and BMI (44.9 kg/m$^2$) matched insulin-resistant obese men. Insulin-resistant obesity is associated with macrophage infiltration into omental adipose tissue. Adapted from [50].

An increased number of macrophages in adipose tissue might also cause an increased systemic concentration of proinflammatory cytokines [80].

In conclusion, these data collectively reinforce the hypothesis that adipose inflammation is an important contributor to insulin resistance in obesity.

# 2. ADIPOSE TISSUE

In mammals, **adipose tissue** exists in two forms, white adipose tissue (WAT) and brown adipose tissue (BAT), with each performing different functions. The primary role of BAT is to store a small amount of fat that can be used, when needed, to produce heat and maintain body temperature [124]. However, WAT is designed to **store** large amounts of **excess energy** in the form of triglycerides for use during periods of food deprivation. In addition, WAT has an **endocrine function** which contributes to the regulation of whole-body energy homeostasis through the secretions of various adipose-derived hormones or adipokines.

## 2.1. Morphology and anatomical distribution of WAT

Adipose tissue is a loose connective tissue composed primarily of adipocytes, but it also contains a variety of other cells, such as preadipocytes, fibroblasts, endothelial cells and macrophages. These other cells make up the stromal vascular fraction (SVF). White adipocytes contain a large lipid droplet that occupies over 90% of the cell volume and is surrounded by a layer of cytoplasm. The nucleus is flattened and located in an eccentric position [125] (**Figure 10**).

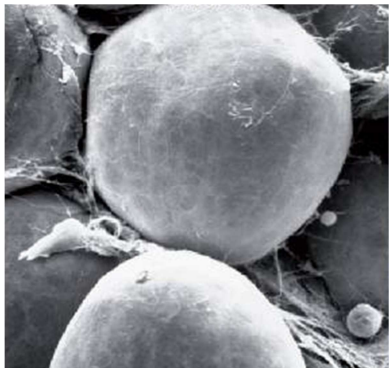

**Figure 10. Scanning electronic microscopy image of WAT [125].**

In healthy individuals, adipose tissue represents 25-31% of women's and 18-24% of men's body weight, and it is located at different anatomical sites [126]. The two major compartments of WAT are located subcutaneously and intra-abdominally (**Figure 11**).

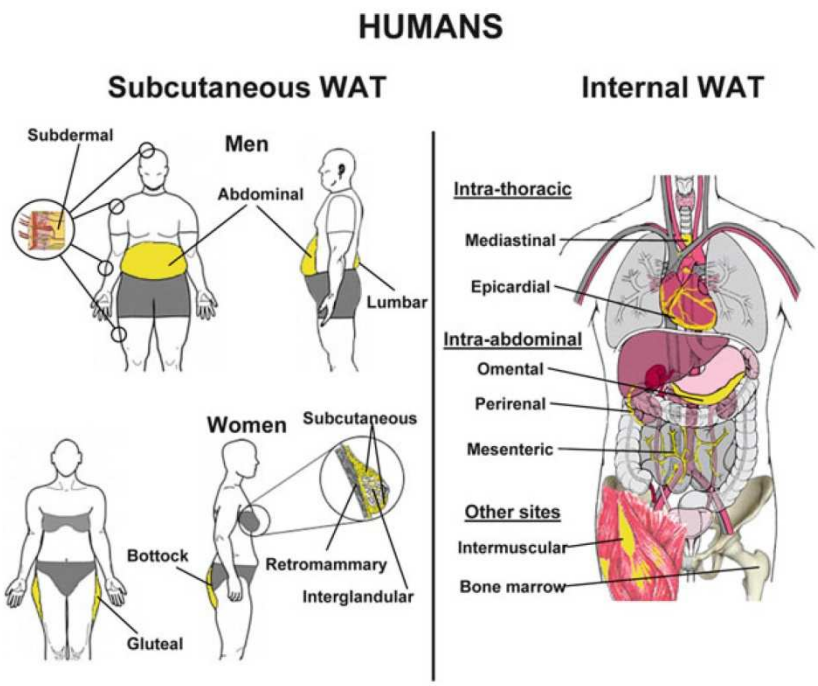


**Figure 11. White adipose tissue distribution in humans.** White adipose tissue is distributed throughout the body. The two major compartments of WAT are located subcutaneously and intra-abdominally, although WAT can be found in other regions such as the intra-thoracic region. Adapted from [126].

As mentioned before, WAT is distributed throughout the body in humans, but this distribution can vary considerably from one individual to another. In individuals with problems controlling their weight, when an increase in body fat accumulation leads to overweight and/or obesity, fat deposition can be increased in specific regions of the body leading to altered fat distribution. These changes have an important impact on metabolism and lead to the development of obesity-related comorbidities.

Several classifications of different types of obesity have been proposed. In the 1940s, Jean Vague proposed the existence of sexual dimorphism as a determining factor for two different patterns of fat distribution in obese patients [127]. Vague classified these two patterns of obesity as **android** (or upper-body) vs **gynoid** (or lower-body) obesity using the brachio-femoral to

adipo-muscular ratio. In 1956, Vague reported that a high brachio-femoral to adipo-muscular ratio in obese individuals (android obesity) was associated with an increased risk of DM2, atherosclerosis, gout and uric calculous, whereas gynoid obesity was not [128].

Three decades later, a new classification was proposed based on the calculated ratio between WC and hip circumference [129,130]. Larsson *et al.* showed that abdominal obesity, determined by a high **waist-hip ratio** (WHR), was associated with an increased risk of myocardial infarction, stroke, and premature death, while no association was found when BMI was used [131]. Interestingly, in this study, individuals with a low BMI but a high WHR exhibited the highest risk of myocardial infarction and premature death [131]. Since then, a large number of studies have recognized that abdominal obesity, as assessed by WHR or simply WC, is associated with adverse health risks, such as IR, DM2, dyslipidemia, hypertension, atherosclerosis, NAFLD, cholesterol gallstones, several cancers, and overall mortality [97,132–136].

## 2.2. Visceral vs subcutaneous adipose tissue

As mentioned previously, it has been recognized for more than 60 years that the cardiovascular risk of obesity and increased body weight are related more to body fat distribution than to total body fat [128]. Individuals with upper abdominal, central or android obesity (**visceral adipose tissue, VAT**) are at a greater risk than those with gluteofemoral, peripheral or gynoid obesity (**subcutaneous adipose tissue, SAT**).

Several theories have been proposed to explain the link between VAT and the increased risk for metabolic complications, such as IR, glucose tolerance, and dyslipidemia. Historically, the "**Portal circulation theory**" has been the most actively discussed. In this theory, it has been noted that VAT drains into the portal vein where FFAs can be accessed by the liver [137]. These high levels of FFAs could stimulate hepatic gluconeogenesis and reduce hepatic insulin sensitivity by decreasing the number of insulin

receptors and altering intracellular insulin signaling. This theory is also supported by the fact that VAT has higher lipolytic rates than SAT and, therefore, releases more FFAs into the portal vein and, consequently, to the liver [138]. In fact, it is well known that in humans, VAT shows a significantly greater lipolytic activity when stimulated by catecholamines than SAT because VAT has higher levels of lipolytic β-adrenergic receptors and lower levels of anti-lipolytic $\alpha_2$-adrenergic receptors compared to the levels in SAT [139–142].

In addition to FFAs, adipokines and cytokines, such as interleukin 1 (IL1), interleukin 6 (IL6), tumor necrosis factor alpha (TNFα), and resistin, which have been associated with reduced insulin sensitivity, are also potential mediators for the portal mechanism of IR [123,143]. These cytokines, whose secretion from AT is increased in obese individuals, are produced at higher levels in VAT than in SAT.

Based on these data, a "**Cell biological theory**" has emerged based on the concept that fat cells in different depots possess different intrinsic properties and possibly have a different developmental origin, which could cause them to be more or less associated with metabolic alterations. This hypothesis is supported by the fact, that at a molecular level, significant differences in the expression of hundreds of genes have been reported between distinct adipose tissue depots in both rodents and humans, and these depot-specific variations in gene expression appear to be intrinsic [144]. Therefore, the intrinsic properties of these depots may also be one of the causes for the association between central obesity and metabolic disorders.

From a metabolic point of view, VAT seems to play a deleterious role during the physiopathology of obesity whereas SAT does not. SAT appears play a "buffering" role in taking up fatty acids and preventing the exposure of other insulin-sensitive tissues to their detrimental effects, which suggests a protective role of SAT in obese individuals [145].

## 2.3. Adipose tissue as an endocrine organ

Classically, the role of WAT was viewed as limited to energy storage. However, in 1953, Kennedy hypothesized that adipose tissue might produce a circulating lipostatic factor that coordinated fat mass and food intake [146]. In 1964, lipoprotein lipase (LPL) was the first protein characterized as being secreted by adipocytes [147]. Furthermore, in 1994, the first adipocyte hormone was discovered with the cloning of leptin [148]. Advances in obesity research have led to the recognition of adipose tissue as an active **endocrine organ** that secretes more than 600 bioactive factors termed adipokines [149].

Adipokines play significant roles in the regulation of appetite and satiety control, fat distribution, insulin sensitivity and secretion, energy expenditure, inflammation, blood pressure, hemostasis, and endothelial function [98,148,150–154]. Many of these factors act locally within the WAT through autocrine/paracrine mechanisms, but others act systemically to influence the function of distant tissues, such as the brain, skeletal muscle, liver, pancreas, and heart [80] (**Figure 12**).

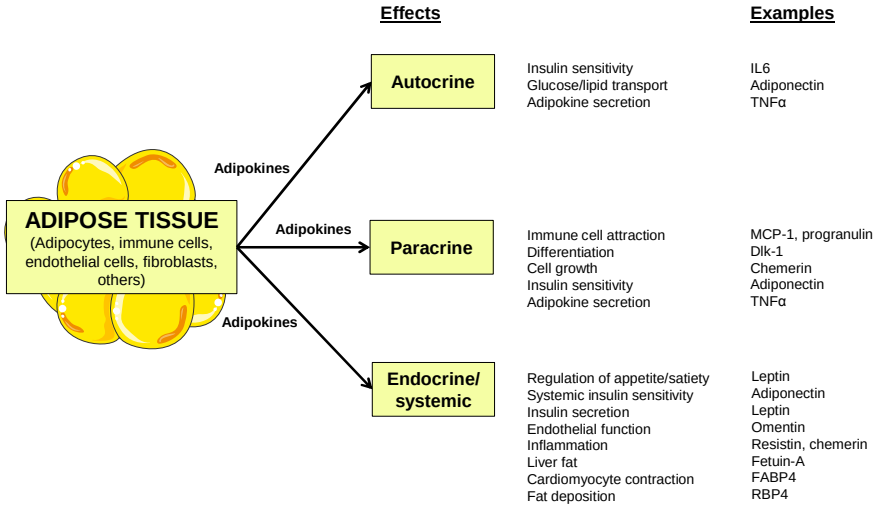


**Figure 12. Effects of adipokines.** Adipokines regulate adipogenesis, adipocyte metabolism, and immune cell migration into adipose tissue via autocrine and

paracrine signaling. Furthermore, adipokines have endocrine/systemic effects that play a role in appetite and satiety control, regulation of energy expenditure and activity, insulin sensitivity and energy metabolism in insulin-sensitive tissues. FABP4, fatty acid binding protein 4; IL, interleukin; MCP1, monocyte chemoattractant protein 1; RBP4, retinol binding protein 4; TNFα, tumor necrosis factor alpha. Adapted from [80]

Among the adipokines discovered, there are molecules that play a role in the inflammatory response, such as interleukins 1, 6, 8, 10, TNFα, transforming growth factor β (TGFβ), interferon-γ, c-reaction protein (CRP), plasminogen activator inhibitor-1, and chemerin. Some adipokines, including RBP4, chemerin, vaspin, fetuin A, omentin, and fatty acid binding protein 4 (FABP4), have been associated with insulin resistance and fatty liver disease [155–157]. However, there are several adipokines that may cause adverse fat distribution, such as RBP4 [158], dipeptidyl peptidase 4 [159], chemerin [160–162], apelin [163], vaspin [157,164,165], endocannabinoids [166,167], fetuin A [168], omentin [157,169], and progranulin [170]. Adipokines may represent links between obesity and various conditions, such as hypertension (e.g., angiotensinogen), endothelial function (e.g., omentin, apelin), hemostasis (e.g., fibrinogen), and immune cell infiltration in adipose tissue (e.g., MCP 1, progranulin and macrophage inflammatory protein 1α) [87,149].

The role of the classical adipokines leptin and adiponectin as mediators linking increased fat mass and/or impaired adipose tissue function to metabolic and cardiovascular diseases are described in detail below.

**Leptin** was discovered in 1994 as the protein product of the ob gene mutation, which causes extreme obesity in the *ob/ob* mouse model [148]. Leptin is almost exclusively secreted from adipocytes and controls food intake and energy expenditure [171]. Activation of leptin receptors in the hypothalamus leads to the repression of orexigenic and induction of anorexigenic pathways, thereby decreasing appetite [171]. Obesity is associated with increased leptin levels, which contributes to the development of IR and MetS [171]. Moreover, leptin increases fatty acid oxidation and decreases triglyceride storage in muscle [171]. In addition to these effects, there may be a direct link between high circulating levels of

leptin and increased cardiovascular risk because it may enhance platelet aggregation and arterial thrombosis, promote angiogenesis, impair arterial distensibility, and induce proliferation and migration of vascular smooth muscle cells [171].

Another important adipokine regulating energy balance and insulin sensitivity is adiponectin. **Adiponectin** was discovered in 1995 [172]. Adiponectin exhibits insulin-sensitizing and anti-atherosclerotic properties [173]. It modulates insulin sensitivity through the inhibition of hepatic glucose production, thereby enhancing glucose uptake in muscle and increasing fatty acid oxidation in both liver and muscle [174]. In contrast to most adipokines, the expression and circulating levels of adiponectin are decreased in obesity and its related comorbidities. Various hormones associated with IR and obesity, including catecholamines, insulin, glucocorticoids, TNFα and IL6, downregulate adiponectin expression and secretion in fat cells *in vitro* [175].

## 2.4. Adipogenesis

**Adipogenesis** is the process of adipocyte formation from precursor cells. Adipocytes are derived from undifferentiated preadipocytes, which undergo terminal differentiation through a complex process orchestrated by a transcriptional cascade involving the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) and the members of the CCAAT/enhancer-binding protein (C/EBP) family [176].

In past years, adipocyte differentiation has been extensively studied using 3T3-L1 and 3T3-F442A preadipocytes cell lines [177,178]. In these cultured preadipocytes, the induction of adipocyte differentiation is under the control of hormonal stimuli by glucocorticoids, cyclic adenosine monophosphate (cAMP), and the insulin/IGF-1 pathways. In cell cultures, this induction occurs during the first 2 days of differentiation and involves a sequence of transcriptional cascades beginning with a transient, high expression of C/EBPβ and C/EBPδ, which promotes the expression of the

transcription factors involved in terminal adipocyte differentiation, PPARγ and C/EBPα [176,177]. Moreover, PPARγ can also be activated by adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein 1 (ADD1/SREBP1). Lastly, PPARγ and C/EBPα cooperate to induce terminal differentiation by increasing the expression of genes involved in the acquisition of a mature adipocyte phenotype, such as the genes for glucose transporter 4 (GLUT4), FABP4, the insulin receptor, and the enzymes involved in triglyceride synthesis, lipolysis and endocrine function [176].

In addition to an enhanced expression of these transcription factors, there is also a downregulation of preadipocyte factor 1 (PREF-1), which has been shown to participate in maintaining the preadipocyte phenotype. The binding of the soluble PREF-1 protein to the putative PREF-1 receptor activates the MAPK kinase/ERK pathway, which in turn, increases the expression of the transcription factor SRY (sex determining region Y)-box 9 (SOX 9). SOX 9 binds to the C/EBPβ and C/EBPδ promoter regions to suppress their transcription and, results in the inhibition of adipocyte differentiation. Because of this pathway, a decrease in PREF-1 expression is required during the early phases of adipogenesis to facilitate adipocyte differentiation [179,180].

A schematic overview of the transcriptional and hormonal induction of adipogenesis is shown in **Figure 13**.

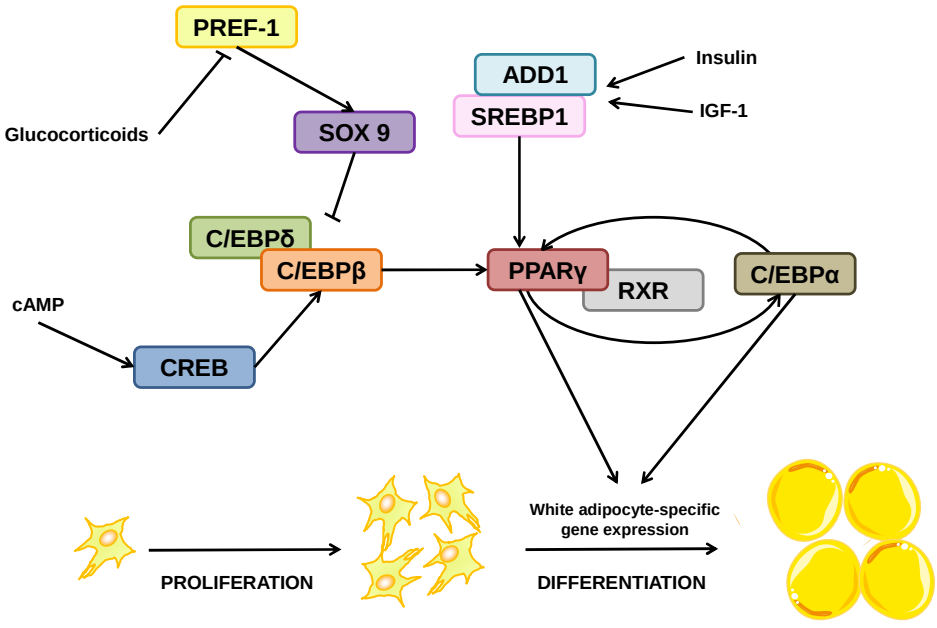


**Figure 13. Schematic overview of the transcriptional and hormonal induction of adipogenesis**. ADD1, adipocyte determination and differentiation-dependent factor 1; C/EBPα, CCAAT/enhancer-binding protein alpha; C/EBPβ, CCAAT/enhancer-binding protein beta; C/EBPδ, CCAAT/enhancer-binding protein delta; CREB, cAMP responsive element binding protein; PPARγ, peroxisome proliferator-activated receptor gamma; PREF-1, preadipocyte factor 1; RxR, retinoid x receptors; SOX 9, SRY (sex determining region Y)-box 9, SREBP1, sterol regulatory element binding protein. Adapted from [176].

## 2.5. Fatty acid metabolism in white adipose tissue

As mentioned, one of the main functions of WAT is the storage of energy in the form of TGs. In white adipocytes, TG accumulation is achieved by *de novo* FA synthesis (lipogenesis) as well as by fatty acid uptake, whereas TG mobilization is accomplished by lipolysis (**Figure 14**).

### 2.5.1. *De novo* fatty acid synthesis (lipogenesis)

**Lipogenesis** ensures the *de novo* synthesis of fatty acids from glucose for storage. This process occurs in WAT and the liver. *De novo* FA synthesis requires the production of cytoplasmic acetyl-coenzyme A (CoA) from the metabolism of glucose. Glucose enters the cell through GLUT4 that is predominantly found in adipocytes. Then, it is metabolized to pyruvate via glycolysis. Under aerobic conditions, pyruvate enters the mitochondria and is transformed by pyruvate dehydrogenase into acetyl-CoA, which then enters the tricarboxylic acid cycle (TCA) to be condensed with oxaloacetate to form citrate. Citrate is released to the cytoplasm and is broken down by ATP-citrate lyase to produce cytoplasmic acetyl-CoA, which is the main substrate of *de novo* FA synthesis. FA synthesis is carried out by the sequential action of two enzymatic systems: acetyl-CoA carboxylase (ACC), which mediates the formation of malonyl-CoA from acetyl-CoA, and the multi-enzyme complex fatty acid synthase (FAS), which mediates the elongation of malonyl-CoA by the successive addition of acetyl-CoA molecules.

*De novo* lipogenesis is controlled by hormones, especially insulin, or by metabolites. Insulin not only stimulates glucose uptake through GLUT4 [181] but also activates pyruvate dehydrogenase [182] and indirectly increases the expression of FAS and ACC [183,184]. The effect of insulin on the expression of lipogenic genes is mainly controlled by sterol regulatory element binding protein 1c (SREBP1c), which has been shown to regulate the expression of several key genes of fatty acid and triglyceride metabolism in cultured

fibroblasts and adipocytes and in the livers of transgenic mice [185,186]. Additionally, SREBP1c has been suggested to be involved in adipogenesis by activating PPARγ [186].

Furthermore, the plasma glucose levels that are directly associated with glucose intake can also stimulate lipogenesis. The effect of glucose on the expression of lipogenic genes is primarily regulated by carbohydrate-responsive-element-binding protein (ChREBP), which induces the expression of the genes of most of the enzymes involved in lipogenesis [187–189].

### 2.5.2. Fatty acid uptake

In addition to *de novo* FA synthesis, FAs can be obtained from circulating triglycerides transported by VLDL produced in the liver or from chylomicrons produced by the absorption of fat in the small intestine. To store FAs in WAT, triglycerides from VLDL and chylomicrons are processed in the extracellular space by lipoprotein lipase (LPL). LPL is secreted by adipocytes and released into blood vessels, where it interacts with VLDL and chylomicrons to liberate FAs and monoacylglycerol (MAG) and facilitate their uptake [190]. Insulin is the major regulator of LPL in WAT. In mature adipocytes, insulin stimulates LPL by increasing its mRNA expression and regulating its activity through both posttranscriptional and posttranslational mechanisms [190].

The FAs generated by the action of LPL on lipoproteins are rapidly taken up by adipocytes. FAs can diffuse passively across the plasma membrane by a mechanism called flip-flop [191,192] or enter actively through certain membrane proteins, including fatty acid translocase (CD36/FAT) [193], caveolin [194], fatty acid transport protein (FATP) [195] and fatty acid binding protein plasma membrane (FABPpm) [196].

### 2.5.3. Triglyceride synthesis

In adipocytes, FA esterification with CoA followed by acylation of the glycerol backbone represent the last steps in the **formation of triglycerides** [197]. FAs can be esterified by the acyl-CoA synthetase activity of FATPs during FA uptake or by long-chain fatty acyl-CoA synthetase which acts in synergy with FATPs [198]. Subsequently, glycerol 3-phosphate acyltransferase (GPAT) catalyzes the addition of acyl-CoA to position 1 of glycerol 3-phosphate to give lysophosphatidic acid (LPA). Then, a second acyl-CoA is added to position 2 of LPA through the action of lysophosphatidate acyltransferase (AGPAT) to produce phosphatidic acid (PA). Next, PA is phosphorylated by phosphatidic acid phosphatase (PAP) to produce diacylglycerol (DAG). Finally, the third acid is added on DAG by several enzymatic activities to produces triacylglycerol (TAG).

### 2.5.4. Lipolysis

During **lipolysis**, the hydrolysis of triglycerides results in the efflux of non-esterified fatty acids (NEFA) and glycerol into the blood stream, which can then be used as a substrate by other tissues. Each FA moiety is sequentially removed from triglyceride to produce DAG, then MAG, and finally glycerol itself. In WAT, this lipolytic cascade is catalyzed by at least three lipases: adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoacylglycerol lipase (MGL), which have been proposed to act sequentially in the conversion of triglyceride to glycerol and three NEFAs [199].

The process starts when epinephrine or glucagon bind to their respective receptors and trigger the activation of adenylate cyclase (AC), which raises cAMP levels and subsequently activates protein kinase A (PKA) [200]. Activated PKA phosphorylates both perilipin (PLIN) and HSL. The phosphorylation of PLIN leads to the release of the ATGL coactivator abhydrolase domain containing 5 (ABHD5, also known as CGI-58) [201,202].

ABHD5 then increases the activity of ATGL, which hydrolyses TG into DAG [203]. Phosphorylated HSL hydrolyzes the DAG produced by ATGL to give MAG.

FFAs are transported to the plasma membrane and bound to adipocyte fatty acid-binding protein (aP2, also known as FABP4). Then, they are transported across the plasma membrane into the circulation by one of several fatty acid transport proteins. The glycerol released through the action of MGL is transported across the plasma membrane via the action of aquaporin 7.

An alternative pathway in the regulation of lipolysis has been proposed. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), which are secreted by the heart, have been reported to stimulate lipolysis in human adipocytes through a cGMP/PKC-signaling pathway leading to the phosphorylation and activation of HSL [204,205]

Lipolysis is a process regulated by hormones. Insulin can inhibit lipolysis through the activation of protein kinase B (PKB, also known as AKT), which hydrolyzes cAMP and reduces PKA activity [206]. Epinephrine (as well as norepinephrine) and glucagon stimulate fatty acid release from triglycerides stored in adipocyte fat droplets, whereas the action of insulin counteracts the actions of these two hormones and induces fat storage.

### 2.5.5. Fatty acid oxidation

**Fatty acid β-oxidation** in mitochondria is a process that shortens the FA into acetyl-CoA, which can subsequently be converted into ketone bodies or can be incorporated into the TCA cycle for full oxidation. To initiate the process, FAs are activated by acyl-CoA-synthetase to acyl-CoA in the cytosol to enable FAs to cross the mitochondrial membrane. While short- and medium-chain FAs can pass the mitochondrial membrane without activation, activated long-chain FAs need to be transported across the membrane in a carnitine-dependent manner [207]. Fatty acyl-CoA is converted to fatty acyl-carnitine by carnitine palmitoyltransferase I (CPT1) in

the outer mitochondrial membrane for translocation into the intermembrane space. Fatty acyl-carnitine is then transported across the inner mitochondrial membrane by carnitine acylcarnitine translocase. Carnitine palmitoyltransferase 2 (CPT2), which is expressed on the inner mitochondrial membrane, converts fatty acyl-carnitine back to acyl-CoA for fatty acid β-oxidation inside the mitochondrial matrix.

In the postprandial state, β-oxidation is suppressed due to the direct control of glucose and insulin over the rate of fatty acid entry into the mitochondria. As described previously, insulin facilitates *de novo* lipogenesis through the upregulation and activation of SREBP1c and the induction of ACC. Malonyl-CoA produced by ACC activity inhibits the activity of CPT1 and thereby decreases the rate of β-oxidation by reducing fatty acid entry into mitochondria [208]. Under fasting conditions, glucagon promotes fatty acid oxidation. Glucagon signaling activates AMP-activated protein kinase (AMPK), which in turn inactivates ACC1 and ACC2 by phosphorylation and results in a blockade of the synthesis of malonyl-CoA [209].

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that act as heterodimers with retinoid X receptors (RXRs) to regulate a broad set of genes involved in lipid uptake, storage, and metabolism, including genes encoding mitochondrial FA oxidation enzymes [210].

In the organism's adaptation to fasting conditions, PPARα acts as master transcriptional regulator of FA utilization [211]. PPARα target genes are involved in FA transport and uptake and in β-oxidation, and their transcriptional activation contributes to FA homeostasis in lipid-metabolizing tissues [211]. Similar to PPARα, PPARδ regulates FA oxidation through the activation of its target genes but also controls glucose uptake by modulating the expression of GLUT4 [211,212].

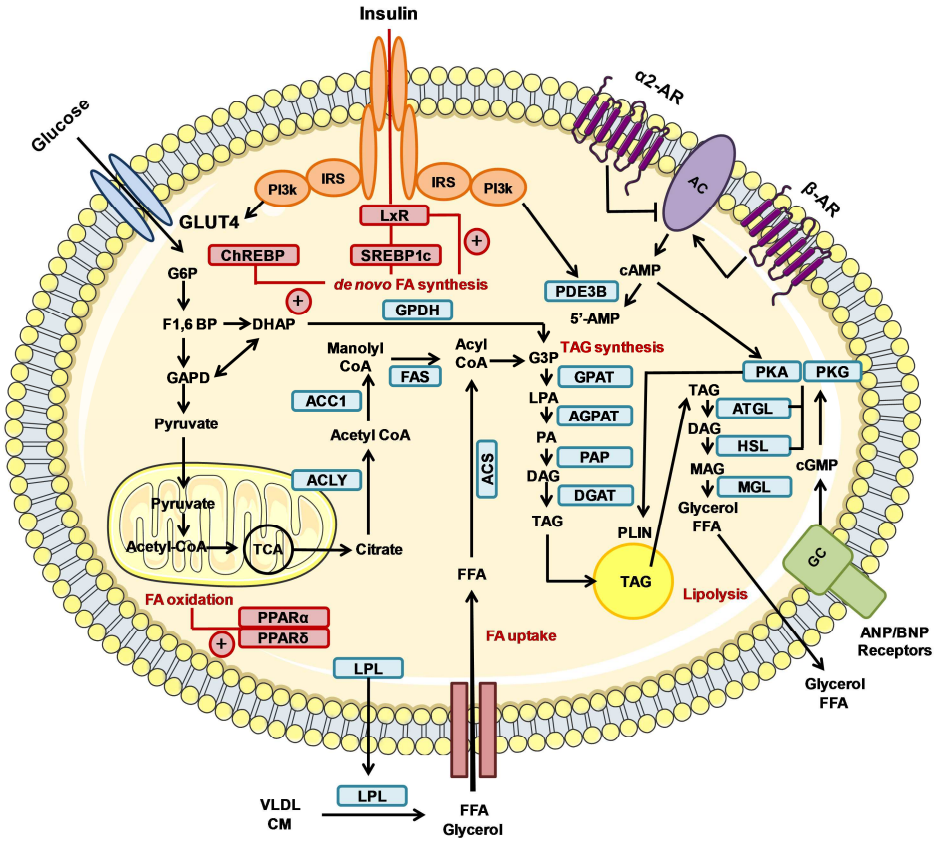


**Figure 14. Fatty acid metabolism in white adipose tissue**. The main metabolic functions of WAT are the storage of energy in the form of triglycerides and the mobilization of this energy when it is required by the body. In WAT, triglycerides can be synthesized following the uptake and metabolism of glucose by the process of *de novo* lipogenesis and/or after the uptake of free fatty acids from the circulation. The triglycerides stored in the adipocyte can be hydrolyzed by the process of lipolysis, which delivers free fatty acids to the circulation. These processes are regulated by the insulin, the adrenergic and atrial natriuretic hormone pathways. α2-AR, alpha 2-adrenergic receptor; β–AR, beta-adrenergic receptor; 5'-AMP, 5'-adenosine monophosphate; AC, adenylate cyclase; ACC1, acetyl-CoA carboxylase 1; ACLY, ATP citrate lyase; ACS, acyl-CoA synthetase; AGPAT, 1-acylglycerol-3-phosphate O-acyltransferase; ANP, atrial natriuretic peptide; ATGL, adipose triglyceride lipase; BNP, brain natriuretic peptide; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ChREBP, carbohydrate-responsive element-binding protein; CM, chylomicrons; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DHAP, dihydroxyacetone phosphate; F1,6BP, fructose 1,6

bisphosphate; FAS, fatty acid synthase; FFA, free fatty acid; G3P, glycerol-3-phosphate; G6P, glucose-6-phosphate; GADH, glyceraldehyde-3-phosphate; GC, guanylate cyclase; GLUT4, glucose transporter 4; GPAT, glycerol-3-phosphate acyltransferase; GPDH, glycerol-3-phosphate dehydrogenase; HSL, hormone sensitive lipase; LPA, lysophosphatidic acid; LPL, lipoprotein lipase; LxR, Liver X receptor; MAG, monoacylglycerol; MGL, monoacylglycerol lipase; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PDE3B, phosphodiesterase 3B; PI3K, phosphatidylinositol 3-kinase; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; PLIN, perilipin; PPARα, peroxisome proliferator-activated receptor alpha; PPARδ, peroxisome proliferator-activated receptor delta; SREBP1c, sterol regulatory element binding protein 1c; TAG, triacylglycerol; VLDL, very low density lipoprotein. Adapted from [126].

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

# III. HYPOTHESIS AND OBJECTIVES

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DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

This doctoral thesis focused on one of the main research lines of the GEMMAIR (Grup d'Estudi de Malalties Metabòliques i Insulin Resistència) research group. GEMMAIR has a track record of over 25 years of experience in the study of liver and chronic metabolic diseases and more than 15 years in the study of the physiopathology of obesity and its associated metabolic diseases.

Justification for the research:

- Obesity is a health epidemic affecting more than 20% of Western populations and has a steadily increasing rate of incidence.
- Obesity significantly increases the risk and worsens the prognosis of many diseases, including diabetes mellitus type 2 (DM2), cardiovascular disease, hyperlipidemia, non-alcoholic fatty liver disease and several types of cancer.
- Not all obese patients have the same risk of developing comorbidities.
- The majority of obese patients have impaired adipose tissue function, which is characterized by adipocyte hypertrophy, hypoxia, and a variety of stresses and inflammatory processes within adipose tissue.
- Adipose tissue dysfunction and ectopic fat accumulation seem to be important factors determining an individual's risk of developing metabolic and obesity-related comorbidities.
- The anatomical distribution of adipose tissue plays an important role in the development of metabolic disorders.
- Subcutaneous and visceral adipose tissues are distinct and produce different metabolic effects.
- Physiological and molecular studies have suggested that fat stored in subcutaneous adipose depots are not directly implicated in the etiology of insulin resistance as this fat appears to play a “buffering”

role in taking up fatty acids and preventing the exposure of other insulin-sensitive tissues to their detrimental effects.

The underlying mechanisms leading to adipose tissue dysfunction and, the different metabolic effects of subcutaneous and visceral adipose tissue in the development of obesity and its related comorbidities are of particular interest in trying to understand the physiopathology of obesity.

We therefore **hypothesized** that the expression of genes and transcription factors involved in the regulation of fatty acid metabolism could be altered in obese patients and that this alteration may be related to adipose tissue dysfunction. As a result, the main objectives of this thesis were to investigate fatty acid metabolism in the subcutaneous and visceral adipose tissue of obese women. To that end, six specific objectives were proposed:

## Study 1: Fatty acid metabolism in morbidly obese women

**1.1** To evaluate the expression of key genes involved in the *de novo* synthesis of fatty acids (LxRα, SREBP1c, ACC1, FAS), the uptake and transport of fatty acids (CD36, FABP4), adipogenesis (PPARγ, adiponectin), fatty acid oxidation (PPARα, PPARδ), and inflammation (IL6, TNFα) in the subcutaneous and visceral adipose tissue of morbidly obese patients and normal-weight healthy subjects.

**1.2** To analyze the protein expression of the genes that are differentially expressed in each group by Western blot analysis.

**1.3** To compare the expression of the genes studied between subcutaneous and visceral adipose tissue.

## Study 2: Fatty acid metabolism in moderately obese women

**2.1** To evaluate the expression of key genes involved in the *de novo* synthesis of FAs (ACC1, FAS), fatty acid oxidation (PPARδ, PPARα) and inflammation (IL6, TNFα) in the subcutaneous and visceral adipose tissue of moderately obese and normal-weight control women to evaluate whether the alterations in fatty acid metabolism in the AT of morbidly obese women found in study 1 are also manifested in moderately obese women.

**2.2** To analyze the protein expression of the genes that are differentially expressed in each group by Western blot analysis.

**2.3** To compare the expression of key genes involved in the *de novo* synthesis of FAs (ACC1, FAS) and fatty acid oxidation (PPARδ, PPARα) in the subcutaneous and visceral adipose tissue of moderately obese and morbidly obese women.

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Esther Guiu Jurado

# IV. RESULTS

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Esther Guiu Jurado

# IV. RESULTS

1. **Downregulation of lipogenesis and fatty acid oxidation in the subcutaneous adipose tissue of morbidly obese women.** *Obesity (Silver Spring)*. 2014 Sep; 22(9):2032-8.

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**Original Article**
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

# Downregulation of Lipogenesis and Fatty Acid Oxidation in the Subcutaneous Adipose Tissue of Morbidly Obese Women

*Teresa Auguet[1,2], Esther Guiu-Jurado[1], Alba Berlanga[1], Ximena Terra[1], Salomé Martinez[3], José Antonio Porras[2], Andra Ceausu[2], Fátima Sabench[4], Mercé Hernandez[4], Carmen Aguilar[1], Joan Josep Sirvent[3], Daniel Del Castillo[4] and Cristóbal Richart[1,2]*

**Objective:** The aim of this study was to analyse the expression of crucial genes in fatty acid metabolism in visceral (VAT) and subcutaneous (SAT) adipose tissue samples from morbidly obese women.
**Methods:** The VAT and SAT expression of key genes in 145 morbidly obese women (MO, BMI $>$ 40 Kg/m$^2$) and 18 normal weight control women by RT-PCR and Western Blot was analyzed.
**Results:** In SAT, the expression levels of the genes related to lipogenesis and fatty acid oxidation were significantly lower in MO than in controls. In VAT, most of the lipogenic genes studied had similar expression levels in MO and control cohort. Regarding inflammation, IL6 was significantly higher in MO in both tissues whereas TNF$\alpha$ mRNA expression was significantly higher only in VAT.
**Conclusions:** Our results indicate that in morbidly obese patients, lipogenesis and fatty acid oxidation are downregulated in SAT, whereas in VAT these pathways are almost unchanged. By contrast, inflammation is induced in both adipose tissues. It is hypothesized that, in this type of extreme obesity, SAT works to limit any further development of fat mass, decreasing the expression of lipogenic and FA oxidative genes whereas VAT depot might have lost this capability.

*Obesity* (2014) **22**, 2032–2038. doi:10.1002/oby.20809

## Introduction

Obesity is a health epidemic affecting more than 20% of Western populations with steadily increasing incidence (1). Obesity significantly increases the risk and prognosis of many diseases, including diabetes mellitus type 2 (DM2), cardiovascular disease, hyperlipidemia, nonalcoholic fatty liver disease, and several types of cancer (2). However, not all obese patients have the same risk of developing these disorders. Adipose tissue dysfunction and ectopic fat accumulation seem to be important factors determining an individual's risk of developing metabolic and cardiovascular comorbidities of obesity (3-5).

Adipocyte functionality is lost during obesity and has been shown to be linked to adipocyte hypertrophy, disequilibrium between lipogenesis and lipolysis, impaired transcriptional regulation of the key factors that control adipogenesis and a lack of sensitivity to external signals, as well as a failure in the signal transduction process, among other factors (6).

Sterol regulatory element binding protein 1c (SREBP1c) is a transcription factor that has an important role in the control of adipogenesis, stimulating the nuclear hormone receptor PPAR$\gamma$, which is the central regulator of adipogenesis and plays a dominant role in fat tissue development (6,7). SREBP1c is now well established as a key

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**Authors' contributions:** TA and EGJ participated in the design of study, in the analysis and interpretation of data and were involved in drafting the manuscript. AB, EGJ, and CA carried out the genetic and protein molecular studies and the immunoassays. XT performed the statistical analysis. SM and JJS are the pathologists who scored liver samples. JAP, FS, AC, and MH made substantial contributions to the conception and design of the study, and to the acquisition of data. DDC and CR revised the draft and gave final approval for publication.

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transcription factor for the regulation of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxilase (ACC1) in liver. However, the mechanisms of lipogenic gene regulation in adipocytes remain unclear (6,8-11). Further studies are needed to investigate whether it has a role in the development of obesity in humans.

The anatomical distribution of adipose tissue plays an important role in the development of metabolic disorders. Subcutaneous (SAT) and visceral (VAT) fat depots are distinct and produce different metabolic effects (12). It is known that VAT adipocytes are more metabolically active, more sensitive to lipolysis and more insulin-resistant than SAT (13). A number of experimental clinical interventions have suggested that SAT may not be implicated directly in the etiology of insulin resistance as it appears to play a "buffering" role in taking up fatty acids and preventing the exposure of other insulin-sensitive tissues to their detrimental effects (14).

The study of VAT and SAT fat depots may help us to understand the physiopathology of the disorders related to obesity. Some studies have analyzed the gene and protein expression or activity levels of some lipogenic enzymes in human adipose tissue and showed that they were lower in obese patients compared with lean subjects (15-20).

Based on that data and to better understand the mechanisms causing or maintaining the adipose tissue dysfunction, the aim of this study was to investigate fatty acid metabolism in VAT and SAT by evaluating the expression of key genes involved in *de novo* synthesis of fatty acids (LxRα, SREBP1c, ACC1, FAS), the uptake and transport of fatty acids (CD36, FABP4), adipogenesis (PPARγ, Adiponectin), fatty acid oxidation (PPARα, PPARδ), and finally, related to inflammation (IL6, TNFα) from morbidly obese (MO) patients and normal-weight healthy subjects.

# Methods

## Subjects

The study was approved by the institutional review board. All participants gave written informed consent for participation in medical research. Visceral (VAT) and subcutaneous (SAT) adipose tissue was analyzed from 163 Spanish women of Western European descent: 145 MO (BMI $>40$ kg/m$^2$) and 18 normal-weight controls (BMI $<25$ kg/m$^2$). Adipose tissue samples were obtained from the MO women who underwent bariatric surgery by laparoscopic gastric bypass, and from normal-weight women who underwent laparoscopic cholecystectomy for benign gall bladder disease or laparoscopic hiatus hernia repair. SAT and VAT biopsies were taken from the superficial right hypochondrial region and from the epiploon region, respectively. Each sample was obtained by the same specialist.

MO women and controls were age matched. The weight of all subjects in the MO group was stable with no fluctuation greater than 2% of body weight for at least 3 months prior to surgery. The exclusion criteria were: (1) patients using antidiabetics or lipid-lowering medications, including PPARα or -γ agonists, (2) diabetic women that were receiving insulin or on medication likely to influence endogenous insulin levels, (3) menopausal and post-menopausal women and subjects receiving contraceptive treatment, and (4) patients who had an acute illness, current evidence of acute or chronic inflammatory or infectious diseases or end-stage malignant diseases.

In the MO group, 59 women had type 2 diabetes mellitus (T2DM), a diagnosis based on ADA guidelines (21).

## Biochemical analyses

A complete anthropometrical, biochemical, and physical examination was carried out on each patient. Body height and weight were measured with the patient standing in light clothes and shoeless. BMI was calculated as body weight divided by squared height (kg/m$^2$). The subjects' waist circumference (WC) was measured with a soft tape midway between the lowest rib and the iliac crest. Laboratory studies included glucose, insulin, glycated haemoglobin (HbA1c), total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides, all of which were analyzed using a conventional automated analyser after overnight fasting. Insulin resistance (IR) was estimated using the homeostasis model assessment of IR (HOMA2-IR) (22).

Circulating levels of TNFRI, TNFRII (Biosource Europe S.A., Nivelles, Belgium), HMW adiponectin (Millipore, Missouri, USA), C-reactive protein (CRP) (Dade Behring, Marburg, Germany), leptin (Biovendor, Modrice, Czech Republic), and IL6 (Quantikine, R&D Systems, Minneapolis, USA) were measured in duplicate using enzyme-linked immunosorbent assays (ELISA) following the manufacturer's instructions.

## RNA isolation and real-time PCR

SAT and VAT samples obtained were conserved in RNAlater (Sigma, Barcelona, Spain) for 24 h at 4°C and then stored at −80°C. Total RNA from SAT and VAT was isolated according to the manufacturer's protocol RNeasy midi kit (Qiagen, Barcelona, Spain). RNA was digested with DNase I (RNase-Free DNase set; Qiagen). First-strand cDNA was synthesized using an equal amount of total RNA with High Capacity RNA-to-cDNA Kit (Applied Biosystems, Madrid, Spain). Real-time quantitative PCR was carried out in a final volume of 20 μl, which contained 10 ng of reverse-transcribed cDNA, 10 μl of 2X Taq Man Fast Universal PCR Master Mix (Applied Biosystems) and 1 μl Taq Man Assay predesigned by Applied Biosystems for the detection of LxRα, SREBP1c, ACC1, FAS, CD36, FABP4, PPARγ, adiponectin, PPARα, PPARδ, IL6 and TNFα gene, and for GAPDH, which was used as the housekeeping gene. All reactions were carried out in duplicate in 96-well plates using the 7900HT Fast Real-Time PCR systems (Applied Biosystems).

## Western blot analysis

Protein levels were evaluated in a subgroup of 24 subjects (MO, $n=12$; Control, $n=12$) from whom enough tissue was available. SAT and VAT samples were homogenized in a medium containing 50 mM HEPES, 150 mM NaCl, 1 mM DTT, 0.1% SDS, and 1% protease inhibitor cocktail (Thermo Scientific, Madrid, Spain). Protein concentrations were determined using a BCA assay kit (Thermo Scientific). For Western blot analysis, equal amounts of protein (35 μg) were separated by SDS/PAGE (7% acrylamide) and transferred onto nylon membranes. Nonspecific binding was blocked by preincubation of the membranes with 5% (w/v) nonfat milk powder in

0.1% PBS-Tween for 1 h. Specific protein expression was detected by incubating with rabbit anti-ACC1 (Cell Signaling Technology, Barcelona, Spain) and rabbit anti-FAS (Cell Signaling Technology) antibodies overnight at 4°C, followed by an incubation with anti-mouse IgG (GE Healthcare, Freiburg, Germany) or anti-rabbit IgG (GE Healthcare) antibodies for 2 h at room temperature and developed with SuperSignal West Pico Chemiluminescent or SuperSignal-Femto Maximum Sensitivity Substrate (Thermo Scientific). The density of specific bands was determined by densitometry and quantified by the Phoretix1D software from TotalLab. The expression pattern of all proteins was normalized by $\beta$-actin (Sigma) adipose expression.

### Statistical analysis

All the values reported are expressed as mean ± S.D (standard deviation) and were analyzed using SPSS/PC+ statistical package for windows (version 19.0; SPSS, Chicago, IL). Differences between groups were calculated using Student's *t*-test or the one-way ANOVA analysis. The strength of association between variables was calculated using Pearson's method for parametric variables and Spearman's $\rho$-correlation test for nonparametric contrasts. *P* values $<0.05$ were considered statistically significant.

## Results

### Baseline characteristics of the subjects in the study

Patients' baseline characteristics given in Table 1 show the mean and SD of the variables of interest. Patients were separated into control (BMI $<25$ kg/m$^2$), and MO subjects (BMI $>40$ kg/m$^2$). Biochemical analyses indicate that MO women had significantly higher levels of HOMA2-IR, fasting glucose, insulin, HbA1c, triglycerides and systolic blood pressure (SBP) than the control group did. Cholesterol HDL (HDL-c) was significantly lower in the MO patients than in the control group.

We further sub-classified the MO cohort according to the presence of diabetes into: diabetic (D, $n = 59$) and nondiabetic (ND, $n = 86$) patients. As expected, the results indicate that glucose (ND = 97.8 ± 1.4, D = 160.0 ± 5.8 mg/dL, $P < 0.001$), HbA1c (ND = 5.1 ± 0.1, D = 7.2 ± 2.7%, $P < 0.001$) and triglyceride levels (ND = 153.5 ± 7.8, D = 186.7 ± 11.4 mg/dL, $P = 0.018$) were higher in the D sub-group compared with ND.

Circulating cytokine levels also varied between normal-weight and obese subjects. Table 1 shows the circulating levels of HMW adiponectin, CRP, TNFRI, TNFRII, leptin and IL6. The results indicate that the circulating levels of CRP and leptin increased in the MO cohort whereas HMW adiponectin levels decreased in this group. Furthermore, adipocytokine levels differed between diabetics and nondiabetics. TNFRI levels were higher in diabetics (ND = 2.8 ± 0.1, D = 3.3 ± 0.2 ng/mL, $P < 0.001$) whereas adiponectin was lower in this group (ND = 3.2 ± 0.6, D = 5.8 ± 0.6 μg/mL, $P < 0.001$).

### Evaluation of the expression of genes related to lipid metabolism and inflammation in SAT and VAT and their protein expression

We analyzed the expression of genes related to *de novo* synthesis of fatty acids (FAs) (LxRα, SREBP1c, ACC1, FAS), the uptake and transport of FAs(CD36, FABP4), adipogenesis (PPARγ, adiponectin), FA oxidation (PPARα, PPARδ), and related to inflammation (IL6, TNFα).

**TABLE 1** Characteristics of the cohort studied

| Variables | Control ($n = 18$) Mean ± SD | Morbidly obese ($n = 145$) Mean ± SD | *P*-value |
|---|---|---|---|
| **Age (years)** | 52.6 ± 309 | 47.0 ± 1.0 | 0.184 |
| **Weight (kg)** | 61.5 ± 2.4 | 122.5 ± 1.6 | **<0.001** |
| **WC (cm)** | 75.7 ± 3.9 | 130.9 ± 1.3 | **<0.001** |
| **BMI (kg/m$^2$)** | 23.0 ± 0.4 | 47.6 ± 0.5 | **<0.001** |
| **Glucose (mg/dl)** | 92.6 ± 2.2 | 123.5 ± 3.5 | **<0.001** |
| **Insulin (mUI/l)** | 6.6 ± 1.1 | 21.2 ± 1.6 | **<0.001** |
| **HbA1c (%)** | 4.6 ± 0.1 | 6.0 ± 0.2 | **<0.001** |
| **HOMA2-IR** | 1.1 ± 0.2 | 2.8 ± 0.2 | **0.012** |
| **SBP (mmHg)** | 127.2 ± 4.6 | 136.5 ± 1.4 | **0.022** |
| **DBP (mmHg)** | 73.6 ± 2.4 | 77.8 ± 1.2 | 0.185 |
| **Total cholesterol (mg/dl)** | 179.7 ± 7.3 | 173.0 ± 2.8 | 0.403 |
| **HDL-C (mg/dl)** | 53.0 ± 4.1 | 39.8 ± 0.8 | **0.007** |
| **LDL-C (mg/dl)** | 103.9 ± 7.3 | 100.4 ± 2.5 | 0.653 |
| **Triglycerides (mg/dl)** | 109.2 ± 14.6 | 168.9 ± 6.8 | **0.001** |
| **Adipo/cytokine levels** | | | |
| **HMW adiponectin (μg/ml)** | 11.9 ± 1.8 | 7.4 ± 4.1 | **<0.001** |
| **IL6 (pg/ml)** | 2.1 ± 0.7 | 3.0 ± 0.3 | 0.848 |
| **Leptin (ng/ml)** | 34.3 ± 13.0 | 255.6 ± 24.0 | **<0.001** |
| **TNFRI (ng/ml)** | 2.5 ± 0.4 | 3.0 ± 0.1 | 0.221 |
| **TNFRII (ng/ml)** | 4.2 ± 0.7 | 5.1 ± 0.3 | 0.195 |
| **CRP (mg/dl)** | 0.7 ± 0.4 | 2.6 ± 0.5 | **0.002** |

*P*-values in bold indicate significant differences respect control group ($P < 0.05$). Data are expressed as mean ± SD.
BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycosylated haemoglobin; HDL-C, high density lipoprotein; HOMA2-IR, homeostatic model assessment 2- insulin resistance; LDL-C, low density lipoprotein; SBP, systolic blood pressure; WC, waist circumference.

We first compared MO and control groups. The results indicate that the visceral mRNA expression of genes related to *de novo* synthesis of FAs in MO were similar to those of control women. Only, FAS mRNA expression was significantly lower in MO (Table 2). Regarding SAT, the results indicate that the mRNA expression levels of LxRα, SREBP1c, ACC1, and FAS were significantly lower in MO (Table 3).

In order to confirm these results regarding gene expression, we also analyzed the protein expression of ACC1 and FAS by Western Blot in both adipose tissues. There were similar results with respect to ACC1 and FAS protein expression and those obtained in the gene expression analysis. ACC1 and FAS protein expression was significantly lower in MO in both adipose tissues (Figure 1).

Additionally, we studied the genes related to the uptake and transport of FAs. In VAT, we found that CD36 and FABP4 mRNA expression were significantly lower in MO compared to controls (Table 2) whereas in SAT, CD36 was significantly higher in the obese group (Table 3).

**TABLE 2** Visceral adipose tissue expression of genes related to *de novo* fatty acid synthesis, fatty acid oxidation, uptake and transport and inflammation in morbidly obese patients

| Gene expression | Control ($N = 18$) | MO ($N = 145$) | $P$-value |
|---|---|---|---|
| **Lipogenesis** | | | |
| LxRα | 0.6 ± 0.17 | 0.5 ± 0.03 | 0.301 |
| SREBP1c | 1.1 ± 0.31 | 0.8 ± 0.04 | 0.091 |
| ACC1 | 1.1 ± 0.26 | 0.8 ± 0.05 | 0.114 |
| FAS | 7.8 ± 2.84 | 1.3 ± 0.1[a] | 0.05 |
| **Fatty acid oxidation** | | | |
| PPARα | 0.5 ± 0.2 | 0.6 ± 0.03 | 0.565 |
| PPARδ | 0.8 ± 0.16 | 0.8 ± 0.04 | 0.859 |
| **Fatty acid uptake and transport** | | | |
| PPARγ | 14.5 ± 4.68 | 14 ± 0.91 | 0.892 |
| CD36 | 40.4 ± 8.92 | 27.7 ± 2.16[a] | 0.05 |
| FABP4 | 418 ± 119.03 | 210.4 ± 17.7[a] | 0.002 |
| **Inflammation** | | | |
| IL6 | 0.5 | 7.6 ± 1.11[a] | <0.001 |
| TNFα | 0.04 | 0.2 ± 0.03[a] | 0.001 |
| AdipoQ | 56.4 | 53.9 ± 3.81 | 0.469 |

MO, morbidly obese subjects.
[a]Indicates significant differences respect control group ($P < 0.05$). Data are expressed as mean ± SD.

**TABLE 3** Subcutaneous adipose tissue expression of genes related to *de novo* fatty acid synthesis, fatty acid oxidation, uptake and transport and inflammation in morbidly obese patients

| Gene expression | Control ($N = 18$) | MO ($N = 145$) | $P$-value |
|---|---|---|---|
| **Lipogenesis** | | | |
| LxRα | 0.4 ± 0.06 | 0.3 ± 0.02[a] | 0.041 |
| SREBP1c | 1.9 ± 0.67 | 0.7 ± 0.07[a] | <0.001 |
| ACC1 | 52 ± 26.87 | 0.6 ± 0.05[a] | <0.001 |
| FAS | 41.8 ± 28.85 | 1.5 ± 0.15[a] | <0.001 |
| **Fatty acid oxidation** | | | |
| PPARα | 12.6 ± 71.15 | 0.6 ± 0.03[a] | <0.001 |
| PPARδ | 14.7 ± 0.58 | 0.6 ± 0.03[a] | <0.001 |
| **Fatty acid uptake and transport** | | | |
| PPARγ | 24.5 ± 9.05 | 16.4 ± 1.24 | 0.374 |
| CD36 | 47.9 ± 4.65 | 93.2 ± 83.53[a] | <0.001 |
| FABP4 | 607.5 ± 147.12 | 605.4 ± 98.4 | 0.991 |
| **Inflammation** | | | |
| IL6 | 3 ± 1.89 | 24 ± 3.55[a] | <0.001 |
| TNFα | 0.1 ± 0.02 | 0.1 ± 0.01 | 0.784 |
| AdipoQ | 51.7 ± 6.77 | 30.7 ± 2.42[a] | 0.009 |

MO, morbidly obese subjects.
[a]Indicates significant differences respect control group ($P < 0.05$). Data are expressed as mean ± SD.

Regarding FA oxidation genes, the results indicate that visceral mRNA expression were similar to those of control women (Table 2), while in SAT, PPARα and PPARδ mRNA expression were significantly lower in MO (Table 3).

We also studied the genes related to adipogenesis. In VAT, we found that PPARγ and adiponectin mRNA expression in MO were similar to those of control women whereas in SAT adiponectin mRNA expression was significantly lower in MO.

Finally, we found that IL6 and TNFα mRNA expression in VAT were significantly higher in MO. In SAT, IL6 mRNA expression was significantly higher in MO.

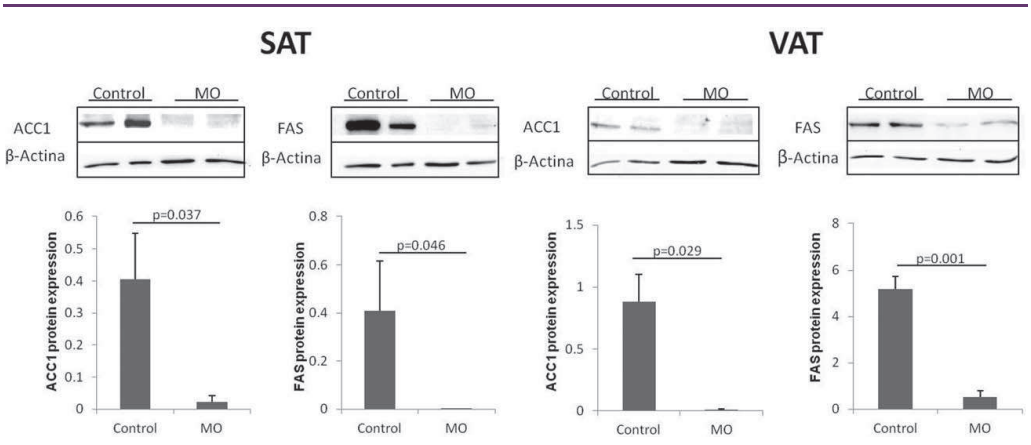


**FIGURE 1** Subcutaneous (SAT) and visceral (VAT) adipose tissue protein expression of lipogenic enzymes in MO patients. Representative Western blot analysis showing ACC1, FAS and β-actin protein expression and bar graphs showing the quantification of bands normalized by values of β-actin bands ($n = 12$ for each group). Results are shown as mean ± SD. $P < 0.05$ are considered statistically significant.

We also compared the expression levels of these genes in diabetic MO women and nondiabetic. We did not find any differences in any of the studied genes (data not shown).

## Comparison between SAT and VAT gene expression

We compared the mRNA expression of genes related to de novo synthesis of FAs, the uptake and transport of FAs, FA oxidation, and related to inflammation between VAT and SAT. In the MO cohort, when we studied *de novo* FA synthesis, the results indicate that ACC1 and LxRα mRNA expression were significantly higher in VAT compared to SAT (ACC1: $P = 0.005$, LxRα: $P < 0.001$), whereas FAS and SREBP1c mRNA expression were similar in both tissues. In contrast, the expression of genes related to FA uptake and transport (CD36 and FABP4) were significantly lower in VAT versus SAT (CD36: $P < 0.001$, FABP4: $P < 0.001$). Regarding FA oxidation, PPARδ and PPARα expression in VAT were higher than in SAT (PPARδ: $P < 0.001$, PPARα: $P = 0.036$). Finally, when we compared the mRNA expression of inflammatory genes between both tissues, the results showed that TNFα expression was higher in VAT ($P = 0.001$). By contrast, IL6 expression was increased in SAT ($P < 0.001$).

We also compared the expression of these genes in VAT and SAT from normal-weight subjects; however, we did not find significant differences between tissues (data not shown).

## Relationship of VAT and sat gene expression with anthropometric and metabolic variables in morbidly obese patients

In VAT, we found a positive correlation between ACC1 expression and leptin levels ($r = 0.301$, $P = 0.035$) and also between FAS expression and CRP circulating levels ($r = -0.270$, $r = 0.033$) in the MO group. LxRα expression correlated positively with IL6 levels ($r = 0.473$, $P = 0.019$). Our results showed positive correlations between PPARδ expression and levels of leptin ($r = 0.304$, $P = 0.042$), TNFRI ($r = 0.441$, $P = 0.002$) and TNFRII ($r = 0.337$, $P = 0.024$). CD36 and adiponectin expression correlated positively with HMW adiponectin levels (CD36: $r = 0.345$, $P = 0.039$; adiponectin: $r = 0.387$, $P = 0.022$). We also found negative correlations between TNFα expression and the levels of IL6 ($r = -0.342$, $P = 0.055$) and CRP ($r = -0.450$, $P = 0.008$). Our results showed that PPARα expression correlated negatively with diastolic blood pressure (DBP) ($r = -0.306$, $P = 0.008$), and also TNFα expression with SBP ($r = -0.359$, $P = 0.034$). Finally, FABP4 expression correlated positively with weight, BMI and WC (weight: $r = 0.377$, $P = 0.021$; BMI: $r = 0.346$, $P = 0.036$; WC: $r = 0.888$, $P = 0.044$).

In SAT, we found a positive correlation between ACC1 expression and HDL-c levels ($r = 0.224$, $P = 0.015$). LxRα and PPARδ expression correlated negatively with WC (LxRα: $r = -0.307$, $P = 0.019$; PPARδ: $r = -0.268$, $P = 0.027$). Our results also showed negative correlations between PPARγ expression and total cholesterol circulating levels ($r = -0.183$, $P = 0.059$), and between PPARα expression and insulin levels ($r = -0.285$, $P = 0.017$). CD36 expression correlated negatively with levels of total cholesterol ($r = -0.371$, $P = 0.020$) and LDL-C ($r = -0.336$, $P = 0.045$). We found negative correlations between IL6 expression and WC ($r = -0.813$, $P = 0.026$), total cholesterol ($r = -0.472$, $P = 0.003$) and LDL-C circulating levels ($r = -0.432$, $P = 0.011$). Also, we found positive correlations between ACC1 expression and HMW adiponectin levels ($r = 0.377$, $P = 0.009$), and between TNFα expression and leptin circulating levels ($r = 0.440$, $P = 0.007$). Finally, PPARγ correlated negatively with levels of TNFRI ($r = -0.318$, $P = 0.020$) and CRP ($r = 0.260$, $P = 0.010$).

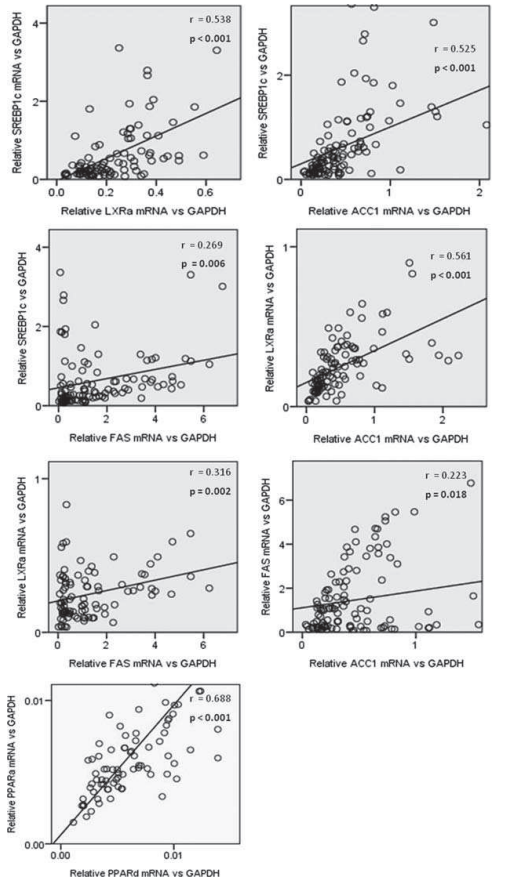


**FIGURE 2** Subcutaneous adipose tissue correlations of genes related to *de novo* fatty acid synthesis and related to fatty acid oxidation in MO patients.

## Correlations between the expression of genes related to *de novo* FA synthesis, FA oxidation, and related to uptake and transport of FAs in VAT and SAT from morbidly obese patients

The analysis of the relationship of genes related to *de novo* FA synthesis indicate that SREBP1c was positively related to LxRα and ACC1 in VAT (LxRα: $r = 0.393$, $P < 0.001$; ACC1: $r = 0.690$, $P < 0.001$). Also, LxRα and ACC1 were strongly related ($r = 0.664$, $P < 0.001$). In SAT, all the genes in this pathway were positively related (Figure 2).

The correlations between the expression of genes related to FA oxidation indicate that PPARα and PPARδ were related in both tissues

(VAT: $r = 0.497$, $P < 0.001$; SAT: $r = 0.688$; $P < 0.001$). Regarding the analysis of the relationship of genes related to the uptake and transport of FAs and adipogenesis, we found that CD36 was related to PPAR$\gamma$ (VAT: $r = 0.673$, $P < 0.001$; SAT: $r = 0.398$, $P = 0.012$) and to FABP4 (VAT: $r = 0.545$, $P = 0.001$) in both VAT and SAT. In addition, PPAR$\gamma$ and FABP4 were also related (VAT: $r = 0.372$, $P = 0.023$; SAT: $r = 0.659$, $P < 0.001$). Finally, the correlations between the expression of genes related to inflammation indicate that IL6 and TNF$\alpha$ were related in VAT ($r = 0.515$, $P = 0.002$).

## Discussion

In this study, we analyzed the expression of crucial genes in fatty acid metabolism in VAT and SAT samples from MO women and normal-weight subjects. To date, few studies have been reported in these two adipose tissues simultaneously.

This study demonstrates that mRNA gene expression of the main enzymes involved in *de novo* fatty acid synthesis (LxR$\alpha$, SREBP1c, ACC1 and FAS) were significantly lower in MO women than those of control group in the SAT depot, whereas in VAT only FAS gene expression was lower. Moreover, the expression of key genes related to fatty acid oxidation (PPAR$\alpha$, PPAR$\delta$) was significantly lower in SAT in MO. However, the gene expressions of CD36, involved in fatty acid uptake and transport, and of IL6, a pro inflammatory factor, were higher in SAT.

Our findings indicate that, in women with extreme obesity, the lipogenic pathway is, at gene and protein expression levels, downregulated in SAT. During a dynamic obesity period, an increase in the lipogenic capacity of adipose tissue is expected. However, our results agree with other authors who suggest that the low expression of lipogenesis pathway in MO cohort, with a lasting fat excess, could be a late adaptative process, aimed at limiting further development of fat mass (23). Interestingly, we also found the same pattern regarding PPAR$\alpha$ and PPAR$\delta$ involved in FA oxidation pathway. They both were downregulated in SAT in MO women. Furthermore, the positive correlations found between the different genes involved in these pathways strengthen their role in the adaptative process of SAT.

Regarding the expression of genes related to the uptake and transport of FAs, CD36 and FABP4 were lower in VAT in MO. A recent study described that FABP4 gene expression was significant lower in MO than in moderately obese or lean subjects in VAT and SAT depot (24). In contrast, we found that CD36 was significantly higher in SAT in the MO group. These results suggest that in MO patients the uptake and transport of FAs could be downregulated only in VAT. Regarding that, it is well known that visceral obesity is associated to IR, attributed in part to the increases in circulating free FAs concentrations (25). Insulin is an anabolic hormone known to direct the storage and utilization of energy in adipocytes (26). When cells become insulin resistant, they lose its relative capacity to uptake glucose and free FAs from bloodstream. This fact could explain the downregulation of CD36 and FABP4 in VAT in the MO group.

Our results support the hypothesis that SAT is "metabolically innocent" and intra-abdominal fat has deleterious consequences. Recent studies have examined the relative correlations of subcutaneous, intra-abdominal and liver fat with fasting insulin, hepatic insulin sensitivity, and dyslipidemia. Only intra-abdominal and liver fat were strongly and independently linked to these variables (27). The SAT appears to play a "buffering" role in taking up FAs and preventing the exposure of other insulin-sensitive tissues to their harmful effects (14). In this context, individuals with different congenital lipodystrophy syndromes, in spite of having a marked reduction in body fat, have severe IR, hepatic steatosis, and severe dyslipidemia (28). This supports the concept that a limitation of SAT expansion leads to IR and subsequent metabolic complications. Besides an inability to store triglycerides in adipocytes, a marked reduction in adipokine production will also contribute to the metabolic derangement in lipodystrophy (29). Data collected on the positive metabolic aspects of SAT and adverse consequences of its deficiency in experimental animals (30) or human lipodystrophy has led to a hypothesis about the desirability capacity of SAT "expandability" to accommodate excess lipid supply and to avoid its spillover into "ectopic" sites (31,32).

Several adipose tissue-secreted proinflamatory products, such as IL6 or TNF$\alpha$, have been shown to induce insulin resistance and are thought to link obesity and type 2 diabetes (33). The increased production and high circulating levels of these products in obesity have led to the view that obese individuals are characterized by a state of chronic low-grade inflammation (13,34-38). As expected, we found that IL6 and TNF$\alpha$ gene expression were significantly higher in MO compared to control group in VAT. In SAT, only IL6 was significantly higher in MO. Therefore, in both tissues, there is a proinflammatory profile in MO women. Interestingly, other authors have described that an increased inflammation is accompanied by a decrease in lipogenesis, in agreement with our results (39).

When we compared the gene expression between SAT and VAT in the MO cohort, we found that the expression of genes related to lipogenesis (ACC1 and LxR$\alpha$), FA oxidation (PPAR$\delta$, PPAR$\alpha$), and inflammation (TNF$\alpha$) were upregulated in VAT compared to SAT. In contrast, regarding to FA uptake and transport (FABP4 and CD36) were upregulated in SAT. These results suggest that the lipid metabolism regulation of the adipose tissue in MO patients differs depending on its localization. In this sense, transplantation of SAT adipose tissue into VAT depots has been performed successfully in mice with beneficial effects, improving glucose tolerance and decreasing plasma insulin concentration and portal plasma triglycerides (40). According to the beneficial effects of SAT described by these authors and our results discussed above, we found that the FA uptake from the blood stream in SAT is increased but the lipogenic and FA oxidation pathways are downregulated.

Our study cohort has made it possible to investigate fatty acid metabolism in VAT and SAT adipose tissue without the interference of such confounding factors as gender or age. However, the results of our study cannot be extrapolated to other obesity groups or to men.

In conclusion, although it is not possible to determine the causality that leads downregulation of lipogenesis and fatty acid oxidation in SAT, the reported results suggest that in this type of extreme obesity, SAT works to limit a further development of fat mass, decreasing the expression of lipogenic and FA oxidative genes. Furthermore, our findings also suggest that SAT adipose tissue might have a protective role in MO patients. Further prospective experiments are needed in order to better understand the deregulation of the pathways studied and the differences between VAT and SAT in morbid obesity. O

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DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

# IV. RESULTS

2. **Downregulation of *de novo* fatty acid synthesis pathway in subcutaneous adipose tissue of moderately obese women.** *Int J Mol Sci*. 2015; 16, 29911–29922.

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# Downregulation of *de Novo* Fatty Acid Synthesis in Subcutaneous Adipose Tissue of Moderately Obese Women

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**Abstract:** The purpose of this work was to evaluate the expression of fatty acid metabolism-related genes in human adipose tissue from moderately obese women. We used qRT-PCR and Western Blot to analyze visceral (VAT) and subcutaneous (SAT) adipose tissue mRNA expression involved in *de novo* fatty acid synthesis (*ACC1*, *FAS*), fatty acid oxidation (*PPARα*, *PPARδ*) and inflammation (*IL6*, *TNFα*), in normal weight control women (BMI < 25 kg/m$^2$, $n$ = 35) and moderately obese women (BMI 30–38 kg/m$^2$, $n$ = 55). In SAT, *ACC1*, *FAS* and *PPARα* mRNA expression were significantly decreased in moderately obese women compared to controls. The downregulation reported in SAT was more pronounced when BMI increased. In VAT, lipogenic-related genes and *PPARα* were similar in both groups. Only *PPARδ* gene expression was significantly increased in moderately obese women. As far as inflammation is concerned, *TNFα* and *IL6* were significantly increased in moderate obesity in both tissues. Our results indicate that there is a progressive downregulation in lipogenesis in SAT as BMI increases, which suggests that SAT decreases the synthesis of fatty acid *de novo* during the development of obesity, whereas in VAT lipogenesis remains active regardless of the degree of obesity.

**Keywords:** moderate obesity; fatty acid metabolism; adipose tissue; *de novo* fatty acid synthesis

## 1. Introduction

Obesity is significantly associated with the development of several comorbidities including type 2 diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome, non-alcoholic fatty liver disease, cardiovascular disease and certain neoplasms [1]. Nevertheless, obesity itself does not necessarily lead to these comorbidities [2–4].

*Int. J. Mol. Sci.* **2015**, *16*, 29911–29922

Not only fat accumulation in ectopic sites but also dysfunction of adipose tissue might play a significant role in defining an individual's risk of developing obesity-related comorbidities [5]. Physiological and molecular studies have suggested that the fat stored in subcutaneous adipose depots are not directly implicated in the development of insulin resistance. It seems to have a "buffering" role due to the fact that it takes up fatty acids (FAs) and prevents other insulin-sensitive tissues from being exposed to their damaging consequences [6]. In this sense, Klein *et al.* showed that obesity-associated metabolic variables were not improved by liposuction (reduction of subcutaneous adipose tissue) [7]. However, reducing visceral adipose tissue by omentectomy combined with gastric banding has positive long-term effects on insulin sensitivity and glucose metabolism [8].

Likewise, deregulation of lipogenesis and FA oxidation contribute to the development of metabolic diseases [9]. The expression of *de novo* FA synthesis enzymes in human adipose tissue have been evaluated in some studies that have found lower mRNA expression in obese patients compared to control subjects [10–15]. With regard to FA oxidation, several reports have shown that activating the peroxisome proliferator-activated receptor aplha (PPARα) in human adipocytes enhanced FA oxidation by inducing the mRNA expression of the genes involved in this pathway [16,17]. Moreover, Wang *et al.* showed that targeted activation of peroxisome proliferator-activated receptor delta (PPARδ) in adipose tissue induces FA oxidation gene expression [18].

In a previous work, we studied the expression of the main genes involved in fatty acid metabolism in adipose tissue of morbidly obese and normal-weight control women [19]. Our findings suggested that, in morbid obesity, SAT prevents the subcutaneous fat mass from developing further. Because not all obese subjects have the same metabolic traits and the mechanisms of adipose tissue dysfunction are not fully understood, the aim of the present study was to use our previous findings to investigate whether the alterations in the fatty acid metabolism of morbidly obese women also manifest in moderately obese women. Consequently, we evaluated the expression of key genes related to *de novo* synthesis of FAs (*ACC1*, *FAS*), FA oxidation (*PPARδ*, *PPARα*) and inflammation (*IL6*, *TNFα*) in the SAT and VAT of moderately obese and normal-weight control women.

## 2. Results

### 2.1. Baseline Characteristics of the Cohort Studied

Subjects were classified according to BMI into control (BMI < 25 kg/m$^2$), and moderately obese patients (BMI 30–38 kg/m$^2$). The patients' baseline characteristics are shown in Table 1. Moderately obese women had significantly higher levels of glucose metabolism variables (fasting glucose, insulin, HbA1c and HOMA2-IR) and triglycerides than the control group. HDL-C was significantly decreased in the moderately obese compared to controls.

Subsequently we sub-classified the moderately obese women according to the presence of diabetes. Obviously, the results indicate that glucose and HbA1c were significantly increased in diabetic patients (D) compared to non-diabetic subjects (ND) (Glucose: ND = 93.67 ± 2.73, D = 172.56 ± 21.58 mg/dL, $p < 0.001$; HbA1c: ND = 5.22 ± 1.83, D = 7.16 ± 1.83, $p = 0.030$).

**Table 1.** Characteristics of the cohort studied.

| Variables | Controls ($n$ = 35) | Moderately Obese Patients ($n$ = 55) | *p-Value* |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| AGE (years) | 49.57 ± 14.17 | 52.94 ± 14.24 | 0.289 |
| WEIGHT (kg) | 57.53 ± 7.17 | 84.49 ± 11.65 | **<0.001** |
| WC (cm) | 76.43 ± 11.42 | 109.85 ± 11.15 | **<0.001** |
| BMI (kg/m$^2$) | 22.28 ± 1.63 | 33.67 ± 2.70 | **<0.001** |
| GLUCOSE (mg/dL) | 86.51 ± 22.96 | 110.61 ± 45.32 | **0.002** |
| INSULIN (mU/L) | 6.84 ± 5.43 | 13.88 ± 9.65 | **<0.001** |
| HbA1c (%) | 4.92 ± 0.64 | 5.49 ± 1.18 | **0.015** |

*Int. J. Mol. Sci.* **2015**, *16*, 29911–29922

**Table 1.** *Cont.*

| Variables | Controls ($n$ = 35) | Moderately Obese Patients ($n$ = 55) | *p-Value* |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| HOMA2-IR | 0.88 ± 0.75 | 1.90 ± 1.38 | **<0.001** |
| SBP (mmHg) | 124.63 ± 18.20 | 130.37 ± 18.11 | 0.157 |
| DBP (mmHg) | 69.8 ± 9.52 | 74.27 ± 11.45 | 0.063 |
| TOTAL CHOLESTEROL (mg/dL) | 181.33 ± 37.57 | 182.92 ± 51.38 | 0.867 |
| HDL-C (mg/dL) | 55.34 ± 14.93 | 43.29 ± 10.86 | **<0.001** |
| LDL-C (mg/dL) | 107.33 ± 31.02 | 113.6 ± 43.37 | 0.482 |
| TRIGLYCERIDES (mg/dL) | 93.57 ± 58.13 | 149.15 ± 82.57 | **0.001** |

*p*-Values in bold indicate significant differences with respect to the control group ($p < 0.05$). BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein; HOMA2-IR, homeostatic model assessment 2-insulin resistance; LDL-C, low-density lipoprotein; SBP, systolic blood pressure; WC, waist circumference.

### 2.2. Evaluation of the Expression of FA Metabolism Genes and Their Protein Levels in SAT and VAT

We performed the expression analysis of genes related to lipogenesis (*ACC1*, *FAS*), FA oxidation (*PPARα*, *PPARδ*) and inflammation (*IL6*, *TNFα*) in human adipose tissue.

To investigate how gene expression was affected by BMI, we conducted analyses in the controls (BMI < 25 kg/m$^2$) and moderately obese women (BMI 30–38 kg/m$^2$). The subcutaneous mRNA expression of genes related to lipogenesis was significantly decreased in moderately obese women compared to control women (Figure 1). The results for VAT indicate that *ACC1* and *FAS* mRNA expression were similar in both groups (Figure 2). To validate these results, we also conducted Western Blot analysis of ACC1 and FAS in both fat depots. The protein analysis showed that ACC1 and FAS protein levels were similar to those obtained in the mRNA expression analysis. ACC1 and FAS protein levels in SAT were significantly lower in moderately obese patients (Figure 3A), whereas in VAT there were no differences between the two groups (Figure 3B).

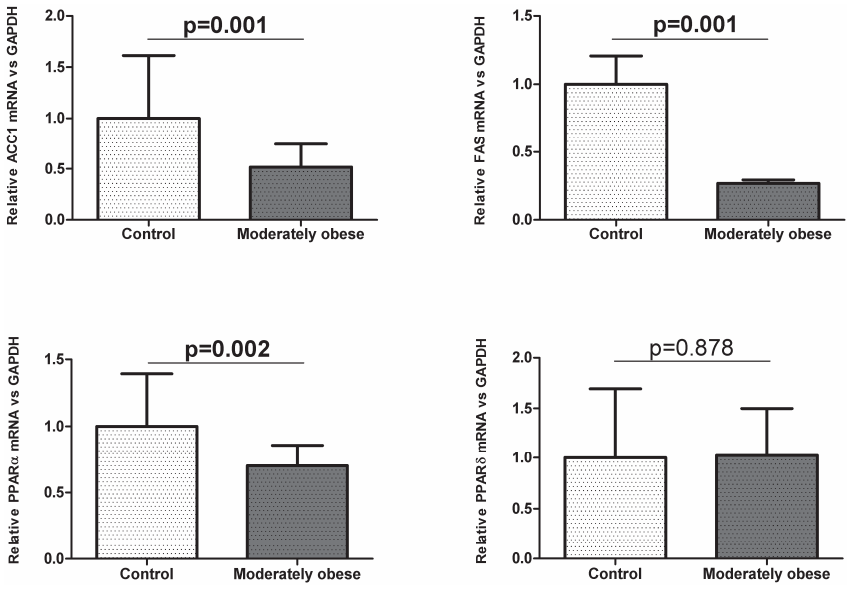


**Figure 1.** *Cont.*

*Int. J. Mol. Sci.* **2015**, *16*, 29911–29922

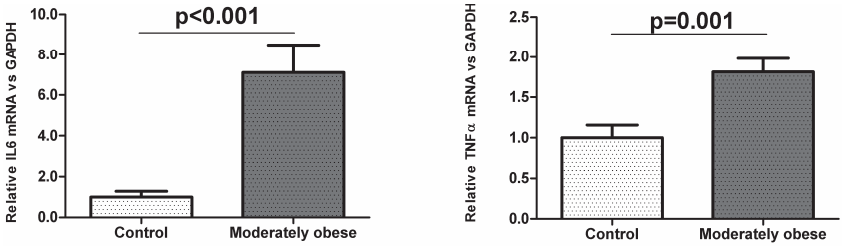


**Figure 1.** Expression of genes involved in lipogenesis, FA oxidation and inflammation in control ($n$ = 35) and moderately obese women ($n$ = 55) in subcutaneous adipose tissue. Student's $t$-test was used to determinate differences between groups. Data are expressed as mean ± SD. The mRNA expression was calculated relative to the control group, whose mRNA expression was set to 1.0. ACC1, Acetyl-CoA carboxylase 1; FAS, Fatty acid synthase; IL6, Interleukin 6; PPARα, Peroxisome proliferator-activated receptor alpha; PPARδ, Peroxisome proliferator-activated receptor delta; TNFα, Tumor necrosis factor alpha.

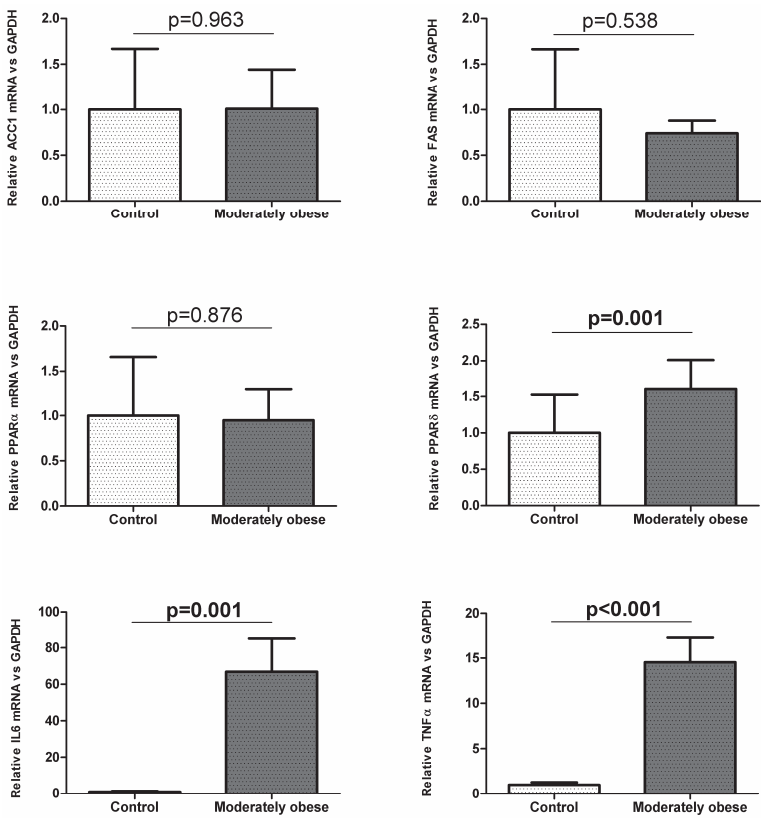


**Figure 2.** Expression of genes related to lipogenesis, FA oxidation and inflammation in control ($n$ = 35) and moderately obese women ($n$ = 55) in visceral adipose tissue. Student's $t$-test was used to determinate differences between groups. Data are expressed as mean ± SD. The mRNA expression was calculated relative to the control group, whose mRNA expression was set to 1.0. ACC1, Acetyl-CoA carboxylase 1; FAS, Fatty acid synthase; IL6, Interleukin 6; PPARα, Peroxisome proliferator-activated receptor alpha; PPARδ, Peroxisome proliferator-activated receptor delta; TNFα, Tumor necrosis factor alpha.

*Int. J. Mol. Sci.* **2015**, *16*, 29911–29922

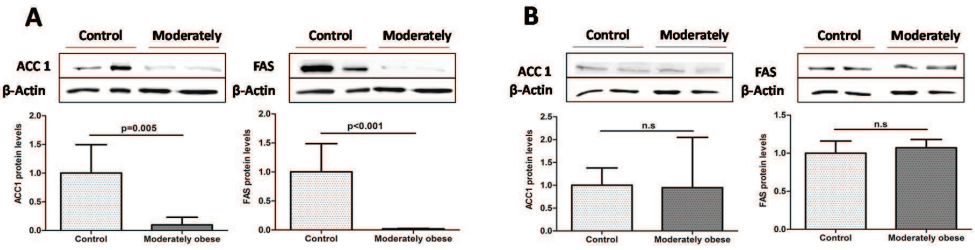


**Figure 3.** Western blot analysis of the main lipogenic enzymes in subcutaneous (**A**); and visceral (**B**) adipose tissue of moderately obese patients. Bar graphs show the quantification of ACC1 and FAS bands normalized by values of β-actin ($n$ = 12 for each group). Student's $t$-test was used to determinate differences between groups. Data are expressed as mean ± SD. ACC1, Acetyl-CoA carboxylase 1; FAS, Fatty acid synthase.

In relation to the FA oxidation genes, our findings showed that visceral *PPARδ* gene expression was significantly higher in moderately obese women than in controls (Figure 2), while subcutaneous mRNA expression was not significantly different in both groups (Figure 1). The subcutaneous mRNA expression of *PPARα* was significantly lower in moderately obese women (Figure 1), whereas in VAT, *PPARα* gene expression was similar in the two groups studied (Figure 2).

Regarding inflammation genes, the results showed that *IL6* and *TNFα* gene expression were significantly increased in the moderately obese women compared to control women in both tissues (Figures 1 and 2).

Finally, the comparison of the mRNA expression of lipogenic and oxidative genes between moderately obese women and the morbidly obese women studied elsewhere [19] showed that the expression of the genes related to lipogenesis (*ACC1*, *FAS*) and FA oxidation (*PPARα*, *PPARδ*) was significantly lower in morbidly obese women than in moderately obese women in the SAT depot ($p < 0.001$). In the VAT depot, *ACC1* mRNA expression was downregulated in morbidly obese women ($p = 0.013$), while *FAS* gene expression was not significantly different in both types of obesity ($p = 0.080$). With regard to FA oxidation, *PPARδ* mRNA expression was lower in morbidly obese women than in moderately obese women ($p < 0.001$), whereas *PPARα* mRNA expression was similar in both groups ($p = 0.124$).

### *2.3. Correlation of VAT and SAT mRNA Expression with Parameters of Obesity and Glucose Metabolism*

In VAT, we found that the oxidative gene *PPARδ* correlated positively with BMI, weight and waist circumference (WC) (Table 2). *PPARα* mRNA expression correlated negatively with HOMA2-IR (Table 2). On the contrary, *IL6* and *TNFα* gene expression correlated positively with HOMA2-IR, insulin, glucose and HbA1c (Table 2).

In SAT, we found that *FAS* mRNA expression has negative correlations with weight, BMI, WC, glucose, insulin and HOMA2-IR (Table 3). One of the key enzymes in lipogenesis, *ACC1*, correlated negatively with weight and BMI (Table 3). Also, our findings showed negative correlations between *PPARα* expression and weight, BMI and WC (Table 3). Furthermore, *PPARδ* correlated positively with HbA1c (Table 3). For the genes involved in inflammation, we found that *IL6* mRNA expression has positive correlations with weight, BMI, WC, glucose, insulin and HOMA2-IR (Table 3). *TNFα* gene expression also correlated positively with weight, BMI, WC, insulin and HOMA2-IR (Table 3).

**Table 2.** Correlations of visceral mRNA expression of genes involved in fatty acid metabolism and inflammation with anthropometric and glucose metabolism parameters.

| Variables | *ACC1* | | *FAS* | | *PPARα* | | *PPARδ* | | *IL6* | | *TNFα* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value |
| WEIGHT (kg) | −0.044 | 0.797 | −0.010 | 0.957 | −0.141 | 0.464 | 0.387 | **0.018** | 0.430 | **0.020** | 0.214 | 0.275 |
| WC (cm) | 0.196 | 0.521 | 0.291 | 0.335 | −0.314 | 0.320 | 0.527 | **0.044** | 0.243 | 0.447 | 0.179 | 0.597 |
| BMI (kg/m$^2$) | −0.027 | 0.874 | −0.044 | 0.812 | −0.146 | 0.450 | 0.464 | **0.004** | 0.459 | **0.012** | 0.351 | 0.067 |
| GLUCOSE (mg/dL) | −0.122 | 0.487 | −0.153 | 0.411 | −0.261 | 0.180 | 0.136 | 0.423 | 0.573 | **0.001** | 0.533 | **0.004** |
| INSULIN (mU/L) | −0.045 | 0.808 | −0.251 | 0.198 | 0.375 | 0.065 | 0.041 | 0.816 | 0.374 | **0.046** | 0.526 | **0.007** |
| HbA1c (%) | 0.105 | 0.587 | −0.212 | 0.298 | −0.031 | 0.887 | 0.221 | 0.233 | 0.496 | **0.002** | 0.427 | **0.042** |
| HOMA2-IR | −0.087 | 0.642 | −0.279 | 0.158 | −0.418 | **0.042** | 0.062 | 0.730 | 0.479 | **0.009** | 0.558 | **0.004** |

BMI, body mass index; HbA1c, glycated haemoglobin; HOMA2-IR, homeostatic model assessment 2-insulin resistance; WC, waist circumference. The strength of association between variables was calculated using Pearson's *r* correlation test. Bold numbers indicate statistically significant correlations ($p$-value < 0.05).

**Table 3.** Correlations of subcutaneous mRNA expression of genes involved in fatty acid metabolism and inflammation with anthropometric and glucose metabolism parameters.

| Variables | *ACC1* | | *FAS* | | *PPARα* | | *PPARδ* | | *IL6* | | *TNFα* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value | *r* | *p*-Value |
| WEIGHT (kg) | −0.483 | **<0.001** | −0.429 | **<0.001** | −0.369 | **0.003** | −0.002 | 0.990 | 0.488 | **<0.001** | 0.247 | **0.030** |
| WC (cm) | −0.366 | 0.072 | −0.388 | **0.031** | −0.475 | **0.026** | 0.162 | 0.400 | 0.451 | **0.018** | 0.493 | **0.005** |
| BMI (kg/m$^2$) | −0.526 | **<0.001** | −0.496 | **<0.001** | −0.483 | **<0.001** | 0.000 | 0.998 | 0.449 | **<0.001** | 0.269 | **0.018** |
| GLUCOSE (mg/dL) | −0.192 | 0.108 | −0.240 | **0.022** | −0.205 | 0.116 | 0.074 | 0.527 | 0.244 | **0.040** | 0.048 | 0.684 |
| INSULIN (mU/L) | −0.216 | 0.081 | −0.298 | **0.013** | −0.180 | 0.185 | 0.182 | 0.132 | 0.275 | **0.028** | 0.264 | **0.028** |
| HbA1c (%) | −0.042 | 0.750 | −0.076 | 0.562 | −0.127 | 0.367 | 0.264 | **0.034** | 0.107 | 0.428 | 0.147 | 0.257 |
| HOMA2-IR | −0.216 | 0.082 | −0.297 | **0.013** | −0.183 | 0.177 | 0.188 | 0.120 | 0.341 | **0.006** | 0.238 | **0.049** |

BMI, body mass index; HbA1c, glycated haemoglobin; HOMA2-IR, homeostatic model assessment 2-insulin resistance; WC, waist circumference. The strength of association between variables was calculated using Pearson's *r* correlation test. Bold numbers indicate statistically significant correlations ($p$-value < 0.05).

*Int. J. Mol. Sci.* **2015**, *16*, 29911–29922

### *2.4. Relationship between the mRNA Expression of Genes Involved in Lipogenesis, FA Oxidation and Inflammation in VAT and SAT*

The associations between the expression of the genes involved in lipogenesis point out that *FAS* mRNA expression was directly related to *ACC1* mRNA expression in both VAT and SAT depots (SAT: $r = 0.822$, $p < 0.001$; VAT: $r = 0.417$, $p = 0.018$). In regard to FA oxidation correlation analysis, the findings showed that *PPARα* and *PPARδ* mRNA expression were not related in both fat tissues. Finally, *IL6* gene expression had a positive correlation with *TNFα* gene expression in both tissues (SAT: $r = 0.314$, $p = 0.004$; VAT: $r = 0.342$, $p = 0.05$).

## 3. Discussion

In the present work, we investigated the expression of crucial genes in fatty acid metabolism in VAT and SAT paired samples from moderately obese and normal-weight women. Although some studies of fatty acid metabolism in human adipose tissue have been published [10,12–14,20,21], the originality of the present work resides in the fact that it provides a validated study of fatty acid metabolism in both adipose tissues simultaneously in moderate obesity.

The main findings of this work show that the gene expression of the main enzymes related to *de novo* fatty acid synthesis (*ACC1*, *FAS*) and *PPARα* was similar in the two groups in VAT, but different in SAT. Their subcutaneous mRNA expression was significantly downregulated in moderately obese women.

It should also be noted that when the mRNA expression of these genes in moderately obese women was compared to the expression in morbidly obese women studied elsewhere [19], we found that the subcutaneous mRNA expression of all the genes studied was lower in morbidly obese women; that is to say, mRNA expression decreases when BMI increases.

In our study, the lipogenic pathway is, at mRNA and protein expression levels, downregulated in the subcutaneous fat depot of moderately obese women. Although an increase of *de novo* FA synthesis is expected in the development of obesity, our findings agree with those of other authors [22,23]. They indicate that the downregulation of the lipogenesis pathway in the obese cohort is a late and adaptive process that prevents the fat mass from developing further. In this sense, mice lacking the lipogenic enzyme FAS in adipose tissue manifested resistance to diet-induced obesity and increased energy expenditure. Also, Lodhi *et al.* found a decreased adipogenesis activity in *FAS* knockdown embryonic fibroblasts [24].

As far as the expression of genes related to FA oxidation was concerned, our results showed that in SAT *PPARδ* gene expression was similar in the two groups studied, whereas in VAT it was upregulated in moderately obese women. In SAT *PPARα* gene expression was downregulated, while in VAT it was similar in the two groups. The decreased FA oxidation in SAT in moderately obese women might be explained, at least in part, because mitochondrial function-related genes are downregulated in male and female obese subjects [25]. Moreover, in morbidly obese patients, MacLaren *et al.* found an increase in lipid storage and lipolytic genes, but a decrease in *de novo* triglyceride synthesis and oxidative genes in SAT [26]. The role of these PPARs in white adipose tissue in the pathophysiology of obesity has yet to be elucidated. New prospective studies are needed to clarify their function in obese adipocytes.

Besides the processes described above, there are also others involved in fat accumulation in white adipose tissue such as FA uptake or lipolysis. It is important to note, that all these processes, occur at different rates and amounts in obese and normal-weight individuals, depending upon the anatomical location of the adipose tissue, and also according to gender and grade of obesity [27].

It is well known that the increased IL6 and TNFα circulating levels in obese patients have led to the conclusion that obesity is characterized by a subjacent chronic low-grade inflammation [28]. In this sense, our results showed increased *IL6* and *TNFα* gene expression in moderately obese women in comparison with the control group in both tissues.

Our results reinforce the hypothesis that SAT, from a metabolic point of view, is less harmful than VAT [29]. Recent studies have analyzed whether subcutaneous, intra-abdominal and hepatic fat were related to insulin resistance and lipidic parameters. Only subcutaneous fat was not significantly correlated to these variables [30]. *In vitro* and *in vivo* studies of the physiology of adipose tissue confirm that lipolysis and fatty acid uptake rates are not the same in SAT as in VAT. SAT appears to be more passive than VAT and to limit the detrimental effects of ectopic fat deposition by the long-term accumulation of excess FAs [6]. Also, subcutaneous fat is related to a favorable adipokine profile [6]. In this respect, individuals with Cushing's syndrome or congenital lipodystrophies tend to have increased metabolic and cardiovascular risk despite having a marked reduction in subcutaneous fat [31,32]. Moreover, several reports have shown that regional subcutaneous fat mass is inversely associated with fasting insulin levels and insulin levels after an oral glucose load, and positively associated with insulin sensitivity [33–36]. In agreement with these results, we found that FAS mRNA expression is inversely associated with insulin, glucose and HOMA2-IR. Data on the beneficial metabolic consequences of SAT, the deleterious effects of its deficiency [37,38] and the positive effects of its transplantation into VAT depots in mice [39] suggests that SAT plays a "buffering" role in obesity due to the fact that it prevents excess supply of lipids from spilling over into "ectopic" sites [40].

Our study cohort allowed us to investigate lipogenic and FA oxidation pathways in SAT and VAT fat depots without the interference of confounding factors like gender or age. Only women were included because it is well known that men and women differ substantially in regard to body composition, energy imbalance, sex hormones and adipokines [41,42]. Moreover, several studies showed sex-specific differences in lipid and glucose metabolism [43]. We were also able to extrapolate the results found in the morbidly obese cohort [19] to the moderately obese cohort. Nevertheless, the results of our study cannot be extrapolated to men.

## 4. Material and Methods

### *4.1. Subjects*

The study was approved by the ethics committee of the Hospital Sant Joan de Reus and all subjects gave written informed consent before taking part in the study. The majority of the patients in the Hospital Sant Joan de Reus who undergo bariatric surgery or laparoscopic cholecystectomy are women. Therefore, adipose tissue samples were from 105 Caucasian women. Of these, 55 were moderately obese (body mass index (BMI) 30–38 $kg/m^2$) and 35 were normal-weight controls (BMI < 25 $kg/m^2$). SAT and VAT samples were obtained from moderately obese patients who had undergone bariatric surgery (patients with BMI $\geqslant$ 37 $kg/m^2$) and laparoscopic cholecystectomy for benign gall bladder disease or laparoscopic hiatus hernia repair (patients with BMI < 37 $kg/m^2$) and from normal-weight subjects who had undergone laparoscopic abdominal surgery (described in detail elsewhere) [19].

The moderately obese and normal-weight women were of similar ages. The body weight of the moderately obese group had not fluctuated be more than 2% for at least three months before surgery. The exclusion criteria were: (1) patients who were taken antidiabetic or hypolipemiant drugs; (2) diabetic women receiving insulin; (3) subjects undergoing contraceptive treatment; (4) patients who had an acute illness, inflammatory or infectious diseases or neoplastic malignant diseases.

Of the moderately obese women, 16% were diagnosed with type 2 diabetes mellitus based on ADA guidelines [44]. These patients were following a dietetic treatment. All the usual exclusion criteria were taken into account.

### *4.2. Biochemical Analyses*

Each of our patients was evaluated with a complete physical, anthropometrical and biochemical assessment. Total cholesterol, HDL-C, LDL-C, triglycerides, glucose, insulin and HbA1c were

measured using a conventional automated analyzer after overnight fasting. Insulin resistance (IR) was calculated using HOMA2-IR [45].

### *4.3. RNA Isolation and Gene Expression*

Total RNA was extracted from SAT and VAT by using the RNeasy mini kit (Qiagen, Barcelona, Spain) and was reverse transcribed to cDNA using the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Madrid, Spain). Real-time quantitative PCR was carried out with TaqMan Assay predesigned by Applied Biosystems for the detection of *ACC1*, *FAS*, *PPARα*, *PPARδ*, *IL6*, *TNFα* and GAPDH gene. All reactions were performed in duplicate using the 7900HT Fast Real-Time PCR systems. SAT and VAT mRNA expression of the genes mentioned above was calculated relative to the mRNA expression of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH).

### *4.4. Western Analysis*

Protein levels of ACC1 and FAS were assayed by Western Blot. Frozen SAT and VAT tissue samples from 24 individuals (MO, $n$ = 12; Control, $n$ = 12) were homogenized in lysis buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% SDS, 100 mM NaF, 30 mM $Na_4O_7P_2$ and 1% protease inhibitor cocktail (Thermo Scientific, Madrid, Spain)). Protein concentration was determined using BCA assay kit (Thermo Scientific). Samples were separated by SDS/PAGE and transferred electrophoretically to nylon membranes. Membranes were blocked by incubation in a solution of 5% skimmed milk and were probed using antibodies against ACC1, FAS and β-actin (Cell Signaling, Danvers, MA, USA). Anti-rabbit IgG or anti-mouse IgG (Thermo Scientific) were used as secondary antibody. Immunodetection of the protein was done using SuperSignal West Pico or Femto Chemiluminescent kit (Thermo Scientific). Finally, band densitometry was analyzed using Phoretix1D software.

### *4.5. Statistical Analyses*

Results are expressed as mean ± SD (standard deviation). Student's $t$-test or one-way ANOVA were carried out to determinate differences between groups. Univariate association was tested by Pearson (parametric variables) or Spearman (nonparametric variables) correlation analysis. We used SPSS/PC+ statistical package (version 22.0; SPSS, Chicago, IL, USA) for the statistical analyses. $p$-values < 0.05 were considered statistically significant.

## 5. Conclusions

The results reported here suggest that, in moderate obesity, subcutaneous fat has a defense mechanism against an excess of fatty acid accumulation by diminishing the expression of lipogenic-related genes, while visceral fat does not. Interestingly, the extrapolation of the results found in the morbidly obese cohort [19] to the moderately obese cohort showed that this downregulation reported in subcutaneous adipose tissue increases as BMI increases. As far as FA oxidation is concerned, future studies are necessary to gain further knowledge about PPARs regulation in white adipose tissue of obese subjects.

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**Author Contributions:** Esther Guiu-Jurado and Teresa Auguet participated in the design of the study, in the analysis and interpretation of data, and in drafting the manuscript; Alba Berlanga, Esther Guiu-Jurado, Sandra Armengol and Carmen Aguilar carried out the genetic and protein molecular studies and the immunoassays; Gemma Aragonès performed the statistical analysis; José Antonio Porras, Rosa Jorba, Fàtima Sabench, Andreu Marti and Mercè Hernàndez made substantial contributions to the conception and

design of the study, and to the acquisition of samples; Daniel del Castillo and Cristóbal Richart revised the draft and gave final approval for publication.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

ACC1, acetyl-coenzyme A carboxylase 1; BMI, body mass index; DM2, diabetes mellitus type 2; FA, fatty acid; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HbA1c, glycated haemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA2-IR, homeostatic model assessment method insulin resistance; IL6, interleukin 6; IR, insulin resistance; LDL-C, low density lipoprotein cholesterol; PPARα, peroxisome proliferator-activated receptor α; PPARδ, peroxisome proliferator-activated receptor δ; PPARγ, peroxisome proliferator-activated receptor γ; SAT, subcutaneous adipose tissue, TNFα, tumor necrosis factor; VAT, visceral adipose tissue; WC, waist circumference.

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# V. SUMMARY OF RESULTS

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

In the first study of this doctoral thesis, we analyzed the expression of some of the key genes related to the *de novo* synthesis of fatty acids (FAs) (LxRα, SREBP1c, ACC1, FAS), the uptake and transport of FAs (CD36, FABP4), adipogenesis (PPARγ, adiponectin), FA oxidation (PPARα, PPARδ), and inflammation (IL6, TNFα) in subcutaneous (SAT) and visceral (VAT) adipose tissue from 145 morbidly obese (MO) women (BMI > 40 kg/m$^2$) and 18 normal-weight controls (BMI < 25 kg/m$^2$).

To investigate gene expression in relation to BMI, we performed analyses of the control and MO women. The results indicate that the visceral mRNA expression of genes related to the *de novo* synthesis of FAs (lipogenesis) in MO women were similar to those of control women. Only FAS mRNA expression was significantly lower in MO women. In SAT, the results indicate that the mRNA expression levels of LxRα, SREBP1c, ACC1 and FAS were significantly lower in MO women than in control women. Interestingly, we found that ACC1 and FAS protein levels were significantly lower in MO women in both adipose tissue types.

Additionally, we studied the genes related to the uptake and transport of FAs. In VAT, we found that CD36 and FABP4 mRNA expression was significantly lower in MO women compared to that in control women, whereas in SAT, CD36 expression was significantly higher in the obese group.

For genes related to FA oxidation, the results indicate that visceral mRNA expression in MO women was similar to that of control women, while in SAT, PPARα and PPARδ mRNA expressions levels were significantly lower in MO women. We also studied the genes related to adipogenesis. In VAT, we found that PPARγ and adiponectin mRNA expression levels in MO women were similar to those of control women, whereas in SAT, adiponectin mRNA expression was significantly lower in MO women.

Finally, we found that IL6 and TNFα mRNA expression in VAT was significantly higher in MO women than in control women. In SAT, IL6 mRNA expression was significantly higher in MO women.

In addition, we compared the mRNA expression of these genes between VAT and SAT in the MO women. The main findings were that the

expression of genes related to lipogenesis (ACC1 and LxRα), FA oxidation (PPARδ, PPARα), and inflammation (TNFα) were upregulated in VAT compared to that in SAT. In contrast, genes related to FA uptake and transport (FABP4 and CD36) were upregulated in SAT.

To investigate whether the alterations in the fatty acid metabolism of morbidly obese women found in study 1 were also present in moderately obese women, we performed a second study to evaluate the expression of key genes related to the *de novo* synthesis of FAs (ACC1, FAS), FA oxidation (PPARδ, PPARα) and inflammation (IL6, TNFα) in the SAT and VAT of 55 moderately obese (BMI 30–38 kg/m$^2$) and 35 normal-weight control women (BMI < 25 kg/m$^2$).

As in study 1, we conducted analyses on the controls and moderately obese women. The subcutaneous mRNA expression of genes related to lipogenesis was significantly decreased in moderately obese women compared to those in control women. The results from VAT indicate that ACC1 and FAS mRNA expression were similar in both groups. Interestingly, the protein analysis showed similar results to those obtained in the mRNA expression analysis. In SAT, ACC1 and FAS protein levels were significantly lower in moderately obese patients, whereas in VAT there were no differences between the two groups.

For the genes related to FA oxidation, our findings showed that visceral PPARδ gene expression was significantly higher in moderately obese women than in controls, while subcutaneous mRNA expression was not significantly different between the two groups. The subcutaneous mRNA expression of PPARα was significantly lower in moderately obese women, whereas in VAT, PPARα gene expression was similar in the two groups studied.

As far as inflammation is concerned, the results showed that IL6 and TNFα gene expression were significantly increased in the moderately obese women in both tissues relative to that in control women.

Furthermore, we also compared the mRNA expression of these genes in moderately obese women and the morbidly obese women studied previously. The main finding of this comparison was that the mRNA

expression of the main enzymes involved in *de novo* fatty acid synthesis (ACC1, FAS) and of the genes related to FA oxidation (PPARα, PPARδ) was significantly lower in morbidly obese women than in moderately obese women in the SAT.

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

# VI. GENERAL DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

As mentioned in the introduction of this doctoral thesis, obesity is a worldwide epidemic and its prevalence continues to rise at an alarming rate [1]. Obesity significantly increases the risk and worsens the prognosis of many diseases, such as type 2 diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome, non-alcoholic fatty liver disease, cardiovascular disease and certain cancers and, consequently, is associated with increased mortality [4,5,44,45]. However, obesity itself does not necessarily lead to these comorbidities [49–51]. There are some obese individuals who, despite having excessive body fat, display a favorable metabolic profile characterized by high insulin sensitivity and favorable lipid and inflammation profiles [55,52–54]. These individuals are known as "metabolically healthy obese" patients.

Adipose tissue dysfunction and ectopic fat accumulation seem to play an important role in determining an individual's risk of developing metabolic and cardiovascular comorbidities of obesity [87]. Physiological and molecular studies have suggested that fat stored in subcutaneous adipose depots are not directly implicated in the development of insulin resistance as this fat appears to play a "buffering" role in taking up fatty acids (FAs) and preventing the exposure of other insulin-sensitive tissues to their detrimental effects [145]. Because of this, reducing the subcutaneous fat mass by liposuction does not ameliorate circulating metabolic and inflammatory variables [213]. However, reducing visceral fat mass by omentectomy combined with gastric banding results in long-term beneficial effects on glucose metabolism and insulin sensitivity [214].

Adipocyte functionality is impaired during obesity and has been shown to be linked to adipocyte hypertrophy, disequilibrium between lipogenesis and lipolysis, impaired transcriptional regulation of the key factors that control adipogenesis and a lack of sensitivity to external signals, as well as a failure in the signal transduction process and other factors [215]. It has been reported that deregulation of adipocyte FA metabolism contributes to the development of metabolic diseases [216–220]. However, the mechanisms involved in the deregulation of FA metabolism in adipocytes remain unclear [220].

Based on this previous research, a better understanding of the underlying mechanism of the deregulation of FA metabolism in adipose tissue during the development of obesity would be valuable in developing better understanding of the physiopathology of obesity. In addition, a study of the different metabolic effects of subcutaneous and visceral adipose tissue might also be particularly helpful in understanding the mechanisms that lead to adipose tissue dysfunction in obese individuals. We therefore decided to investigate the expression of crucial genes that play significant roles in the metabolism of fatty acids in visceral (VAT) and subcutaneous (SAT) adipose tissue samples from an extensive cohort of women. Although some studies of fatty acid metabolism in human adipose tissue have been published [221–226], the novelty of the present studies lie in the fact that they provide validated studies of fatty acid metabolism in these two adipose tissues simultaneously in different obese cohorts.

In the first study, we analyzed the expression of key genes involved in the *de novo* synthesis of fatty acids (LxRα, SREBP1c, ACC1, FAS), the uptake and transport of FAs (CD36, FABP4), adipogenesis (PPARγ, adiponectin), FA oxidation (PPARα, PPARδ), and inflammation (IL6, TNFα) from morbidly obese patients (MO) and normal-weight healthy subjects. This study demonstrates that mRNA expression of the main enzymes involved in the *de novo* fatty acid synthesis (LxRα, SREBP1c, ACC1 and FAS) were significantly lower in MO women than those of the control group in the SAT depot, whereas in VAT, only FAS gene expression was lower. The downregulation of lipogenesis was confirmed by evaluating the protein levels. Moreover, the expression of key genes related to fatty acid oxidation (PPARα, PPARδ) was significantly lower in MO women than in the control group in SAT. However, the gene expression of CD36, which is involved in fatty acid uptake and transport, and of IL6, a pro-inflammatory factor, were higher in MO women in SAT.

Our results indicate that the lipogenic pathway in women with extreme obesity is, at the level of gene and protein expression, downregulated in SAT. During dynamic obesity, the lipogenic capacity of adipose tissue is expected to increase. However, our findings agree with those of other

authors [227,228] who suggest that the low expression of the lipogenesis pathway genes in obese cohorts could be a late and adaptive process that prevents the subcutaneous fat mass from developing further. Interestingly, mice lacking the lipogenic enzyme FAS in adipose tissue manifested a resistance to diet-induced obesity and showed an increase in energy expenditure. Additionally, Lodhi et al. found a decrease in adipogenic activity in FAS knockdown embryonic fibroblasts [229]. Furthermore, the positive correlations found in our first study between the different genes involved in this pathway strengthen their role in the adaptive process of SAT.

For the expression of genes involved in FA oxidation, our results showed that subcutaneous PPARα and PPARδ mRNA expression was significantly lower in the MO group. In agreement with our results, Mardinoglu *et al.* found that mitochondrial function-related genes are downregulated in male and female obese subjects [230]. Moreover, MacLaren *et al.* found an increase in lipid storage and lipolytic genes, but a decrease in *de novo* triglyceride synthesis and oxidative genes, in SAT of morbidly obese patients [231]. The role of these PPARs in white adipose tissue in the physiopathology of obesity has yet to be elucidated and further studies are needed to clarify their function in obese adipocytes.

For the expression of genes related to the uptake and transport of FAs, we found that CD36 and FABP4 mRNA expression was lower in VAT in MO women than the control group. In agreement with this, a recent study showed that FABP4 gene expression was significantly lower in MO subjects than in moderately obese or lean subjects in VAT and SAT depots [232]. In contrast, we found that CD36 mRNA expression was significantly higher in SAT in the MO group than in the control group. One explanation for these trends may be that SAT seems to have a “buffering” role because it takes up FAs and prevents other insulin-sensitive tissues from being exposed to their damaging consequences [145]. These results suggest that the uptake and transport of FAs in MO patients may be downregulated only in VAT. In support of this, it is well documented that visceral obesity is associated with insulin resistance and attributed in part to the increase in circulating free

FAs concentration [233,234]. Insulin is an anabolic hormone known to direct the storage and utilization of energy in adipocytes [235]. When cells become insulin resistant, they lose their relative capacity to take up glucose and free FAs from the bloodstream. This fact could explain the downregulation of CD36 and FABP4 in VAT in the MO group.

In addition to free FAs, several adipose tissue-secreted proinflammatory products, such as IL6 and TNFα, have been shown to induce insulin resistance and are thought to link obesity and type 2 diabetes [123,143,236]. The increased production and high circulating levels of these products in obesity have led to the view that obese individuals are characterized by a state of chronic low-grade inflammation [37,234,237]. As expected, we found that IL6 and TNFα gene expression were significantly higher in MO women compared to that in the control group in VAT. In SAT, only IL6 was significantly higher in MO women. Therefore, in both tissues, there is a proinflammatory profile in MO women. Interestingly, other authors have described that increased inflammation is accompanied by a decrease in lipogenesis, which is in agreement with our results [238].

Our findings reinforce the hypothesis that SAT is “metabolically innocent” and that VAT has deleterious consequences [94]. Recent studies have analyzed the relative correlations of subcutaneous, intra-abdominal and liver fat with fasting insulin, hepatic insulin sensitivity, and dyslipidemia. Only subcutaneous fat was not strongly and independently linked to these variables [239]. *In vitro* and *in vivo* studies of the physiology of adipose tissue confirm that lipolysis and fatty acid uptake rates are not the same in SAT as in VAT. SAT appears to be more passive than VAT and to limit the adverse effects of ectopic fat deposition by the long-term entrapment of excess fatty acids [145]. Subcutaneous fat is also associated with a beneficial adipokine profile [145]. In this respect, individuals with Cushing’s syndrome or congenital lipodystrophies, who have a marked reduction in subcutaneous fat, tend to have increased metabolic and cardiovascular risk [240,241]. Moreover, several reports have shown that a regional subcutaneous fat mass is inversely associated with fasting insulin levels and insulin levels after an oral glucose load and positively associated with insulin sensitivity [242–245]. Data on the

positive metabolic aspects of SAT, the adverse consequences of its deficiency in experimental animals [246] and in human lipodystrophy [247], and the beneficial effects of its transplantation into VAT depots in mice [248] has led to a hypothesis about the beneficial capacity of SAT "expandability" to accommodate any excess supply of lipids and to prevent them from spilling over into "ectopic" sites [88]. This suggests that SAT plays a "buffering" role in obesity.

When we compared gene expression between SAT and VAT in the MO cohort, we found that the expression of genes related to lipogenesis (ACC1 and LxRα), FA oxidation (PPARδ, PPARα), and inflammation (TNFα) were upregulated in VAT compared to that in SAT. In contrast, expression was upregulated for genes related to FA uptake and transport (FABP4 and CD36) in SAT. These results suggest that the regulation of lipid metabolism by the adipose tissue in MO patients differs depending on its localization. Transplantation of SAT adipose tissue into VAT depots has been performed successfully in mice with beneficial effects and has improved glucose tolerance and decreased plasma insulin concentration and portal plasma triglycerides [248]. Furthermore, the upregulation of the genes involved in FA uptake and transport in SAT suggests that, in obese individuals, SAT plays a protective role by preventing other insulin-sensitive tissues from exposure to free FAs.

This study has made it possible to investigate fatty acid metabolism in VAT and SAT adipose tissue without the interference of such confounding factors as sex [249] or age. However, the results of our study cannot be extrapolated to other obesity groups or to men.

To partially address this limitation and evaluate whether the alterations in the fatty acid metabolism in AT of morbidly obese women found in study 1 were also manifested in other obesity groups, we analyzed the expression of key genes involved in the *de novo* synthesis of FAs (ACC1, FAS), fatty acid oxidation (PPARδ, PPARα) and inflammation (IL6, TNFα) in the SAT and VAT of moderately obese women in a second study.

Our study demonstrates that the mRNA expression of the main enzymes involved in *de novo* fatty acid synthesis (ACC1, FAS) and PPARα was

significantly lower in moderately obese women than in the control group in the SAT depot, but was similar between the two groups in VAT. It should also be noted that this study enabled us to compare the mRNA expression of these genes between the two obese cohorts studied. Interestingly, we found that the subcutaneous mRNA expression of all the genes studied was lower in morbidly obese women compared to that in moderately obese women. This suggests that the mRNA expression of these genes decreases as BMI increases.

As mentioned before, it is well known that the increased production and high circulating levels of IL6 and TNFα induce insulin resistance and are thought to be associated with obesity and its related comorbidities [237]. As expected, our results showed increased IL6 and TNFα gene expression in moderately obese women compared to that of the control group in both tissues.

The results of the second study strengthen the hypothesis that SAT is "metabolically innocent" and that VAT has deleterious consequences, as explained above [94].

This study allowed us to extrapolate the results found in the morbidly obese cohort to the moderately obese cohort. However, they cannot be extrapolated to men.

In summary, the main finding of this doctoral thesis is that there is a progressive downregulation in *de novo* fatty acid synthesis in SAT during the development of obesity. Although it was not possible to determine what causes this downregulation in SAT, the results reported here suggest that, in obesity, SAT has a defense mechanism against excess fatty acid accumulation that acts by preventing the subcutaneous fat mass from developing further by decreasing the expression of lipogenic genes, whereas VAT may have lost this mechanism. Further prospective studies are needed to better understand FA oxidation and examine how PPARs are regulated in the white adipose tissue of obese patients.

# VII. CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
Esther Guiu Jurado

1. The expression of key genes involved in the *de novo* fatty acid synthesis (LxRα, SREBP1c, ACC1, FAS) was downregulated in morbidly obese women (MO) compared to that of the normal-weight control group in the subcutaneous adipose tissue (SAT), whereas in the visceral adipose tissue (VAT), only FAS was downregulated in morbidly obese women.

2. ACC1 and FAS protein levels were significantly lower in SAT of MO women.

3. The expression of key genes related to fatty acid oxidation (PPARα, PPARδ) was significantly lower in SAT of MO women.

4. In VAT, we found that the expression of the genes involved in fatty acid uptake (CD36 and FABP4) was significantly lower in MO women compared to that of controls women, whereas in SAT, the expression of CD36 was significantly higher in the obese group.

5. Gene expression and protein levels of the main enzymes of the *de novo* fatty acids synthesis (ACC1, FAS) were downregulated in moderately obese women relative to those of the control group in the SAT.

6. Only PPARα mRNA expression was significantly lower in SAT in the moderately obese cohort.

7. There was a proinflammatory profile in both adipose tissues of the two obese cohorts studied.

8. The expression of the main enzymes involved in *de novo* fatty acid synthesis (ACC1, FAS) and of the genes related to FA oxidation (PPARα, PPARδ) was significantly lower in morbidly obese women than in moderately obese women in the SAT.

9. There is a progressive downregulation of *de novo* fatty acid synthesis in SAT during the development of obesity. This suggests that in obesity, SAT has a defense mechanism against an excess of fatty acid

accumulation that prevents the subcutaneous fat mass from developing further by decreasing the expression of lipogenic genes.

# VIII. REFERENCES

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Esther Guiu Jurado

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UNIVERSITAT ROVIRA I VIRGILI
DEREGULATION OF FATTY ACID METABOLISM IN THE ADIPOSE TISSUE OF OBESE WOMEN
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# IX. ANNEX

UNIVERSITAT ROVIRA I VIRGILI
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## Publications

**Downregulation of *de novo* fatty acid synthesis lipogenesis pathway in subcutaneous adipose tissue of moderately obese women.**
Esther Guiu-Jurado, Teresa Auguet, Alba Berlanga, Gemma Aragonès, Carmen Aguilar, Fàtima Sabench, Sandra Armengol, José Antonio Porras, Andreu Martí, Rosa Jorba, Mercè Hernández, Daniel Del Castillo3, Cristóbal Richart. *Int J Mol Sci*. 2015; 16, 29911–29922.

**Elevated autophagy gene expression in adipose tissue of obese humans: A potential non-cell-cycle-dependent function of E2F1.**
Haim Y, Blüher M, Slutsky N, Goldstein N, Klöting N, Harman-Boehm I, Kirshtein B, Ginsberg D, Gericke M, Guiu Jurado E, Kovsan J, Tarnovscki T, Kachko L, Bashan N, Gepner Y, Shai I, Rudich A. *Autophagy*. 2015 Sep.

**Interleukin-17A Gene Expression in Morbidly Obese Women.**
Fernando Zapata-Gonzalez, Teresa Auguet, Gemma Aragonès, Esther Guiu-Jurado, Alba Berlanga, Salomé Martinez, Andreu Martí, Fátima Sabench, Mercé Hernandez, Carmen Aguilar, Joan Josep Sirvent, Rosa Jorba, Daniel Del Castillo and Cristóbal Richart. *Int J Mol. Sci.* 2015, *16*, 17469-17481.

**Role of metabolic lipases and lipotoxicity in the development of non-alcoholic steatosis and non-alcoholic steatohepatitis.**
Berlanga A*, Guiu-Jurado E*, Porras JA, Aragonès G, Auguet T. *Clin Investig Arterioscler.* 2015. *These authors contributed equally.

**Altered fatty acid metabolism-related gene expression in liver from morbidly obese women with non-alcoholic fatty liver disease**
Auguet T, Berlanga A, Guiu-Jurado E, Martinez S, Porras JA, Aragonès G, Sabench F, Hernandez M, Aguilar C, Sirvent JJ, Del Castillo C, Richart C. *Int J Mol Sci.* 2014;15(12):22173-22187.

**Molecular pathways in non-alcoholic fatty liver disease.**
Berlanga A*, Guiu-Jurado E*, Porras JA, Auguet T. *Clin Exp Gastroenterol*. 2014;7(1):221-239.*These authors contributed equally.

**Downregulation of lipogenesis and fatty acid oxidation in the subcutaneous adipose tissue of morbidly obese women.**
Auguet T*, Guiu-Jurado E*, Berlanga A, Terra X, Martinez S, Porras JA, Ceausu A, Sabench F, Hernandez M, Aguilar C, Sirvent JJ, Castillo DD, Richart C. *Obesity (Silver Spring). 2014;22(9):2032-2038.* *These authors contributed equally.

**Endocannabinoid receptors gene expression in morbidly obese women with nonalcoholic fatty liver disease.**
Auguet T, Berlanga A, Guiu-Jurado E, Terra X, Martinez S, Aguilar C, Filiu E, Alibalic A, Sabench F, Hernández M, Del Castillo D, Richart C. *Biomed Res Int.* 2014;2014:502542.

**Clinical and adipocytokine changes after bariatric surgery in morbidly obese women.**
Auguet T, Terra X, Hernández M, Sabench F, Porras JA, Orellana-Gavaldà JM, Llutart J, Guiu-Jurado E, Berlanga A, Martinez S, Aguilar C, Castillo DD, Richart C. *Obesity (Silver Spring)*. 2014;22(1):188-194.

**Adipocytokine levels in women with anorexia nervosa. Relationship with weight restoration and disease duration.**
Terra X, Auguet T, Agüera Z, Quesada IM, Orellana-Gavaldà JM, Aguilar C, Jiménez-Murcia S, Berlanga A, Guiu-Jurado E, Menchón JM, Fernández-Aranda F, Richart C. *Int J Eat Disord*. 2013;46(8):855-861.

**Long-term changes in leptin, chemerin and ghrelin levels following different bariatric surgery procedures: Roux-en-Y gastric bypass and sleeve gastrectomy.**
Terra X, Auguet T, Guiu-Jurado E, Berlanga A, Orellana-Gavaldà JM, Hernández M, Sabench F, Porras JA, Llutart J, Martinez S, Aguilar C, Del Castillo D, Richart C. *Obes Surg*. 2013;23(11):1790-1798.

## Congress attendance

**Downregulation of *de novo* lipogenesis and fatty acid oxidation in subcutaneous adipose tissue of moderate obese women**

Guiu-Jurado E, Berlanga A, Auguet T, Aragonés G, Sabench F, Martí A, Aguilar C, Armengol S, del Castillo D, Richart C.

Type of participation: Poster.

22nd European Congress on Obesity (ECO). Prague (Czech Republic) 2015.

**miR33a/b AND miR122 hepaticexpression in obese patients with non-alcoholic fatty liver disease**

Berlanga A, Guiu-Jurado E, Auguet T, Aragonés G, Martinez S, Aguilar C, Sabench F, Armengol S, Alibalic A, del Castillo D, Richart C.

Type of participation: Poster.

22nd European Congress on Obesity (ECO). Prague (Czech Republic) 2015.

**The role of BMP2 in the pathophysiology of obesity**

M. Unthan, E. Guiu-Jurado, T. Wohland, D. Schleinitz, K. Ruschke, M. Kern, B. Gutsmann, N. Klöting, A. Tönjes, M. Stumvoll1, M. Scholz, M. Blühe, P. Kovacs.

Type of participation: Oral Presentation.

European Association for the Study of Diabetes (EASD) Annual Meeting. Stockholm (Sweden) 2015.

**Interleukin-17A gene expression in morbidly obese women: Relationship with interleukin-6**

T. Auguet, G. Aragonès, F. Sabench, E Guiu-Jurado, M. Hernández, A. Berlanga, A. Molina, C. Aguilar, D. del Castillo, C. Richart.

Type of participation: Poster.

20th World Congress of the International Federation for the Surgery of Obesity and Metabolic Disordes (IFSO). Vienna (Austria). 2015.

**ACC1 and FAS mRNA expression in visceral stromal vascular fraction and mature adipocytes from morbidly obese women**

E Guiu-Jurado, T. Auguet, F. Sabench, A. Molina, A. Berlanga, G. Aragonès, C. Aguilar, D. del Castillo, C Richart.

Type of participation: Poster.

20th World Congress of the International Federation for the Surgery of Obesity and Metabolic Disordes (IFSO). Vienna (Austria). 2015.

**Expresión génica de la Interleuquina-IL17A en mujeres obesas mórbidas: Relación con la IL-6.**

T. Auguet, G. Aragonès, A. Muñoz, E Guiu-Jurado, F. Sabench, A. Berlanga, M. Hernández, C. Aguilar, D. del Castillo, C. Richart.

Type of participation: Poster.

XVII Congreso de la Sociedad Española de Cirurgía de la Obesidad y de las Enfermedades Metabólicas Y de la Seccion de Obesidad de la AEC. Vitoria (Spain) 2015.

**Estudio de la expresión génica de la fracción estromal vascular y de los adipocitos maduros en pacientes obesos mórbidos**

E Guiu-Jurado, T. Auguet, F. Sabench, A. Berlanga, E. Raga, G. Aragonès, M. Hernández, C. Aguilar, C. Richart, D. del Castillo.

Type of participation: Poster.

XVII Congreso de la Sociedad Española de Cirurgía de la Obesidad y de las Enfermedades Metabólicas Y de la Seccion de Obesidad de la AEC. Vitoria (Spain) 2015.

**The role of BMP2 in the pathophysiology of obesity** (Diabetologie und Stoffwechsel 2015; 10 - P288 DOI: 10.1055/s-0035-1549794)

M Unthan, E Guiu-Jurado, K Ruschke, D Schleinitz, M Kern, B Gutsmann, N Klöting, A Tönjes, M Stumvoll, P Kovacs, M Blüher.

Type of participation: Poster.

Diabetes Kongress 2015 – 50. Jahrestagung der DDG. Berlin (Germany) 2015.

**Adipose tissue expression of transcription factors and lipogenic enzymes in morbidly obese women.**

Esther Guiu-Jurado, Alba Berlanga, Ximena Terra, Teresa Auguet, Josep Maria Orellana Gavaldà, José Antonio Porras, Fátima Sabench, Mercé Hernandez, Carmen Aguilar, Joan Josep Sirvent, Salomé Martinez, Daniel del Castillo, Cristóbal Richart.

Type of participation: Poster.

XXXVI Congreso de la Sociedad Española de Bioquímica y Biología Molecular (SEBBM). Madrid (SPAIN) 2013.

**Liver expression of transcription factors and lipogenic enzymes in morbidly obese women with non-alcoholic fatty liver disease.**

Alba Berlanga, Esther Guiu-Jurado, Teresa Auguet, Ximena Terra, Josep Maria Orellana Gavaldà, Salomé Martinez, José Antonio Porras, Fátima Sabench, Mercé Hernandez, Carmen Aguilar Crespillo, Joan Josep Sirvent, Daniel Del Castillo, Cristóbal Richart Jurado.

Type of participation: Poster.

XXXVI Congreso de la Sociedad Española de Bioquímica y Biología Molecular (SEBBM). Madrid (SPAIN) 2013.

**El sistema endocannabinoide en obesidad mórbida y hepatopatía grasa asociada. Correlación de receptors CB1 y CB2 con la enfermedad del hígado graso no alcohólica.**

E.Filiu, A.Ceausu, T. Auguet, E. Guiu-Jurado, M. Milián, J. Ramos, M. Espuis, C. Richart.

Type of participation: Poster.

XXXIV Congreso Nacional de la Sociedad Española de Medicina Interna (SEMI) and XXIX Congreso de la Sociedad Andaluza de Medicina Interna (SADEMI). Málaga (SPAIN) 2013.

**Estudio de los cambios en los niveles de adipoquinas después de la cirugía bariátrica y su relación con los cambios clínicos y metabólicos.**

X. Terra, F. Sabench, T. Auguet, J.A. Porras, M. Hernández, J.M. Orellana, C. Aguilar, E. Guiu, C. Richart Jurado, D. Del Castillo Déjardin.

Type of participation: Poster.

1er Congreso médico-quirúrgico de la obesidad**.** Madrid (ESPAÑA) 2013.

**Longitudinal changes in adipo/cytokine levels after bariatric surgery: preoperative concentrations as predictors of weight reduction and insulin sensitivity recovery.**

Ximena Terra, Josep Maria Orellana-Gavaldà, Teresa Auguet, Esther Guiu, Alba Berlanga, Fàtima Sabench, Carmen Aguilar, Mercè Hernández, Daniel del Castillo and Cristobal Richart.

Type of participation: Poster.

22nd IUBMB- 37th FEBS Congress. From Single Molecules to Systems Biology. Sevilla (SPAIN) 2012.

**The Role of Liver X Receptor Alpha in non-alcoholic liver disease.**

Josep Maria Orellana-Gavaldà, Ximena Terra, Teresa Auguet, Alba Berlanga, Esther Guiu, Fàtima Sabench, Carmen Aguilar, Salomé Martínez, Mercè Hernández, Daniel del Castillo and Cristobal Richart.

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22nd IUBMB- 37th FEBS Congress. From Single Molecules to Systems Biology. Sevilla (SPAIN) 2012

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