CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

The worldwide increase in obesity prevalence that we have been observing in the last two decades is associated with a dramatic increase in cardiovascular disease (CVD) and type 2 diabetes mellitus (DM) incidence. It has been estimated that both CVD and type 2 DM will account for at least three-fourth of the mortality by the year 2020. The recognition that health risk is related not only with obesity but also with body fat distribution has opened new investigation fields. In this sense, both overall obesity markers, as well as regional adipose tissue (AT) compartments contributions to health risk have been increasingly studied. Indeed, abdominal obesity is related with an increased CVD and type 2 DM risk, as well as an unfavourable metabolic profile.

Therefore, besides the unequivocal research necessity regarding the mechanisms underlying these associations, which seem to be mediated by insulin resistance (IR), it is also imperative to study which are the most effective strategies to prevent weight gain or to promote a sustainable long-term weight loss. Furthermore, it seems also relevant to study another particular sensible body composition area related with the methods to assess the body composition changes promoted by weight loss. In this context, the main purpose of this investigation thesis is to study the relationships between body composition markers and health risk in overweight and obese women.

In the general introduction (*Chapter 1*) are reviewed the most recent evidence concerning the obesity relationships with metabolic disturbances and IR, which raise CVD and type 2 DM risk. The major underlying mechanisms connecting metabolic syndrome with morbidity and mortality are examined to better understand the separate abdominal and thigh adipose and muscle tissue contributions to an increased risk. The main characteristics of effective weight management strategies and the consequent body composition changes are also described in this chapter. Simple anthropometric markers which have been used in

epidemiological settings to identify obesity and body fat distribution, such as waist and hip circumference seem to present different metabolic roles in heath risk. However, the underlying mechanisms are not totally understood and further research needed.

In this chapter, it is also reviewed the most relevant mechanisms influencing ectopic fat storage and the metabolic consequences of lipotoxicity. In this context, evidence linking central and peripheral adipose and muscle tissue markers with lipid infiltration in lean body tissues and organs is also examined. In addition, adipose tissue metabolism, as well as regional AT depots relevance to the production of several inflammatory and atherothrombotic cytokines are at the forefront of research and are additionally addressed in this chapter. At last, recent evidence demonstrating the skeletal muscle contribution to metabolic risk prevention is also reviewed.

Chapter 2 presents a detailed description of the methodology used in the present investigation.

In *Chapter 3, 4*, and *5* are presented the original research contributions. The separate associations of waist circumference (WC) and hip circumference (HC) to major metabolic syndrome components, as well as to proinflammatory and atherothrombotic disturbances are studied in *Chapter 3*. In this chapter is also examined the independent relations of each thigh adipose and muscle tissue compartment to the previously referred metabolic syndrome features.

In *Chapter 4* it is analyzed the relationships between both abdominal and thigh AT compartments with atherogenic, inflammatory and thrombotic risk factors.

In *Chapter 5* we seek to examine the relevance of each abdominal and thigh adipose and muscle tissue compartment to liver fat storage. Additionally, the associations of liver fat with metabolic syndrome risk factors are also studied.

An integrative discussion of these studies, addressing the interrelationships between metabolic syndrome clinical outcomes and both anthropometric and computed tomography (CT)-measured body composition variables is presented in *Chapter 6*.

At last, *Chapter 7* summarizes the main findings of this thesis.

1.1. Obesity

After the Second World War, an unexpected milestone in lifestyle and eating behaviors took place in most industrialized societies. A new emerging food industry, having in common the production of high fat and carbohydrate processed food, easily available and more cheap, was responsible for a drastic transformation in the eating habits. These strong advertising campaigns, allied with a concomitant reduction in caloric expenditure through daily physical activity, mainly resulting from a new variety of immobilizing technology advances, lead to dramatic changes in caloric balance, and, thus, in body weight and body composition. These transformations have carted a cluster of specific health consequences, which have been propagated to the entire population in an evident way. Almost fifty years after these huge changes in eating and lifestyle patters, approximately 1.2 billion people all over the world are overweight and at least 300 million are already obese[1]. Unfortunately, it is expected that this number will increase, as a new worldwide generation of young population, already overweight and obese, reaches adulthood.

Obesity has been associated to several other diseases, such as type 2 diabetes DM, dyslipidemia, hypertension, coronary heart disease (CHD), osteoarthritis, gallbladder, and liver disease. Unfortunately, obesity has been assuming an epidemic profile across the world. In the American population, several cross-sectional health examinations surveys have been developed since 1960. In adults aged from 20 to 74 years, the National Health and Examination Survey (NHANES) have reported an alarming increase in obesity prevalence

from 14.6% (1960-1962) to 32.2% (2003-2004)[2]. Furthermore, not only the overweight prevalence among US children and adolescents has been increasing but also morbid obesity has risen almost three-fold from 1900 to 2000[3].

Conversely, Europe and several other emerging economies have been reporting obesity prevalence values ranging between 15-20%. Moreover, the estimated obesity prevalence in Europe has risen from 15-25% for women and 10-20% for men in 1995[4], to recent values as high as 31% and 26%, respectively. In our country, almost half of Portuguese population is overweight or obese[5]. In the Asia-Pacific region, it has also been reported an increase of both obesity and type 2 DM prevalence[6], which can be primarily explained by an increase in central obesity.

The prevalence increase in obesity and related comorbidities has been accelerating the healthcare and socio-economical costs. In the US, the obesity-related costs during 1999 were estimated to be approximately 24 billion dollars[7], reaching almost 75 billion dollars during 2003, which represented 5-7% of total healthcare costs[8]. The global costs, including both direct and indirect expenses (lower productivity due to illness or premature death) ascended to 100 billion dollars per year[9]. In addition, the estimated direct costs of obesity were comparable to those observed in both CHD and type 2 DM, being however more costly than both stroke and hypertension[10]. Other countries like Canada and Australia have also been reporting an increase in health care expenditures related with obesity[11, 12]. In fact, obesity is a major risk factor to the development of several other chronic diseases. A recent study estimated that 17% of hypertension, 61% of the type 2 DM, and 17% of the CHD direct costs are directly attributable to obesity[10]. Europe has also raised the obesity estimated costs in about 1-5% of total healthcare expenditures, which puts obesity among the most costly diseases[1].

The total expected lifetime medical care costs related to the treatment of numerous chronic diseases, such as dyslipidemia, type 2 DM, hypertension, and several other CVD seem to increase concomitantly with body mass index (BMI) elevation[13, 14]. Compared with lean persons, and independently of gender, overweight raises lifetime healthcare costs of the referred comorbidities by 20%; class I obesity raises them by 50%, and class II seems to promote a nearly 100% increase[14]. In this sense, evidence has been demonstrating that obesity is a major health concern, representing tremendous medical, social and economical costs, not only in the US but also worldwide.

The initial simple thought that obesity was only related to an imbalance between energy intake and expenditure has been changing due to the ever increasing number of studies demonstrating that physiological, genetic and behavioral factors may also play crucial roles in its aetiology. All taken into account, evidence suggests that this global epidemic phenomenon requires proper and specific management strategies in order to overcome the most challenging healthcare problem of this new century.

1.2. Obesity management

Obesity management through medical or pharmacological intervention, nutrition and exercise are now at the forefront of research. When defining obesity based only in BMI criteria[15, 16], some obese individuals (BMI $\geq$30 kg/m^2) can be classified has being at higher risk despite their metabolic health, which might justify the weak results obtained in previous weight loss strategies based primarily on pharmacotherapy. To date, obesity pharmacological agents available have been formulated to suppress appetite, to reduce both energy intake and nutrients absorption, and to increase energy expenditure. Based on guidelines proposed by the National Institutes of Health (NIH) to assess and treat obesity[17], pharmacotherapy should only be considered in appropriate patients who were unable to achieve an adequate weight

loss after 6 months of lifestyle therapy, and who had a BMI≥30 kg/m^2, or BMI≥27 kg/m^2 with concomitant obesity-related comorbidities. The main goal for all individuals with BMI greater than 25 kg/m^2 should be primarily placed in lifestyle modification, involving a weight loss program which should include appropriate diet, physical activity and behavior therapy (**Figure 1.1)**. It has been suggested that the first goal is trying to achieve a moderate weight loss (5-10%) and maintain at least this magnitude of weight loss in a long-term perspective[18].

	BMI Category (kg/m^2)				
Treatment	25-26.9	27-29.9	30-34.9	35-39.9	≥40
Diet, Exercise and Behavior Therapy	+	+	+	+	+
Pharmaco-therapy		With co-morbidities	+	+	+
Surgery				With co-morbidities	+

Figure 1.1. Overweight and obesity management guidelines. Adapted from NIH[17].

Data resulting from the Framingham Offspring Study demonstrated that small reductions in body weight were associated to significant reductions in several CHD risk factors, including hypertension, hypertriglyceridemia, hypercholesterolemia and hyperglycemia[19]. During the 16 years that this study lasted, a reduction in patient's weight by 2.3 kg or more decreased significantly the health risk by 40% in women and 48% in men. However, when patients gain 2.3 kg or more over same period, a significantly increase in those risk factors was observed by 37% and 20%, respectively. Another study developed with 114 obese patients with type 2 DM demonstrated that even a modest weight loss as small as 2.5% was sufficient to reduce both fasting glycemia, fasting insulin, and hemoglobin A1c (Hb A1c) in a dose dependent way, improving simultaneously both insulin

sensitivity and glycemic control[20]. In an excellent meta-analysis which reviewed 70 clinical trials, moderate weight loss promoted markedly changes in plasma lipids concentrations, inducing a more favorable metabolic profile[21].

The energy imbalance that leads to overweight and obesity can be, in part, attributed to behaviors promoting both an energy expenditure reduction, as well as an increase in caloric intake. The behavioral modification therapy applied to weight loss interventions has been used as a standard therapeutic strategy for the last 25 years[22], being recommended as one of the first approaches to obesity treatment. Usually developed by several types of healthcare providers, such as psychologists, behavioral therapists, dieticians or exercise physiologists, it tries to encourage patients to make healthier lifestyle choices through eating and physical activity habits modification[23]. The most successful models usually include stimulus control and self-monitoring of both eating and physical activity behaviors [18, 22]. In obese adolescents, the cognitive-behavioral approach has been used to help patients to become more assertive in coping with social stigmas, enhance their self-esteem and body image[24]. Behavioral therapies have been increasingly study in the obesity context. However, despite these issues are not a major concern of our work, is it important to note that regardless the approach followed, the behavioral interventions should promote the development self realistic goals, adapted to one's limitations and easy to evaluate, as well as a reasonable program to reach those goals. This program must respect self adaptation and, hence, should be controlled in order to promote successful experiences which are crucial for future changes in patient's lifestyle. In addition, special attention should be paid in strategies that could prevent relapse, helping the patient to make small and incremental changes rather than large and drastic ones.

In this sense, recent studies have investigated the behavioral predictors associated with long-term successful weight loss. Results obtained from the National Weight Control

Registry (NWCR) developed with 3000 patients who, on average, have maintained a 32 kg weight loss during 6 years indicated that low-calorie (1300 to 1400 kcal/d) and low-fat diets (20% to 25% of daily energy intake), regular self-monitoring of body weight and food intake, regular physical activity (2500 to 3000 kcal/week), and eating breakfast every day were the specific predictors associated with successful long-term weight loss[25-27]. In this study, only patients that have maintained a weigh loss superior to 13.6 kg for at least one year were included. Another recent study developed in our laboratory have investigated the principal pretreatment predictors of short-term weight loss in 140 healthy overweight or obese premenopausal women[28]. Previous dieting, weight loss attempts, pre-treatment body image dissatisfaction, and low self-motivation were inversely associated with future weight loss, and thus, were identified as negative predictors. These results constitute new insights in the predictors of weight outcomes and suggest that behavioral and psychological prediction models should be further investigated in order to consubstantiate and modulate weight loss strategies and treatment decisions for overweight and obese women[28].

Besides behavioral therapy, diets are still the most common strategy to manage overweight and obesity. Several reference public health institutions have been publishing dietary guidelines, such as the American Heart Association (AHA)[29] and the American College of Sports Medicine (ACSM). Indeed, ACSM has published an important position stand addressing the appropriate intervention strategies for weight loss and prevention of weight regain for adults[18]. According to ACSM, the available evidence reviewed have highlighted the relevance of dietary fat intake reduction for long-term weight loss, which is in accordance with the obesity treatment recommendations published by the National Heart, Lung, and Blood Institute (NHLBI)[30]. However, it is still not clear the optimal macronutrient diet composition to promote a healthy, sustainable long-term weight loss despite the wide range of dietary approaches known[18]. Although evidence has been demonstrating that both

carbohydrate and protein intake can affect thermogenesis and satiety, further research is needed in this area.

Together with dietary recommendations, the increase in daily energy expenditure through physical activity and exercise is another obesity management strategy. Some institutions like ACSM have been recommending that significant health benefits in overweight and obese patients can be achieved with the participation of at least 150 min (2.5 h) of moderate intensity exercise per week[18]. Patients should progress from this initial goal to a defiant exercise volume of 200 to 300 min (3.5 to 5 h) or up to 2000 kcal per week. In fact, evidence has been demonstrating that volumes of this magnitude may facilitate the long-term weight loss maintenance. However, it is noteworthy that the recommended volume and intensity of exercise for sedentary adults who initiate a weight loss programme is far different from the amount that can be sustainable achieved in later programme phases. Regarding exercise intensity, it seems that moderate-intensity (55 to 69% of maximal heart rate - HR_{max}) aerobic exercise can be beneficial in a weight management context[18]. To date, there are few studies obtained from randomized trials that support the recommendation of more vigorous (higher than 70% of HR_{max}) exercise intensities to long-term weight management[18].

On other hand, exercise prescription should be adapted to patient's needs, tastes and capacity to maintain. Moreover, several behavioral strategies can be very helpful to promote exercise adherence and maintenance. In order to overcome some potential adherence and maintenance exercise barriers such as dislike or lack of time to engage continuous sets of exercise, intermittent exercise bouts can be prescribed as an effective alternative[31]. Similarly, the ACSM recommend that resistance exercise should be included in weight loss programs due to its ability to improve muscular strength and endurance, both important muscular qualities with significant impact in daily functional tasks.

However, the increase of daily energy expenditure can not be only reduced to exercise prescription. Lifestyle modification should focus not only the physical activity reinforcement, but also the sedentary lifestyle limitation. In this sense several behavioral modifications that should be reinforced. People are more likely to adhere to exercise if they have positive attitudes towards it, if they perceive social pressure, and if they believe that they will be successful[32].

The debate related with successful long-term weight management programs is now at the forefront of research in scientific community. Despite the current dissertation will only address cross-sectional data, our work is part of a large comprehensive group-based weight management program designed to promote healthy lifestyle changes, to improve dietary habits, and to increase physical activity. Together with this cross-sectional analysis, the longitudinal data will hopefully allow further insights related with obesity and body composition associations with health risk.

1.3. Heath risk: metabolic syndrome and cardiovascular disease

Obesity seems to represent the driving force behind the prevalence explosion in cardiovascular diseases and type 2 DM, leading to the assumption of a "global pathobesity crisis"[33]. Both CVD and diabetes are on track to assume them selves as the main comorbidities that can raise mortality in the 21st century. The scientific community has been making important efforts to understand the underlying pathophysiological mechanisms and to identify the contributing risk factors for these diseases. As the evidence was being collected it became clearer that a cluster of CVD risk factors were often simultaneously present with obesity, type 2 DM, hypertension and hyperlipidemia[34, 35]. This risk factor clustering was additionally confirmed by observations showing that insulin resistance[36, 37] (i.e. resistance to insulin stimulated glucose), early described by Himsworth[38], and

hyperinsulinemia, both key features of type 2 DM[39], were also associated with hyperlipidemia[40, 41], hypertension[42, 43] and obesity[43, 44]. These relationships combined with the evidence that most of the subjects with the CVD risk factor clustering had one of these two abnormalities led the scientific community to propose the existence of a common pathophysiological condition, which was primarily defined as "insulin resistance "[44] or "metabolic" syndrome[34].

These early concepts were unified by Reaven[45], who postulated that IR and the consequent compensatory hyperinsulinemia could predispose patients to type 2 DM, hyperlipidemia and hypertension[40], creating the basis for the CVD development. Despite the list disorders identified by the "syndrome X" proposed by Reaven, obesity was not included yet. During the last three decades, many studies have been documenting the clustering of CVD risk factors and their important relationship with IR[46-49], creating a body of evidence which leaded the development of several "metabolic" syndrome definitions[50-53]. These definitions have been proposed to extend and clarify previous concepts, such as "insulin resistance" syndrome[44], "syndrome X"[45], and "deadly quartet visceral fat" syndrome[54].

Despite the controversy regarding the several questions that are still waiting to be answered, the term "metabolic syndrome" has now taken hold in the medical literature and was recently defined and institutionalized, albeit differently, by the World Health Organization (WHO)[52] and the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP III) [50, 55]. Additionally, other organizations, such as International Diabetes Federation (IDF), have also developed a similar but not identical definition[51]. In spite of the fact that metabolic syndrome pathogenesis as well as each one of its features are still not fully understood, several studies have been suggesting a new global approach defined by a cluster of several obesity-related complications. This group includes abnormalities such as atherogenic dyslipidemia, defined by a combination of

low concentrations of high-density lipoprotein cholesterol (HDL-C) appearing together with higher triglycerides (TG), apolipoprotein B (apo B), and small dense low-density lipoprotein cholesterol (LDL-C) concentrations. Hypertension, IR with or without glucose intolerance (i.e. impaired fasting glucose -IFG or impaired glucose tolerance - IGT) and, a proinflammatory and prothrombotic state are some of the disturbances also included in this group.

However, despite the fact that these health agencies have been proposing that obesity, operationally defined by BMI[30, 56, 57], is closely related with the incidence of several chronic diseases and metabolic syndrome features[15, 16], only some of those definitions include obesity markers. The NCEP-ATP III Panel[58] has recently provided a new metabolic syndrome operational definition, which, in fact, seems to be the most often used in the literature (**Table 1.1**). This definition diagnoses metabolic syndrome based on the presence of at least 3 of the 5 required criteria[58].

Table 1.1. Metabolic syndrome definitions

ATP III definition	***WHO definition***
Any three or more of the following criteria:	Type 2 diabetes mellitus *or* IFG [≥6.1 mmol/L (110 mg/dl)] *or* IGT *or* insulin resistance (glucose uptake below lowest quartile under hyperinsulinemic-euglycemic conditions) and at least two of the following criteria:
Abdominal obesity Waist circumference Men > 102 cm Women > 88 cm	Obesity Waist-to-hip ratio Men > 0.9 cm Women > 0.85 cm *or* BMI >30 kg/m^2
Triglycerides ≥ 150 mg/dL (1.7 mmol/L)	Triglycerides ≥ 150 mg/dL (1.7 mmol/L)
Blood pressure ≥130/85 mmHg	Blood pressure ≥140/90 mmHg
HDL-C Men > 40 mg/dL (1.0 mmol/L) Women > 50 mg/dL (1.3 mmol/L)	HDL-C Men > 35 mg/dL (0.9 mmol/L) Women > 40 mg/dL (1.0 mmol/L)
Fasting Glucose> 110 mg/dL (6.1 mmol/L)	Urinary albumin excretion rate > 20 μg/min or Albumin-to-creatinine ratio ≥ 30 mg/g

More recently, a revision of this ATP III definition has extended the pathogenesis of metabolic syndrome to AT related disorders and to a new variety of independent metabolic risk factors such as proinflammatory and atherothrombotic abnormalities[55].

Despite some similarities, the WHO suggests a different operational definition to diagnose metabolic syndrome, listing, for example, waist-to-hip ratio (WHR) instead of waist circumference (WC). In addition, microalbuminuria is listed in the WHO criteria but not in the ATP III, and insulin resistance, as measured by hyperinsulinemic-euglicemic clamp, is also relevant for WHO but not for the ATP III. Furthermore, while WHO recognize any IR marker, only impaired fasting glycemia is addressed by ATP III.

The IDF has also proposed a new metabolic syndrome definition based on the presence of large WC, gender and ethnic group specific, plus any two of four listed ATP III cut-points[51]. Based on a recent American Diabetes Association (ADA) recommendations[59], IDF definition updated the fasting glycemia cut-points from 110 mg/dL to 100 mg/dL, reducing also the WC cut-points. Additionally, the IDF consensus group has also highlighted in its rationale the necessity of developing more research in order to determine the predictive power of several metabolic syndrome features for diabetes and CVD, such as atherogenic dyslipidemia, abnormal fat distribution, vascular deregulation, hormonal factors, dysglycemia, and proinflammatory and prothrombotic states[51].

Despite the wide range of possible combinations when diagnosing metabolic syndrome, an increased WC is commonly present. However, healthcare professionals are still perplexed about the enormous heterogeneity found in obese patients. While some can be easily included in these definitions, others show a relatively “normal” metabolic risk profile despite the presence of substantial fat accumulation. In addition, there are moderately overweight individuals who can be diagnosed with a cluster of metabolic disturbances ultimately responsible for increasing the risk of future CVD and type 2 DM. Furthermore, it

was recently proposed a new formal definition for the concept of metabolically obese normal-weight[60] individuals, originally advanced more than 20 years ago[61], which have come to raise the problem complexity.

Since metabolic syndrome aetiology is far from being fully understood, it is very difficult to decide which specific features should be included in an operational definition. Although both the ATP III and WHO definitions can generally diagnose the same patients, studies have been reporting important differences. In a recent NHANES[62], the ATP III and the WHO definition identified similar percentages of metabolic syndrome (23.9 vs. 25.1%, respectively). However, approximately 15 to 20% of the patients were classified by one definition as having the syndrome, but not by the other. Similarly, other authors have also identified differences in metabolic syndrome prevalence rates[63]. We found in our study different prevalence rates of metabolic syndrome when using the ATP III or IDF definitions (13.7 vs. 18.5%, respectively). Regardless the definition used, it is estimated that at least 47 million Americans could have metabolic syndrome[37].

There is growing evidence showing that patients with metabolic syndrome have an increased risk of incident diabetes[64] and CVD[65-69], which is not totally surprising, as several traits of the metabolic syndrome are important major diabetes and CVD risk factors[70-73]. However, there is some uncertainty whether the presence of the metabolic syndrome increases the health risk to a greater degree than the risk associated with the presence of each one of its features[74]. A recent study reported that the presence of the metabolic syndrome is responsible for atherosclerosis increase in the carotid artery to a greater degree than the risk expected solely by its individual components[75]. Another recent analysis of participants in two large lipid lowering clinical trials showed that the metabolic syndrome was related with an elevated risk of major coronary events[76].

However, there are some exceptions to this large body of evidence documenting the relationship between CVD and metabolic syndrome. A recent study conducted in nondiabetic American Indians reported that ATP III definition predicted the risk of diabetes, but neither predicted CVD independently of other very well established CVD risk factors[77]. In another study conducted in women with suspected CVD, the metabolic syndrome did not increase the CVD risk when no angiographically coronary artery disease was present[78].

Another important issue when addressing the relationship between CVD and metabolic syndrome relies on the fact that some definitions do not consider important CVD risk factors such as physical activity, gender, age, and family history. In addition, several proinflammatory and atherothrombotic markers associated with CVD are also not included[50]. The main role of inflammation in atherogenesis is already known[79], being C-reactive protein (CRP) one of the most important independent CVD predictors[80]. Additionally, some adipose tissue-derived cytokines, known as "adipocytokines" or "adipokines", such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) have also been linked with hypertension, dyslipidemia, and IR[81]. Other atherothrombotic molecules such as plasminogen activator inhibitor-1 (PAI-1)[82-84] and fibrinogen[83, 84] are still not included in the definitions despite their CVD predictive value. There is also a growing interest in other adipocytokines such as adiponectin, which is a strong independent inverse predictor of CVD[85, 86], being also inversely associated with hypertension[87], TG, LDL-C[88], IR features[89], and inflammatory markers[81].

To date, it is still unclear if the substitution or inclusion of any of these features would improve the predictive value of the metabolic syndrome. In fact, some of the studies here reviewed[65-68] demonstrated that the risk remained significant even after adjustment to several other conventional factors, suggesting that, if included in the operational definition, the predictive values may improve.

Although the diagnosis of metabolic syndrome points to a better prevention of diabetes and CVD, it is necessary to obtain more conclusive data about the outcome of aggressive interventions that could address healthy weight, physical activity habits, and serum risk factors control. Even modest lifestyle changes can substantially reduce type 2 DM and CVD risk. However, it has to be clarified if even modest changes towards weight loss and a healthy lifestyle are the most effective way of dealing with the metabolic syndrome, and therefore, preventing its CVD and diabetes endpoints.

1.4. Weight loss: body composition changes and health risk reduction

Body composition modifications promoted by weight loss have been increasingly study. Together with adipose and muscle tissue changes, modifications in metabolic and health risk elapsed from weight loss have also been largely examined. In this context, a recent study analyzed in 33 upper body obese men the effects of diet only (DO) and diet combined with either aerobic (DA) or resistance (DR) exercise on body composition distribution[90]. Skeletal muscle and abdominal adipose tissue depots were measured by magnetic resonance imaging (MRI). All groups have similarly decreased their body weight, abdominal subcutaneous adipose tissue (Ab SAT) and visceral adipose tissue (VAT) volumes. In the three groups, the VAT relative reduction was greater than the observed in Ab SAT, being uniform across all groups. However, both exercise groups have demonstrated a greater Ab SAT reduction when compared with that verified in the femoral-gluteal region. While DA group have increased their peak O_2 uptake, only DR revealed improvements in muscular strength, suggesting that diet combined with exercise could promote better lean tissue preservation, higher Ab SAT mobilization, as well as functional capacity improvements.

More recently other studies were developed by the same research group in order to overcome the absence from literature of random controlled trials comparing the efficacy of diet- or exercise-induced weight loss and exercise without weight loss on obesity reduction in both obese men[91] and women[92]. Abdominal AT compartments, skeletal muscle mass and insulin sensitivity were measured. Body weight decreased in weight loss groups by 8% and 6.5% respectively, being unchanged in the exercise without weight loss and control groups. Both men and women exercise-induced weight loss groups have improved their cardiorespiratory fitness (CRF), revealing also greater reductions in total body fat, total abdominal AT, Ab SAT and VAT mass[91, 92]. However, both diet weight loss and exercise without weight loss groups have presented greater reductions in total body fat and abdominal fat mass when compared with the controls. In women, while visceral fat have similarly decreased within all treatment groups, only the exercise-induced weight loss group have improved insulin sensitivity[92]. In men, similarly to the exercise-induced weight loss group, diet weight loss group have also improved glucose disposal[91]. These improvements in insulin sensitivity, reaching approximately 60%, are superior to those reported in response to some glucose-lowering drugs, such as metformin (improving approximately 25%)[93], highlighting the exercise relevance in IR management. However, in the absence of weight loss or a VAT reduction, the beneficial exercise effects on insulin sensitivity area were quickly attenuated, being therefore necessary to maintain the exercise adherence[91].

In the study which included 54 premenopausal obese women, the exercise-induced weight loss group presented greater reductions in total and abdominal fat, and greater improvements in CRF when compared with diet-induced weight loss group[92]. The fact that only the exercise-induced weight loss group had improved insulin sensitivity, combined with the evidence that even in the exercise without weight loss there were substantial reductions in total and abdominal fat have provided strong support to a daily exercise recommendation

(e.g. 30 a 60 minutes per day of brisk walking) as an effective strategy to reduce obesity and IR in women. The similar results obtained in men have also provided basis for recognition that diet restriction and exercise are effective methods to decrease body weight and to reduce and prevent abdominal obesity.

In another 12-month exercise program developed with older women, similar reductions in total and abdominal obesity were also reported, being an approximately 2 kg reduction in body weight associated with a 7% decrease in visceral fat[94]. Similarly, it was recently reported in overweight and obese nondieting men and women that ~45 minutes of exercise, performed 4 days per week, promoted a markedly reduction in both total and abdominal fat[95]. In another study developed with 30 men, exercise performed ~40% to 60% of maximal oxygen uptake (VO_{2max}) promoted markedly reductions in total body weight and body fat[96]. The results obtained in these trials suggest that 45 to 60 minutes of exercise without caloric restriction, performed at ~65% to 80% of HR_{max}, can promote substantial body weight and obesity reductions independently of gender, which can mostly be attributed to a significant lipolysis increase in abdominal AT depots[97]. However, few studies have documented that overweight or obese individuals can, after losing a substantial amount of body weight, maintain body weight for several years[26, 98, 99]. The individuals who have successfully maintained a reduced weight had in common, together with regular exercise, a low-fat and a calorie restricted diet[26, 98]. These common characteristics, resulting from the combination of regular exercise with dietary modifications, are also responsible for the selective changes in abdominal and thigh body fat and muscle distribution that have been observed.

Contrarily to these results demonstrating substantial reductions in body weight and obesity, previous meta-analysis[100-102] focusing the efficacy of exercise, isolated or combined with dietary restrictions as a useful method for obesity management, have concluded that

exercise alone can promote modest weight loss (1 to 2 kg) in overweight individuals. Similar conclusions were also proposed by several randomized trials which have observed modest abdominal AT reductions independently associated with physical activity[13]. In an rigorously controlled trial recently developed, exercise performed 5 days/week, resulting in caloric expenditure of about 2200 kcal/week, could prevent weight gain in overweight and obese women[103]. Although women were resistant to weight loss in response to exercise, visceral fat decreased by 5%. However, exercise without changes in diet has produced significant reductions in body weight, total body fat, and visceral fat in men, probably due to a higher negative energy balance achieved[103].

Other previous studies developed by the same scientific group have also showed no significant decreases in women body weight when participating in exercise without energy restriction[104, 105]. The absence of body weight changes in women after a 10 month exercise period, with energy expenditure exceeding 2000 kcal/week, raises the necessity of identifying the characteristics of the women that did not lose weight after this very substantial effort in order to better identify the individuals who may benefit from exercise, or to understand the amount of exercise needed to promote a weight reduction and prevent weight gain. Although several authors[31, 106] have been suggesting that prevention of weight regain is associated with 280 to 450 exercise minutes per week, performed in a moderate intensity, the previous results indicate that 225 minutes per week of moderate exercise may prevent weight regain in women and reduce body weight in men[103].

Despite recent recommendations to prevent weight gain in adult subjects seem to indicate the necessity of accumulating at least 60 minutes of moderate continuous exercise per day[107] (which could be a barrier to exercise adherence and participation for most individuals), evidence is pointing out that short, intermittent bouts of exercise, more easily attainable, may also promote a weight reduction [31, 108]. Other health institutions such as

WHO have also been publishing exercise recommendations to weight management based on a physical activity level (PAL)[109]. Obtained dividing the total energy expenditure by the resting metabolic rate, the PAL recommended to prevent weight gain ranges from 1.6 to at least 1.75. Indeed, it was recently observed in a clinic-based cohort study with 2501 men at risk of weight gain that a shift from a low PAL<1.45 to a moderate 1.45-1.6 or high (PAL>1.6) level was necessary to induce weight loss over time[110]. According to these authors, it may be necessary to increase or maintain a daily PAL>1.6 to maintain body weight in middle-age men, which can be attainable, for example, with 45 to 60 min of brisk walking.

It is also noteworthy that physical activity effectiveness on body weight loss and weight regain prevention depends also on the macronutrient composition of the post-exercise diet[111]. In fact, some studies have shown that, following a bout of exercise, if individuals are offered a high-fat diet, they consume more energy than when offered a low-fat diet. If exercise is followed by a low-fat diet, a short term negative energy balance is more easily attainable[111].

Physical activity may also promote a health risk reduction even without weight loss. Despite evidence is suggesting a dose-response relationship between physical activity caloric expenditure and the weight loss magnitude, which is relatively clear in short term interventions associated with high levels of energy expenditure (>2000 kcal/week)[112], it was already reported in a 6-month exercise programme, consisting of 90 min exercise sessions, performed 4 to 5 times per week at 55% of maximal aerobic capacity, significant improvements in metabolic profile of obese women who gained 2.0 kg of weight and 2.8 kg of fat during the intervention period[113]. On the other hand, despite the small impact of resistance training observed in weight reduction, selective benefits in muscle tissue distribution have been observed[114]. It has also noteworthy that not only structured exercise,

performed on a daily basis, is important in obesity reduction. It has been suggested that non-exercise activity thermogenesis (NEAT), defined as the physical activity energy expenditure not associated with exercise, can represent an important role in weight gain prevention[115, 116]. Hence, it is clear that exercise should be a part of any metabolic syndrome and obesity intervention, regardless of whether the primary outcomes for success are body-weight related or health-related.

More recently, new approaches to reduce health risk have been investigated. The efficacy of transcendental meditation (TM) on metabolic syndrome features, such as blood pressure, lipoprotein profile, IR determined by homeostasis model assessment (HOMA)[117], brachial artery reactivity, and cardiac autonomic nervous system activity (CANSA) was studied in a random trial with 103 subjects with stable coronary heart disease[118]. After 16 weeks of intervention, the TM group improved blood pressure and IR components, as well as CANSA compared with the control group which has only received health education. These encouraging results obtained with new approaches capable of modulating the neuro-humoral pathways related to stress response and capable of improving CVD risk factors opened a new interesting investigation field which needs further research.

1.5. Waist circumference and hip circumference: relations with health risk

The overwhelming group of evidences presented makes clear that obesity, and, especially abdominal obesity is a major clinical and public health concern and need to be addressed in order to reduce health risk and mortality. It seems evident that BMI, besides its usefulness in an epidemiologic context, when screening obesity on an individual level, needs to be coupled with other anthropometric markers more capable to discriminate regional fat accumulation and predict obesity-related health risks. In fact, the association of waist circumference with BMI predicts health risk better than BMI alone[119]. Furthermore, WC and

HC, both easy assessment anthropometric markers, seem to reflect different aspects of body composition, CVD and type 2 DM risk assumption[120-122]. Recent studies have reported that central and peripheral fat accumulation configures two different phenotypes of body composition. Prospective studies have shown that abdominal obesity predicts not only a higher risk of future type 2 DM[123, 124], but it can also be considered as a major risk factor for impaired glucose metabolism disturbances[123, 125, 126] and for CVD related mortality[126, 127].

Several population studies have been using WC as the best anthropometric marker to indicate a central obese phenotype. Some of these studies, developed with sophisticated imaging techniques such as CT and MRI, both able to discriminate Ab SAT from VAT, have demonstrated that WC was the most reliable predictor of VAT (**Figure 1.2**)[126, 128]. Similarly, another seven year longitudinal study developed with women revealed that WC was the anthropometric index better correlated with VAT changes over time[129].

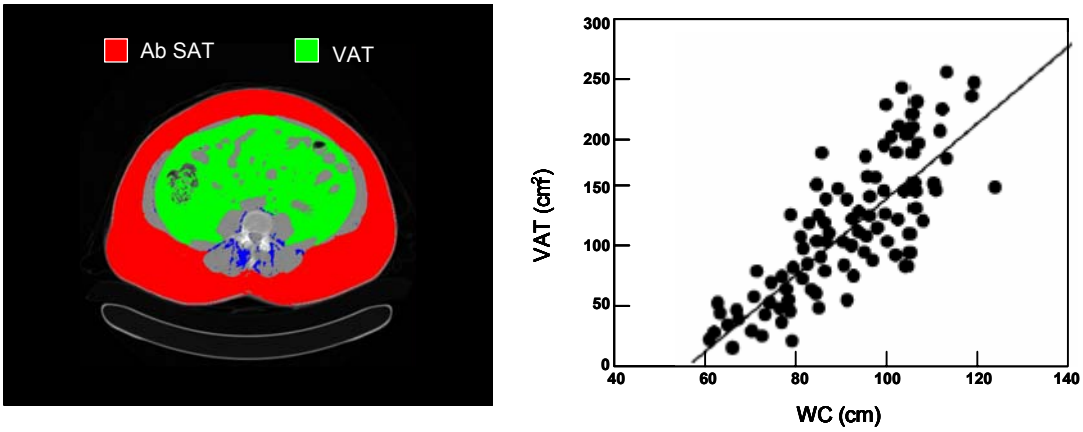

Figure 1.2. Correlation between WC and VAT, measured by CT[126].

These studies confirming that WC was a better VAT predictor were additionally extended by those showing that WC is also the best anthropometric predictor of metabolic syndrome disturbances[124, 130], hypertension and several CVD[131] such as CHD[126, 128, 132]. The underlying pathophysiologic mechanisms may be related to increased lipolysis in abdominal AT depots, inducing liver glucose and very-low density lipoproteins (VLDL) synthesis.

Concomitantly, the skeletal muscle decreases its substrates utilization, contributing to hyperglycemia and hypertriglyceridemia, to an increase of small and dense atherogenic LDL, and to a reduction in HDL-C. These metabolic cascade is complemented with a blood pressure (BP) increase and with the onset of a low chronic inflammatory state[133].

The evidence demonstrating the existence of an atherogenic metabolic triad in visceral obese patients, composed by hyperinsulinemia, increased apolipoprotein (apo) B and small and dense LDL plasma concentrations, which substantially contribute to an increased risk of CVD and type 2 DM, have constituted the basis for the "hypertriglyceridemic waist" concept suggestion[134]. Although proposed only in men, it has been advanced a simple clinical diagnosis algorithm, with cut-points for WC (≥90 cm) and TG (≥2.0 mmol/L)[135], which could be a helpful tool to be used by the majority of physicians and other healthcare professionals in central obesity screening and management[134]. In a recent five year longitudinal study with initially asymptomatic middle aged men, the presence of the atherogenic triad in visceral obese patients promoted and 20-fold increased in future risk of CHD[136]. Using these criteria to investigate the role of hyperglycemia in the modulation of angiographically assessed CHD, another recent study has reported that CHD risk was markedly higher among subjects with both hypertriglyceridemic waist and glucose intolerance compared with the normoglycemic group with low TG and WC values[137]. In the absence of hypertriglyceridemic waist, impaired fasting glucose did not remain a significant predictor of CHD[137].

Although this approach seems to be an efficient and promising strategy to central obesity screening and, hence, to CVD and type 2 DM risk assumption, it has to be considered that there are gender and ethnic group differences regarding WC, body fat distribution patterns and metabolic disturbances[134]. Therefore, despite the concept of "hypertriglyceridemic waist" has been tested for their ability to screen atherogenic triad in

visceral obese men[135], the absence of prospective studies addressing the association of serious cardiovascular endpoints with the atherogenic triad in women, and the recognition that age and menopause are related with selective VAT deposition which increases CHD risk[129], highlight the relative weakness of this screening algorithm when applied to women.

For a given WC, accumulation of fat in the hips and legs is independently associated with lower glucose metabolism disturbances[125, 138-141], predicting lower risk of future type 2 DM[138, 142, 143] and CVD related mortality[120, 127, 142, 144]. In fact, a larger HC has been associated with a better glucose tolerance profile, lower plasma lipid concentrations, and lower incidence of type 2 DM[122, 138-140] and some CVD endpoints[120, 144], independently of WC. In a very recent study, these associations previously observed in Caucasian subjects were extended to other ethnic groups. The results demonstrated that, in all groups, for a given WC, a larger HC was associated with lower TG and glucose concentrations in both genders, and higher HDL-C values in women[145]. In addition, both in a prospective follow-up study and in cross-sectional settings, it has been observed a protective effect of higher fat leg accumulation, measured by dual x-ray absorptiometry (DXA) in IR, CVD risk factors, and in the severity degree of aortic calcification[146-148]. It was suggested that these independent and opposite associations could configure a potential protective role of a larger HC, if WC is taking into account, to a less atherogenic body composition and metabolic profile[122, 149]. Some of these studies have also reported that HC was inversely associated with abdominal AT compartments, fat-free mass and overall body fat distribution markers. Despite the underlying mechanisms are still a matter of important scientific debate, it has been proposed that both WC and HC may play different roles in risk assumption based on different physiological mechanisms[141].

Regarding to the HC role, it seems that both thigh adipose and muscle tissue can contribute in different ways to the phenomenon[122, 138]. Regardless the existence of limited

evidence addressing the specific role of peripheral fat mass (PFM) and, more specifically, thigh AT in metabolic risk prediction, it has been reported that a large HC is an independent predictor of lower diabetes and CVD related mortality[120, 144]. Another study developed with women have also reported that leg fat deposition was inversely related with both atherogenic lipid markers and glucose metabolism disturbances[120, 142]. Furthermore, recent studies with elderly women have also shown that PFM can assume an independent dominant antiatherogenic effect[146]. In women with the same percentage of central fat mass (CFM) but significantly higher PFM, the metabolic profile was more favorable and the aortic calcification progression was slower than that verified in women with central obesity, suggesting that fat localization is more important than obesity *per se* in atherogenesity and in CVD risk[146, 147]. This relative protective role of PFM in health risk may be, in part, explained by an insulin sensitization effect mediated by adiponectin[150, 151]. Moreover, adiponectin concentrations seem to be significantly elevated in women with general obesity compared with those with central obesity[152]. Additionally, it has been reported in postmenopausal women that hyperadiponectinemia can also mediate not only an antiatherogenic and anti-inflammatory effect but may also promote a reduction in body weight increase tendency, attenuating the DM progression[153, 154].

On the other hand, low peripheral exposure to estradiol appears to be the key feature for maintained adiponectin secretion from PFM[152]. Increased or prolonged exposure of body fat mass to estradiol may promote IR, involving adiponectin inhibition[155]. In addition, evidence have showed that low sex hormone-binding globulin (SHBG) can increase the risk of gestational diabetes, highlighting the contribution of increased PFM exposure to sex steroids[156]. Women with lower PFM have higher SHBG and lower estradiol when compared to women with an abdominal obesity phenotype[157], suggesting the existence of several differences between visceral and subcutaneous adipocytes regarding to the enzymatic

conversion of the steroid hormones (peripheral aromatization). In this context, subcutaneous adipose tissue (SAT), the AT depot that mostly contribute for circulating adiponectin (owing anti-atherogenic, anti-inflammatory and insulin-sensitizing effects), may mediate the protective associations observed in these studies examining PFM[157].

The evidence reviewed so far leave clear that, when screening obesity, the relevance of assessing both WC and HC seems to be unquestionable and should be proposed as an integrant part of an efficient assessment and treatment algorithm. However, there are still many questions that need to be answered regarding the underlying mechanism behind the opposite associations verified. For example, it is not known if these inverse associations observed when WC is taken into account remain significant with other specific metabolic syndrome features, such as proinflammatory and atherothrombotic markers. In addition, the independent role of the different thigh AT and muscular compartments to this metabolic cascade is still not clear. Those are questions that we will try to address in the present work.

1.6. Visceral adiposity and health risk

Epidemiological studies conducted in recent years have come to the conclusion that comorbidities linked with obesity were more closely associated with the location of fat depots rather than to the total amount *per se*[134, 158, 159]. It has been shown that VAT is associated with the development of several obesity-related comorbidities such as dyslipidemia, type 2 DM, hypertension, coronary atherosclerosis and CVD[124, 158]. In the past decade, the study of abdominal AT distribution by imagiologic methods, such as MRI and CT[160] demonstrated the relevance of visceral adiposity to IR and glucose intolerance[161], dyslipidemia, and several other metabolic syndrome clinical features, including coagulation abnormalities, proinflammatory disturbances and microalbuminuria[162-164].

As previously reviewed, IR seems to play a crucial role as it seems to link all of these metabolic complications[134]. In fact, the literature has been pointing out two major perspectives to explain the underlying pathophysiologic mechanisms behind metabolic syndrome in abdominal obese patients. While some authors have been proposing that neuroendocrine perturbations, mediated by the hypothalamic-pituitary-adrenal (HPA) axis and stimulated by central factors, the so-called stress, are responsible for IR, as well as central obesity[165, 166], others have been suggesting a "portal" hypothesis[167], focusing the relevance in the increased lipolytic activity of visceral adipocytes. These lipolytic activity is responsible for increased delivery of free fatty acids (FFA) into portal vein, which reach the liver, promoting hepatic function disturbances, IR and several other metabolic abnormalities[164, 168, 169]. In the first case, it is known that HPA axis stimulation during prolonged periods can alter cortisol secretion, inhibit steroid and growth hormones production and activate sympathetic nervous centers. These metabolic alterations induce dysfunctions in several regulatory mechanisms, mediating the onset of several metabolic syndrome clinical features[170, 171]. On the other hand, due to increased activity of visceral adipocytes β_3-adrenergic receptors and lipogenic enzyme concentrations[172], catecholamine-induced lipolysis is highest in these adipocytes[173], increasing the rate of VAT-derived nonesterified FFA drained directly into the liver through portal vein. According to the "portal" hypothesis, visceral obesity leads to accelerated mobilization of fatty acids[174-176], promoting a large number of undesirable effects on the liver and in the hormonal regulation of lipolysis. In these hepatic disturbances are included hyperinsulinemia, glucose intolerance and dyslipidemia, gluconeogenesis, hepatic lipogenesis, and impaired insulin clearance[177]. Additionally, these mechanism disturbances may also impair α_2-adrenorecpetors function in visceral adipocytes, increasing simultaneously the β_3-adrenoreceptors activity[163].

In this sense, this group of evidence has been suggesting that the "portal" hypothesis should be considered as a strong candidate for the explanation of hepatic IR in presence of visceral adiposity. Additionally, recent observations derived from fat-fed dog model have added further insights related with the still missing molecular support of these hypothesis[174]. In fact, it was demonstrated that increased gene expression promoted by both lipid fat storage and lipolysis in visceral adipocytes was accompanied by an elevated rate-limiting gluconeogenic enzyme expression in the liver[174].

Prospective studies have been demonstrating that abdominal adiposity, mainly VAT, is the major risk factor for the development of type 2 DM[177-179]. Obese individuals with higher VAT present lower HDL-C concentrations (which are responsible for the total cholesterol/HDL-C ratio elevation commonly observed), increased fraction of atherogenic, small and dense LDL-C, increased apolipoprotein B100 concentrations, and hypertriglyceridemia[136, 180]. Another recent prospective study developed with middle aged men showed that in visceral obese individuals, the presence of the atherogenic triad (increased hyperinsulinemia, increased fraction of small and dense LDL, and apo B concentrations), was associated with a 20-fold increase in CHD risk[136]. The dyslipidemic metabolic profile, the postprandial hyperlipidemia, and liver fat storage are not the unique characteristics of a visceral obese phenotype[181]. In these individuals, endothelial dysfunctions and atherothrombotic abnormalities, mediated by increased concentrations of PAI-1 and fibrinogen are often present. These atherothrombotic abnormalities combined with the simultaneously present inflammatory disturbances[134], such as increased CRP and adipocyte-derived TNF-α and IL-6 plasma concentrations, reinforce the VAT contribution to a heightened type 2 DM and CVD risk[175, 182].

It was already observed in obese women that trunk fat mass, assessed by DXA, was robustly associated with IR[183]. Another study developed in our laboratory with 62 healthy

Caucasian men have also observed that subcutaneous central fat was associated with several CVD risk factors, independently of total body fat mass and CRF[184]. However, DXA does not discriminate VAT from Ab SAT. To overcome this methodological handicap, the debate was recently re-centered on the separate contributions of VAT and Ab SAT to IR aetiology and health risk. While some researchers demonstrated that, independently of VAT, Ab SAT was highly correlated with IR[91, 161, 185] and several CVD risk factors[184], others have reported close associations between VAT and IR[84, 186, 187], independently of Ab SAT. However, a recent cross-sectional study with men and women have reported similar associations between IR and both VAT and Ab SAT[185]. Another study using ultrasound as a tool for measuring Ab SAT, VAT and hepatic steatosis have reported positive associations between both two abdominal AT markers and the degree of liver fat infiltration[188].

In order to clarify this debate, it has been suggested that these discrepancies could be solved by subdividing abdominal subcutaneous AT according to its histological and metabolic differences[158] (**Figure 1.3**). If anatomical fascial plane within Ab SAT is used as reference[189], it is possible to subdivide Ab SAT into superficial compartment, characterized by small tightly packed lobules, and deep compartment, with larger and more irregularly distributed lobules[190].

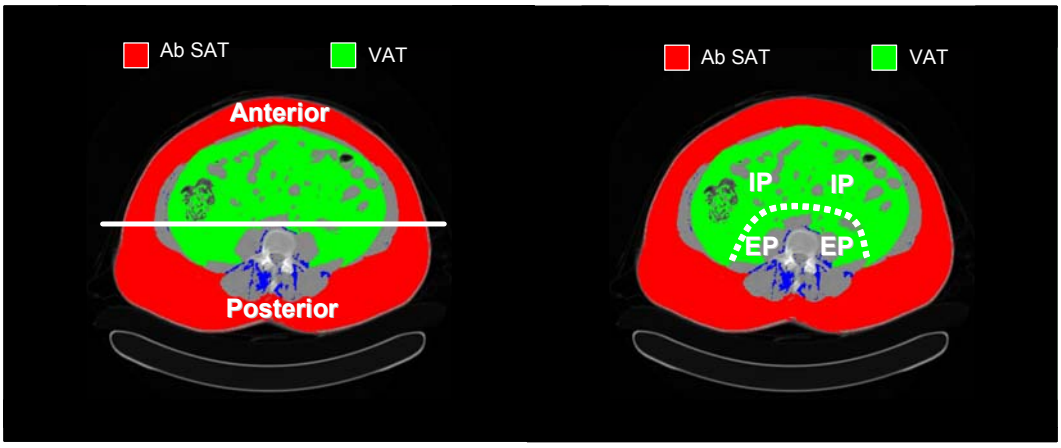

Figure 1.3. Subdivisions of Ab SAT and VAT according to different anatomical criteria. IP, intraperitoneal; EP, extraperitoneal.

Besides the histological differences, animal studies have been proposing that deep Ab SAT present a higher the metabolic activity when compared with superficial Ab SAT adipocytes[191]. Interestingly, recent in vivo evidence suggests that the lipolytic activity of superficial anterior Ab SAT is higher than deep posterior Ab SAT[192]. Although preliminary evidence with human volunteers seems to refute the animal model hypothesis, some studies have reported that deep Ab SAT was highly associated with IR, both in men and women[158, 193].

Other types of Ab SAT subdivisions have been proposed based upon different anatomical criteria. Previous studies with obese women have observed that posterior subcutaneous fat, analogous to deep SAT in men, was independently associated with IR[194]. Similar subdivision can be done in VAT using peritoneum boundary as reference criteria, identifying intraperitoneal and extraperitoneal depots (**Figure 1.3**). Both depots have been directly related with glucose intolerance[187]. Considering this large group of evidence, it seems evident the necessity of further research to clarify the independent associations of each abdominal AT compartment to metabolic and health risk.

1.7. Thigh adiposity and health risk

Abdominal adiposity is an important body composition feature related with IR and CVD in obese and type 2 DM. However, until very recently, little was known about the thigh AT association with health risk. In fact, several studies did not found associations between thigh fat and metabolic disturbances. Moreover, studies using DXA to measure thigh fat mass[183] or lower-body adiposity[195] did not observe associations with IR in overweight and obese women. Furthermore, other studies using CT to measure cross-sectional thigh AT did not found associations with glucose tolerance[196] and with plasma lipoprotein concentrations[197].

More recently, thigh AT located beneath the fascia lata and between muscular fibbers was, for the first time, identified as a body composition marker of IR in both obese and type 2 DM patients[198]. On the contrary, thigh subcutaneous AT, considerably larger than subfascial AT (comprising more than three-thirds of thigh AT area), was not a significant predictor of insulin metabolism disturbances[198]. Similarly, in a study developed with 23 men and women with type 2 DM it was observed that thigh subfascial adiposity was significantly correlated to skeletal muscle IR[199].

A posterior study developed by the same scientific group in a large sample of elderly men and women with hyperinsulinemia, type 2 DM, and IGT have also reported that increased intermuscular AT was significantly related with higher fasting insulin concentrations in both genders, even in normal weight individuals[200]. However, these associations did not remain significant in obese subjects. Furthermore, it was already reported that both subfascial and subcutaneous AT areas were inversely associated with muscle tissue attenuation, reflecting a higher lipid infiltration within the muscle fibber, which was confirmed by muscular biopsy[201].

In this context, recent evidence is suggesting that with obesity there is an increase of thigh AT compartments areas, being the AT interspersed around the thigh muscle fibbers a significant correlate of IR, and thus, a predictor of higher atherogenic risk. Furthermore, despite the observations showing that women with a lower ratio of visceral to mid-thigh AT areas generally present a more favourable metabolic risk profile, including higher HDL2-C values, and lower insulin, apo B, and TG concentrations[202] very little in known about the independent relevance of each thigh AT compartment to metabolic syndrome inflammatory ant atherothrombotic features. In this sense, we will examine, in the present work, some of these relationships in overweight and obese women.

1.8. Skeletal muscle tissue and health risk

Muscle tissue is another important thigh body composition compartment that seems also to predict health risk. As compared to normal weight individuals, evidence has been demonstrating that obesity and type 2 DM change muscle tissue biochemistry and composition[203]. In these patients, skeletal muscle, assessed by CT, presents lower attenuation values, representing a lower tissue density elapsed from increased intramyocyte lipid content. This reduced muscle tissue attenuation is a significant correlate of IR, even after adjustment for total body or visceral adiposity[185]. Several studies using different assessment approaches have been reporting that, in obesity and type 2 DM context, intramuscular fat storage is associated with IR[203-207].

In healthy subjects, muscle tissue is able to switch from predominant lipid oxidation verified in fasting conditions, which is accompanied by an augmented FFA uptake from circulation, to increased glucose uptake and oxidation in insulin-stimulated conditions, accompanied by a reduction in lipid oxidation[208]. This ability, recently defined as "metabolic flexibility"[209], seems to be a key feature in the regulation of muscle energy metabolism. However, this capacity seems to disappear with both obesity and type 2 DM[210]. Indeed, it has been advanced that the excess of TG infiltration within the muscle fibber is responsible for the metabolic inflexibility found in these patients. With ectopic muscle fat storage, glucose oxidation is compromised in response to insulin. Furthermore, lipid oxidation rates seem also to not increase during the transition to fasting conditions as it would be expected[210]. Based on these findings, it has been proposed that reductions in lipid oxidation metabolism may mediate myocyte lipid accumulation.

However, it seems that weight loss can change lipid muscle tissue content. Recent studies which have addressed the impact of weight loss in muscle tissue metabolism have reported that, concomitantly with weight and abdominal AT depots reduction, weight loss in

obese men and women can reduce muscle lipid content and improve insulin sensitivity[201, 211, 212]. Further studies have suggested that after weight loss, while muscle tissue fatty acid metabolism impairments persist in fasting conditions, there is an improvement in insulin suppression of lipolysis and lipid oxidation[213, 214]. Therefore, weight management interventions, specially through physical activity, should be able to modify skeletal muscle in obese and type 2 DM patients in order to increase lipid oxidation capacity and improve metabolic flexibility.

On the other hand, recent evidence has been demonstrating that thigh muscle tissue is also related with health risk through cytokines metabolism. Although most information about cytokines comes from studies examining tissue injury and acute infections, a growing group of evidence is emphasizing the relative contribution of muscle tissue during exercise to IL-6 production. Contrarily to the proinflammatory effects exerted by AT-derived IL-6, linking acute phase proteins to visceral obesity and, thus, to IR and atherosclerosis[215], it was recently demonstrated that skeletal muscle-derived IL-6, produced during exercise, can be primarily considered has an anti-inflammatory cytokine[216]. Furthermore, it was already observed that this muscular-derived IL-6 present several other biological effects, including inhibition of TNF-α production, stimulation of both AT lipolysis[217] and endogenous glucose synthesis[218], and muscle insulin sensitivity improvements during exercise[218-220].

In this sense, concomitantly with its crucial role in FFA oxidation and energy metabolism flexibility regulation, very recent observations have been demonstrating that thigh muscle tissue can be also responsible for releasing IL-6, an anti-inflammatory cytokine associated with favorable effects in both adipocytes and hepatocytes. This cytokine seems to present a relevant role in glucose homeostasis during exercise and in the downregulation of some proinflammatory cytokines. The combination of these two major effects, impaired in

obesity and type 2 DM patients, may confer additional protection against IR and atherosclerosis[218].

1.9. Ectopic fat storage and adipokines metabolism

Already defined as lipid accumulation outside the "classical" AT depots[221], ectopic fat storage pathogenicity has been increasingly study in order to understand the underlying mechanisms linking metabolic risk to lipid accumulation in different organs and lean tissues, such as skeletal muscle, heart, kidneys, liver and pancreas. It is known that small intracellular lipid reserves in nonadipocytes are very important to ensure basic functions like cellular membrane integrity maintenance and intracellular signaling[222]. However, when these tightly balance is braked by lipid overload deposition, lipotoxicity and lipoapoptosis are likely to appear, assuming a potential aggressive role that can lead to lean body mass destruction[223].

Several studies have been linking ectopic fat storage with IR and type 2 DM[33, 224-227]. Based on this evidence, they proposed new pathophysiological pathways responsible for the ectopic fat storage. These mechanisms reside not only in an adipocyte proliferation failure[228, 229], reducing the "buffer" protection capacity of lean body tissues against excessive exposure to circulating FFA, but may also reside in oxidation capacity (mitochondrial oxidative and phosphorylative activity) impairments in these tissues or organs[230, 231]. The recognition of these ectopic fat storage mechanisms together with the compelling evidence demonstrating that AT is an important endocrine organ suggests a new health risk approach in obesity context. In fact, this emerging paradigm combined with observations showing that total body fat mass and subcutaneous AT are also important to IR[232-235], independently of VAT, and the inexistence of clear evidence highlighting VAT as the primary cause of IR[236], may reallocate the Randle "portal" hypothesis to a secondary plane.

For a long time, AT was classified as a storage tissue, serving the purpose of extending survival during recurrent cycles of famine[237], allowing a surplus caloric storage as TG in white adipocytes, and preventing future periods of less caloric intake. Similarly, when normal, healthy individuals consume excessive calories, this surplus fuel is also stored as TG. As tissues of lean body mass lack a storage substrate capacity compatible to caloric needs, AT assume a primordial role in the body fat-storage ability.

However, in addition to this energy-storing capacity, adipose tissue seem to play an important role in cytokines production, a group of hormone-like substances, growth factors and other bioactive products, which can promote physiologic metabolism alterations not only in AT itself (autocrine stimulation) but also in several other distant lean body tissues and organs[224]. Therefore, the initial notion of AT as a storage tissue was cracked as new evidence were demonstrating its capacity in producing an ever increasing number of adipokines. Moreover, recent studies have been highlighting the tremendous AT metabolic activity, working in a complex network of endocrine, paracrine and autocrine signals that influence the response of several tissues, including liver, pancreas, heart, and skeletal muscle[238].

It has been demonstrated that these adipokines may represent a wide range of metabolic roles, affecting insulin sensitivity, lipid and carbohydrate metabolism, as well as immunologic and inflammatory metabolic responses[239]. Due to the fact that peripheral IR seems to be the major link between metabolic syndrome disturbances and body composition changes, the adipokines study has become a novel and challenging scientific domain. Some adipokines seem to protect against lipotoxic lean tissues damage[240]. Specific cell dysfunctions induced by lipid storage can result from a conjunction of several factors including lipid overloading mediated by excessive FFA delivery to those tissues, higher FFA synthesis promoted by *de novo* lipogenesis occurring in both adipocytes and nonadipocytes (due to glucose overload), and lower oxidation capacity of circulating FFA[241, 242]. In

addition, other studies have also pointed out the relevance of some adipokines in the prevention of lipid-induced programmed cell death, also known as lipoapoptosis[243]. Indeed, it has been reported that adipokines such as leptin[244, 245] and adiponectin[246] can protect against ectopic fat storage, and thus, avoid the harmful effects of lipotoxic cell damage (**Figure 1.4**).

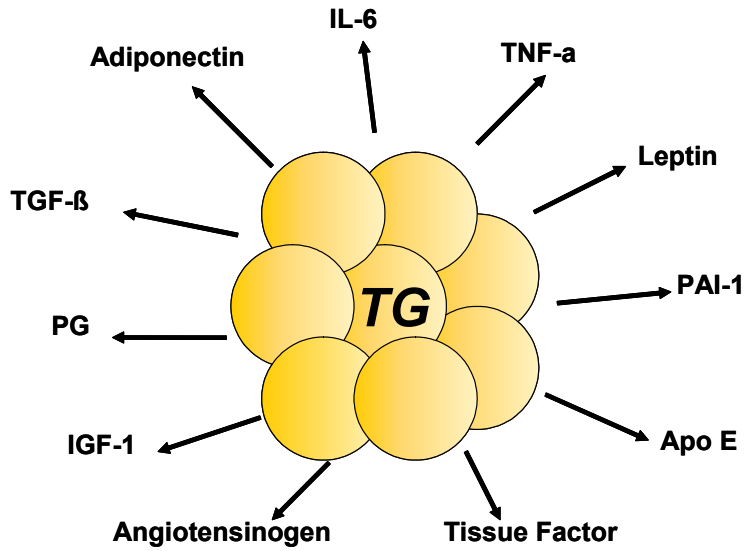

Figure 1.4. Adipocyte as an endocrine organ - dynamic view. Apo E, apolipoprotein E**;** IGF-1, insulin growth factor-1; TGF, transforming growth factor-β; PG, prostaglandin; TNF-a, tumor necrosis factor-alpha.

1.9.1. Leptin

The underling mechanisms of the anti-steatosis effect carried out by leptin is still disputed. To date, it is well established that leptin produced in adipocytes can control the feeding behavior through the transmission of satiety signals via hypothalamic centers, particularly, by sympathetic outflow[221]. Furthermore, it is known that early in obesity, previous expanded adipocytes increase their leptin secretion, attempting to enhance FFA oxidation in lean tissues by up-regulating peroxisome proliferator-activated receptor-gamma (PPAR-γ) coactivator 1-α, responsible for mitochondrial biosynthesis, as well as to reduce the lipogenic cascade enzymes activity[221] (primarily mediated by activation of AMP-activated protein kinase - AMPK).

However, deficient production or unresponsiveness to leptin blunts these protective attempts, resulting in ectopic fat storage and consequent lipoapoptosis. Lipid-induced cell damage may involve more than one pathway[247], and lipoapoptosis differences between pancreatic islets and myocardiocytes have already been identified (besides ceramide involvement, nitric oxide pathway has not been implicated[248] in heart cells as verified in pancreas[249]). Hence, there are alternative pathways which may vary according to tissues specificity and conditions. Pancreatic ß-cells and myocardiocytes seem to be the most sensible cells to ectopic fat accumulation[221]. It has been observed that heart ectopic fat storage may promote lipotoxic cardiomyopathy[250]. In addition, the increased liver and pancreas lipid content can lead to glucose intolerance and type 2 DM[251]. In a recent study with congenital lipodystrophic mice, leptin reversed IR and type 2 DM[252], suggesting that leptin could have relevant effects on glucose metabolism and insulin sensitivity.

Hypoleptinemia is probably common in visceral obesity but it has not been properly recognized yet. Despite abdominal obese subjects produce more leptin than normal weight individuals, maybe the amount produced is not enough to provide the necessary anti-steatosis effect. Moreover, while VAT undersecretes leptin, subcutaneous adipocytes produce higher amounts of leptin, providing better a higher anti-steatosis protection[253]. Unresponsiveness to leptin is probably the most common cause of liporegulatory failure and metabolic syndrome.

In addition, it has been suggested that hyperleptinemia and increased ectopic fat storage that normally occur with aging are responsible for the evidence reporting that almost everyone become leptin resistant, after a certain age[254, 255]. Therefore, despite the existence of several questions waiting to be answered, it can be ultimately assumed that metabolic syndrome disturbances may reflect a failure of a system designed to promote intracellular

lipohomeostasis and to prevent lipotoxicity in lean body mass or organs in overnourished individuals.

1.9.2. Adiponectin

Adiponectin, a collagen-like circulating protein, is the most abundant adipokine, accounting for 0.01% of total plasma[256], and seems also to play an important role in lipid regulation. Clinically healthy women have higher adiponectin concentrations compared to those verified in men[256]. Similarly to leptin, evidence has shown that adiponectin can activate AMPK-activated kinase, with anti-lipotoxic action[257], exerting also a protection against several metabolic syndrome features[258-260].

Adiponectin has several biofunctions, including anti-atherosclerotic, anti-diabetic and anti-inflammatory effects. Indeed, it was recently suggested that adiponectin may represent an important protective role in CHD risk[261], revealing also an anti-atherosclerotic protection which seems to be mediated by its stronger capacity to inhibit the expression of the adhesion molecules (intracellular adhesion molecule-1, vascular cellular adhesion molecule-1 and E-selectin) to endothelial walls during atherogenic cellular phenomena[262, 263]. Adiponectin also seems to suppress the monocytes attachment to endothelial walls, an early event in atherosclerotic vascular process[263, 264]. Furthermore, adiponectin can prevent the consequent two process phases, mainly by inhibiting the expression of macrophages scavenger receptor, therefore decreasing oxidized LDL uptake and foam cell formation[265], and inhibiting the proliferation and migration of smooth muscle cell[266].

Besides these evidence obtained from vascular cellular function studies, other studies have additionally confirmed the protective role of adiponectin in CVD[267]. Indeed, when carotid arteries endothelium was injured by a balloon catheter in rats, it was observed an adiponectin accumulation in the vascular walls[267].

Another two studies developed in subjects with essential hypertension revealed inverse associations between adiponectin concentrations and mean systolic, and diastolic blood pressure[263, 268]. These observations unanimously suggest that adiponectin may have a significant role in protection against vascular damage, hypertension, atherosclerosis and CHD.

It has also been proposed an adiponectin mediation role in obesity-related IR, once it was found to be inversely associated with the degree of adiposity and positively associated with insulin sensitivity in Pima Indians[259] (a specific cohort characterized by a high prevalence of obesity accompanied with diabetes). Similar results were also obtained in other populations[269]. Furthermore, adiponectin administration to both lipoatrophic and obese rodents can increase insulin-induced tyrosine phosphorylation of the insulin receptor and thus, increase whole-body insulin sensitivity evaluated by glucose disposal rate[260]. In addiction, adiponectin seems also to enhance insulin sensitivity through AMPK activation, facilitating GLUT-4 translocation and glucose transport. Hyperadiponectinemia has been associated with a lower type 2 DM risk, probably due to its lipid oxidation and insulin-sensitizing properties, both in muscle and liver[260, 269]. In this sense, muscle insulin sensitization is one of the mechanisms by which adiponectin improves glycemia control. However, in a recent study with mice, the administration of recombinant adiponectin during a euglycemic clamp enhanced suppression of hepatic gluconeogenesis, while muscle glucose uptake remained unaltered[270].

Plasma adiponectin concentrations are lower in individuals with IGT or type 2 DM patients compared with healthy individuals[89]. Additionally, it is known that diabetic subjects with macroangiopathy have lower adiponectin concentrations than those without that complication[271]. On the other hand, in healthy humans, independently of the obesity degree, hyperadiponectinemia predicts a lower incidence rate of type 2 DM[272], reinforcing the notion

that adiponectin has a relevant role in insulin metabolism. However, despite some observations have been demonstrating that hypoadiponectinemia may predispose to IR and type 2 DM, the importance of visceral fat accumulation in hypoadiponectinemia aetiology is not totally clear.

Unlike other adipokines, plasma adiponectin concentrations decrease in obesity and increase after weight loss[271, 273]. Both in diabetic as well as in nondiabetic subjects, weight loss have significantly raised adiponectin[271]. This singularity is also verified in its associations with regional AT compartments. Contrarily to leptin, which is known to increase with BMI and with subcutaneous adiposity[274], adiponectin is inversely correlated with BMI, as well as with VAT. The mechanism responsible for the lower adiponectin concentrations in visceral obese subjects has not been clarified yet, but it has been suggested that TNF-α may inhibit adiponectin activity[275]. In this context, it seems that the increase of several adipokines concentrations, such as TNF-α and PAI-1, in conjunction with visceral adiposity and central obesity-related hypoadiponectinemia may constitute the major background conditions to vascular changes, atherosclerotic progression and IR. However, it is also important to considerer that sexual hormones, including progesterone, estrogen and androgen may alter plasma adiponectin concentrations and contribute to gender differences in the described protective mechanisms. Further investigation is also required in this area.

The endocrine AT functions recognition was also supported by the increasing number of observations highlighting the contribution of several adipokines to IR development, to low chronic inflammatory state onset, and to atherosclerotic and thrombotic disturbances appearance. Contrarily to leptin and adiponectin which have been both implicated in several protective mechanisms, PAI-1, TNF-α, IL-6 and acute phase proteins such as CRP seem to be relevant for IR aetiology. Moreover, evidence has been suggesting that these cytokines

are implicated in a large number of metabolic cascades that can, ultimately, result in a cluster of metabolic syndrome abnormalities.

1.9.3. PAI-1

Recent studies focusing gene expression in both visceral and subcutaneous adipocytes demonstrated that, when all AT genes already known were taken into account, the frequency of genes responsible for encoding secretory proteins was higher in VAT[276]. This observation may explain the relative higher contribution of visceral adipocytes to adipokines production, although some of them do not fit in the cytokine classical definition. The genes for PAI-1, a single chain glycoprotein belonging to the serine protease inhibitors, as well as genes for heparin binding endothelial growth factor-like growth factor were found to be overexpressed in VAT[277, 278]. Indeed, VAT seems to be a relevant source of PAI-1[279].

On the other hand, it is known that fibrin deposition in endothelial artery cells may contribute to atherosclerotic plaque growth by cell proliferation stimulation and facilitation of LDL acumulation[280]. Hypofibrinolysis can promote atherothrombosis by elevating fibrin deposition. However, plasmin, a protease which catalyzes fibrin destruction in presence of some activators, such as t-PA and urokinase may attenuate this process[281, 282]. PA1-1 has a strong tendency to establish a covalent binding with t-PA, resulting in a rapid t-PA activity inhibition and, therefore, fibrinolysis impairment. Thus, increased PAI-1 concentrations can lead to fibrinolysis impairment and to thrombosis progression[283]. Consequently, PAI-1 is considered an important risk factor for CHD[284] and myocardial infarction[285], representing also a relevant role in the development of thrombotic and atherosclerotic disorders that are commonly associated with visceral adiposity.

However, the relative contribution of elevated PAI-1 plasma concentrations, often observed in visceral obese subjects, to atherothrombosis and metabolic syndrome

development can not be analyzed separately from other proinflammatory adipokines. In light of recent studies, rather than directly, VAT appears to be indirectly involved in PAI-1 concentrations elevation through metabolic disorders usually associated with central obesity. Indeed, despite the recent observation that TNF-α could be the link between IR and elevated PAI-1 in obese mice[286], these observation is not unequivocal and conflicting results have been presented, both in human, and in cultured adipocytes.

Regardless the intense debate, it was already observed in a very recent study which investigated the regulatory effects of both TNF-α and IL-6 on PAI-1 gene induction in human AT, that TNF-α, rather than IL-6, promoted an elevation in PAI-1 mRNA in the subcutaneous adipose tissue[287]. In addition, TNF-α, overproduced by AT in obese[288] and IR[289] patients, decreases tyrosine kinase activity of the insulin receptor, reducing insulin sensitivity, which seem to be one of the reasons responsible for its possible intervention in obesity and diabetes[290, 291]. On the other hand, it is known that adiponectin, also important in IR aetiology, interferes with TNF-α signalling in endothelial cells, inhibiting monocytes adherence to vascular walls[263]. In obesity context, this group of evidence suggests that PAI-1, accompanied by TNF-α and hypoadiponectinemia may be involved in the pathogenesis of obesity-related metabolic disorders, contributing to IR, hypertension and atherothrombosis.

PAI-1 concentrations are also elevated in type 2 DM, which seem to contribute for a reduction the fibrinolytic activity and an increase in the risk of endothelial complications often observed in these patients[292]. Furthermore, several studies have been reporting positive associations between PAI-1 and several obesity-related comorbidities. Higher PAI-1 concentrations have been associated with IR[82] and hyperinsulinemia, both in obese[293] and type 2 DM patients[292]. However, it is still unclear if insulin acts directly or via IR to enhance circulating PAI-1 concentrations. Independent associations between dyslipidemia, mainly VLDL, and PAI-1 concentrations have also been observed[294, 295]. However, in another study

addressing PAI-1 activity in three hypertriglyceridemic subjects with different degrees of IR, the relation between hypertriglyceridemia and PAI-1 was not always present[296]. Several metabolic disorders like increased TG, FFA, insulin or glucose concentrations, rather than one feature only may be essential for PAI-1 elevation. In line with this hypothesis, it has been suggested that only the combination of hyperglycemia and hypertriglyceridemia was able to enhance plasma PAI-1 concentrations, rather than TG or insulin alone[297].

Interestingly, it was also observed an association between PAI-1 and gamma glutamyl transferase (GGT)[298] in insulin resistant obese patients with fatty liver, suggesting that hepatic steatosis could also be related with increased PAI-1[299]. Another study has also identified an association between fatty liver assessed by ultrasonography and PAI-1, which was mediated by concomitant changes in insulin and TG plasma concentrations[300]. In addition to the major metabolic syndrome components contribution to raise PAI-1 concentrations observed in obese or in type 2 DM patients, it seems that non-alcoholic fatty liver disease (NAFLD) could be faced as another important body composition metabolic syndrome feature related with PAI-1 overexpression. Elevated concentrations of GGT were found to be associated with hypertension[301]. Besides the verified association between GGT and PAI-1, increased PAI-1 concentrations have also been found in hypertensive patients[302]. However, it was suggested that IR rather than hypertension was the responsible for these higher values. In addition, higher PAI-1 concentrations could also be found in hypertensive patients without IR features[303], suggesting that this association can be found independently of IR.

To gain further insights regarding the relationship between PAI-1 and hypertension, two recent studies demonstrated that both preadipocytes and adipocytes could express angiotensinogen and other renin-angiotensin system enzymes, including the expression of angiotensin II[304, 305]. It was then hypothesized that AT, as an important source of angiotensin

II, could be the link between elevated PAI-1 concentrations and hypertension. However, *in vivo*, the potential interaction between renin-angiotensin system and fibrinolysis has not been elucidated yet.

Despite evidence has been highlighting the metabolic disturbances contribution to PAI-1 elevation, recent weight management intervention studies have reported a significant reduction in PAI-1 concentrations after weight loss, mainly induced by caloric restriction, in obese patients[306, 307]. Moreover, this reduction was uniquely related with the weight loss degree, rather than plasma insulin and TG variations[306, 307]. Briefly, these results suggest a direct contribution of AT to the increased PAI-1 concentrations observed in obese subjects.

However, the question about the relative relevance of each AT compartments to these associations it's far to be fully understood. It was hypothesized that VAT may link obesity, IR and PAI-1. On the other hand, weight loss, both in obese men and women, can induce PAI-1 reductions, independently of VAT, suggesting that the link between PAI-1 and VAT could be much more complex[308] and related with the expression of several other adipokines, like TNF-α. The conflicting results obtained regarding the TNF-α role in the regulation of PAI-1 expression in AT and the absence of a clear demonstration that AT is able to secret larger amounts of PAI-1 *in vivo* leave open an interesting field for future research. The recognition that VAT is associated with hypertension, hypertriglyceridemia, hyperinsulinemia, IR, and fatty liver, combined with the evidence highlighting the VAT contribution to the increase of PAI-1 concentrations may explain, at least in part, the described associations between this adipokine and some of the obesity-related comorbidities.

1.9.4. TNF-α

TNF-α seems to mediate IR via serine phosphorylation of insulin receptor substrate-1 (IRS-1) in skeletal muscle, inhibiting the ability of insulin to stimulate GLUT-4 translocation

to the cell membrane, which may reduce the glucose uptake in muscle fibbers[309]. Additionally to this impairment in insulin sensitivity, TNF-α seems not only to inhibit the lipoprotein lipase (LPL) activity, but may also induce adipocyte lipolysis[310]. The consequent FFA plasma elevation may contribute to IR progression[311]. These observations have supported the notion that TNF-α is the main adipokine responsible for IR in both obesity and metabolic syndrome context.

Although the mechanisms of TNF-α mediating IR are still unclear, TNF-α is becoming an attractive target for pharmacological intervention focused in protecting patients against obesity-induced IR[312]. It should be interesting to understand if pharmacological TNF-α suppression could improve insulin sensitivity and influence AT metabolism as it seems to be achieved by weight management programs. In fact, in a 6-month weight management intervention designed to improve dietary habits and increase physical activity (3 times/week, 30 min, 60-80% VO_{2peak}), concomitantly with a significant weight loss, it was observed a reduction in serum TNF-α concentrations in IGT subjects, as well as in diabetic patients [313]. However, because there was weight loss, it was difficult to determine the independent contribution of exercise to the observed TNF-α reduction.

On the contrary, another recent study combining aerobic exercise (60% VO_{2peak} for 60 min) and resistance training (1 set/week, 1 set=8-12 repetitions) for 10 weeks, reported a tendency to the increase of TNF-α concentrations, which was significantly related to a decrease in VAT[314]. Another study developed with diabetic patients and weight-matched controls revealed that both short and acute exercise bouts (60% VO_{2peak} for 25 min) did not have any effects on TNF-α concentrations in both groups[310]. Similar results were found after prolonged endurance exercise (6 h) in trained men[315]. Because TNF-α is an inflammatory cytokine produced by a variety of cells, the exercise effects on TNF-α are difficult to distinguish. Based in the available data, it is difficult to infer an unequivocal trend.

Therefore, research should be also developed in this area to shed light upon the independent exercise associations with this adipokine.

1.9.5. IL-6

Evidence has been demonstrating a close relationship between TNF-α and IL-6. TNF-α, which present a higher mRNA expression (1.7-fold) in subcutaneous rather than in omental adipocytes[316], is an important stimulus to AT-derived IL-6 production, increasing up to 60-fold its concentrations[317]. Moreover, in conjunction with sympathetic nervous system, TNF-α, IL-6, and leptin maintain an imbricate and relevant cross-talk in IR aetiology (**Figure 1.5**).

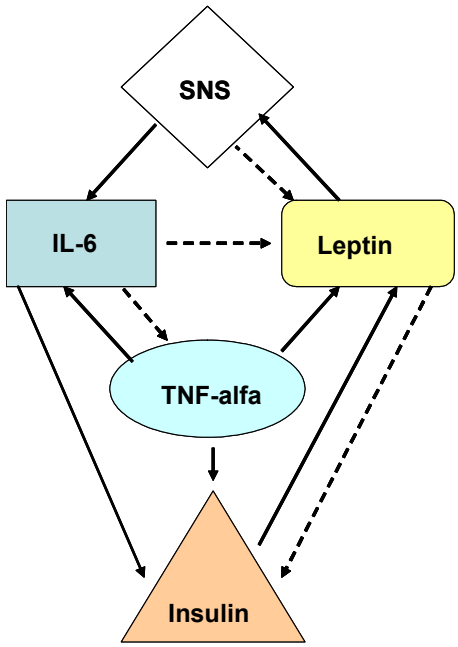

Figure 1.5. Cross-talks between the main adipokines and interaction with sympathetic nervous system (SNS) and insulin. Solid lines indicate stimulation; dashed lines indicate inhibition. Adapted from Fruhbeck et al.[238].

Similarly to TNF-α, IL-6, is also overexpressed in the AT of obese[318] and type 2 DM[319] patients, share a group of functional and structural similarities, including receptor homology (via Janus kinases or signal transducers and activators of transcription – STAT)[320]. This multifunctional cytokine can be produced by many different types of cells

besides AT, such as immune system cells, endothelial and stromal-vascular cells, fibroblasts, myocytes, and some endocrine cells[321].

About one third of the total circulating IL-6 is produced in AT[322], being its secretion by omental adipocytes threefold superior to the registered in the subcutaneous adipocytes. Together with TNF-α and IL-1, IL-6 present relevant contributions in sepsis syndrome, being the amount and speed of their release related with the occurrence of septic shock and, eventually, with mortality[323].

Several other IL-6 biological effects are already known, including stimulation of CRP hepatic production, stimulation of HPA axis, and thermogenesis regulation[215]. Additionally, it has been reported that IL-6 can not only directly stimulate adrenal cortisol release, but can also stimulate the production of hypothalamic corticotrophin-releasing hormone (CRH) and pituitary adrenocorticotropic hormone (ACTH)[324]. It also known that catecholamines can regulate IL-6, leptin, and TNF-α adipocyte expression[322]. In this sense, the evidence reviewed suggest that IL-6 may link inflammation, obesity, stress, and CHD[215].

Besides the proinflammatory effects, adipose-derived IL-6 can also influence muscular energy metabolism. In order to clarify some of these biological roles, several studies have been developed, mainly with animal models. Moreover, a recent study has demonstrated that IL-6 pre-treatment can promote an IRS-1 activity inhibition, impairing GLUT-4 membrane translocation, which reduce insulin-stimulated glucose uptake in skeletal muscle[325]. In addition, the IL-6 pre-treatment has also blunted the insulin ability to suppress hepatic glucose production[325]. The combination of these findings with the observations demonstrating that IL-6 is inversely associated with insulin sensitivity[289], indicate that IL-6 may be also important in IR pathophysiology.

However, recent studies in humans have reported inconclusive results. One these studies observed that acute IL-6 administration did not impair whole-body glucose disposal,

nor skeletal muscle glucose uptake[326], while another identified a stimulatory effect on FFA oxidation and lipolysis[327]. Other studies demonstrated that plasma IL-6 elevation can downregulate adipocyte LPL activity[328, 329]. These observations are particularly important in obesity context. Indeed, the IL-6 overexpression in omental adipocytes combined with an increased lipolysis rate can promote a FFA influx to the liver, increasing hepatic TG secretion[330], contributing to the hypertriglyceridemia observed in visceral obese patients.

Despite only recently addressed, exercise seems to have an influence on AT-derived IL-6 production. In a study with athletes, it was observed, after 3 hours of cycling aerobic exercise (60% of VO_{2max}), an elevation of adipocyte IL-6 mRNA expression[331]. However, this adipocyte IL-6 gene expression in response to exercise was blunted by carbohydrate ingestion, suggesting that glucose disposal can affect IL-6 production in AT.

In light of the available studies showing conflicting results, it seems that IL-6 effects in energy metabolism should be further investigated. Moreover, research should be developed to understand the underlying relationships between this adipokine and both sympathetic nervous system and neuroendocrine pathways, as well as its biological effects in skeletal muscle energy and hepatic insulin metabolism.

New evidence demonstrating that skeletal muscle fibbers may also produce large amounts of IL-6 as response to exercise (up to 100-fold)[332], instead of other muscle tissue or liver cells, as previously postulated, have raised the analysis complexity. Only recently was demonstrated a serum myocyte-derived IL-6 elevation during the execution of one leg exercise contractions[333]. Posterior immunohistochemical studies have clear and unequivocally demonstrated a myocyte IL-6 production following exercise[334]. In fact, it is very well demonstrated that IL-6 plasma concentrations rise up to more 100-fold during concentric exercise[335]. Similar observations were made following a marathon race, being this increase associated with muscle damage[336]. Additionally, eccentric exercise can produce the

same amount of IL-6, which seem also to be associated with muscle damage[337]. However, recent studies reported that IL-6 also rise with one straight concentric exercise without muscle damage, being this muscular production a direct function of the exercise stimulus intensity and duration[218, 337]. Further investigations developed by the same scientific group have concluded that working muscles produce IL-6 during exercise as a consequence of contraction *per se*, glycogen depletion and an altered energy turnover[338].

Furthermore, groups of chemical stimulus capable of inducing myocyte-derived IL-6, including some inflammatory cytokines such as TNF-α were already identified. However, myocytes are able to produce IL-6 even in the absence of inflammatory cytokines[339]. Despite the necessity of additional research, it was already described an association between muscle IL-6 mRNA overexpression and a reduction of glycogen disposal in the muscle fiber[340]. During exercise, the sympathetic-adrenergic response is exacerbated when there is a lower intra-fibber glucose disposal. It was postulated that epinephrine may stimulate myocyte-derived IL-6 production via β-adrenergic receptors stimulation. However, posterior work have demonstrated that it is not the case[341]. This observation lead the investigators to shift their interest to sarcoplasmatic reticulum calcium effect on IL-6 synthesis[342]. To date, the available evidence seem to highlight the stimulatory effect exerted by the combination of sarcoplasmatic reticulum calcium with a lower muscular glucose disposal on muscle IL-6 mRNA expression[220].

The myocyte-derived IL-6 seems present different metabolic functions when compared with AT-derived IL-6[217, 220] (**Figure 1.6**). Recent studies have demonstrated that muscular IL-6 seems to exert opposite effects to those promoted by adipose and immune system-derived IL-6.

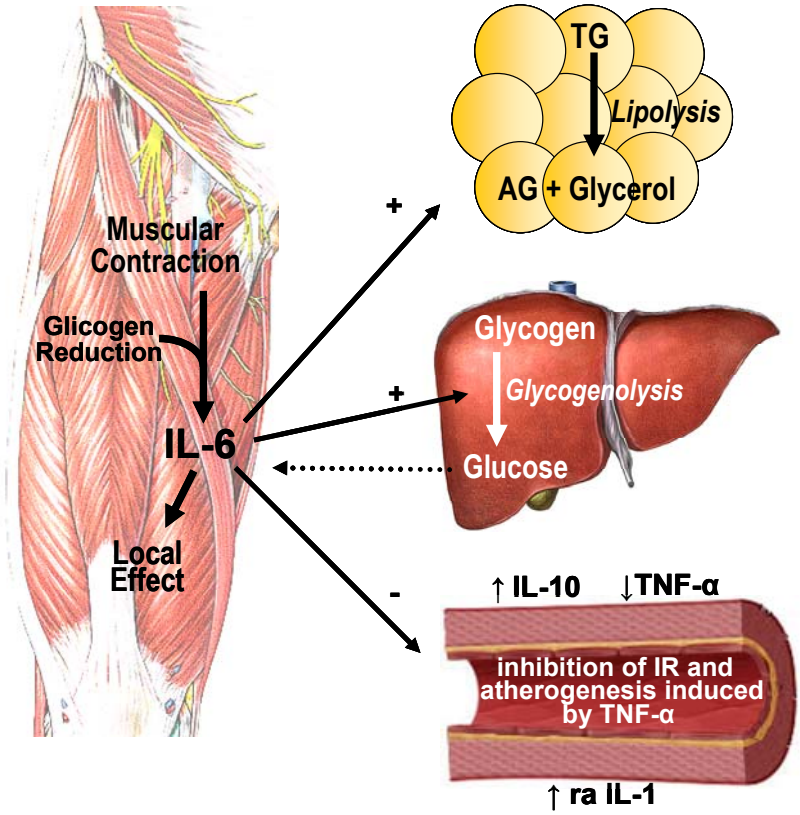

Figure 1.6. Muscle-derived IL-6 (myokine) biological effects. Adapted from Pedersen at al.[217].

Moreover, muscular IL-6 may activate anti-inflammatory mechanisms[217, 219], stimulate AT lipolysis[217]and endogenous glucose production[218], and promote a muscle insulin sensitizing effect during exercise[218]. In addition, it seems also to inhibit TNF-α production. The recognition of muscle-derived IL-6 specific and beneficial biological effects combined with the knowledge that TNF-α expression in AT is relevant for IR and increased risk of atherosclerosis and type 2 DM[343], suggest that TNF-α, rather than IL-6, may be the link to the low chronic inflammatory state associated with metabolic syndrome. Indeed, both TNF-α and IL-1 β, locally produced, are in fact the first two proinflammatory cytokines secreted in the cytokine cascade when local inflammation is established, stimulating the IL-6 production[344]. Normally, this local inflammatory response is accompanied by a systemic response known as the acute phase response, which includes the secretion of several hepatocyte-derived proteins such as CRP. However, recent observations have suggested that

muscle derived-IL-6 may also inhibit the effects and the synthesis of these proinflammatory cytokines[345]. In addition, unlike IL-1 and TNF- α, it seems that muscle IL-6 does not up-regulate major inflammatory mediators such as nitric oxide, thereby protecting against IR and atherosclerosis[345].

Hence, evidence demonstrating a new type of cytokines produced in muscle tissue, fulfilling the criteria of an exercise factor, create the basis for the study of the skeletal muscle as an endocrine organ, capable of producing "myokines"[346]. The myokines study assume another crucial challenge to the understanding of energy metabolism and the relationships established between AT compartments, liver, pancreas, and the HPA in the obesity and metabolic syndrome context.

1.10. Liver fat storage and health risk

Evidence has been associating abdominal obesity with liver fat storage, which seems to predict metabolic risk, independently of abdominal and visceral adiposity[347, 348]. Moreover, these studies have reported that liver fat is a significant predictor of fasting insulin, fasting glycemia, and TG plasma concentrations, independently of VAT or Ab SAT. In this context, these observations suggest that, like VAT or Ab SAT, liver fat should also be considered as another important independent predictor of major metabolic syndrome clinical features. Indeed, liver fat storage or hepatic steatosis is a body composition modification often observed in obese or type 2 DM patients, which may predispose to hepatic steatohepatitis, clinically defined as liver fat infiltration accompanied with lobular inflammation, hepatocyte ballooning degeneration and a low chronic inflammatory state[188, 349].

The ratio of mean liver to spleen Hounsfield units (HU), defined as liver-to-spleen ratio (LSR), has been advanced as a reliable index of liver fat[350]. When LSR<1, its is

clinically assumed the presence of fatty liver[164, 168]. According to the "portal" hypotheses, the increased influx of nonesterified VAT-derived FFA may promote liver fat storage, which has been associated with several important metabolic disturbances, including liver FFA synthesis, insufficient FFA beta-oxidation, hepatocytes proliferation failure, and increased liver TG-rich lipoproteins secretion (i.e. VLDL)[221, 349, 351].

Recent overviews have been demonstrating an association between ectopic fat storage and both IR and type 2 DM, creating the basis for the recognition of a "push and pull" pathophysiologic pathway of ectopic fat storage[224]. In the liver fat storage context, the hepatic metabolism disturbances are primarily accompanied with IR, leading to hepatic steatosis, the first stage of NAFLD. Furthermore, liver fat infiltration combined with a "second hit" induced by proinflammatory adipokines capable of inducing lobular, parenchymal, periportal or portal necroinflammation and fibrosis, may result in non-alcoholic steatohepatitis (NASH), a more severe stage of NAFLD, which can progress to irreversible end-stage liver disease[352, 353].

A wide spectrum of hepatic morphological and physiological conditions ranging from mild fat storage and inflammation to cell degeneration, fibrosis and cirrhosis, with or without the presence of Mallory hyaline bodies have been included in the NAFLD definition. In morbidly obese patients, when nonesterified FFA and IR co-exist with increased production of several acute-phase proteins and adipokines, as well as with inflammatory cytokines cell infiltration, the risk of developing inflammation, liver fibrosis, cirrhosis, and consequent progression to NASH is significantly higher[352, 353]. In addition, the imbalance between anti-inflammatory and proinflammatory cytokines (leptin, adiponectin vs. TNF-α, CRP, IL-6), may trigger the formation of reactive oxygen species and intrahepatocyte lipid peroxidation[354], increasing the liver injury degree. However, it is not yet totally established whether these metabolic mechanisms are responsible for the inflammatory state or if this

inflammatory response in the liver is evoked by other metabolic stimulus capable of inducing hepatocyte disturbances. Moreover, it is still not clear if liver fat may predict a proinflammatory profile, independently of abdominal obesity. Knowing the close relations between inflammatory disturbances and atherogenesis, it is also important to extend this knowledge to the associations of liver fat with atherothrombotic disturbances.

A recent review has observed that only in 12% of severe obesity patients liver biopsy was normal[355]. The most common abnormality observed in these patients was related with fatty changes, followed by portal inflammation. The liver lipotoxicity promoted by elevated VAT-derived FFA can induce IR and increase liver transaminases, CRP, TNF-α, IL-6, and IL-8 production[290], reinforcing the metabolic conditions which permit the steatosis progression to NASH. However, recent evidence have been challenging the "portal" hypothesis, suggesting that subcutaneous AT, considerably larger than VAT, and extraperitoneal AT depots can also release FFA into systemic circulation, which may have grater importance to IR pathogenesis due a higher contribution to nonesterified FFA plasma pool[235, 236].

In fact, the combination of these recent findings with the new concept of "ectopic fat storage" has opened a new scientific research area related to the study of the adipocyte as an important dynamic endocrine organ. Recent evidence demonstrated that obesity can promote not only fat accumulation within the liver, but also in the pancreas, skeletal muscle and heart, affecting not only the local tissue metabolism but also more complex regional metabolism cross-talks.

Despite the association between visceral adiposity and liver fat is considerably more understood, little is known about the liver fat associations with thigh adipose and muscle tissue compartments. To our knowledge, few studies have addressed this particular question. A recent study developed with eighty-three men and women with type 2 DM has observed

that fatty liver, meaning lower LSR, was negatively associated not only with VAT but also with thigh subfascial adipose tissue (TSFAT), independently of VAT and BMI[164]. The authors have proposed that, more than assuming these results has conclusive evidence indicating a causative role of TSFAT in fatty liver pathogenesis it seems that both TSFAT and fatty liver were two special body composition markers related with IR pathogenicity in type 2 DM patients. No further associations were found between any other thigh AT and muscle depots and fatty liver in this small sample of DM patients.

However, it has been suggested that femoral-gluteal AT, presenting more lipoprotein lipase activity, may play an important protective role against ectopic fat storage. Functioning as "buffer" for circulating FFA, this AT depots can be important to counteract the tendency for lean tissue lipotoxicity prevention[356]. In this sense, the absence of more conclusive results leaves a wide open field to the investigation of the relationships between thigh body composition markers and liver fat storage in overweight and obesity context.

1.11. Aim of this investigation

The term "metabolic syndrome" has been applied to risk factors clustering that often accompany obesity and increased type 2 DM and CDV risk. This cluster includes hypertension, hyperglycemia, atherogenic dyslipidemia, and a proinflammatory and thrombotic state. Metabolic syndrome pathophysiology is multifactor, being obesity and IR the two major underlying risk factors. The health risk associated with obesity is best defined by an increased WC, reflecting abdominal obesity. Despite its genetic component, IR is usually secondary to obesity.

Factors such as physical inactivity, age, hormonal dysfunctions and genetic disturbances can affect metabolic syndrome risk factors. However, it seems that the worldwide increase of metabolic syndrome and CVD prevalence is largely related with the

obesity increase, which we have been observing in the last two decades. This obesity exacerbation seems to be driven by sedentary lifestyles combined with high caloric and atherogenic diets. Therefore, metabolic syndrome and CVD pathophysiology study can not be made without the understanding of their relationships with obesity and body composition distribution.

Aims

- To examine the independent contributions of WC and HC to major metabolic syndrome components, proinflammatory and atherothrombotic disturbances. Furthermore, the separate associations of each thigh adipose and muscle tissue compartment, measured by CT, to the previously referred metabolic risk factors are also examined.
- To study the independent relationships between both abdominal and thigh AT compartments with lipid metabolism and IR markers, as well as with inflammatory and atherothrombotic risk factors.
- To investigate the separate associations of abdominal and thigh adipose and muscle tissue compartments to liver fat. Additionally, the independent associations of liver fat with metabolic syndrome inflammatory and atherothrombotic features are also studied.

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