The color of fat and its central role in the development and progression of metabolic diseases

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Role of WAT and BAT in adiposopathy

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Abstract

Excess caloric intake does not always translate in an expansion of the subcutaneous adipose tissue (SAT) and increase in fat mass. It is now recognized that adipocyte type (white, WAT, or brown, BAT), size (large vs small) and metabolism are important factors for the development of cardiometabolic diseases. When the subcutaneous adipose tissue is not able to expand in response to increased energy intake the excess substrate is stored as visceral adipose tissue or as ectopic fat in tissues as muscle, liver and pancreas. Moreover, adipocytes become dysfunctional (adiposopathy, or sick fat), adipokines secretion is increased, fat accumulated in ectopic sites like muscle and liver, alters insulin signaling and increases the demand for insulin secretion. Thus, there are some subjects that despite having normal weight have the metabolic characteristics of the obese (NWMO), while some obese expand their SAT and remain metabolically healthy (MHO).

In this paper we have reviewed the recent findings that relate the metabolism of adipose tissue and its composition to metabolic diseases. In particular, we have discussed the possible role of dysfunctional adipocytes and adipose tissue resistance to the antilipolytic effect of insulin on the development of impaired glucose metabolism. Finally we have reviewed the possible role of BAT vs. WAT in the alteration of lipid and glucose metabolism and the recent studies that tried to stimulate browning in human adipose tissue.

Introduction

Obesity is a recognized risk factor for metabolic diseases like type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD) and also for cardiovascular diseases (CVD). It has been recognized that the major risk factor for the development of metabolic diseases is the distribution of fat accumulation, rather than the total amount of fat (1, 2). The recent studies have shown that the adipose tissue is not simply a deposit of lipids for energy storage but also interacts with other organs through secretion of adipokines and free fatty acids (FFA) (3). Moreover, different types of adipocytes have been identified: white (WAT), brown (BAT) and the recently discovered beige (BeAT) (4-6). The main characteristic of the brown and beige adipocytes is the presence of mitochondria and the possibility of fat burning with the production of heat (i.e., non-shivering thermogenesis). The amount of brown and beige adipocytes is minimum compared to white, and thus their contribution to total energy expenditure is probably irrelevant in humans. On the other hand white adipose tissue, and especially its location (subcutaneous vs intra abdominal), is relevant for the determination of the associated risk of cardiometabolic diseases. In the following paragraphs we have reviewed the recent findings that relate adipose tissue metabolism and composition to metabolic diseases. In particular, we have discussed the possible role of adipose tissue dysfunction and its resistance to the antilipolytic effect of insulin on the development of impaired glucose metabolism. Finally we have reviewed the possible role of BAT vs. WAT in the alteration of lipid and glucose metabolism and the recent studies that tried to stimulate browning in human adipose tissue.

1. Different Characteristics of the Adipocyte

Three different types of adipocytes with different characteristics were identified: white (WAT), brown (BAT) and beige (BeAT) (**Figure 1).** White adipocytes are the most abundant, they are large with a big lipid droplets full of triglycerides and are located mainly in subcutaneous adipose tissue (SAT, corresponding approximately to 85% of total fat), visceral (VAT) and mediastinal (MED) adipose tissue (7). In WAT, adipocytes act both as energy storage and as endocrine organ since WAT produces and releases adipokines like leptin, adiponectin, interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), monocyte chemo attractant protein-1 (MCP-1) and fatty acid binding protein 4 (FABP4) (4, 8-10).

BAT depots are easily recognized for their dark color due to the presence of mitochondria (**Figure 1**). The size of BAT is significantly smaller than WAT, and in rodents BAT is located between the

shoulder blades (interscapular BAT). In humans the location is similar to rodents, mainly around the neck (11), but it is recognizable only when stimulated, for example by cold exposure (4, 12, 13). The main characteristic of BAT is mitochondrial abundance and the unique expression of the uncoupling protein 1 (UCP- 1) that generates heat by non-shivering thermogenesis, i.e., with generation of heat by fat oxidation but with low production of ATP (6). Moreover, BAT is highly innervated by sympathetic nervous system that triggers the activation of these adipocytes. It has been suggested that activation in BAT might contribute to systemic lipid metabolism by increasing fat oxidation and energy expenditure (EE) through heat production (14). However, the contribution of BAT to total EE in humans is mild, considering the size of this depot.

Beige adipocytes (also known as brite or inducible brown adipocytes) are found in rodents within subcutaneous and peri-renal WAT depots (4, 15-17). In humans they have been found inter-scattered within WAT (18), mainly in SAT but also in visceral and mediastinal fat (19, 20). Beige adipocytes are functionally very similar to BAT and may emerge in specific WAT depots ("beiging") in response to various stimuli including sustained cold exposure (5) and catecholamines (19, 21). Interesting BeAT were discovered within epicardial fat, where they might protect the heart against cold but also produce ATP (22). The origin of BeAT is unknown, but it probably derives from the differentiation of pre-adipocytes, as recently demonstrated in vitro using human pre-adipocytes (23). BeAT responds to chronic cold exposure and long-term treatment with agonists of peroxisome proliferator-activated receptor-γ process often referred to as the 'browning' of WAT (24-26).

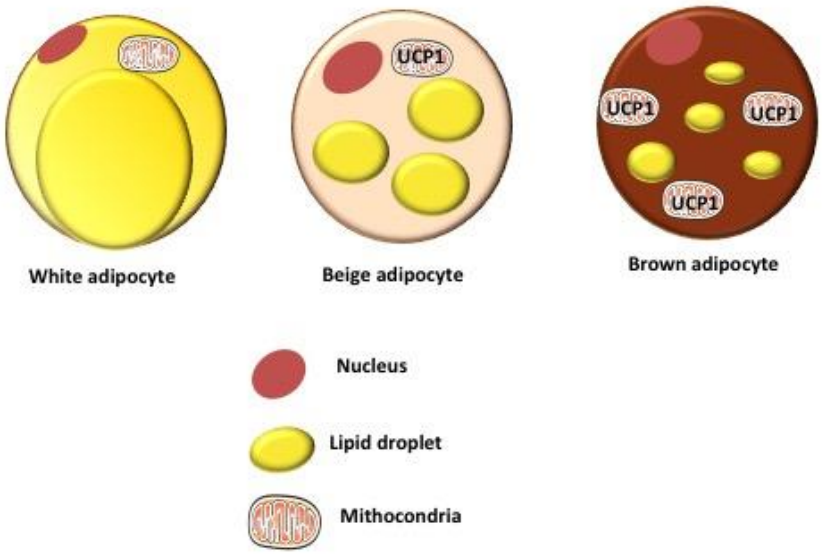

Figure 1:**The Different Types of Adipocytes**

White (WAT), brown (BAT) and beige (BeAT). WAT are large cells characterized by a large lipid droplet within the cytoplasm, with few mitochondria and the nucleus moved to the edge. BAT have numerous but small lipid droplets in the cytoplasm, a central nucleus and many mitochondria. The BeAT phenotype is in between. Only BAT and BeAT contains the uncoupling protein-1 (UCP1) the protein responsible for heat production.

2. When the adipocyte becomes dysfunctional

Obesity is determined by the increase in fat mass. One of the principal functions of adipocytes is storing and releasing lipid, so obesity is the response to excess caloric intake. Obesity is in part determined by the capacity of adipocytes to change their sizes. WAT can expand to store excess fatty acids into TG (lipogenesis), or tighten when stored lipids are hydrolyzed (lipolysis) (**Figure 2**), e.g., under fasting condition or exercise (27).

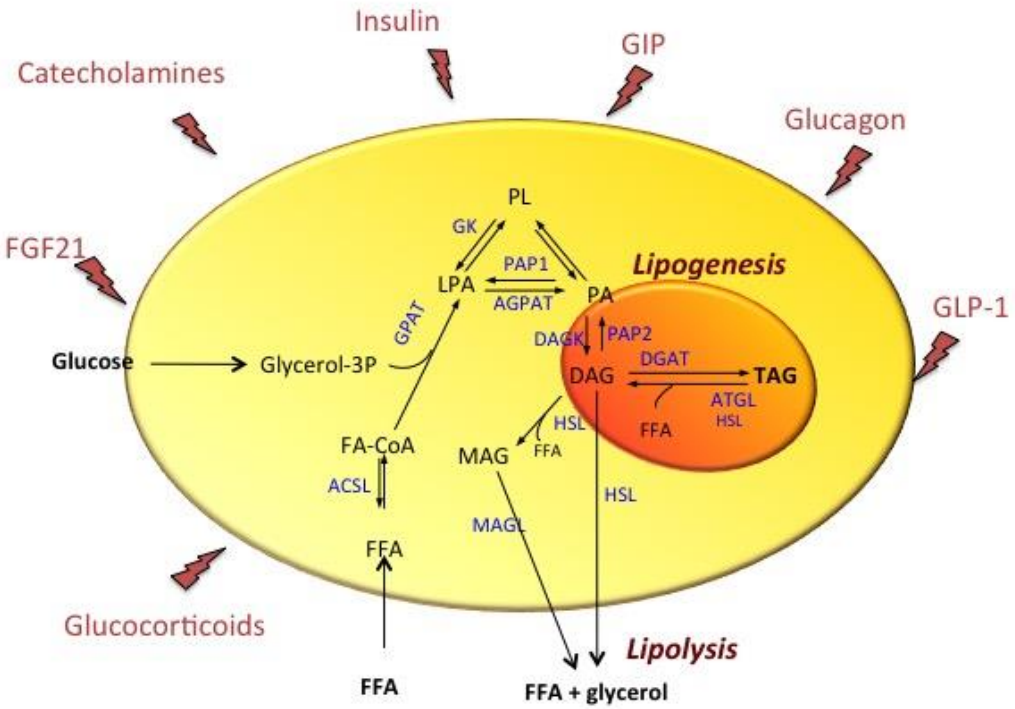

Figure 2: Lipid Metabolism in Adipocytes

A) Lipogenesis: Triglycerides (TG) are synthesized from the esterification of FFA-CoA and Glycerol-3-phosphate (G3P). Diacylglycerol acyltransferase (DGAT) catalyzes conversion of DAG to TAG.

B) Lipolysis: The first step of triglycerides (TG) hydrolysis is carried out by adipose triglyceride lipase (ATGL) that form diacylglycerols (DAG); hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MAGL) then release free fatty acids (FFA) and glycerol.

Several molecules control lipid metabolism: insulin promotes lipid storage; catecholamines stimulate lipolysis; glucagon like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) regulate lipolysis and fatty acid oxidation; glucocorticoids stimulate lipolysis during fasting but promote adipogenesis in postprandial state; fibroblast growth factor 21 (FGF21) suppresses fasting lipolysis, but stimulates lipolysis during feeding and might promote browning; the effect of glucagon is controversial.

Abbreviations: glycerol-3-phosphate acyltransferases (GPAT), acylCoA acylglycerol-3-phosphate acyltransferases (AGPAT) and phosphohydrolases (PAP-1 and PAP-2) attend to obtain lysophosphatidic acid (LPA) and diacylglycerols (DAG).

WAT expansion occurs through the enlargement of adipocyte size (hypertrophy) or the increase in adipocyte number (hyperplasia) (28, 29). Since adipocyte size is well correlated with increased insulin resistance (9, 30) it has been hypothesized that WAT becomes dysfunctional when it cannot expand in response to excess caloric intake it, thus promoting the storage of excess lipids into visceral fat and ectopic fat (e.g. in liver muscle and pancreas, **Figure 3**). For many years the phenotype carrying small subcutaneous adipocytes was considered at lower risk to develop insulin resistance, given that these potentially hyperplastic adipocytes could enlarge by increasing the lipid droplets, thus maintaining a good homeostasis (9). However, this has been proven to be not always the case (31).

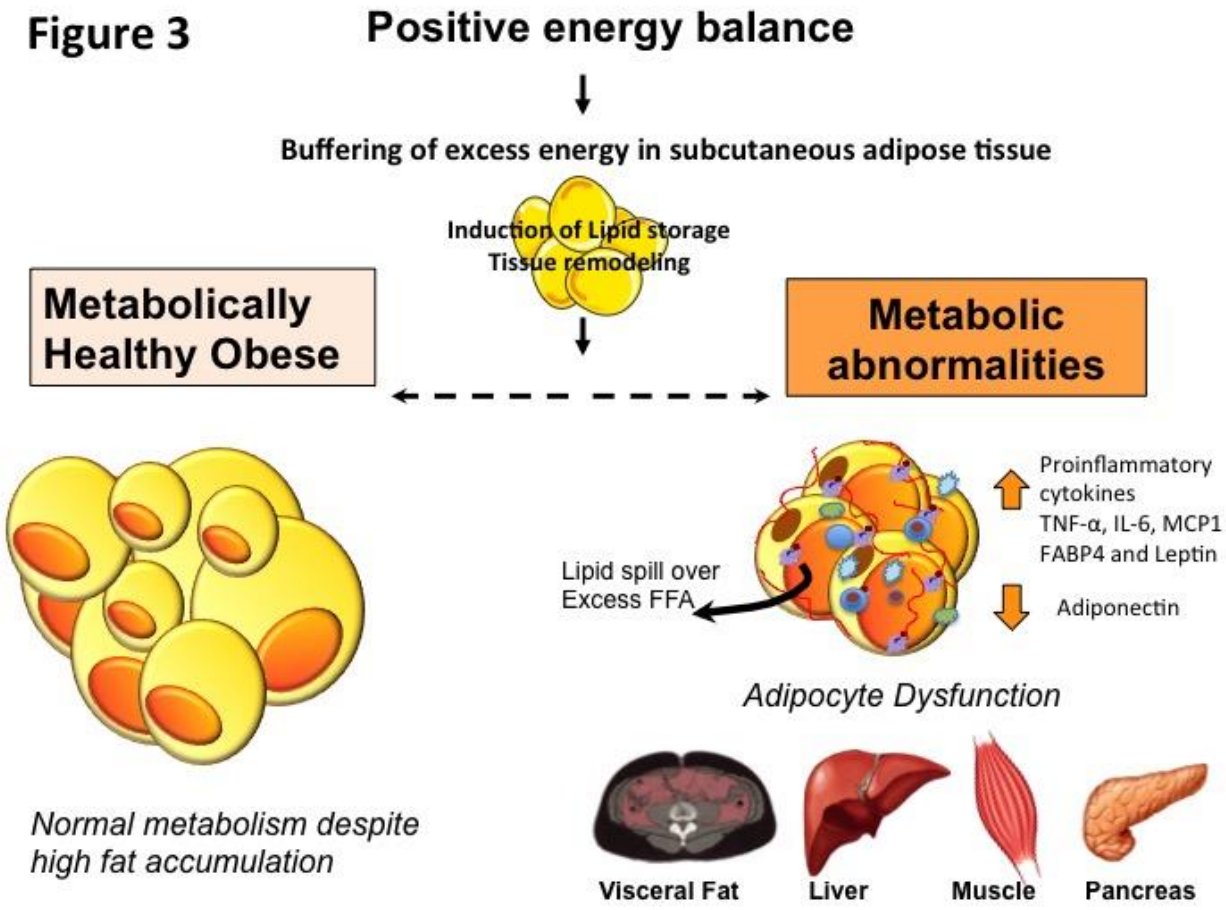

Figure 3: Effects of Positive Energy Balance

In presence of excess energy intake (positive energy balance) the adipose tissue increases lipid storage. A healthy subcutaneous adipose tissue can expand by increasing the number of new adipocytes and enlarging existing adipocytes. When adipocytes cannot enlarge they become dysfunctional, pro-inflammatory cytokines are released and excess lipids that cannot be stored accumulates in visceral fat, liver, muscle and pancreas.

Dysfunctional adipocytes release pro-inflammatory adipokines, such as tumor necrosis factor-α (TNF-α) and monocyte chemo attractant protein-1 (MCP-1). MCP-1 contributes to macrophage infiltration in the adipose tissue, insulin resistance and NAFLD (32) while TNF-α promotes FA mobilization from adipose tissue to oxidative tissues (33). This has led to the definition of adiposopathy, or sick fat (34). Moreover, dysfunctional WAT is resistant to the anti-lipolytic effect of insulin, and is responsible for fatty acid overflow and development of lipotoxicity that in turns determines alteration in glucose and lipid metabolism (35). Studies with overfeeding did not help to clarify the impact of dysfunctional WAT. Alligier et al. have shown that healthy lean subjects

respond differently to overfeeding: subjects in which the subcutaneous fat has a defective regulation of lipid storage-related genes (e.g., DGAT2, SREBP1c, and CIDEA) stored most of the excess calories in visceral rather than peripheral fat despite a similar increase in body weight (36). These results are in agreement with the current hypothesis that presence of small subcutaneous adipocytes protects against insulin resistance and metabolic diseases. However, the study by Johannsen et al. showed opposite results (31). They studied the effect of 8 weeks of excess energy and lipid intake on adipocyte size and expansion in young healthy men: lean subjects with smaller adipocytes responded with a rapidly and not protective adipocyte remodelling, and despite expansion of subcutaneous fat they developed insulin resistance and released more inflammatory marker while subjects with larger subcutaneous adipocytes had less insulin resistance and visceral fat accumulation, maybe due to a reduced expandability of these cells (31). It is likely that other factors are involved, possibly genetic predisposition to type 2 diabetes or in general to ectopic fat deposition (3, 35, 37, 38).

Independent of size, a dysfunctional adipocyte has impaired capacity to store and release free fatty acids generating high fatty acid overflow that leads to lipotoxicity and excess fat accumulation in ectopic sites with consequent risk of mitochondrial dysfunction, oxidative stress due to the formation of reactive oxygen species, cell apoptosis, and metabolic diseases such as type 2 diabetes, dyslipidemia and non-alcoholic steatohepatitis (**Figure 3**).

2. Excess lipolysis causes muscle insulin resistance and beta cell dysfunction

Excess FFA overflow is the main cause of insulin resistance and metabolic dysfunction (**Figure 3**). They derive from adipose tissue (from TG hydrolysis, **Figure 2**), hepatic de novo lipogenesis (DNL), or spillover from plasma TG (3). Excess FFA accumulates into organs like liver, muscle and pancreas determining lipotoxicity and impaired insulin signalling (3). Increased plasma FFA levels are associated with increased lipid synthesis in muscle cells, in particular increased levels of diacylglycerol (DAG), the first step of TG synthesis, ceramides and long-chain fatty acyl-coenzyme A (CoA) (39).

Earlier studies showed that FFA infusion reduces basal and insulin-stimulated muscle glucose uptake by inhibiting insulin signaling (39-41). Lipid infusion during the hyperinsulinemic clamp decreases muscle ATP synthesis (42), impairs insulin-stimulated activation of phosphoinositol-3 kinase (PI3K), pyruvate dehydrogenase kinase, isozyme 1, RAC-alpha serine/threonine-protein kinase (also known as proto-oncogene c-Akt), endothelial nitric oxide synthase (eNOS) (43),

activate transcription factors such as nuclear factor-kB (NF-κB) and inflammatory processes (39). Moreover, acute and chronic lipid infusions alter glucose stimulated insulin secretion, by enhancing insulin secretion to counteract peripheral insulin resistance and stimulated muscle glucose uptake (41, 44). However, in subjects with family history of diabetes and at risk of T2D the insulin secretion is impaired and results in hyperglycemia (44). The same type of response was observed in human islets incubated with fatty acids (45).

Several hormones, beside insulin, control lipolysis through direct or indirect pathways, i.e., catecholamines and glucagon (**Figure 2**). Their secretion is often altered in insulin resistant states. While insulin promotes lipid storage, catecholamine promotes lipolysis, while the effect of glucagon is controversial. Catecholamines exert the most potent action to promote this catabolic pathway and stimulate lipolysis (46, 47). Glucagon might also act as a lipolytic hormone since in vitro it stimulates breakdown of triglycerides from lipid droplets as demonstrated by the increased release of glycerol (48). However, other studies have shown that glucagon reduces the release of FFA and their peripheral oxidation (49, 50), so it is possible that glucagon might also promote FFA re-esterification **(Figure 2).** Lipid metabolism is regulated also by glucocorticoids (GCs) a class of steroid hormones of which cortisol is the most important. GCs act through the glucocorticoid receptors (GRs) that are expressed in all tissues (51, 52). GCs act on adipose tissue metabolism by modulating the expression of lipid genes in the adipose tissue, stimulating both the synthesis (lipogenesis) and breakdown (lipolysis) of adipocyte triglycerides (51, 53). GCs under basal or fasted conditions stimulate lipolysis and decrease lipogenesis, while in postprandial state when insulin secretion is increased, GCs action pair with insulin signaling and increase lipogenesis (52). GCs stimulate lipolysis and FFA release by activating both intracellular hormone sensitive lipase (HSL) and also intravascular lipoprotein lipase (LPL) (51). Excess GCs is associated with adipogenesis and adipocyte hypertrophy; in the Cushing's syndrome patients display expansion of visceral adipose tissue and depletion of subcutaneous adipose tissue (51).

Among further factors that regulate adipose tissue metabolism there are the gastrointestinal hormones **(Figure 2),** glucagon like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (54). GIP receptors (GIPR) are very abundant in adipose tissue and GIP promotes glucose uptake and insulin signaling in adipocytes. However, in adipocytes from obese subjects the interaction GIP/GIPR is impaired as well as the effect of GIP (55). GLP-1 effect on adipocytes is milder also because the half life of GLP-1 is very short (about 2 minutes). However, GLP-1 receptor agonists (GLP-1 RA) are a new class of antidiabetic drug that bind to GLP-1 receptors and with a

much longer half life (the drug is given subcutaneously daily or once weekly). GLP-1RAs is associated with weight loss, mediated in part by the decrease adipose tissue lipogenesis and the improvement in the antilipolytic effect of insulin, while in the liver they increase fat oxidation (54, 56). Fibroblast growth factor 21 (FGF21) has antilipolytic effects in 3T3-L1 cells (57). Studies in FGF21-KO mice have shown that FGF21 suppresses fasting lipolysis, but stimulates lipolysis during feeding; through this it might preserve peripheral insulin sensitivity (58).

3. Impaired insulin action in the adipose tissue

Insulin acts not only in muscle and liver, where its main role is the regulation of glucose uptake, but also in adipose tissue where it regulates lipid metabolism. Insulin promotes adipogenesis, by increasing FA uptake and esterification, synthesis of TG and inhibits lipolysis. The dose response of insulin vs lipolysis and insulin vs plasma FFA concentration has been initially evaluated by Groop et al in 1995 by using the hyperinsulinemic euglycemic clamp and labelled palmitate infusion (59). Groop et al showed that the suppression of FFA during insulin infusion follows the hyperbolic shape (59). Thus, the degree of antilipolytic effect of insulin, i.e. the degree of adipose tissue insulin resistance, can be estimated by the product of FFA x insulin (60) or by the product of rate of lipolysis x insulin (61-63), the so-called adipose tissue insulin resistance index (Adipo-IR) (**Figure 4).**

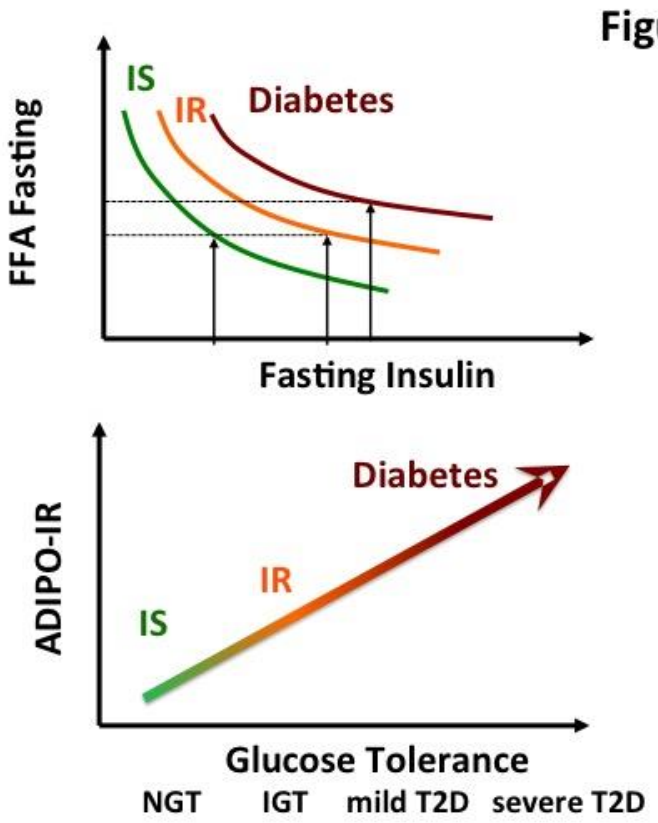

Figure 4: The Insulin-FFA Relationship.

As insulin concentration increases, lipolysis and plasma FFA concentrations are suppressed following a hyperbolic curve. Since the formula that describes the hyperbola is y=const/x, the product FFAxInsulin can be used as an index of adipose tissue-insulin resistance (Adipo-IR). In presence of insulin resistance for the same insulin levels lipolysis is less suppressed and circulating FFA levels are higher. As a result the curve is shifted to the right and the Adipo-IR index is higher in IR than in IS. The index is increased as the glucose tolerance worsens and subjects become glucose intolerant (IGT) or diabetics (T2D) (data from reference (65))

Higher levels of insulin in the blood are observed in insulin resistant subjects (**Figure 4)** since there is a greater demand of insulin secretion by the beta cells to facilitate peripheral glucose uptake (64, 65). In subjects with insulin resistance, e.g., obese, type 2 diabetes, NAFLD etc., the dose-response insulin-lipolysis is shifted to the right (**Figure 4)** and the Adipo-IR is increased (59, 61, 65). It has been hypothesized that most of the metabolic abnormalities are due to a dysfunctional subcutaneous adipose tissue (SAT) that cannot expand to store excess lipids. This dysfunctional SAT releases more FFA during fasting state (**Figure 3**) and the excess lipids are then stored ectopically, mainly in visceral fat, but also in the liver. In line with this hypothesis the Adipo-IR has

been found increased proportionally to visceral and hepatic fat, as reviewed in (66). We have shown that the Adipo-IR is increased in subjects with impaired glucose tolerance (IGT) or T2D (65) and with NAFLD (67). Drugs like the PPAR-gamma agonists (68, 69) or GLP-1 receptor agonists (70) are able to improve the Adipo-IR. Moreover, it has been shown that Adipo-IR is associated with the severity of NAFLD and especially with the degree of liver fibrosis (67); the improvement in liver histology after pioglitazone treatment is associated with the reduction in Adipo-IR (68, 69, 71).

4. Adipose tissue remodeling: the metabolically healthy vs metabolically abnormal subjects

Not all obese subjects have the most common cardio-metabolic abnormalities, such as abnormal glucose and lipid levels, insulin resistance, systemic inflammation and increased blood pressure plus increased waist circumference (listed in **Table 1**). Considering that a "healthy" subcutaneous adipose tissue can expand becoming a buffer for excess circulating substrates, it has been hypothesized the presence of a phenotype defined as "metabolically healthy obese" (or MHO). These subjects, despite being obese, have close to normal insulin sensitivity and lipid homeostasis (38, 72-74). MHO individuals do not have dyslipidemia or hyperglycemia and are protected from metabolic and cardiovascular comorbidities (38, 75). On the other hand, non-obese subjects despite being lean might have metabolic syndrome and cardio-metabolic diseases. The reason is not completely known but the MHOs tend to expand their subcutaneous adipose tissue mass, by increasing the number of adipocyte. Phenotypically, they usually have small size adipocytes (31, 76) compared to obese insulin resistant subjects that have are hypertrophic and hypoxic adipocytes with macrophage infiltration (38, 77, 78) (**Figure 2**). Conversely, individuals with normal body weight when they have abdominal obesity and/or fatty liver are often characterized by insulin resistance (IR), hyperinsulinemia, hyperglycemia, impaired glucose tolerance (IGT), hypercholesterolemia and hypertriglyceridemia, and were defined as "Metabolically Obese Normal Weight", (MONW) (79). A recent meta-analysis showed that MHO have less risk than MONW but they are at higher risk than healthy non obese subjects (75). These characteristics in lean individuals mark a departure from common patterns in which metabolic disease is a consequence of weight gain (79). Accumulation of visceral fat is often found in MONW, (34). The analysis of Pou et al has shown that non obese subjects with metabolic syndrome are more likely to have visceral fat and that in the Framingham study over 40% of men and women had increased visceral fat despite an average BMI of 27 kg/m2 in women and 28 kg/m2 in men (80). Also fatty liver is associated to an increased risk of both CVD and T2D, and this is explained by the fact that

subjects with NAFLD are insulin resistant (in muscle, liver and adipose tissue), secrete more lipoproteins, triglycerides, fibrinogen and c-reactive protein (66). The same metabolic abnormalities can be observed in non-obese NAFLD (61).

Adipocyte size might be implicated in the protection of MHO individuals from the adverse effects of obesity since hypertrophy of both subcutaneous and omental adipocytes was increased in T2D (81) and correlated with the degree of fatty liver and the risk of the progression from hepatic steatosis to fibrosis (82).

The prevalence of body size phenotypes was studied by Wildman et al. that examined over 5000 participants of the National Health and Nutrition Examination Surveys 1999-2004 (72), see Table 2 for the criteria used to identify MHO. Wildman et al showed that among US adults over 30% of obese patients are MHO with near normal insulin sensitivity and without metabolic syndrome, according to the IDF criteria, excluding waist circumference (72). However, this prevalence can vary between 10% and 50% according to the criteria used for the definition of MHO since there is not a common agreement (38). In **Table 2** we have reported the most used criteria for the definition of MHO, as proposed by Wildman (72). Of note, in the Wildman's study the cut off for HOMA-IR was 5.13, but this seems quite high. We therefore propose a lower cut-of as HOMA-IR>2.0 that corresponds to the upper 95th percentile for two population-based cohorts, the Programme for Prevention of Type 2 Diabetes in Finland [FIN-D2D; n = 2849]) and (FINRISK 2007 [n = 5024] (83)).

5. Browning of WAT as a possible aid to fight metabolic diseases

Brown adipocytes (BAT) and their thermogenesis capacity have been first described in rodents and only later they have been discovered in humans, where, however, BAT is limited to neck supraclavicular, suprarenal, paraaortic and pericardial areas and is recognizable only when activated by cold exposure (4). Although BAT contribution to human energy expenditure (TEE) is minor, browning has been proposed as a new possible target to fight obesity and improve whole body metabolism and TEE (5, 19, 84).

In animal studies browning of WAT was associated with weight loss and improved metabolic outcome (5). BAT transplantation in ob/ob mice decreased weight gain, total and hepatic fat, but also increased the expressions of β-adrenergic receptors and gene related fatty acid oxidation related in subcutaneous and epididymal (EP) WAT (85). Thus, the differentiation of WAT into BAT or BeAT has emerged as a promising way to induce energy expenditure and has been proposed as

a possible tool to counteract obesity and insulin resistance. WAT browning is activated by Beta-adrenoceptor and by cold temperatures, i.e. below thermoneutrality (86) (**Figure 5**). In humans, acute cold exposure activates BAT especially around the neck and in the supraclavicular area (reviewed in (4)) and increases energy expenditure in proportion to BAT activation (87, 88). Using PET it has been shown that cold stimulates activation of BAT and increases fatty acid oxidation in this depot, but not in skeletal muscle or subcutaneous adipose tissue (88). Although it is well established that cold activates BAT, this is not the best way for browning of SAT. After 10 days of exposure to low temperature (15–16°C for 6 hours a day) cold acclimation increased upper body BAT size and activity but did not promote browning of subcutaneous adipose tissue (89). Lean subjects increased non-shivering thermogenesis but no change was observed in resting energy expenditure (REE) (89). An even more extreme cold exposure (10°C for 2 hours a day, 5 d/week for 4 weeks) increased BAT volume by 45% and fractional glucose uptake (90).

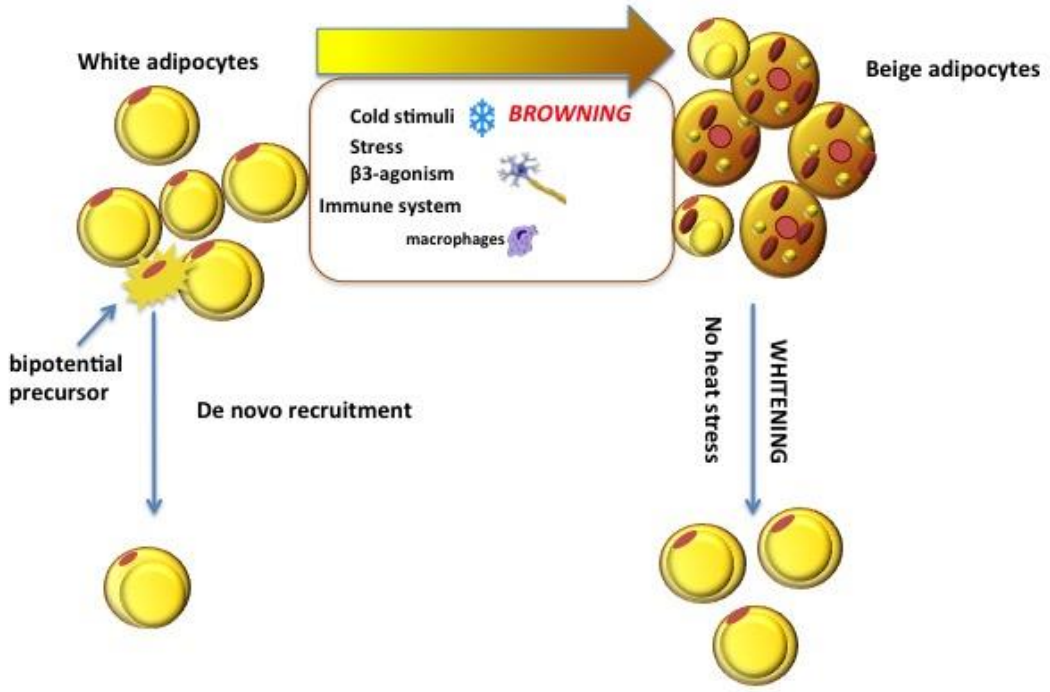

Figure 5: **Browning of white adipocyte**

Several mechanisms have been associated with the activation of BAT and the stimulation of browning of WAT. The origin of BeAT is unknown, but it probably derives from the differentiation

of pre-adipocytes. Among the browning stimuli cold exposure is one of the strongest: exposure to low temperature leads to the activation of adaptive thermogenesis and heat production via uncoupling protein-1 (UCP-1). Other stimuli include response to stress, catecholamines and cortisol.

Increased stress has recently been associated to activation of supraclavicular brown fat (91). Stress is associated to increased secretion of cortisol and catecholamines and the two hormones are released also during the stress induced by exposure to low temperature. During cold exposure, the glucocorticoid prednisolone increased BAT activation measured by PET, supraclavicolar skin temperature and energy expenditure (92). Thus, it is likely that the trigger for browning is not simply the cold exposure but rather the adrenergic secretion stimulated by low temperature. It is well established that catecholamines are potent activators of BAT and their release is stimulated by stress or cold exposure, the same stimuli that can activate BAT. In response to cold, norepinephrine is released from the sympathetic nervous system and binds to beta adrenergic receptors on brown and beige adipocytes; this leads to the activation the adenylyl cyclase/cAMP/protein kinase A (PKA) signaling cascade required for the hydrolysis of intracellular TG. PKA phosphorylates and activates cAMP response element-binding protein (CREB) resulting in enhanced transcription of UCP1 and peroxisome proliferator-activated receptor-gamma (93). Kuji et al. observed that patients with high plasma catecholamines, due to pheochromocytoma, during a PET/CT exams have increased FDG uptake in cervical, paravertebral, mediastinal, and perirenal regions, suggesting activation of BAT (94). After surgical removal of the tumor also the FDG uptake disappeared. Later Frontini et al. observed that half of the visceral fat samples of patients with pheochromocytoma contains BAT or BeAT cells (19) indicating that when WAT is subjected to adrenergic stimulation is able to promote direct transformation into brown adipocytes. Contrary to mice, in humans gene expression related to browning is more prevalent in visceral than in subcutaneous WAT (95), but whether chronic exposure to low temperature promotes browning of visceral tissue is still not known. These results were confirmed by Sidossis et al. that showed that in patients exposed to a prolonged adrenergic stress, such as burn trauma, subcutaneous WAT contains multilocular UCP1-positive adipocytes with increased mitochondrial density and respiratory capacity (84).

Other factors might promote browning. FGF21 has been proposed as an activator of BAT to stimulate non shivering thermogenesis and also to promote browning of WAT (96, 97). FGF21

secretion is increased after exposure to cold and FGF21 treatment upregulated human adipocyte brown fat gene/protein expression and thermogenesis in a depot-specific manner (98). Since obesity (both in humans and mice) is characterized by high plasma levels of FGF21 (99, 100), it is likely that the target tissues are resistant to the effect of this cytokine. Glucagon-like peptide (GLP-1), glucagon (GCG), and oxyntomodulin (OXM) have been also implicated inBAT activation and browning (101). All these peptides originate from proglucagon and are involved in both glucose and lipid metabolism (102). The administration of the GLP-1, GCG or OXM directly into the brain activates BAT and thermogenesis mainly through cerebral GLP-1 receptors (101), particular those present in the hypothalamus (103).GLP-1 receptors agonists (GLP-1 RA) therapy is associated with weight loss and both liraglutide and exenatide (two currently approved GLP-1 RA) have been shown to activate cerebral GLP-1R (56) and to increase energy expenditure (103). However, it is still not clear if the browning effect of these factor is direct or mediated by their action of lipolysis, since fatty acids are potent activators of uncoupling protein 1 (UCP1) beside being the fuel of BAT mitochondria (104)

6. Activation of BAT for the improvement of lipid and glucose metabolism

Several studies indicate that BAT activation has positive metabolic effects beyond energy expenditure, in particular on lipid and glucose metabolism. Increased ^{18}F-deoxy-glucose (FDG) uptake after cold exposure was the first demonstrations of BAT activation in humans and FDG uptake has been considered a marker of BAT activation (4, 12, 88, 105, 106). Even if FFA, not glucose, are used as substrate for BAT thermogenesis (104), these studied showed that BAT metabolizes glucose. FDG uptake in BAT is significant only during cold exposure; nevertheless fractional glucose uptake in BAT is much higher than in skeletal muscle or cervical subcutaneous fat (107). It is likely that glucose is used as a substrate for lipid synthesis rather than for oxidation, despite mitochondria abundance in BAT.

Insulin is very important for BAT metabolism. In BATIRKO mice, i.e., lacking insulin receptors only in brown adipocytes, BAT size is reduced and these mice have impaired glucose tolerance mainly due to reduced beta cell mass and impaired insulin secretion (108). BAT is sensitive to the effect of insulin, at least regarding glucose metabolism: in conditions of both fasting and insulin resistance BAT glucose uptake was found reduced (105). This is an interesting observation since many metabolic diseases are associated with insulin resistance. It has not been elucidated if the reduction in BAT glucose uptake was due to substrate competition and increased FFA uptake and

oxidation, given that metabolic disease are also associated to excess lipolysis and adipose tissue IR.

BAT activation appears to stimulate peripheral lipid mobilization and oxidative disposal, presumably to accommodate the increased demand of FFA for thermogenesis. As previously said, FFA are the only substrate for BAT activity, i.e., oxidation and thermogenesis, but in addition FFA are the main activators of UCP1 (104). FFA derive mainly from intracellular lipolysis (**Figure 2**) but it is possible that BAT uses circulating FFA when intracellular fuel sources are depleted, for example by cold exposure (104). The recent paper by Blondin et al. evaluated how BAT glucose and lipid metabolism changed after inhibition of lipolysis by oral ingestion of niacin (NiAc) (107). The authors observed a reduction in BAT not only of FFA oxidation (measured by 11C-acetate) but also of glucose uptake (107). Interestingly the reduced FFA flux determined an increase in glucose uptake in the myocardium, probably because this organ that generally relies of FFA as a substrate, shifted to glucose as a source of energy.

Cold acclimation reduced, although not significantly, the plasma levels of FFA and triglycerides in the study by van der Lans (89). Cold exposure increases also BAT fatty acid uptake and oxidation as shown by PET studies employing the long-chain fatty acid PET tracer, ^{18}F-fluoro-thiaheptadecanoic acid (FTHA) (88). The same authors studied the effects of mild cold stimulation (18 °C) on dietary fatty acid (DFA) tissue extraction and oxidation in non-cold-acclimated men; after a standard liquid meal containing the long-chain fatty acid PET tracer FTHA, they observed that fractional (not total) DFA extraction in BAT was much greater than in skeletal muscle or white adipose tissue (109). Chondronikola et al showed that 5-8h cold exposure increases whole-body energy expenditure, glucose homeostasis, and insulin sensitivity (87) and that prolonged cold exposure stimulates genes associated with lipolysis but only in BAT and not in WAT when compared to thermoneutral conditions, (110), although whole body lipolysis, FFA oxidation and TG-FFA cycling were all increased. Taken together these results suggest that BAT, despite its small size, might play a role in whole body lipid metabolism and postprandial lipid handling, if not direct at least indirect. In fact, the contribution of BAT to total lipid oxidation or clearance of DFA is minor compared to muscle and WAT due to the small size of human BAT. It is still to be proven if BAT “signals” the peripheral tissue to increase lipolysis, e.g. by releasing specific adipokines. Some cytokines as FGF21 are secreted by BAT in murine models, but not in humans. Moreover, high catecholamine concentrations stimulate peripheral lipolysis and FFA release (46).

BAT activation has been proposed also for the reduction of plasma lipoprotein and cholesterol

levels and prevention of atherosclerosis. Thus, it has been hypothesized that once BAT is activated and uses up the TG stored in lipid droplets for the production of energy, BAT lipid storage becomes short; circulating FFA are then reabsorbed by BAT to restore the lipid droplets or to be used for oxidation. Increased plasma triglycerides and TRL are major risk factors for metabolic diseases and atherosclerosis. TRL are synthesized in the liver and secreted as VLDL; once secreted, the TG core is depleted by circulating LPL that releases FFA (spillover) that are taken up by the adipose tissue and the liver (3).

Animal studies have shown that BAT activation improves lipoprotein metabolism by delipidating TG-rich lipoproteins (TRL) (111-113). Recent evidences from animal and in vitro models have indicated that BAT can play an important role in TG clearance (111). Activation of BAT in hypertriglyceridemic mice reduces plasma triglyceride, illustrating the importance of BAT in lipoprotein metabolism (111). Lipoprotein lipase (LPL), which is highly abundant in BAT, can promote hydrolysis of TRLs. Moreover, once BAT is activated by cold there is a down-regulation in the expression of Angiopoietin-like 4 (ANGPTL4), a protein that inhibits LPL activity; this leads the activation of AMPK, enhancing plasma LPL activity and uptake of plasma triglyceride-derived fatty acids (112). Matsushita et al. showed that subjects with detectable BAT have lower total plasma cholesterol and LDL-C than subjects without detectable BAT (114) and De Lorenzo et al., showed that 90 days daily exposure to cold (14°C) for 20 minutes reduced total cholesterol, LDL-C, and BMI in hypercholesterolemic individuals (115). Whether this mechanism is important in humans for the regulation of lipoprotein metabolism and the risk for atherosclerosis has yet to be proven.

7. Conclusions

It is now recognized that adipocyte size, type and metabolism are important factors for the development of metabolic diseases including non alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D). Not all obese subjects develop NAFLD and/or T2D, some remain metabolically healthy (MHO). On the other hand, there are some subjects that despite having normal weight have the metabolic characteristics of the obese (NWMO), like insulin resistance, dyslipidemia and/or impaired glucose metabolism. The plasticity and composition of adipose tissue (WAT vs BAT) seems to play a major role in promoting these metabolic alterations and current research is focusing on the possibility to increase browning of WAT not only to prevent/reduce obesity, but also to improve the cardiometabolic status of patients (either obese or non obese) with metabolic alterations. Understanding how and why NWMO develop cardiometabolic diseases and why MHO

are somehow protect is important to prevent these diseases.

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Conflicts

The authors report no conflict of interest related to this article.

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Table 1 Cardiometabolic abnormalities*

Cardiometabolic abnormalities	**Definition**
Elevated blood pressure	Systolic/diastolic blood pressure ≥130/85 mm Hg or antihypertensive medication use
Elevated triglyceride level	Fasting triglyceride level ≥150 mg/dL
Decreased HDL-C level	HDL-C level < 40 mg/dL in men or < 50 mg/dL in women or lipid-lowering medication use
Elevated glucose level	Fasting glucose level ≥100 mg/dL or antidiabetic medication use
Insulin resistance	HOMA-IR > 2.0 (ie, the 95th percentile in healthy subjects)#
Systemic inflammation	hsCRP level >0.1 mg/L (ie, the 90th percentile)

*Modified from Wildman et al 2008

according to Isokuortti et al 2017

Table 2. Definition of metabolically healthy vs. metabolically abnormal subjects*

Weight Categories	**Definition**
Normal weight: BMI < 25.0	
metabolically healthy	BMI < 25.0 and < 2 cardiometabolic abnormalities
metabolically abnormal	BMI < 25.0 and ≥ 2 cardiometabolic abnormalities
Overweight: BMI 25.0-29.9	
metabolically healthy	BMI 25.0-29.9 and < 2 cardiometabolic abnormalities
metabolically abnormal	BMI 25.0-29.9 and ≥ 2 cardiometabolic abnormalities
Obese: BMI ≥ 30.0	
metabolically healthy	BMI ≥ 30.0 and < 2 cardiometabolic abnormalities
metabolically abnormal	BMI ≥ 30.0 and ≥ 2 cardiometabolic abnormalities

*According to Wildman et al 2008