REVIEW

Metabolic inflexibility of white and brown adipose tissues in abnormal fatty acid partitioning of type 2 diabetes

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Type 2 diabetes (T2D) is characterized by a general dysregulation of postprandial energy substrate partitioning. Although classically described in regard to glucose metabolism, it is now evident that metabolic inflexibility of plasma lipid fluxes is also present in T2D. The organ that is most importantly involved in the latter metabolic defect is the white adipose tissue (WAT). Both catecholamine-induced nonesterified fatty acid mobilization and insulin-stimulated storage of meal fatty acids are impaired in many WAT depots of insulin-resistant individuals. Novel molecular imaging techniques now demonstrate that these defects are linked to increased dietary fatty acid fluxes toward lean organs and myocardial dysfunction in humans. Recent findings also demonstrate functional abnormalities of brown adipose tissues in T2D, thus suggesting that a generalized adipose tissue dysregulation of energy storage and dissipation may be at play in the development of lean tissue energy overload and lipotoxicity.

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INTRODUCTION

Metabolic inflexibility has been described as the inability to switch from lipid oxidation during fasting to carbohydrate oxidation in the postprandial state. Although this concept was originally described in the context of the disturbance of glucose metabolism in insulin resistance,¹ there is evidence that the metabolic inflexibility of lipid fluxes is also implicated in the pathogenesis of type 2 diabetes (T2D) and obesity (Figure 1). The physiological regulation exerted by white adipose tissues (WATs) on fasting and postprandial lipid kinetics has been investigated for decades. During fasting, lipolysis of triglycerides (TGs) contained in white adipocytes contributes to the majority of the circulating nonesterified fatty acid (NEFA) pool. Quantitatively, this process is mainly driven by intracellular lipolysis in the abdominal subcutaneous adipose tissues (SCATs), followed by femoral SCAT and visceral adipose tissues (VATs).² During the postprandial state, insulin inhibits intracellular lipolytic activity in WATs, promotes the transcriptional activation of lipoprotein lipase (LPL), its translocation from adipocytes to the AT capillary bed and its activation.^{3,4} These activation mechanisms are set into play in a matter of minutes to hours and lead to hydrolysis of dietary TG from chylomicrons preferentially in adipose tissues.^{5,6} However, only a fraction of the fatty acids produced from this process in adipose tissue capillary beds are esterified and trapped in adipocytes. Actually, the relative adipose tissue fatty acid storage efficiency is dependent on the concentration of NEFA in the interstitium and on insulin stimulation. Indeed, up to 50% of the fatty acids generated from circulating TG lipolysis in adipose tissues readily recirculate into the systemic bloodstream and contribute to raise postprandial NEFA.⁷ This process, suspected of being implicated in lean tissue fatty acid overexposure and lipotoxicity, was coined 'NEFA spillover' by Unger and Zhou.⁸ During the past decades, the inability of the WAT to properly regulate NEFA fluxes in obesity and T2D has been highlighted in several comprehensive reviews.^{9–13}

On the other hand, brown adipose tissue (BAT) is a specialized tissue, the function of which is to produce heat. BAT is highly vascularized and richly innervated by terminal fibers arising from postganglionic neurons of the sympathetic nervous system (SNS). Until recent studies showing ¹⁸Fluoro-deoxyglucose (¹⁸FDG) uptake in BAT using positron emission tomography coupled to computed tomography (PET/CT), BAT was thought to have metabolic importance only in small mammals and newborn humans. However, we now know that adult humans have several discrete areas of active BAT depots.^{14–16} In this regard, BAT may have a more important role than initially thought, emerging as an important regulator of whole-body energy homeostasis. WAT is the primary site of energy storage, whereas BAT is specialized in energy expenditure. For this reason, the balance between WAT and BAT could possibly affect systemic energy balance and may contribute to the development of obesity and insulin resistance. Some studies show that obese and diabetic subjects have a lower prevalence of spontaneously metabolically active BAT compared with lean, young and healthy subjects.^{15,16}

This short review focuses on the mechanisms of WAT dysfunctions and their effects on the regulation of fatty acid fluxes toward lean organs. We will also examine whether some of these mechanisms may be shared by BAT and may contribute to the observed association between obesity and diabetes and reduced spontaneous metabolic BAT activity.

DOES IMPAIRED WAT STORAGE LEAD TO OVEREXPOSURE OF LEAN TISSUES TO FATTY ACIDS?

We have systematically reviewed all studies published that quantified dietary fatty acid storage in adipose tissues in humans.¹² From this review, we concluded that storage in adipose tissues is stimulated by higher meal fatty acid content and by consumption of a previous meal. In most studies, storage per mass

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Figure 1. Schematic representation of WAT metabolic inflexibility in T2D. (a) Reduction of dietary fatty acid uptake in WAT, probably mediated by LPL-dependent (1) and LPL-independent (2) mechanisms, contributes to postprandial dietary fatty acid spillover toward lean organs (3). Downregulation of β_2 -AR impairs catecholamine-induced lipolysis and the release of NEFA during fasting (4). The combination of these defects leads to reduced intracellular TG turnover in WAT (5). Stimulation of β_1 -AR and β_3 -AR promotes UCP-1 and PGC-1a expression (6) and then leads to mitochondrial biogenesis, higher thermogenic capacity and trans-differentiation of white adipocytes into brown-like adipocytes. Classical BAT depots (b) preferentially use intracellular pool of lipids as an energy source for cold-induced thermogenesis (7). Insulin increases glucose uptake in BAT but does not necessarily induce thermogenesis (8). Adrenergic stimulation increases lipolysis and UCP1 and PGC1a expression (9). The increase in intracellular NEFA inhibits adenyl cyclase activity as a negative feedback (10). It is still unknown whether the observed reduction in spontaneously metabolically active BAT in obesity and T2D results from abnormalities in insulin and/or catecholamine signaling that are analogous to those observed in WAT. Red arrows: trends of changes in insulin resistance and obesity. Dashed arrows: metabolic pathways under investigation. Red question marks: possible mechanisms of metabolic dysfunction in insulin resistance and obesity.

of adipose tissue is higher, intermediate and lower in abdominal, gluteal and femoral SCAT, respectively. Dietary fatty acid storage also appears higher in VAT vs SCAT. Postprandial increase in adipose tissue blood flow could have a role in the postprandial increase in fatty acid storage, although the increase in WAT blood flow is more closely related to glucose than fatty acid uptake.^{17,18} Clearly, insulin-mediated LPL stimulation is implicated in dietary fatty acid storage in WAT.^{12,13} There is, however, evidence for a significant role of insulin-stimulated post-LPL fatty acid trapping in this process.^{19,20} This is further demonstrated by our studies using intravenous heparin + intralipid infusion (that 'clamps' LPL activity) without and with nicotinic acid administration (that inhibits intracellular lipolysis) and without and with an euglycemic hyperinsulinemic clamp showing insulin-mediated reduction of plasma NEFA appearance rate independent of LPL and intracellular lipolysis.^{21,22}

WAT from insulin-resistant individuals without or with T2D displays reduced dietary fatty acid storage.^{7,23,24} This impaired storage is also associated with reduced postprandial WAT blood flow and glucose uptake.⁷ Insulin-resistant subjects display increased spillover of NEFA from circulating TG in subcutaneous WAT.²⁵ By using the heparin + intralipid experimental paradigm without or with nicotinic acid administration and without or with an euglycemic hyperinsulinemic clamp, we demonstrated that healthy offspring of two parents with T2D have reduced insulin-mediated suppression of plasma NEFA appearance and reduced insulin-stimulated storage of plasma NEFA compared with healthy individuals without a family history of T2D.²² This defect is, however, not fully established in these prediabetic individuals and is more readily apparent in the postprandial state in patients with established T2D.²⁶ The main determinant of abnormal postprandial NEFA metabolism in our studies was the degree of abdominal adiposity. An interesting feature of WAT metabolic dysfunction is

the increased release of lactate found in obese people with T2D compared with nondiabetic obese subjects²⁷ or in obese women with hyperinsulinemia compared with healthy lean women.²⁸ Furthermore, healthy normoglycemic nonobese subjects with at least two first-degree relatives with T2D have increased net lactate release from WAT per fat cell during an euglycemic hyperinsulinemic clamp compared with matched control subjects without or with a family history of T2D.²⁹ Increased release of lactate by WAT is due to the relative adipose tissue hypoxia observed in obese, insulin-resistant subjects and may be responsible for the metabolic abnormalities and reduced capacity for adipocyte expansion observed in these individuals.^{30–32}

Is there any evidence of a link between ectopic fat deposition and impaired WAT storage of meal fatty acids? By using PET/CT, we showed that subjects with T2D do not display increased net skeletal muscle plasma NEFA uptake rate despite increased plasma NEFA levels and turnover during the postprandial state.³³ This paradoxical finding was likely explained by reduced postprandial skeletal muscle blood flow in T2D, as we found a direct relationship between muscle fractional uptake of plasma NEFA and muscle blood flow in the latter study. Therefore, at least in skeletal muscle, plasma NEFA tissue uptake is also regulated by local tissue mechanisms. By using magnetic resonance spectroscopy with ¹³C-labeled meal fatty acids, Ravikumar *et al.*³⁴ showed increased liver and skeletal muscle dietary fatty acid deposition over 9 h postprandially in subjects with T2D. In the later study, there was no significant difference in postprandial plasma NEFA or TG, although NEFA or TG production rates were not measured. The ¹³C magnetic resonance spectroscopy method used by Ravikumar *et al.* can only measure nonoxidative dietary fatty acid tissue deposition in liver and skeletal muscles. Furthermore, this method cannot assess net dietary fatty acid uptake simultaneously in all organs.

To address these limitations, we established and validated a novel method based on oral administration of ^{18}F -fluoro-6-thiaheptadecanoic acid ($^{18}\text{FTHA}$), a positron-emitting long-chain fatty acid analog, with sequential PET/CT scanning in humans.³⁵ $^{18}\text{FTHA}$ has a 110-min radioactive half-life and incorporates into nonoxidative fatty acid metabolic pathways and gets trapped into the mitochondrial matrix during its oxidation.³⁶ These characteristics make $^{18}\text{FTHA}$ an ideal tool for the measurement of net, cumulative tissue uptake of fatty acids, especially if delivered slowly over a few hours, as is the case for dietary fatty acids. In our study,³⁵ we demonstrated that $^{18}\text{FTHA}$ is exclusively absorbed through incorporation into chylomicron-TG at a similar rate to tritiated oleate. In healthy men, we found gradual uptake of dietary fatty acids over 3 to 4 h in most organs, with higher uptake in the liver, followed by the heart, kidneys, VAT and then resting skeletal muscles and subcutaneous WAT. By using this novel method, we recently compared subjects with impaired glucose tolerance (IGT) and healthy controls.³⁷ As expected on the basis of previous findings by others,^{7,23,24} dietary fatty acid uptake expressed per volume of tissue was reduced in VAT and abdominal SCAT in IGT individuals. We found that increased waist circumference, increased body mass index and increased level of insulin resistance were associated with reduced WAT dietary fatty acid storage. We furthermore demonstrated that the degree of hepatic steatosis (as assessed by CT) was significantly correlated with reduced WAT dietary fatty acid uptake. However, net liver dietary fatty acid uptake was not different between IGT and control individuals, suggesting that impaired dietary fatty acid storage in WAT may not be directly responsible for ectopic liver fat deposition in prediabetic individuals. We also found no increase in dietary fatty acid uptake in skeletal muscles of subjects with IGT. Our findings lend indirect support for a possible role of impaired fatty acid oxidation in ectopic fat deposition in skeletal muscles and the liver in prediabetic and diabetic individuals. *De novo* lipogenesis is also a very likely mechanism for ectopic fat deposition in the liver. Detailed discussions on impaired fatty acid oxidation and *de novo* lipogenesis are available elsewhere.^{12,38–40}

We found a very significant increase in myocardial dietary fatty acid uptake in subjects with IGT that was associated with subclinical reduction in left ventricular systolic and diastolic function.³⁷ The latter finding was also observed in a T2D rat model.⁴¹ We did not find an association between increased myocardial dietary fatty acid uptake and reduced WAT storage. Again, this points toward other local tissue regulatory processes that, in addition to impaired WAT fatty acid storage, may drive dietary fatty acid utilization in lean organs. In the heart, increased expression and function of *LPL*, *Cd36* and carnitine palmitoyl-transferase-1 in diabetes are likely to be involved in this phenomenon.^{4,41}

INSULIN AND CATECHOLAMINE RESISTANCE IN WAT

Lipolysis in WAT is mainly controlled by the intracellular pool of cyclic adenosine monophosphate and the subsequent activation of protein kinase A (PKA). Active PKA phosphorylates hormone-sensitive lipase and perilipin-1, which allows their translocation to the lipid droplets. Adipose triglycerides lipase (ATGL) catalyzes the conversion of TG in DAG and is the rate-limiting enzyme for NEFA release into the bloodstream. PKA does not directly interact with ATGL, but perilipin-1 phosphorylation leads to the recruitment of CGI-58 and the activation of ATGL (reviewed in Jocken and Blaak⁴²). Phosphorylation of AKT/PKB by insulin promotes the degradation of cyclic adenosine monophosphate by phosphodiesterase 3B (PDE3B) and inhibits lipolysis. As insulin resistance is an important pathophysiological feature of T2D, resistance to the antilipolytic action of insulin in WAT has been proposed to explain the increased NEFA appearance observed in these subjects. This

hypothesis has been recently challenged by a systematic review that showed only a modest difference of plasma NEFA concentrations between lean ($n = 953$) and obese ($n = 1410$) subjects.⁴³ NEFA release (in kg) of WAT negatively correlates with body mass index and fasting insulinemia. The latter data suggest that obese subjects have slower adipose tissue lipolytic rates and exclude WAT-reduced insulin-mediated suppression of intracellular lipolysis as the main mechanism for elevated plasma NEFA in prediabetic and diabetic subjects.

Resistance to catecholamine action in WAT is one possible explanation for the observed downregulated fasting intracellular lipolytic in insulin resistance and diabetes.^{44,45} A reduction of β_2 -adrenergic receptor (β_2 -AR) density in adipocytes isolated from obese individuals may explain reduced catecholamine-stimulated adipose tissue lipolysis in the face of higher tissue noradrenaline (NA) turnover.^{44,46} Recently, radiocarbon dating was used to measure residence time of acylglycerols in abdominal subcutaneous WAT and to approximate intracellular TG turnover in adipocytes from healthy and obese individuals.⁴⁷ Whereas reduced WAT intracellular removal (lipolysis) is associated with obesity and insulin resistance, reduced WAT storage was more associated with severe hyperlipidemia in the latter study. Moreover, obese subjects show a significant increase in adipose tissue lipid residence time and greater lipid storage rate per year than healthy lean subjects. This highlights the fact that WAT fatty acid lipolysis and storage are differently regulated and may confer different metabolic risk profiles during the development of obesity. The Arner group also previously reported that impaired adipogenesis is observed in subjects with a genetic predisposition for T2D but not in those predisposed for obesity.⁴⁸ *Ex vivo*, the lipolytic capacity of large adipocytes is higher in response to NA and atrial natriuretic peptide stimulation and during inhibition by insulin when compared with small adipocytes from the same subject.⁴⁹ Taken together, these data suggest that obese insulin-resistant subjects have a blunted response to catecholamine-induced lipolysis in WAT per kg of fat mass, which is not dependent on adipocyte hypertrophy but is related to the reduction in the expression of β_2 -AR.

Another important aspect is the neuronal control of lipolysis by the SNS. Hypothalamic leptin and insulin resistance have been associated with many complications observed in obesity such as inflammation and dyslipidemia (reviewed in Belgardt and Bruning⁵⁰ and Scherer and Buettner⁵¹). Insulin stimulation in the mediobasal hypothalamus was found to hyperpolarize Agouti-related peptide and pro-opiomelanocortin neurons in a PI3K-dependent manner. This reduces SNS outflow and NA release to the WAT and may thus contribute to the reduction of lipolysis.⁵² This system is balanced by leptin signaling in the brain, which increases SNS tone to the WAT. Leptin and insulin mediate their effects through PI3K activation, although they induce different electrophysiological responses in different populations of pro-opiomelanocortin neurons. Moreover, leptin suppresses PI3K signaling in Agouti-related peptide neurons. At the moment, it is not yet clear how central insulin and leptin resistance may have a role in the catecholamine resistance of WAT in humans.

THERMOGENIC CAPACITY AND INSULIN/CATECHOLAMINE RESISTANCE

Thermogenic capacity is mainly determined by adrenergic stimulation, which triggers the differentiation of brown adipocytes from myogenic precursors (*Myf5*+) in classical BAT depots. Moreover, the differentiation of white and brown preadipocytes (*Myf5*-) and/or the trans-differentiation of white adipocytes into brown-like adipocytes also occur in WAT depots. Several proteins involved in the transcriptional control of uncoupling protein 1 (*UCP1*), mitochondrial biogenesis and metabolic substrate oxidation are induced by NA stimulation and PPAR γ agonists.⁵³ In

rodents, treatment with a β_3 -AR agonist during 6 days increases the number of brown adipocytes and oxidative metabolism in WAT.⁵⁴ Activation of PKA signaling by adrenergic stimulation stimulates lipolysis but also activates p38 MAP kinase pathways that increase the transcription of UCP1 and PGC1A.⁵⁵ The differentiation of new brown adipocytes from mesenchymal stem cells is dependent on COX2 activity, which is rate limiting for prostaglandin synthesis under NA stimulation⁵⁶ and cold exposure.⁵⁷ Interestingly, cotreatment of mice with β_3 -AR agonist and hormone-sensitive lipase inhibitor reduces intracellular lipolysis and increases the transcription of genes associated with the brown adipocyte phenotype.⁵⁸ This suggests that adenylyl cyclase activity may be reduced by intracellular levels of NEFA or lipid metabolites, thus reducing the browning of WAT.

In addition to catecholamine signaling, activation of insulin and IGF-1 pathways are necessary to induce brown adipogenesis. Necdin and its downstream targets, Pref1 and Wnt10a, inhibit brown fat cell differentiation. Insulin-mediated phosphorylation of the insulin receptor substrate-1 reduces Necdin expression and triggers clonal expansion of murine brown preadipocytes.^{59,60} Interestingly, white preadipocytes isolated from diabetic patients present some defects in insulin signaling and in molecular events regulating the early steps of white adipogenesis.⁶¹ These data suggest that insulin resistance occurs in white preadipocytes and may have a role in impaired WAT storage capacity through promoting reduction in adipogenesis. Although obese and diabetic individuals display reduced BAT capacity as assessed by the total volume of ¹⁸FDG BAT activity during PET/CT scanning,¹⁶ a link between impaired insulin/IGF-1 signaling in brown fat precursors (Myf5+ or Myf5-) and a reduction in BAT thermogenic capacity is not yet established.

BAT ACTIVATION AND METABOLIC DYSFUNCTION

BAT depots are richly innervated by sympathetic efferent fibers.⁶² The release by SNS nerves of NA in the vicinity of BAT adipocytes not only enhances thermogenic activity but also increases the capacity (cell proliferation, mitochondriogenesis and synthesis of UCP1) of brown fat cells to produce heat. SNS activation is the physiological trigger of brown adipocyte thermogenesis. Conditions such as cold exposure or overfeeding increase NA turnover rate in BAT. Consistently, cold exposure and overfeeding do not lead to increased thermogenic activity in mice lacking β -AR (β -less mice).⁶³ The inbred Lou/C rat, originating from the Wistar strain, has been previously described as a model of resistance to the development of obesity.⁶⁴ Lou/C rats display higher expression of β_3 -AR in inguinal WAT and show enhanced capacity to maintain body temperature following acute cold exposure after 8 weeks of high-fat diet.⁶⁵ Diet-induced obese mice have chronically elevated temperature in interscapular BAT during both the light and dark period at thermoneutrality without change in locomotor activity.⁶⁶ These animals display selective resistance to leptin's anorexic effect, whereas leptin's stimulation of sympathetic neuronal activity to BAT is normal. It should be noted that although the main endogenous system activating BAT is β_3 -AR stimulation through the SNS,⁶⁷ and despite the effects observed in rodents,⁶⁸ β_3 -agonists have not proved to be useful in the treatment of obesity in humans.⁶⁹ β_1 -AR stimulation is, however, able to induce thermogenesis in animals lacking β_3 -AR.⁷⁰ Furthermore, mesenchymal stem cells isolated from human subcutaneous WAT (hADSC) differentiate into brown adipocytes in response to dobutamine and β_3 -agonist stimulation even if β_3 -AR expression is 30-fold lower than that of the β_1 -AR.⁷⁰

The best way to activate BAT in humans in the light of currently available studies is cold exposure. Although spontaneously active BAT is visible on ¹⁸FDG PET scanning in only 4 to 7% of individuals,^{15,16} all human studies conducted to date have shown that cold-induced BAT glucose uptake occurs in 60 to 100% of

adult humans.^{71–73} However, showing glucose uptake in BAT is not sufficient to demonstrate its role in thermogenesis. We recently established a unique experimental setup to make this demonstration.⁷⁴ By using a cooling suit with thermal sensors and electromyography electrodes disposed over the participants' body, we carefully reduced skin temperature down to the shivering threshold (2 to 4% maximal voluntary muscle contraction) while performing indirect calorimetry and PET/CT scanning with ¹¹C-acetate to determine total body and supraclavicular BAT energy expenditure during cold exposure and at room temperature. We determined that cold exposure resulting in $\sim 3.8^\circ\text{C}$ reduction of skin temperature in healthy men leads to approximately 250 kcal excess energy expenditure over 3 h and a significant increase in BAT energy expenditure. By using ¹⁸FDG and ¹⁸FTHA together with stable and radioactive glucose and palmitate tracer intravenous infusion, we determined that $\sim 1\%$ of circulating glucose and NEFA were consumed by BAT in this condition, for a total of less than 5 kcal in excess energy expenditure. However, we found a significant reduction in BAT TG content on the basis of an increase in CT radio density of BAT. Our results suggest that BAT is implicated in cold-induced thermogenesis in healthy humans. At least acutely, cold-induced BAT thermogenesis appears to be driven by intracellular lipolysis with very rapid reduction of BAT TG content. The group of van Marken Lichtenbelt also demonstrated cold-induced activation of BAT blood flow in humans, supporting the notion of significant BAT thermogenic activity.⁷³ The latter study reported insulin-mediated stimulation of BAT glucose uptake without a simultaneous increase in BAT blood flow, suggesting that glucose uptake and thermogenic activity can be differentially regulated in BAT.

By using an air cooling system to cold-expose individuals down to the shivering threshold, the group of van Marken Lichtenbelt also recently showed BAT glucose uptake in only 3 out of 15 morbidly obese individuals.⁷⁵ In the latter study, these investigators also demonstrated cold-induced increased energy expenditure only in those individuals with demonstrated presence of BAT. One limitation of the latter study was that BAT-negative subjects had higher core temperatures. Whether a similar degree of cold stimulation was achieved in all subjects is therefore uncertain. The cause for reduced BAT metabolic activity in obese and diabetic individuals remains unknown.

CONCLUSION

From this review, we can conclude that impairment of both insulin-mediated storage of dietary fatty acids and catecholamine-stimulated intracellular lipolysis occurs in WAT in prediabetic and diabetic individuals. However, the former may be more specific to diabetogenic forms of obesity, whereas the latter may occur during all forms of excess adiposity. Impaired dietary fatty acid storage in WAT may not, however, be the critical factor leading to ectopic fat deposition in prediabetic and diabetic states, as numerous local tissue factors further modulate lean tissue exposure to fatty acids. Although intracellular lipolysis has a critical role in BAT thermogenesis, whether there is a specific reduction of SNS-mediated BAT activation has yet to be demonstrated in humans. Impaired insulin-mediated recruitment and lipid storage in BAT is a very attractive hypothesis, but more studies are required to demonstrate whether the typical WAT metabolic abnormalities are also present in BAT from diabetic individuals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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