TARGETING ADIPOSE TISSUE LIPID METABOLISM TO IMPROVE GLUCOSE METABOLISM IN CARDIOMETABOLIC DISEASE

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ABSTRACT

With Type 2 diabetes mellitus and cardiovascular disease prevalence on the rise, there is a growing need for improved strategies to prevent or treat obesity and insulin resistance, both of which are major risk factors for these chronic diseases. Impairments in adipose tissue lipid metabolism seem to play a critical role in these disorders. In the classical picture of intracellular lipid breakdown, cytosolic lipolysis was proposed as the sole mechanism for triacylglycerol hydrolysis in adipocytes. Recent evidence suggests involvement of several hormones, membrane receptors, and intracellular signalling cascades, which has added complexity to the regulation of cytosolic lipolysis. Interestingly, a specific form of autophagy, called lipophagy, has been implicated as alternative lipolytic pathway. Defective regulation of cytosolic lipolysis and lipophagy might have substantial effects on lipid metabolism, thereby contributing to adipose tissue dysfunction, insulin resistance, and related cardiometabolic (cMet) diseases. This review will discuss recent advances in our understanding of classical lipolysis and lipophagy in adipocyte lipid metabolism under normal and pathological conditions. Furthermore, the question of whether modulation of adipocyte lipolysis and lipophagy might be a potential therapeutic target to combat cMet disorders will be addressed.

Keywords: Lipolysis, lipophagy, cardiometabolic disease, obesity, adipose tissue, insulin resistance, Type 2 diabetes, lipid metabolism.

INTRODUCTION

Obesity and related insulin resistance are major risk factors for cardiometabolic (cMet) disorders including Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Increased fat mass is associated with increased mortality rates, mainly due to vascular diseases.[1] Adipose tissue is the most important organ for lipid storage in the human body, in which lipids are stored mainly in the form of triacylglycerol (TAG) in intracellular lipid droplets (LD). Subcutaneous adipose tissue (SAT) serves as a buffer to store lipids in times of excess energy intake (e.g. after meal ingestion) and to release non-esterified free fatty acids (FFA) for use by oxidative tissues (e.g. skeletal muscle, heart, and liver) in times of energy demand (e.g. fasting, exercise). In obesity, the adipose tissue depot is enlarged to a size that exceeds its storage capacity; lipid overflow results in increased fat deposition outside the SAT (i.e. visceral adipose tissue, skeletal muscle, heart, and liver).

Substantial evidence indicates that this is associated with the development of obesity-associated insulin resistance and cMet diseases.[2] Indeed, metabolically healthy (insulin sensitive) obese subjects have significantly lower visceral fat mass, a decreased liver fat content, less macrophage infiltration, and a smaller adipocyte size, both in visceral and subcutaneous fat depots, compared to insulin resistant obese subjects.[3] It is

interesting to note that surgical removal of either visceral[4-6] or subcutaneous fat[7] does not affect cardiovascular (CV) and metabolic risk factors, suggesting that adipose tissue function, rather than fat mass per se, determines cMet risk.[8]

Classical lipolysis and the recently discovered alternative pathway for lipid breakdown, lipophagy, largely determine intracellular lipid turnover. Therefore, understanding depot-specific regulation of both pathways under normal and pathological conditions is crucial to develop novel therapeutic strategies to prevent or treat obesity-associated cMet disorders. In this review, we will discuss the current knowledge about the potential involvement of classical lipolysis and lipophagy in adipocyte lipid metabolism under normal and pathological states, and highlight potential therapeutic targets.

REGULATION OF ADIPOCYTE LIPID METABOLISM BY INTRACELLULAR LIPOLYSIS AND LIPOPHAGY

Intracellular Lipolysis: the Classical Way of Fat Breakdown

Intracellular or cytosolic lipolysis is the process via which stored TAG is hydrolysed in order to provide sufficient energy in times of increased energy demand (e.g. fasting or exercise). The complexity of its regulation has been investigated extensively and is illustrated in Figure 1A. Up to a decade ago, when natriuretic peptides (NPs) entered the lipolytic picture, catecholamines, secreted by the adrenal medulla and sympathetic nervous system, were considered to be the sole physiological lipolytic agents (Figure 1A). In general, visceral adipocytes are more sensitive to catecholamine-induced lipolysis compared with subcutaneous adipocytes due to differences in the expression of adrenoceptor subtypes and key lipolytic proteins.[9-12]

Sengenes et al.[13] has shown that atrial (ANP), brain-type, and C-type NPs, produced in the myocardium and central nervous system, are potent activators of human lipolysis. Physical exercise increases plasma ANP levels, which is accompanied by an increased lipid mobilisation to serve as subsequent substrate in oxidative tissues (e.g. skeletal muscle).[14,15] Although data on depot-specific differences in NP-sensitivity are limited, two studies have suggested that NP-sensitivity is comparable between the visceral and SAT.[12,16]

In the postprandial state, lipolysis is suppressed due to a rise in insulin, which is the major anti-lipolytic hormone in human adipocytes (Figure 1A). In contrast to catecholamine-mediated lipolysis, insulin does not seem to have a direct anti-lipolytic effect on NP-mediated lipolysis.[17,18] Adipocytes from visceral adipose tissue (VAT) are more insulin resistant than subcutaneous adipocytes, and smaller adipocytes tend to be more insulin sensitive, while large (hypertrophic) adipocytes become more insulin resistant.[19-21] Besides insulin, gut-derived short chain fatty acids (SCFA), formed after fermentation of dietary fibres, have a potent anti-lipolytic effect, suggesting metabolic cross-talk between the gut and peripheral lipid metabolism (Figure 1A).[22,23] Recent data have shown that metabolites produced by the adipocyte, such as lactate and β-hydroxybutyrate, exert anti-lipolytic effects via inhibitory G-coupled receptors, suggesting the importance of autocrine regulation of adipocyte lipolysis.[24,25] Finally, preliminary evidence suggests that adipose tissue oxygen tension may be involved in the regulation of adipose tissue lipolysis.[26]

In summary, two major lipolytic hormones (e.g. catecholamines and NPs) and several anti-lipolytic hormones, of which insulin is the most potent, regulate human fat cell lipolysis. In the last decade, tremendous progress has been made by the discovery of several regulatory proteins, adding remarkable complexity to the regulation of classical intracellular lipolysis.

Lipophagy: an Alternative Pathway for Lipid Breakdown Enters the Picture

Autophagy is a homeostatic mechanism functioning as a 'self-digestion' system that degrades unnecessary or dysfunctional cellular components to generate essential nutrients in times of energy deprivation to ensure cellular survival. Although autophagy is largely viewed as a non-selective process, three recent studies[27-29] have clearly implicated autophagy in selective degradation of LD in adipocytes and subsequent lipid hydrolysis, both under basal and β-adrenergically stimulated conditions, a process termed lipophagy. As illustrated in Figure 1B, the three major steps in this alternative pathway for lipid breakdown - including autophagosome formation, lysosomal degradation, and mitochondrial oxidation of the lysosomal lipid products - are tightly regulated by phosphorylation and nuclear translocation of transcription factor EB (TFEB).[30]

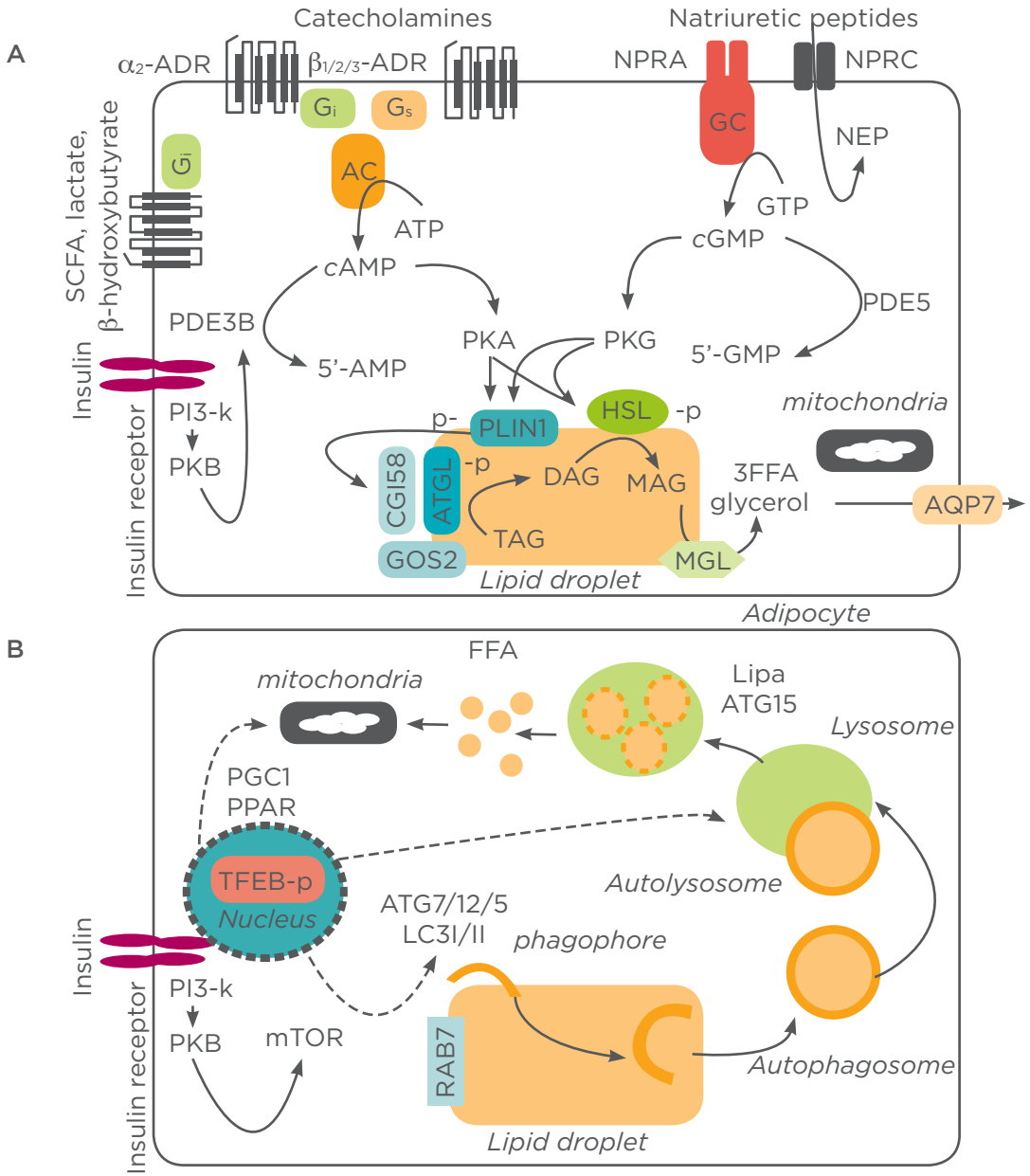

Figure 1: Schematic illustration of: A) the regulation of classical lipolysis in adipocytes; B) Lipophagy. Catecholamines signal via α and β-adrenoceptors, and NPs exert their effect via NPRA and the scavenging receptor NPRC. Subsequent phosphorylation of lipid droplet associated proteins including PLIN1, HSL, and ATGL ensures complete hydrolysis of stored triacylglycerol (TAG) in one glycerol and three free fatty acid (FFA) molecules. Insulin increases PDE3B activity, which converts cAMP in 5'-AMP, decreasing PKA activity and subsequent HSL phosphorylation. Lipophagy, is tightly regulated by phosphorylation and nuclear translocation of transcription factor EB (TFEB).

AC: adenylate cyclase; ADR: adrenoreceptor; ATG: autophagy-related gene proteins; ATGL: adipose triglyceride lipase; ATP: adenosine triphosphate; AQP7: aquaporin 7; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; DAG: Diacylglycerol; FABP4: fatty acid binding protein 4; GC: guanylyl cyclase; Gi: inhibitory G protein; Gs: stimulatory G protein; GTP: guanosine triphosphate; HSL: hormone-sensitive lipase; LC3I/II: microtubule-associated protein light chain 3 (mammalian homologue of ATG8); Lipa: lysosomal lipase; MAG: monoacylglycerol; MGL: monoglyceride lipase; mTOR: mammalian target of rapamycin; NPs: natriuretic peptides; NEP: neutral endopeptidases; NPRA/C: natriuretic peptide type A and C receptor; PDE3B/5: Phosphodiesterase 3B and 5; PGC1: PPAR co-activator type 1; PI3-k/PKB: phosphoinositide/protein kinase B; PLIN1: perilipin 1; PPAR: peroxisome proliferator-activated receptor; RAB7: ras-related protein 7; SCFA: short-chain fatty acid.

ADIPOSE TISSUE LIPID METABOLISM IN OBESITY AND CVDS

Elevated Basal but Blunted Catecholamine-Stimulated Lipolysis

Obesity is the most often studied clinical condition regarding pathophysiological aspects of lipolysis. As far as *in vivo* whole-body lipolysis under fasting conditions (basal lipolysis) is concerned, this rate may be increased in obesity because of the increased total adipose tissue mass. However, if obese adipose tissue would release FFA at the same rate as lean adipose tissue then circulating FFA would be much higher than the observed 20-30%, suggesting that FFA concentrations are not elevated in proportion to the increase in fat mass.[31] Indeed, others[32] and ourselves,[33] have demonstrated that fasting lipolysis expressed per unit of fat mass is rather reduced in obesity. This was accompanied by downregulation of the expression of several key lipolytic enzymes.[33,34] *In vitro*, basal spontaneous lipolysis expressed per number of adipocytes is higher in obese compared to lean adipose tissue[35] and subcutaneous versus visceral adipocytes.[36]

Adipocyte enlargement (hypertrophy), as observed in human obesity, is associated with increased macrophage infiltration, chronic low-grade inflammation, and release of pro-lipolytic cytokines (e.g. tumour necrosis factor alpha), which may contribute to the enhanced basal lipolysis.[37,38] Since humans are in the post-prandial state most of the day, insulin-mediated inhibition of adipose tissue lipolysis (ATL) is a major regulator of basal lipolytic rate. Insulin-mediated suppression of ATL per unit of fat mass is normal[39] or slightly attenuated in obese individuals,[40-43] suggesting that chronic hyperinsulinaemia cannot overcome the increase in whole-body lipolysis.

Others,[35,44] and ourselves,[33] have clearly shown that *in vitro* and *in vivo* catecholamine-induced lipolysis is blunted in SAT of obese subjects, which persists after significant weight loss.[45] This was also shown in normal weight subjects with obesity among first-degree relatives.[46] The blunted catecholamine-mediated lipolytic response supports the observation that adipocyte lipid turnover is decreased in human obesity,[47,48] which might be an important primary factor in the development of increased fat stores in obese subjects.[49] On the other hand, visceral adipocyte lipolysis, induced by catecholamines, is increased and strongly correlates with CV and metabolic risk factors in obesity.[50] These data support the 'portal hypothesis',[51] postulating that the liver in obese subjects is directly exposed to an increased release of FFA derived from visceral lipolysis (≈10-50%) into the portal vein.[52-54]

With respect to NP-induced lipolysis, data are scarce. However, reduced circulating NP levels[55] and a defective *in vivo* ANP-mediated lipolytic response in SAT from young overweight/obese subjects has been observed.[56] This may partly be explained by upregulation of the scavenging receptor, NP receptor C, in SAT of obese subjects.[57] In contrast, patients with chronic heart failure, with elevated circulating NP levels, show a preserved,[58] or even increased, catecholamine and ANP-mediated lipolytic response in subcutaneous adipocytes,[59] possibly contributing to the development of cardiac cachexia.[60]

In summary, obesity is characterised by an increased basal and a blunted catecholamine and NP-stimulated lipolysis in subcutaneous adipocytes, while catecholamine sensitivity in the visceral depot is increased. This altered lipid turnover may be an early factor in the development of increased fat stores in obesity and associated cMet complications.

Defective Regulation of Autophagy

Under normal physiological conditions, adipocytes rely mainly on cytosolic lipolysis, while lipophagy may become more important in pathophysiological conditions to maintain lipid homeostasis (Figure 2A). Indeed, autophagy markers and fluxes appear to be elevated in the cardiometabolically unhealthy VAT depot of obese insulin-resistant and T2DM subjects, and these markers are reduced following weight loss.[61-65] Furthermore, autophagy markers and fluxes are increased in adipose tissue of lean mice upon caloric restriction (CR), whereas they decrease in obese mice,[65] suggesting defective nutritional and hormonal regulation of adipose tissue autophagy in obesity. Interestingly, adipose tissue of adipose triglyceride lipase (ATGL) deficient mice showed increased lipophagy,[66] suggesting lipophagy might be upregulated in order to compensate for the reduced expression and activity of cytosolic lipases in obesity. On the other hand, autophagy is involved in adipocyte differentiation.[29] Therefore, it could be primarily elevated in order to accommodate expansion and growth of adipocytes to deal with the increased lipid availability in obesity.

As illustrated in Figure 2B, induction of autophagosome formation will increase delivery of lipids to lysosomes, which may accumulate to a toxic level in this organelle if subsequent lysosomal hydrolysis and mitochondrial oxidation are not adapted accordingly to accommodate the increased lipid cargo. This hypothesis is supported by the observation that upregulation of autophagy, in ATGL deficient mice, is accompanied by increased lysosomal lipid accumulation and severe metabolic complications.[67] Furthermore, upregulation of Lipa - an enzyme involved in lysosomal lipid hydrolysis - in adipose tissue of severely obese individuals has recently been shown, suggesting increased processing of the excess lysosomal lipid cargo.[68] Finally, excessive lipid delivery and accumulation in lysosomes evoked lysosomal destabilisation, cell apoptosis, and a subsequent inflammatory response in 3T3-L1 adipocytes,[69] supporting the view that increased autophagy and inadequate handling of the lipid cargo may contribute to adipose tissue inflammation, which has been linked to obesity-associated insulin resistance (Figure 2B).

In summary, lipophagy might be increased in adipose tissue of obese subjects as a compensatory mechanism to deal with increased lipid availability. A disbalance between autophagosome formation, lysosomal degradation, and mitochondrial oxidation is proposed to be one of the putative mechanisms that may contribute to an inflammatory response, which may lead to obesity-related insulin resistance in humans (Figure 2).

ADIPOCYTE LIPID METABOLISM: A TARGET TO PREVENT CMET DISORDERS

Modulation of Classical Lipolysis

Lifestyle interventions are the most effective way to improve lipid metabolism and to prevent the development of T2DM and subsequent CV events.[70-73] However, long-term outcomes of a dietary and physical activity programme for older adults and for those with significant comorbidities (e.g. heart failure) remain to be improved. Therefore, research is increasingly aimed at identifying natural and/or pharmacological CR and exercise mimetics.[74]

Inhibition of ATL might be a therapeutic strategy to limit excess FFA release, thereby alleviating the development of insulin resistance and cMet abnormalities.[75] On the other hand, a diminished ATL could favour the development of obesity through retention of lipids within adipocytes. The interest in anti-lipolytic drugs has been illustrated, for instance, by nicotinic acid (NA), which has been used for decades as a lipid-lowering drug.[76,77] However, NA shows receptor-independent effects, and the use of the drug has been restricted due to upper-body skin flushing.[78-81] Therefore, the search for alternative drugs with anti-lipolytic effects has led to the synthesis of selective hormone-sensitive lipase (HSL) inhibitors.[82] Although data are scarce, reduced plasma FFA and glucose levels have been demonstrated in diabetic rats treated with a selective HSL inhibitor.[83] Recently, Girousse et al.[84] showed that systemic glucose tolerance was improved in mice treated for 7 days with a HSL inhibitor and haploinsufficient HSL +/- mice, possibly through induction of adipocyte *de novo* lipogenesis (DNL).[84] Evidence is accumulating that adipose tissue DNL might significantly contribute to whole-body insulin sensitivity,[85,86] possibly via secretion of beneficial lipids (lipokines), by adipose tissue upon activation of lipogenesis.[87] In addition to selective inhibition of HSL, recent data report on the development of a selective inhibitor of ATGL, atglistatin, highlighting the development of selective lipase inhibitors to correct defects in lipid metabolism for the treatment and prevention of cMet diseases.[88]

It has been shown that intravenous acetate administration decreases plasma FFA concentrations and improves insulin sensitivity in humans.[89] These data suggest that modulation of systemic SCFA levels by colonic fermentation of certain types of dietary fibres might affect systemic lipolysis, and therefore, improve insulin sensitivity and cMet health, by reducing adipose tissue FFA efflux.[90,91] Nevertheless, to optimise the effectiveness of this type of nutritional intervention, further studies are required since the effects may depend on the type and amount of SCFA produced.

In contrast to the anti-lipolytic approach with selective lipase inhibitors and SCFAs, several sympathomimetic agents have been used to treat obesity because of lipolytic, thermogenic, and anorectic effects.[92] However, the earlier use of non-selective β-adrenergic compounds was associated with adverse reactions such as tachycardia and tremor. The discovery of a β_3-adrenoceptor expressed in white and brown adipose tissue gave new impetus to the field.[93,94] However, activation of lipolysis and browning by β_3-agonists in human

white adipose tissue have, so far, not provided promising results due to the low abundance of β_3-adrenoceptors in human adipose tissue compared to rodents, difficulties of extrapolating *in vitro* data, CV side-effects, and receptor desensitisation.[95-97] Recent data have shown that, next to catecholamines, NPs are able to enhance human skeletal muscle mitochondrial function and induce browning in human adipocytes.[98,99] Furthermore, inhibition of NP degradation and increasing the cyclic adenosine monophosphate/cyclic guanosine monophosphate content, via inhibition of neutral endopeptidases (NEP, neprilysin) and phosphodiesterases (PDE), has demonstrated only limited beneficial cMet effects.[100] Therefore, research is currently focused on dual angiotensin converting enzyme (ACE)/NEP inhibitors (LCZ696),[101] having both CV and metabolic effects. So far, the limited available data of PDE and ACE/NEP inhibition on adipose tissue lipid metabolism are not conclusive and warrant further investigation.[102-104]

In summary, modulation of classical lipolysis recently regained interest in the treatment of obesity-related insulin resistance by the development of selective ATGL, HSL, NEP, and PDE inhibitors. However, to prevent excessive gain or loss in body weight, tissue FFA turnover (uptake, esterification, and oxidation) should be adapted accordingly.

Modulation of Lipophagy

The potential involvement of the lipophagy pathway in adipocyte lipid metabolism makes it an attractive target for the prevention and treatment of cMet disorders. However, before considering manipulation of the adipose tissue lipophagy pathway for therapeutic purposes, a better insight into its role in pathophysiology is warranted.

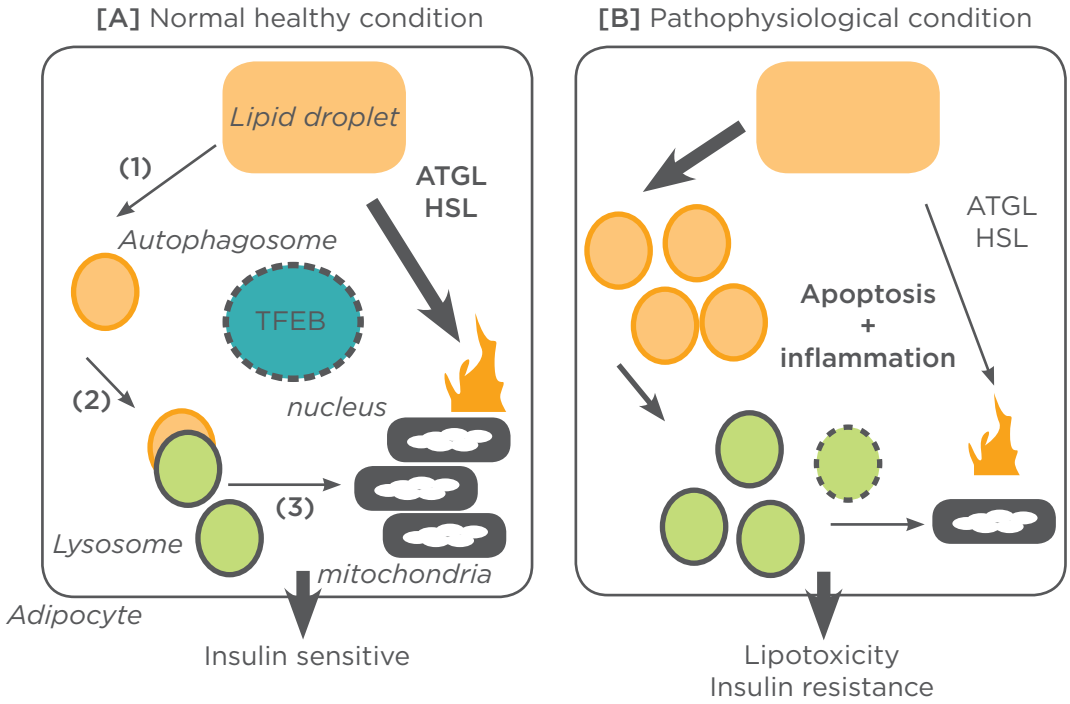

Figure 2: Putative mechanism for impaired adipocyte lipid metabolism in obesity. Under normal physiological conditions (panel A), adipocytes rely mainly on cytosolic lipolysis for hydrolysis of stored TAG. Under pathological conditions (e.g. obesity), autophagy is increased to compensate for the lack in cytosolic lipolysis (panel B). Phosphorylation and nuclear translocation of TFEB regulates all three major steps in this alternative pathway: 1) autophagosome formation; 2) lysosomal lipid hydrolysis; and 3) mitochondrial oxidation. Impaired fine-tuning of all three steps prevents flawless progression of lipids through this pathway, resulting in toxic accumulation of lipids in lysosomes. This might elicit lysosomal destabilisation and cell apoptosis and a subsequent inflammatory response, playing a crucial role in the development of obesity-associated insulin resistance.

ATGL: adipose triglyceride lipase; HSL: hormone-sensitive lipase; TFEB: transcription factor EB.

Recently, we have shown that dietary polyphenols, including resveratrol and epigallocatechin-3-gallate, found naturally in red wine and green tea, have CR-like effects in overweight humans.[105,106] Interestingly, our microarray data showed that resveratrol supplementation affected the expression of the master of lipophagy TFEB and improved adipose tissue function in humans.[105,107] However, it needs to be determined whether lipophagy-mediated lipid catabolism in adipose tissue is directly involved in the potential beneficial metabolic effects of polyphenols. Finally, it has been shown that autophagy might regulate lipid accumulation by controlling the balance between white and brown adipose tissue mass, which favours lipid oxidation and increases systemic insulin sensitivity by limiting FFA efflux.[27,29,108] Overall, we propose that the success of modulating lipophagy, as a potential strategy in the management of obesity, is largely dependent on the fine tuning of all three steps in this pathway, namely autophagosome formation, lysososmal breakdown, and final mitochondrial oxidation of the lipid cargo (Figure 2).

CONCLUSION AND PERSPECTIVE

Research over the last decade has substantially increased our understanding, but also added complexity to the regulation of adipose tissue lipid metabolism in cMet diseases. Increased basal and desensitisation of catecholamine and NP-stimulated adipose tissue lipolysis, due to downregulation of the expression of the key lipolytic enzymes, is a hallmark of human obesity (Figure 2). However, there is no straightforward relationship between fat mass, systemic FFA flux, and the development of insulin resistance and cMet diseases. Nevertheless, the interest in anti-lipolytic drugs, which have been used for decades as a lipid-lowering agent, recently regained interest by the development of selective HSL and ATGL inhibitors. Partial inhibition of HSL shows promising effects, preventing extra weight gain by reshaping FFA fluxes and improving systemic glucose metabolism via stimulation of adipose tissue DNL.[84] However, long-term human clinical trials using selective ATGL and HSL inhibitors are lacking.

In contrast to this anti-lipolytic approach, the effect of increasing NP and catecholamine sensitivity/signalling, using NEP or PDE inhibitors, on lipid metabolism needs to be investigated in more detail. Importantly, exaggerated inhibition or activation of ATL may result in excessive weight gain or the development of cachexia when tissue FFA uptake, esterification, and oxidation are not adapted accordingly. In addition, the alternative pathway for adipocyte lipid breakdown, lipophagy might be an interesting target for treatment. Increased autophagy, as observed in obese adipose tissue, might be a compensatory mechanism for an impaired classical lipolysis, and contribute to the development of systemic insulin resistance when all steps in this pathway are not aligned with each other (Figure 2). Thus, fine-tuning all three steps in the autophagy-lysosomal-mitochondrial pathway in human adipose tissue may be critical regarding treatment outcome. For this reason, components with dual/multiple action on lipid metabolism might hold promise for future treatment of cMet disorders.

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