ALTERATIONS IN PLASMA AND TISSUE LIPIDS ASSOCIATED WITH OBESITY AND METABOLIC SYNDROME

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ABSTRACT

The MS (metabolic syndrome) is a cluster of clinical and biochemical abnormalities characterized by central obesity, dyslipidaemia [hypertriglyceridaemia and decreased HDL-C (high-density lipoprotein cholesterol)], glucose intolerance and hypertension. Insulin resistance, hyperleptinaemia and low plasma levels of adiponectin are also widely related to features of the MS. This review focuses on lipid metabolism alterations associated with the MS, paying special attention to changes in plasma lipids and cellular fatty acid oxidation. Lipid metabolism alterations in liver and peripheral tissues are addressed, with particular reference to adipose and muscle tissues, and the mechanisms by which some adipokines, namely leptin and adiponectin, mediate the regulation of fatty acid oxidation in those tissues. Activation of the AMPK (AMP-dependent kinase) pathway, together with a subsequent increase in fatty acid oxidation, appear to constitute the main mechanism of action of these hormones in the regulation of lipid metabolism. Decreased activation of AMPK appears to have a role in the development of features of the MS. In addition, alteration of AMPK signalling in the hypothalamus, which may function as a sensor of nutrient availability, integrating multiple nutritional and hormonal signals, may have a key role in the appearance of the MS.

INTRODUCTION

White adipose tissue, the body's major energy store, is composed of TAGs (triacylglycerols), produced mainly by FAs (fatty acids) derived from chylomicrons and circulating VLDLs [very-LDL (low density lipoproteins)], released through the action of LPL (lipoprotein lipase), an insulin-stimulated enzyme. In addition, glucose provides the glycerol backbone for TAG. In humans, FAs can also be synthesized from glucose, although the rate of synthesis is lower than in rodents. Adipose tissue is also able to release NEFAs [non-esterified FAs ('free FAs')];

Key words: AMP-dependent kinase (AMPK), fatty acid oxidation, hypothalamus, lipid, metabolic syndrome, non-esterified fatty acid (NEFA), obesity.

Abbreviations: ACC, acetyl-CoA carboxylase; AgRP, agouti protein; AMPK, AMP-activated protein kinase; apo, apolipoprotein; CE, cholesterol ester; CETP, CE transfer protein; CPT-1, carnitine-palmitoyl transferase-1; D6D, Δ6 desaturase; FA, fatty acid; FAS, FA synthase; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HL, hepatic lipase; HSL, hormone-sensitive lipase; IR, insulin resistance; LC-CoA, long-chain acyl-CoA; LCFA, long-chain FA; LDL, low-density lipoprotein; LPL, lipoprotein lipase; MS, metabolic syndrome; MUFA, monounsaturated FA; NEFA, non-esterified FA; NPY, neuropeptide Y; P13K, phosphoinositide 3-kinase; PL, phospholipid; PPAR, peroxisome-proliferator-activated receptor; PUFA, polyunsaturated FA; SFA, saturated FA; TAG, triacylglycerol; Tri-C, triacsin C; VLDL, very-LDL.

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during lipolysis, TAGs are hydrolysed in a reaction catalysed by HSL (hormone-sensitive lipase), which is, in turn, regulated by numerous factors and hormones. Catecholamines, by binding to β-adrenergic receptors, stimulate lipolysis, whereas insulin inhibits this process [1].

TAG accumulation is enhanced by the preferential channelling of nutrients into adipose tissue, rather than into muscle or other tissues for fairly immediate oxidation. Overall, it would seem reasonable to assume that alterations which limit lipolysis and oxidation of FAs, and those which stimulate lipogenesis (the two processes are often linked), are the cause of, or at least are related to, obesity [2–4]. A number of published findings point to a link between human obesity and genetic defects affecting the lipolytic pathway [5]. Oxidation of FAs depends on the availability of substrates and on co-adjuvant mechanisms for their transport into mitochondria. In obese subjects with a predominance of visceral fat, increased lipolytic activity triggers the release of NEFAs, prompting the subsequent hormonal and metabolic changes observed in obesity, such as hyperinsulinaemia, hypoadiponectinaemia and increased fibrinogen and PAI-1 (plasminogen-activator inhibitor) levels, and impaired haemostasis due to increased apoB (apolipoprotein B), decreased apoA-I (apolipoprotein A-I) and hypertriglyceridaemia [6]. However, as stated by Smith et al. [7], sustained changes in lipolysis could never occur dissociated from sustained changes in synthesis. Otherwise, the mass of TAGs within the adipocyte would soon be depleted.

Obesity is associated with the so-called MS (metabolic syndrome) [6] characterized by hyperinsulinaemia and peripheral resistance to the action of insulin, glucose intolerance or Type 2 diabetes, hypertriglyceridaemia, decreased HDL-C [HDL (high-density lipoprotein) cholesterol] and other changes associated with risks of cardiovascular disease, such as arterial hypertension [8–10]. IR (insulin resistance) would appear to be the major common finding in subjects with obesity, glucose intolerance or Type 2 diabetes, hypertension or dyslipidaemia, and it has been claimed to be the initial factor triggering a metabolic cascade which is also influenced by genetic and environmental factors [8,11–13]. The combination of IR and hyperinsulinaemia greatly increases the likelihood of developing a cluster of closely related abnormalities, and the resulting clinical diagnoses can be considered to make up the IR syndrome [8].

Findings common to central obesity and the MS include, in addition to increased VLDL and indeed TAG, the presence of small-dense LDL particles, increased apoB (apolipoprotein B), decreased apoA-I (apolipoprotein A-I) and impaired haemostasis due to increased fibrinogen and PAI-1 (plasminogen-activator inhibitor) [14–17]. Likewise, the cholesterol content of HDL declines, whereas that of VLDL and LDL increases [18].

The present review focuses on some of the major alterations in plasma lipids in obesity and their involvement in the MS, and also addresses the main cellular lipid alterations, especially FA oxidation, occurring in liver, adipose tissue and muscle tissue. Attention is also paid to the LCFA (long-chain FA)-mediated mechanisms by which the hypothalamus controls energy homoeostasis, and to alterations linked to obesity and the MS.

PLASMA LIPOPROTEINS AND THE MS

The MS can best be explained by viewing abdominal adipose tissue as an endocrine organ that releases excess NEFAs and adipokines into the circulation. First, increased blood NEFAs inhibit the uptake of glucose by muscle [19]. Although the pancreas manufactures extra insulin, there is not enough to counter the hyperglycaemia, thus explaining the paradox of fasting hyperglycaemia despite increased plasma insulin levels, which is known as IR [20]. Hyperglycaemia and increased circulating NEFAs provide the correct substrates for increased manufacture of TAGs by the liver [21]. Release of NEFAs by adipocytes is greater in central obesity than in lower-body obesity, with no concomitant increase in oxidation by peripheral tissues [22,23]. The ‘portal theory’ posits one of the major mechanisms behind the dyslipidaemia, which is the increased flux of NEFAs from adipose tissue to the liver via the portal vein when visceral TAG stores are increased, which is related to IR and the lack of inhibition of HSL [24]. Individuals afflicted by the MS are often viscerally obese and insulin-resistant. Under these circumstances, the failure of insulin to suppress HSL stimulates the release of NEFAs from lipolytically active visceral fat. This increased flux of NEFAs, channelled to the liver via the portal circulation, stimulates hepatic TAG synthesis, apoB100 formation and, ultimately, the assembly and secretion of VLDL [25]. NEFAs promote increased TAG synthesis in the liver, which can lead to the secretion of VLDL (Figure 1).

In peripheral tissues, VLDL particles are exposed to LPL, which hydrolyses the TAG of VLDL particles, generating NEFAs. Under normal conditions, these NEFAs are taken up by muscle and adipose tissue for energy use or storage. The resulting remnant particles are hydrolysed further by HL (hepatic lipase) to form LDL. In contrast, in individuals afflicted by the MS, the failure of insulin to activate LPL favours the accumulation of TAG-rich lipoproteins in the circulation [23,26], which in turn enhances the exchange of TAG from TAG-rich lipoproteins to LDL and HDL, with a reciprocal transfer of cholesteryl esters to TAG-rich lipoproteins. TAG-enriched cholesteryl ester-depleted LDL are better substrates for HL-mediated TAG lipolysis, which in turn leads to the formation of small-dense LDL (Figure 2). Mechanistically, small-dense LDL particles enter the arterial wall more easily, bind to arterial wall proteoglycans more avidly and are highly susceptible to oxidative modification, leading to macrophage uptake, all of which may contribute to increased atherogenesis [27].
Elevated LC-CoA (long-chain acyl-CoA) tissue levels are involved in the increase in plasma TAG concentrations associated with IR. In the liver, LC-CoA stimulates the synthesis of TAG-rich lipoproteins, whereas in peripheral tissue they reduce plasma lipoprotein clearance via inhibition of LPL [28]. Elevated plasma apoC-III concentration is also a feature of dyslipidaemia in the MS that contributes to the kinetic defects in apoB metabolism [29]. ApoC-III is a protein that inhibits LPL, favouring the accumulation of TAG-rich lipoproteins. (Figure 2). A recent study has shown that some polymorphisms in the APOC3 and LPL genes might have a small effect on apoB levels in the Central European Caucasian population with dyslipidaemia associated with the MS [30].

A low HDL-C level is even more common in patients with IR than hypertriglyceridaemia. Two mechanisms explain why increased plasma TAG is almost always associated with reduced HDL levels: (i) CETP (cholesterol ester transfer protein) mediates the transfer of cholesterol from HDL to the apoB-containing lipoproteins; and (ii) enzymes, such as HL and endothelial lipase, are up-regulated in the MS and, therefore, promote hypercatabolism of HDL, which leads to the generation of small-dense LDL particles and a decrease in HDL2-C [28] (Figure 2).

The ‘portal theory’ proposes a link between visceral adipose tissue to IR and the MS and is based on the direct effects of NEFAs on the liver. Intra-abdominal tissue...
In the liver, NEFAs (FFA) provide enough energy for glucose production. In addition, high levels of NEFAs are toxic to pancreatic β-cells and promote insulin resistance in muscle. Adipocytes are much more insulin-resistant than their subcutaneous counterparts, suggesting, as we commented before, that NEFA delivery to the liver via the portal vein is increased when visceral TAG stores are increased [24]. Elevated NEFA levels increase hepatic gluconeogenesis and lower peripheral tissue glucose uptake, prompting a further increase in the hyperinsulinaemia typically found in the MS [31,32] (Figure 3). Studies involving healthy adult volunteers undergoing short-term starvation have shown that NEFAs inhibit the degradation, but not the synthesis, of hepatic glycogen and stimulate gluconeogenesis [31]. Moreover, it was found in the early 1960s that NEFAs restrain glucose use in muscle, as increased production of acetyl-CoA in muscle tissue mitochondria inhibits pyruvate dehydrogenase, a glucose-oxidation-limiting enzyme [13]. Although certain NEFA levels are believed to stimulate insulin secretion, elevated levels are reported to have few effects on in vivo insulin secretion [33]. It has been reported previously that NEFAs interfere with insulin signalling, thereby stimulating various PKC (protein kinase C) isoforms, which block cellular insulin signalling mechanisms and inhibit glucose transport [34]. Moreover, an inverse correlation between PI3K (phosphoinositide 3-kinase) activity, a key enzyme in the insulin signalling cascade, and plasma NEFA concentrations has been reported [35]. Also, it has been shown that elevated intracellular LC-CoA levels are associated with IR, and that these compounds are the equivalent of NEFAs at the intracellular level [36,37]. Accumulation of free radicals derived from mitochondrial oxidation of LC-CoA gives rise to endothelial dysfunction and the gradual decline in insulin production by β-cells [38,39]. Functional defects in pancreatic β-cells have been identified even prior to diabetes diagnosis, especially in individuals with central obesity and the MS. β-Cell regulation is governed by central processes and by signals such as NEFAs. Elevated NEFA concentrations are toxic to pancreatic β-cells, inducing their apoptosis, accelerating pancreas failure and favouring progression to diabetes [24,40]. In some cases, increased insulin secretion aimed at maintaining blood sugar levels within a normal range may prompt a functional decrease in β-cells, leading eventually to irreversible failure [41] (Figure 3).

**CHANGES IN PLASMA AND TISSUE FA COMPOSITION AS RELATED TO THE MS**

The serum FA composition has been shown to predict the risk of diabetes and cardiovascular disease, and has been closely related to components of the MS. Prospective studies are, however, needed to investigate the role of FA composition in the development of the MS. A number of studies in both animal obesity models and adult obese humans report changes in FA composition in plasma.
as well as in liver, muscle and adipose tissue lipids. Higher levels of SFAs (saturated FAs) and lower levels of \( n-3 \) PUFAs (polyunsaturated FAs) are associated with obesity in the MS [42–47].

Rossner et al. [42] reported that, in obese patients, the most marked differences were decreased relative contents of \( \text{C}18:2\n−6 \) (\( \gamma \)-linoleic acid) in serum TAGs, PLs (phospholipids) and CE\( \text{s} \), whereas \( \text{C}18:3\n−3 \) (linolenic acid) was decreased in TAGs and CE\( \text{s} \), with reciprocal increases in \( \text{C}16:0 \) (palmitic acid) and \( \text{C}16:1\n−7 \) (palmitoleic acid) in TAGs and CE\( \text{s} \). Similarly, an increased proportion of \( \text{C}16:0 \) and a low proportion of \( \text{C}18:2\n−6 \) have been reported in obese subjects with the MS [46]. Interestingly, the proportion of \( \text{C}18:2\n−6 \) in the TAG fraction has been positively associated with plasma fasting insulin and DBP (diastolic blood pressure) and SBP (systolic blood pressure). On the other hand, the proportion of \( \text{C}18:3\n−3 \) has been associated negatively with apoB concentrations and positively with LDL diameter, whereas the proportion of \( \text{C}18:3\n−6 \) was associated negatively with plasma TAG, DBP, SBP and plasma fasting insulin and positively with HDL-C and LDL diameter [47]. A study by Klein-Platat et al. [48] in overweight adolescents found similar correlations between FA composition and some MS-related parameters. In that study [48], normal-weight adolescents had lower SFA levels in PLs and CE\( \text{s} \), and higher \( \text{C}22:5\n−3 \) (docosahexaenoic acid) levels than obese adolescents, who also had an increase in \( \text{C}16:1\n−7 \), attributed to de novo lipogenesis. In obese subjects, the PUFA/SFA ratio and \( \text{C}18:2\n−6 \) levels were associated positively with HDL-C, whereas the PUFA/SFA ratio in PLs and CE\( \text{s} \) was associated inversely with IL-6 (interleukin-6). \( \text{C}18:2\n−6 \), \( \text{C}20:5\n−3 \) (eicosapentaenoic acid) and CE\( \text{s} \) were also inversely related to CRP (C-reactive protein). This would suggest that a high intake of \( n-3 \) PUFAs may protect obese subjects against the MS and inflammation associated with obesity [49]. Moreover, lower proportions of the PL \( \text{C}20:4\n−6 \) (arachidonic acid) have been reported in serum from obese humans and in liver from obese Zucker rats, implying impaired D6D (\( \Delta^6 \) desaturase) activity or, alternatively, an abnormality in the catabolism or tissue distribution of this FA in obesity [43,44].

Changes in tissue FA composition largely reflect changes in plasma PL composition. However, there may be site-specific differences in FA composition of adipose tissue. A study in obese adults found that SFAs were higher and MUFAs (monounsaturated FAs) perivisceral than in subcutaneous fat. Moreover, central obesity was positively associated with \( n-6 \) PUFAs and inversely associated with \( n-3 \) PUFAs, which in turn had a negative correlation with apoB and TAG. Thus adipose tissue composition may govern alterations in certain obesity risk biomarkers [50]. Certain changes in the composition and endogenous synthesis of tissue FAs may predict the development of the MS. High activity of SCD-1 (stearoyl-CoA desaturase 1) and D6D, and low activity of D5D (\( \Delta^5 \) desaturase), have been associated with the MS [51]. Thus, although the quantity and quality of dietary fat clearly contribute to changes in FA composition, other hormonal or genetic factors may also be involved.

Given the fact that individuals with MS features might benefit more from a diet that is low in carbohydrate, but with a greater proportion of fat, the qualitative composition of dietary fat may be of importance for individuals with the MS. Diets rich in FAs, mainly SFAs and trans-FAs, as well as carbohydrate-rich diets, favor an acute increase in IR, independent of adiposity [52]. In addition, \( \text{C}18:2\n−6 \) and \( \text{C}22:4\n−6 \), which are consumed in relatively higher amounts in Western diets, may contribute to IR in adulthood [53]. Moreover it has been suggested that dietary \( n-3 \) PUFAs, although they do not appear to influence IR, due to the anti-inflammatory and anti-atherogenic properties, may benefit individuals with the MS [54].

### CELLULAR FA OXIDATION AND THE MS

#### Role of adipokines

Ravussin and Smith [55] have put forward an alternative to the classical paradigm or ‘portal hypothesis’ to explain the features of the MS. The ‘endocrine’ paradigm, developed in parallel with the ectopic fat storage syndrome hypothesis, posits that adipose tissue secretes a wide variety of endocrine hormones and adipocytokines that regulate energy metabolism and, especially, lipid metabolism. From this viewpoint, adipose tissue plays a critical role as an endocrine gland, affecting the functions of distant organs including the CNS (central nervous system), skeletal muscle and liver. Hormone changes may thus precede any change in metabolites such as NEFAs or plasma glucose.

Leptin is currently considered the major liporegulatory hormone [56], maintaining normal intracellular lipid homoeostasis in the same way that insulin is required for glucose homoeostasis. Almost 10 years ago, leptin was identified as the circulating hormone that informs the brain about the abundance of body fat, thus enabling food intake, metabolism and endocrine physiology to match the body’s nutritional status [57,58]. It has been suggested that leptin promotes weight loss by suppressing appetite and stimulating metabolic activity. In theory, however, there appear to be sound reasons for doubting that its primary function is to prevent obesity. In the first place, there is little evidence to show that the pressures to which humans have been subjected in the course of evolution were aimed at preventing an accumulation of body fat. In fact, evidence appears to suggest the reverse, that increased body fat might be seen as a survival mechanism or a defence against periods of scarcity or famine [59].
It appears that the primary function of leptin, the protein product of the ob gene, is not to prevent obesity by acting on the hypothalamus, but to avoid metabolic damage to non-adipose tissues by allowing body fat to accumulate in fat cells during excess caloric intake through a direct effect on leptin receptors, as adipocytes are the only cells adapted to this purpose. This points to the critical role of leptin as an antisteatotic hormone [60,61].

If it were not for this normal and physiological antisteatotic activity of leptin, surplus FAs during excess caloric intake would flood non-adipose tissues, mainly pancreatic islet β-cells and heart/muscle cells, causing organ dysfunction, lipotoxicity and lipopoptosis. Just as insulin regulates tolerance of a carbohydrate-rich diet by directing glucose to the relevant target cells, leptin increases tolerance of a fat-rich diet, protecting key non-adipose tissues against potential lipotoxicity by increasing FA oxidation [62]. Research suggests that, at the molecular level, the end product of the HPA (hypothalamic–pituitary–adrenal) axis (cortisol) is the major local factor involved in inducing the expression of the so-called leptin-resistance factors, which inhibit leptin signalling in non-adipose tissues (muscle, liver, β-cells, heart and kidney) [63]. Indeed, tissue steatosis appears to be reverted or prevented by appropriate leptin signalling [64,65].

Subcutaneous adipose tissue synthesizes more leptin and displays a greater affinity for insulin, exerting greater antilipolytic effects; visceral adipose tissue is more metabolically active, has a higher rate of TAG turnover, releases more NEFAs, is more insulin-resistant and has more adrenergic, androgenic and glucocorticoid receptors. Thus impaired lipid metabolism may be an early manifestation of IR and the MS, subsequently giving rise to changes in blood sugar levels [66,67].

Leptin, by binding to its cell-membrane receptor OB-R, induces the phosphorylation of a protein known as STAT-3 (signal transducer and activator of transcription-3) which, when activated, penetrates the nucleus and regulates the transcriptional activity of leptin-controlled genes. Leptin therefore down-regulates the activity of lipogenic transcription factors, mainly PPAR-γ2 (peroxisome-proliferator-activated receptor-γ2) and, in liver cells, the sterol-regulatory-element-binding protein SREBP-1c [4]. It thus induces decreased expression of the lipogenic enzymes ACC (acetyl-CoA carboxylase) and FAS (FA synthase), and increased expression of key enzymes involved in FA oxidation, such as ACO (acyl-CoA oxidase) and CPT-1 (carnitine-palmitoyl transferase-1), particularly in adipose tissue. At the same time, leptin activates AMPK (AMP-activated protein kinase) which, in turn, inactivates ACC, an enzyme involved in the initial phase of TAG and FA synthesis, by phosphorylation and blocks its expression, lowering the formation of malonyl-CoA. This is the key to the antisteatotic effect of leptin. Inhibition of malonyl-CoA formation, in turn, activates expression of CPT-1, thus prompting adequate mitochondrial FA oxidation [21,68] (Figure 4). Leptin also enhances the intracellular expression of PGC-1α (PPAR-γ co-activator 1α), thus increasing mitochondrial enzyme activity for FA oxidation and mitochondrial biogenesis. In cases of leptin resistance, as it occurs in obesity and the MS, AMPK fails to inhibit ACC, leading to overexpression of malonyl-CoA and increased synthesis of TAGs and FAs, and simultaneous blocking of FA oxidation through inhibition of CPT-1 [62,68].

Studies involving incubation of muscle cells with leptin have shown that leptin stimulates FA oxidation in skeletal muscle by activating AMPK, which leads to the activation of CPT-1, thus enhancing the access of FAs to mitochondria [68]. Early activation of AMPK occurs by leptin acting directly on muscle, whereas later activation depends on leptin functioning through the hypothalamic–nervous system axis [21,69,70], as discussed below.

Adiponectin is another peptide hormone secreted in large amounts by adipocytes, which also displays clear antisteatotic activity in non-adipose tissues, together with major insulin-sensitizing, anti-atherogenic and anti-inflammatory properties [71–73]. Plasma concentrations are inversely correlated with the amount of body fat in obesity, Type 2 diabetes and, in general, in all states characterized by IR, including coronary heart disease [74]. Plasma adiponectin levels correlate negatively with BMI (body mass index), insulin and TAG levels, and positively with HDL-C, in obese adults [75]. Adiponectin also enhances whole-body insulin sensitivity by increasing FA oxidation, prompting a decline in circulating FA levels as well as in muscle and liver TAGs [76] (Figure 5).

Adiponectin stimulates insulin receptor tyrosine kinase activity by activating oxidative phosphorylation mediated by UCPs (uncoupling proteins). Adiponectin-knockout mice fed on a carbohydrate-rich diet develop IR and display impaired PI3K activity [77]. Like leptin, adiponectin also activates AMPK, stimulating glucose utilization and FA oxidation [(21,78–80), but see [79a]] (Figure 4). Administration of full-length or globular adiponectin in mice increases AMPK-dependent phosphorylation in skeletal muscle; in the liver, this activation is achieved only with the full-length form [81]. As indicated above, AMPK activation prompts an increase in acyl-CoA oxidation. The adiponectin receptors adipor1 and adipor2 have been identified and cloned in muscle and liver cells respectively; expression of adipor1 is associated with increased phosphorylation of AMPK, ACC and p38 MAPK (mitogen-activated protein kinase), whereas, in liver cells, there is an increase in phosphorylation of AMPK and ACC. Adiponectin also increases the activity of PPAR-α, a transcription factor.
Figure 4 Regulation of leptin- and adiponectin-mediated cellular FA oxidation
Leptin and adiponectin activate AMPK, which, in turn, inactivates ACC by phosphorylation and blocks its expression, lowering the formation of malonyl-CoA. Inhibition of malonyl-CoA formation, in turn, activates CPT-1, thus prompting adequate mitochondrial FA oxidation. P, phosphorylation; TG, TAG.

expressed in the liver, which plays an essential role in regulating FA oxidation ([21,79], but see [79a]).

Role of LCFAs in the hypothalamus
LCFAs in certain areas of the hypothalamus may act as a sensor for nutrient availability, integrating multiple hormonal and nutritional signals. LCFAs are transported through the bloodstream bound to albumin, or packaged into chylomicrons or other lipoproteins. LCFAs can cross the blood–brain barrier by simple diffusion and, once inside the cell, are transformed into CoA esters (LCFA-CoA) by ACS (acyl-CoA synthetase). Intracellular LCFA-CoA content depends on the amount formed and its use, either for lipid biosynthesis or for mitochondrial $\beta$-oxidation. This latter process requires CPT-1 to transport LCFA-CoA into the mitochondria. $\beta$-Oxidation is regulated by the availability of malonyl-CoA, a potent CPT-1 inhibitor. Malonyl-CoA derives largely from acetyl-CoA, which is, in turn, the end-product of glycolysis [37,81], and, by this means, cell lipid and carbohydrate availability are controlled. As indicated above, malonyl-CoA formation from acetyl-CoA is catalysed by ACC, which is allosterically inhibited via phosphorylation of AMPK.

Circulating lipids, particularly LCFAs, may regulate appetite and glucose production by prompting an increase in intracellular LCFA-CoAs in the hypothalamus, and the alteration of this homeostatic mechanism may be related to central obesity and the MS. LCFA-CoAs appear to initiate a hypothalamic satiety signal by activating neuronal pathways to reduce food intake and glucose production. In a study carried out in baboons, intravenous injection of a lipid emulsion was sufficient to reduce food intake; circulating lipids (TAGs, glycerol and LCFAs) prompted a satiety signal, regardless of changes in plasma insulin levels or intestinal absorption of nutrients [82]. Intracerebroventricular administration of C18:1$\text{c}^{-9}$ (oleic acid) markedly inhibits food intake and liver glucose production. Administration of this LCFA also inhibits the hypothalamic expression of orexigenic peptides, such as NPY (neuropeptide Y) and AgRP (agouti protein), and the expression of glucose-6-phosphatase in the liver [83,84]. Interestingly, administration of the short-chain FA C8:0 (octanoic acid), which does not require CPT-1 for mitochondrial access and subsequent oxidation, fails to reproduce the potent effect of intracerebroventricular C18:1$\text{c}^{-9}$ [85].

Elevation of circulating LCFAs may duplicate the hypothalamic LCFA-CoA pool, thus creating a satiety signal. This hypothesis is borne out by the finding that the increase in LCFA-CoA can be inhibited by intrahypothalamic infusion of the pharmacological
ACS inhibitor Tri-C (triacsin C); moreover, central administration of Tri-C prevents circulating LCFAs from restricting liver glucose production [86]. It may be concluded that hypothalamic accumulation of LCFA-CoA, rather than delivery of FAs to mitochondria for β-oxidation, constitutes the first hypothalamic signal leading to the inhibition of food intake and glucose production (Figure 6). Therefore the availability of cellular LCFA-CoA in the hypothalamus may modulate these hypothalamic signals, so that either genetic or biochemical inhibition of CPT-1 will increase hypothalamic LCFA-CoA levels while decreasing the expression of peptides NPY and AgRP, leading to the eventual inhibition of food intake and diminished liver glucose production [87].

AMPK plays a major role in hypothalamic mechanisms for controlling energy homeostasis. A number of studies have shown that hypothalamic AMPK may regulate food intake. Manipulation of hypothalamic nucleus activity in rats has proven sufficient to alter feeding behaviour [69]. Leptin, like other anorexigenic compounds such as insulin and C-75, blocks AMPK activity in the hypothalamus, whereas the orexigenic hormone ghrelin increases AMPK expression [70]. Although little is known about the mechanisms by which hypothalamic inhibition of AMPK prompts decreased expression of orexigenic peptides (NPY and AgRP) leading to reduced food intake, it would appear that decreased AMPK activity gives rise to an increase in hypothalamic malonyl-CoA levels through activation of ACC. Increased malonyl-CoA levels might inhibit CPT-1 activity, and thus inhibit food intake due to the accumulation of LCFA-CoA [83,85] (Figure 6). AMPK may also modulate hypothalamic transcription of neuropeptides, regardless of its effect on ACC or malonyl-CoA [70]. Leptin resistance, as occurring in obesity and the MS, would contribute to a lack of inhibition of food intake through AMPK.

FAS is an enzymatic complex required for FA synthesis that utilizes malonyl-CoA. Double fluorescence in situ has shown that FAS co-localizes with NPY in neurons in the arcuate nucleus, thus demonstrating its presence in the hypothalamus and its role in modulating food intake at neuronal level [88]. Two FAS inhibitors, cerulenin and C-75, have been shown to reduce food intake and body weight in rodents [89,90]. Moreover, central administration of C-75 in fasted mice increases hypothalamic levels of malonyl-CoA and blocks the expression of fasting-induced NPY and AgRP [91]. The increase in malonyl-CoA levels prompted by C-75 appears to take place through two mechanisms: (i) accumulation of substrate due to FAS or (ii) inhibition of AMPK activity, giving rise to increased conversion of acetyl-CoA into malonyl-CoA by ACC [91].
CONCLUSIONS AND FUTURE DIRECTIONS

Central obesity is the main cause of the MS, which, in turn, is associated with numerous alterations in plasma lipids and tissue lipid metabolism. In the liver, increased synthesis of VLDL and thus of TAG is accompanied by decreased HDL synthesis. This, in conjunction with decreased TAG clearance by peripheral tissues, leads to increased plasma TAG levels. There are also changes in plasma FA profiles, with a decrease in \( n-6 \) and \( n-3 \) PUFAs relative to SFAs and an increase in \( C_{16:1n-7} \) due to endogenous synthesis. At the same time, obesity and MS-related hormonal changes, including increased insulin synthesis and peripheral IR, increased leptin synthesis and decreased adiponectin synthesis by adipose tissue, lead to diminished FA oxidation. AMPK is a key enzyme in regulating FA metabolism not only in adipose and muscle tissue, but also in the hypothalamus, where it appears to play a critical role in regulating energy homoeostasis. Further research is needed to establish the relationship between hormones, adipokines and lipids in the development of obesity and the MS. Findings emerging from other studies about the behaviour of lipid metabolism and adipose tissue could modify the future evolution and treatment in obesity and the MS.

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