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Ceramides as the molecular link between impaired lipid metabolism, saturated fatty acid intake and insulin resistance: are all saturated fatty acids to be blamed for ceramide-mediated lipotoxicity?

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Abstract

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that has reached epidemic proportions worldwide, posing a huge treat on people's health and quality of life. From a pathogenetic prospective, T2DM is driven by insulin resistance defined as a blunted response of tissues to insulin which leads to chronic hyperglycaemia. Mechanistically, lipotoxicity and particularly the intracellular accumulation of ceramides in the skeletal muscle and the liver, is a primary metabolic aberration underpinning insulin resistance. Indeed, intracellular ceramide accumulation can hamper insulin signal transduction pathway thereby promoting insulin resistance. This review will provide an updated overview of the metabolic defects underlaying ceramide buildup and the molecular mechanism by which ceramides imping upon insulin signalling. Additionally, the role of specific ceramide subspecies as potential biomarkers for T2DM and the role of both long- and medium-chain saturated fatty acids as a modulator of ceramide metabolism will be discussed.

Key words: ceramides: insulin resistance: lipotoxicity: long-chain saturated fatty acids: medium-chain saturated fatty acids: type 2 diabetes mellitus

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Introduction

Obesity represents a global health pandemic affecting both developed and, increasingly, developing countries[1]. The burden posed by obesity on people's health and the health care systems worldwide is a direct consequence of its life-threatening comorbidities. Indeed, obesity is not to be merely considered as an abnormal excess of body fat, instead the excess of body weight and the overexpansion of the adipose tissue trigger a plethora of pathophysiological derangements that disrupt cardiometabolic health[2]. In support of this, obesity is closely associated with the onset of type 2 diabetes (T2DM)[3], cardiovascular diseases[4], non-alcoholic fatty liver disease[5], certain types of carcer[6] and mental health issues[7].

In terms of its pathophysiology, obesity arises from a long-term positive energy balance which is the direct consequence of energy overconsumption, fostered by the ready availability of cheap, highly palatable, nutrient-dense foods and physical inactivity[8–11]. These are two pivotal paradigms which have been fuelling obesity and its comorbidities in high-income countries and are now spreading to low- and middle-income countries due to rapid behavioural and cultural changes prompted by the pressure of globalisation[12]. Remarkably, obesity develops despite the human subject's body being equipped with a sophisticated and finely tuned centre for the control of energy balance, located in the hypothalamus, which is tasked to match energy intake and expenditure to one another to maintain body weight within a tight range[13]. This is achieved by the ability of the hypothalamus, and particularly the arcuate nucleus, to sense peripheral hormonal, nervous and nutritional cues related to the nutritional status and mount physiological responses to maintain energy homoeostasis[13–16]. Nonetheless, this homoeostatic system appears to fail in obesity, thereby predisposing to weight and adiposity gain[17]. From a nutritional perspective, long-chain saturated fatty acids and sugar represent the primary nutritional culprits in promoting hypothalamic dysfunction[18,19].

Thus, obesity arises as a consequence of nutrient oversupply, with this energy excess being stored in the adipose tissue in the form of triglycerides. The adipose tissue triglycerides represent an energy reservoir to be used in response to fasting or increased energy demand, as in the case of prolonged physical activity. In particular, the adipose tissue releases free fatty acids as a result of triacylglycerols lipolysis which, in turn, is a finely tuned process under the control of hormonal, nervous and nutritional inputs[20]. Therefore, under physiological conditions, this allows the correct matching of free fatty acid supply to and demand of extra adipose tissues. However, this tight physiological regulation of lipid metabolism becomes impaired in obesity, with a mismatch between fatty acid supply to metabolically active tissues and their ability to catabolise them[21–24]. In this regard,

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adipose tissue dysfunction represents a pivotal driver of free fatty acid oversupply to metabolically active tissues such as the skeletal muscle and liver. Indeed, dysfunctional adipocytes are characterised by insulin resistance with promotes the disinhibition of lipolysis leading to an uncontrolled release of free fatty acids from the adipose tissue[25]. Uncontrolled fatty acid spill over from the adipose tissue and fatty acids derived from chylomicrons and VLDL, in the face of impaired fatty acid oxidative capacity and reduced energy demand, promote the ectopic accumulation of lipid in tissue not suited for lipid storage, namely skeletal muscle[26], liver[27], pancreas[28] and heart[29], a phenomenon termed lipotoxicity[30]. Lipotoxicity, in turn, has been proposed as one of the key effectors underlaying the link between obesity and its comorbidities, therefore representing a putative target to improve cardiometabolic health[31]. However, not all the lipid species appear to be detrimental when accumulating in non-adipose tissues. In keeping with this, while triacylglycerols intracellular accumulation does not appear to promote insulin resistance[32,33]; the opposite is true for ceramides which have been shown to hamper insulin signalling and impair insulin sensitivity[34–37].

Saturated fatty acids have been widely proposed as a pivotal metabolic culprit in fostering obesity[18] and insulin resistance[38], with ceramides being one of the potential mediators driving the metabolically detrimental effects of these dietary fatty acids[36,39]. In light of this, the aim of this review is to provide an overview on the molecular mechanisms underpinning the effects of ceramides on insulin resistance as well as their role as an innovative biomarker for T2DM. Additionally, this manuscript will discuss the role of saturated fatty acid quality in the modulation of ceramide metabolism.

Ceramide biosynthesis and breakdown

Ceramides are the precursor of complex sphingolipids that are integral components of cell membranes. Cellular ceramide content is regulated by hormonal, metabolic and inflammatory cues which include substrate availability, specifically palmitoyl-CoA, the CoA-derivate of the long-chain saturated fatty acid palmitic acid, pro-inflammatory cytokines, adiponectin and fibroblast growth factor 21 signalling[37,40]. Ceramide *de novo* synthesis takes place in the endoplasmic reticulum (ER) and begins with the condensation of palmitoyl-CoA and the amino acid serine catalysed by serin-palmitoyl transferase. The resulting product, 3-ketosphinganine, is then converted to sphinganine by 3-ketosphinganine reductase. The next step is represented by a *N*-acylation reaction mediated by a family of enzymes termed ceramide synthases, which encompasses six isoforms. In light of this, part of this step provides a wide variety of dihydroceramides differing in their acyl chain length are synthetised, ranging from 14 to 34 carbon atoms. The final step of the *de novo* ceramide synthesis is catalysed by dihydroceramide desaturases which introduces a 4,5-trans-double bond into the dihydroceramide molecules forming ceramides[37,41]. These enzymatic reactions, particularly those mediated by ceramide synthases, give raise to a variety of ceramide pool which potentially confer different pathophysiological roles to these

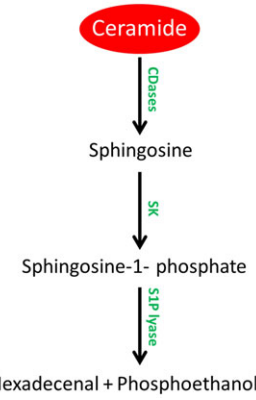

Fig. 1. Ceramide catabolic pathway. Ceramides can be catabolised by acid, neutral and alkaline ceramidases, as well as the intrinsic ceramidase activity of the adiponectin receptor. A ceramide is deacylated with the consequent formation of sphingosine which is phosphorylated by sphingosine kinases 1 and 2. The resulting sphingosine-1-phosphate, via the intervention of sphingosine-1-phosphate lyase, is finally cleaved with the release of hexadecenal and phosphoethanolamine. CDases, ceramidases; SK, sphingosine kinases; S1P lyase, sphingosine-1-phosphate lyase

sphingolipids and is most likely influenced by the availability of specific fatty acids as well as the ceramide synthase substrate preferences[42].

Ceramides can also be synthetised following alternative pathways, namely the catabolism of sphingomyelin and the salvage pathway. The former pathway generates ceramides via the sphingomyelinase-mediated catabolism of sphingomyelins[43], whereas the latter is based on the recycling of sphinganine derived from ceramidase-induced ceramide catabolism[44]. A further pathway responsible for ceramide synthesis, albeit less frequent, relies on the hydrolysis of glycosphingolipids by glycosidases[45]. Once synthetised, ceramide can be exported to the Golgi apparatus where head groups are added to the 1-hydroxyl position to produce ceramide-1-phosphate, sphingomyelin or glycoceramides as extensively reviewed elsewhere[46].

Beside their *de novo* synthesis and regeneration, the metabolism of ceramides also includes a catabolic branch (Figure 1). Indeed, ceramides can be breakdown by ceramidase-mediated diacylation leading to the formation of sphingosine, which, in turn, is converted to sphingosine-1-phosphate by specific cytosolic sphingosine kinases. Sphingosine-1-phosphate is finally broken down to hexadecenal and phosphoethanolamine by sphingosine-1-phosphate lyase localised in the ER (Figure 1)[43]. Ceramide degradation is mediated by ceramidases which in mammals include five proteins classified according to their pH optimum in acid, neutral and alkaline ceramidases[41]. Beside these classical ceramides, the adiponectin receptor has also been reported to possess intrinsic ceramidase activity which is induced by adiponectin binding[47,48].

The impact of ceramides on insulin resistance and the onset of type 2 diabetes mellitus

Ceramides are at the forefront of metabolic dysfunction integrating nutrient overload, particularly in the form of long-

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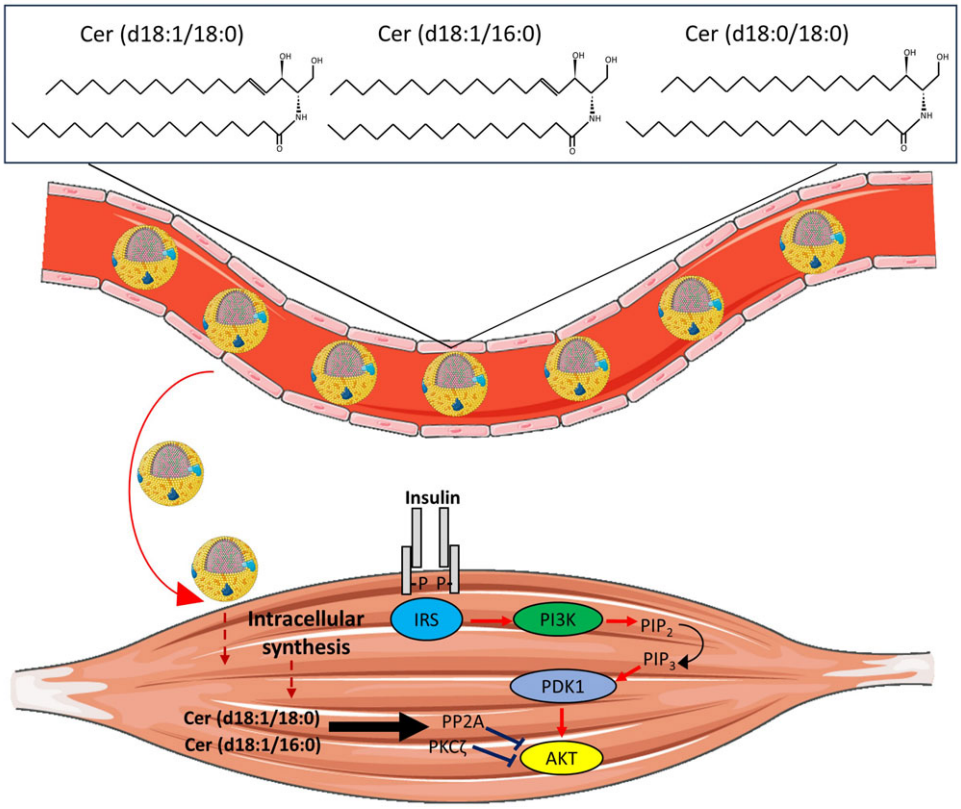

Fig. 2. Circulating ceramides and dihydroceramides packed in lipoproteins and their effect on insulin resistance. Circulating Cer(d18:1/16:0) and Cer(d18:1/18:0), as well as dihydroceramides, of which Cer(d18:0/18:0) is exemplified here, are up-regulated in individuals with insulin resistance and suffering from T2DM. Circulating ceramides packed in LDL and VLDL can target metabolically active tissues, such as the skeletal muscle, to promote insulin resistance along with intracellularly synthetised ceramides. Ceramides hamper intracellular insulin signal transduction by promoting protein kinase Cζ-mediated phosphorylation and inhibition of AKT. To a similar extent, the activation of protein phosphatase 2A by ceramides leads to the dephosphorylation and inhibition of AKT. This figure was created using smart.servier.com

chain saturated fatty acids, obesity and insulin resistance. Indeed, these lipotoxic lipid species have been reported to disrupt insulin signal transduction pathway and promote insulin resistance[36,39], trigger metabolic inflammation[40,49,50] and disrupt energy and glucose homeostasis by inducing hypothalamic dysfunction[18,51], which not surprisingly are all pivotal features of obesity.

From a mechanistic perspective, ceramides have been shown to hamper insulin signalling via different mechanisms. The first relies on ceramide-induced activation of protein kinase Cζ which, by phosphorylating AKT/protein kinase B on a regulatory site of Pleckstrin homology domain, inhibits its translocation to the plasma membrane[52,53] (Figure 2). On the contrary a further mechanism of ceramide-induced insulin resistance involves the activation of protein phosphatase 2A and the subsequent dephosphorylation and inactivation of AKT/protein kinase B[54] (Figure 2). Additionally, ceramides are able to impair insulin signalling by targeting the insulin receptor substrate. This effect is mediated by ceramide-induced activation of the double-stranded RNA dependent protein kinase – c-jun N-terminal kinase (JNK) axis which culminates with the phosphorylation of insulin receptor substrate on serine 307 and its inhibition[55]. Finally, ceramides are also able to disrupt insulin signal transduction pathway by upregulating the transcription factor Pbx-regulating protein 1 and its cofactor p160 and favouring their interaction. In turn, Pbx-regulating protein 1–p160 interaction hampers insulin signalling leading to a decrease in glycogen synthesis and glucose uptake by L6 myotubes[56]. These mechanistic data are extensively supported by *in vivo* studies both in human subjects as well as animal models. Indeed, myriocin-mediated inhibition of serin palmitoyl transferase, the first and rate-limiting enzyme in the ceramide *de novo* synthesis pathway, improved systemic saturated fatty acid and dexamethasone-induced insulin resistance[57], as well as enhanced adipose tissue insulin sensitivity in rats fed a high-fat diet[58]. Furthermore, specifically targeting of ceramides 4,5-trans double bond in their sphingoid backbone by whole body or liver and/or adipose tissue dihydroceramide desaturase 1 knock-out countered insulin resistance and hepatic steatosis induced by either a high-fat diet or leptin deficiency, indicating that the deleterious effects ascribed to ceramides are not dependent of other lipid intermediary in the ceramide biosynthetic pathway[39]. Remarkably, the effects of the inhibition of ceramide production are not limited to insulin resistance, with myriocin preventing the onset of fasting hyperglycaemia, and the decline in β-cell mass in Zucker diabetic fatty rats[57]. Thus, apart from improving insulin sensitivity, the inhibition of ceramide synthesis also exerts a protective effect on pancreatic β cells, a further

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tissue target of lipotoxicity[59], thereby preventing the progression from prediabetes to full blown T2DM[57]. The protective effects of the inhibition of ceramide accumulation upon high-fat diet feeding does not appear to be tissue specific as similar improvements in insulin sensitivity were also reported upon specific inhibition of ceramide synthesis in skeletal muscle[60] or brown adipose tissue[61].

The evidence relative to the metabolic effects of ceramide in humans are limited by the impossibility to perform pharmacological or genetic manipulation to decrease ceramide synthesis as reported in animal models. However, this does not negate the metabolically detrimental effects of ceramide in humans. Indeed, circulating ceramides, particularly circulating C16:0 ceramide, correlated with insulin resistance assessed using the homoeostasis model assessment: insulin resistance[62]. This also held true relative to the accumulation of ceramide in metabolically active tissues. In agreement with this, an increase in ceramide levels in the adipose tissue correlated with hepatic lipid content and insulin resistance independently of obesity[63]. Furthermore, an increase in C16:0 ceramide and the up-regulation of ceramide synthase 6 was reported in the adipose tissue of obese individuals and positively correlated with adiposity, hyperglycaemia as well as insulin resistance assessed by euglycemic hyperinsulinemia clamps[61]. The buildup of ceramides has also been reported in the skeletal muscle and, not surprisingly, also in this case it correlated with insulin resistance[64–66]. Most importantly, lifestyle, such as physical activity, or pharmacological interventions (metformin and pioglitazone), as well as bariatric surgery apart from improving glycaemic control also resulted in a decrease in intramyocellular and circulating ceramide levels[66–69] supporting the notion that a decrease in muscular and circulating ceramide levels goes hand in hand with improved insulin resistance. However, the cause–effect relationship between ceramides and insulin resistance in humans remains to be fully elucidated. Indeed, it cannot be excluded that improved insulin sensitivity in response to the aforementioned interventions may be exquisitely dependent on improved lipid metabolism of which a decrease in ceramide levels may only be a read out. Furthermore, albeit unlikely, the improvement in insulin sensitivity may be a cause, rather than a consequence, of the decrease in tissue and circulating ceramide levels. Nonetheless, the animal studies described that fat provides sufficient support to the conundrum that ceramides represent a pivotal driver of insulin resistance.

Nevertheless, the fact that not all ceramides are created equal must not be overlooked. In line with this notion, the metabolically detrimental effects described above appear to be ceramide specific. Indeed, evidence from animal models suggest that lowering C18:0 ceramide by knocking out ceramide synthase 1 both within the skeletal muscle and globally improved glucose homoeostasis[60]. Nevertheless, lowering C16:0 ceramide by targeting ceramide synthase 5 and 6 in the skeletal muscle did not improve insulin sensitivity in high-fat-fed animals[60]. On the contrary, other evidence, always collected as part of animal studies, supported the role of C16:0 ceramide as being instrumental for the development of insulin resistance[61,70]. This was also corroborated in human subject studies reporting that in the adipose tissue, the *de novo* synthesis of C16:0 ceramide was a major driver of insulin resistance[71]. While the involvement of specific ceramide species in promoting insulin resistance remains a matter of debate, indistinctly lowering ceramide levels by dihydroceramide desaturase 1 knock out has metabolically beneficial effects of insulin resistance and hepatic steatosis[39].

Ceramide accumulation within the hypothalamus contributes to impaired peripheral metabolic health and obesity

The metabolically detrimental effects of ceramide accumulation are not limited to peripheral tissues, as described so far. Indeed, ceramide buildup has also been reported in the hypothalamus of rodents fed a high-fat diet leading to a deterioration of metabolic health[72]. Hypothalamic lipotoxicity, and particularly ceramide accumulation, is not without metabolic consequences. In support of this, direct hypothalamic infusion of C6 ceramide, a cell permeable ceramide, induced hypothalamic ER stress and inflammation[51], two pivotal mechanisms responsible for promoting insulin and leptin resistance, posing the pathogenetic basis for obesity and impaired glycaemic control[73–76]. As such, animals receiving intracerebroventricular C6 ceramide administration displayed a decrease in insulin sensitivity and developed obesity secondary to decreased energy expenditure and brown adipose tissue thermogenesis[51]. Remarkably, these effects were countered by inhibition of hypothalamic ER stress which, not only normalised body weight, but also reversed central ceramide-induced peripheral insulin resistance[51], supporting the notion that ER stress represents an effector of lipotoxicity. These effects were recapitulated in obese Zucker rats which are generally characterised by hypothalamic ER stress and ceramide accumulation. In these animals, inhibition of ER stress was sufficient to improve their metabolic phenotype marked by an improvement in insulin as well as leptin resistance, reduced body weight gain and hepatic steatosis[51]. In consideration of this, besides their role in insulin resistance, ceramides may also promote obesity. This was confirmed by the protective effect of ceramide synthase 6 knock out against high-fat diet-induced obesity[61]. Always in support of the metabolically detrimental role of ceramide accumulation in the hypothalamus, inhibition of its synthesis within the hypothalamus resulted in an improvement in insulin sensitivity, an effect that was also confirmed on cultured hypothalamic neurons[77]. Additionally, the inhibition of ceramide synthesis within the hypothalamus improved glycaemic control by enhancing glucose-stimulated insulin secretion and pancreatic β-cell mass[77]. Thus, ceramides appear to impair metabolic health and particularly insulin sensitivity by accumulating both in peripheral tissues and in the hypothalamus, with these effects being presumably dependent on the activation of protein kinase Cζ, protein phosphatase 2A, metabolic inflammation and ER stress[36,40,51,78]. Particularly, ER stress may be at the interface between lipotoxicity and metabolic dysfunction. However, ER stress may mediate the deleterious effect of ceramide in concert with inflammation given the cross-talk between these pathophysiological responses[79] and the ability of ceramides to trigger both[49,51]. Nevertheless, ER stress may not be responsible for the detrimental effects of ceramide on

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insulin resistance in *in vitro* skeletal muscle models. This possibility is supported by the fact that the inhibition of ER stress is not sufficient to rescue palmitic acid-induced impairment in insulin signalling in C2C12 and human myotubes[80,81].

As described thus far, ceramides represent a particularly strong candidate in linking obesity, dysfunctional lipid metabolism and insulin resistance. In addition, the inhibition of ceramide synthesis has been reported to exert beneficial effects on a full spectrum of obesity and insulin resistance-associated cardiometabolic aberrations, including metabolic dysfunction-associated steatotic liver disease, cardiomyopathy, atherosclerosis and hypertension[39,57,82–84]. In light of this, modulating lipid metabolism to counter ceramide synthesis represents a promising therapeutic strategy to improve cardiometabolic health.

Ceramides as a biomarker of insulin resistance and progression to type 2 diabetes mellitus

The deleterious effects of ceramides on skeletal muscle insulin signalling stem beyond their intracellular synthesis and may be dictated by plasma ceramides which are primarily derived from the liver in both rodents and humans[85] (Figure 2). These hepatic-synthetised ceramides travel in the blood stream packed in LDL and VLDL[86] (Figure 2) and have been reported to target the skeletal muscle to elicit insulin resistance[50]. Indeed, infusion of LDL containing C24:0 ceramide in lean mice induced an impairment in insulin signalling via AKT, thereby hampering insulin-mediated glucose uptake in skeletal muscle[50]. The ability of LDL-derived ceramides to elicit insulin resistance may also hold true in humans as the transport of ceramide in LDL is increased in obese and type 2 diabetic individuals and correlated with insulin resistance[50]. This notion is also supported by the fact that insulin resistance, assessed by homoeostasis model assessment: insulin resistance, is positively associated with saturated ceramides[87].Thus, an intracellular mismatch between fatty acid supply and oxidation may not be the only driver of ceramide accumulation, but these lipotoxic lipid species may also act in an endocrine fashion to target extra hepatic tissues as demonstrated for skeletal muscle[50]. In consideration of the ability of circulating ceramides to trigger insulin resistance and the positive correlation between circulating ceramides and the onset of insulin resistance[50,88], these sphingolipids are emerging as a predictive marker of insulin resistance and progression to T2DM. Additionally, total dihydroceramides were reported to be elevated up to 9 years prior to the diagnosis T2DM[89] as well as in obese and type 2 diabetic individuals[90] and are associated with the severity of metabolic dysfunction-associated steatotic liver disease[91], further supporting ceramide and the metabolites in its biosynthetic pathways as potential novel prognostic biomarkers of T2DM. Furthermore, not only serum dihydroceramides correlate with insulin resistance, but they are also able to impair insulin signalling in primary myotubes[90]. However, the ability of dihydroceramides to directly promote insulin resistance remains controversial[39]. This further confirms the implication of ceramide metabolism, particularly its anabolism, as a pivotal driver of insulin resistance and impaired glycaemic control, suggesting that defects in ceramide homeostasis may prevent the onset of overt T2DM.

Ceramides link fatty acid oversupply to insulin resistance

Energy overload is *a sine qua non* condition to shift ceramide synthesis beyond cellular physiological requirements as a precursor of complex sphingolipids[43] and promotes its metabolically detrimental accumulation[92]. Particularly, excess fatty acid supply represents a pivotal trigger for ceramide synthesis which takes place as a compensatory mechanism to buffer the excess fatty acid supply and prevent their accumulation and disruption of cellular the bilayer structure[92]. Fatty acids, upon entry into the cells, are activated via the esterification with a CoA molecule to generate acyl-CoAs whose metabolic fate is dictated by the cellular energy status. In the condition of negative energy balance, acyl-CoAs are shuttled into the mitochondria via the carnitine system to be β-oxidised to generate energy and heat. When energy needs are met, fatty acids are diverged towards the synthesis of triacylglycerols, the main form of energy storage in the body along with glycogen, and glycerophospholipids which are key components of cellular lipid bilayers. However, when fatty acid supply overcomes intracellular storage capacity, fatty acid excess is funnelled towards the synthesis of ceramides[92,93].

The effect of ceramides on lipid metabolism

Ceramide accumulation intracellularly is able to modulate lipid metabolism[92,93]. In support of this, ceramides can rewire lipid metabolism to increase fatty acid uptake and promote their storage in the form of triglycerides. In line with this, lowering ceramide levels in the liver or adipose tissue by overexpressing ceramidases in animal models resulted in a down-regulation in CD36[94], suggesting ceramide being able to increase fatty acid uptake. Similarly, knocking out dihydroceramide desaturase 1, thereby lowering ceramide content in the liver, was sufficient to dampen hepatic lipid intake[39]. This effect appears to be dependent upon ceramide-induced protein kinase Cζ activation as the overexpression of a dominant-negative form of this enzyme was sufficient to abrogate the effects of ceramides on fatty acid uptake[94]. Apart from increasing CD36-mediated fatty acid uptake, ceramides also increase fatty acid esterification via the up-regulation of sterol response element binding proteins and the consequent indication of genes involved in triacylglycerols synthesis[95]. Additionally, ceramides decrease the uptake of glucose[96] and amino acids[97,98], possibly to favour the catabolism of fatty acid as the main energy substrate, even though it is also true that C16:0 ceramide impairs mitochondrial function[70] and may therefore negatively impact fatty acid catabolism. In line with this, ceramides, and particularly C16:0 ceramide accumulation, have been shown to impair mitochondrial respiration and the activity of electron transport chains II[70] and IV[99] *in vitro*, leading to a decrease in β-oxidation as reported in cultured hepatocytes[70]. Always in agreement with their ability to affect lipid metabolism, ceramides inhibit isoproterenol-induced phosphorylation of adipocyte hormone sensitive lipase[39] with this effect being a potential mechanism by which these sphingolipids may counter further oversupply of NEFA to metabolically active tissues. Nevertheless, despite this appearing a protective mechanism to prevent fatty acid oversupply to liver and skeletal muscle for example, ceramide

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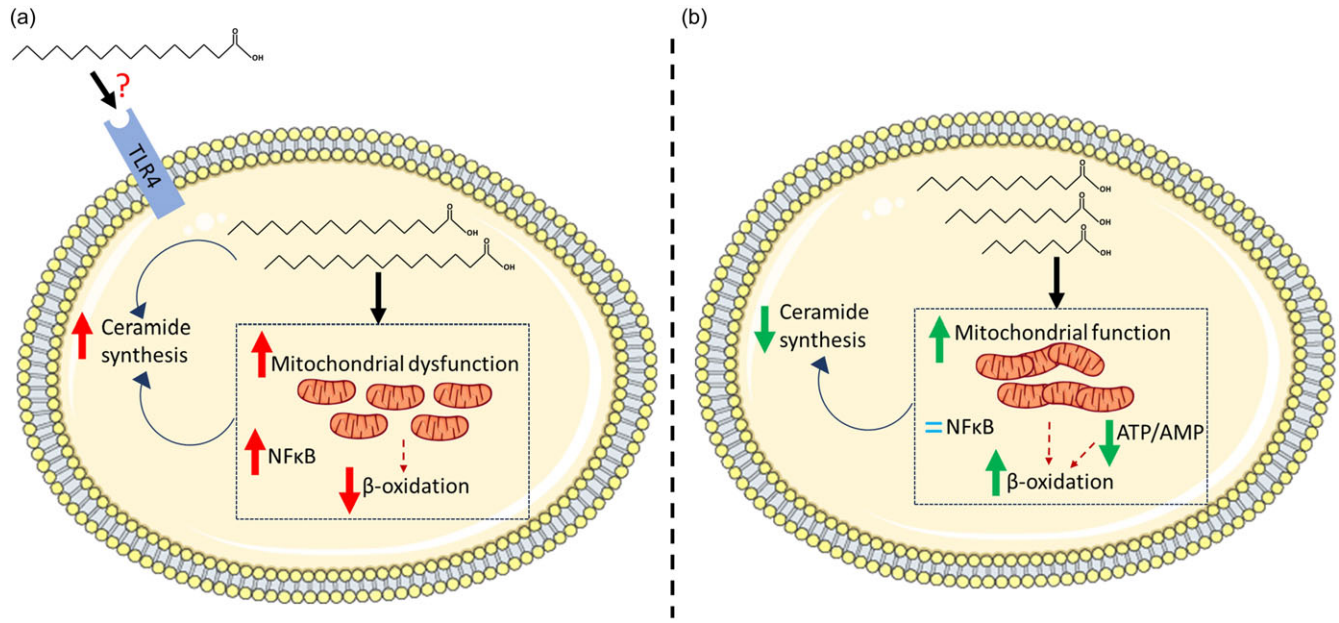

Fig. 3. Modulation of ceramide synthesis by long- and medium-chain saturated fatty acids. Long- and medium-chain saturated fatty acids modulated intracellular ceramide accumulation differently. (a) Long-chain saturated fatty acids, and particularly palmitic acid (represented here), promote ceramide synthesis by providing the building blocks for the synthesis of these sphingolipids. Furthermore, Long-chain saturated fatty acids activate pro-inflammatory responses, including the induction of the NFκB signalling, which in turn, has been reported to foster ceramide synthesis. Long-chain saturated fatty acids have also been shown to induce mitochondrial dysfunction, with a consequent impairment of β-oxidation thereby resulting in an increase in intracellular fatty acid available to be funnelled towards ceramide synthesis. (b) Contrarily to Long-chain saturated fatty acids, medium-chain saturated fatty acids such as lauric, capric and caprylic acid (represented here), may prevent intracellular ceramide accumulation by improving mitochondrial oxidative metabolism which, in concert with a decrease in the ATP/AMP ratio, contribute to sustaining β-oxidation thereby decreasing the availability of fatty acids to be directed towards ceramide synthesis. Additionally, as opposed to long-chain saturated fatty acids, medium-chain saturated fatty acids are unable to induce inflammatory responses which, in turn, contribute to preventing intracellular ceramide accumulation. This figure was created using smart.servier.com

accumulation within adipocytes may contribute to increase adipocyte size and impair mitochondrial respiration[100]. In agreement with this, genetic approaches aimed at lowering adipocyte ceramide levels led to a decrease in adipocyte size, increased mitochondrial function and enhanced insulin sensitivity[39,100]. Thus, on one hand ceramides increase fatty acid uptake and intracellular storage and influence adipocyte lipid metabolism possibly to buffer fatty acid excess and the potential perturbation of cell membranes by the detergent-like effects of NEFA; however, on the other hand these mechanisms contribute to increasing intracellular lipid storage and adipocyte hypometabolism, thereby paving the way for the development of insulin resistance[93].

Long- versus medium-chain saturated fatty acids as the dietary drivers of ceramide synthesis

As already discussed, fatty acid overload is the main driver of ceramide synthesis. However, not all fatty acids promote ceramide synthesis, a paradigm which is in line with the fact that not all fatty acids are metabolically detrimental[101,102]. Indeed, long-chain saturated fatty acids, as opposed to mono- and poly-unsaturated fatty acids, increase circulating ceramide levels[101,103,104] in human subject feeding trials, an effect that was accompanied by insulin resistance[101,104] (Figure 3a). The accumulation of ceramides was also demonstrated in the skeletal muscle and liver of rats upon the infusion of lard oil, mainly composed of long-chain saturated fatty acids, and was paralleled by insulin resistance[57]. The ability of long-chain saturated fatty acids to promote ceramide buildup may be dependent on an increase in serine palmitoyltransferase and a concomitant decrease in ceramidase activity. The induction of serine palmitoyltransferase activity by long-chain saturated fatty acids is not surprising given that palmitoyl-CoA, the CoA derivate of palmitic acid, along with serine, is a substrate for this enzyme[105]. Long-chain saturated fatty acids are also able to trigger inflammatory response in a variety of tissues[106–111] and inflammation itself has been proposed as one of the triggers of ceramide synthesis, thus representing a further mechanism linking saturated fatty acids and ceramide buildup[40] (Figure 3a). Indeed, the chronic low-grade inflammation typical of obesity not only represents a key pathophysiological mechanism underpinning the cardio-metabolic complications linked with obesity, but also promotes the synthesis of ceramide[40]. This may be at the basis of a vicious cycle in which metabolic inflammation promotes the synthesis of ceramide and ceramide itself fuels inflammation[40], with both stimuli hampering insulin signal transduction pathway[36,112,113]. In support of the nexus between inflammation and ceramide synthesis, in cell cultures cytokines stimulate the synthesis of ceramide by promoting the expression of genes involved in *de novo* ceramide synthesis and sphingomyelin breakdown[114–116]. This effect has been further confirmed *in vivo* as demonstrated by the ability of TNFα to increase ceramide levels in epithelial cells via the activation of

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neutral sphingomyelinase[117]. Always in line with the ability of inflammation to modulate ceramide metabolism, toll-like receptor 4 activation resulted in increased ceramide synthesis[118] in primary macrophages, while its pharmacological inhibition led to a decrease in skeletal muscle ceramide levels[119]. Particularly, the toll-like receptor 4 appears to mediate the synergistic effects of its cognate ligand, lipopolysaccharide and palmitic acid in mediating ceramide buildup[94]. However, the mechanisms underpinning this synergy between lipopolysaccharide and palmitic acid remain to be fully elucidated, also in consideration of the fact that the ability of palmitic acid to act as a toll-like receptor 4 agonist remains controversial[120–122].

As already anticipated, ceramides also impact metabolic health by accumulating in the hypothalamus. In light of this, it is not surprising that diet may also impact hypothalamic ceramide accumulation. Indeed, a high-fat diet rich in long-chain saturated fatty acids promoted ceramide accumulation within the hypothalamus[72] with this effect appearing to be sex specific and more marked in male rodents[123]. These *in vivo* data are also supported by *in vitro* studies in hypothalamic neuronal cell cultures confirming the ability of long-chain saturated fatty acids to promote ceramide accumulation not only in peripheral tissues, but also within the hypothalamus. Saturated fatty acids, and particularly palmitic acid, promoted C16:0 ceramide accumulation within neuronal hypothalamic cell lines with this effect being accompanied by inflammatory responses[121,124] and impaired insulin signalling[77]. Remarkably, increasing fatty acid oxidation in hypothalamic neurons not only lowered ceramide levels with cultured hypothalamic neurons, but also mitigated inflammation[124]. To a similar extend, specifically inhibiting ceramide synthesis using pharmacological or molecular tools was sufficient to rescue hypothalamic insulin signalling[77], confirming the role of ceramides as pivotal mediators of the metabolically detrimental role of long-chain saturated fatty acids also with the hypothalamus.

Nevertheless, the effects of saturated fatty acids on ceramide accumulation appear to be chain-length specific as long- but not medium-chain saturated fatty acids are able to increase ceramide synthesis. Indeed, animals fed a high-fat diet, when switched to a high-fat diet supplemented with medium-chain triacylglycerols (C8 and C10), not only experienced an improvement in metabolic health independently of changes in adiposity but also displayed a decrease in hepatic ceramide content[125] (Figure 3b). This effect may be dependent upon the ability of medium-chain triglycerides to down-regulate the expression of key enzymes involved in ceramide biosynthesis and sphingomyelin hydrolysis, namely ceramide synthase 6 and sphingomyelin phosphodiesterase 3, respectively[125]. Another potential mechanism underpinning these effects may be dependent on the ability of medium-chain saturated fatty acids to modulate mitochondrial oxidative metabolism and the fact that, compared with long-chain saturated fatty acids, they are more effectively β-oxidised[126]. With regard to their impact on mitochondrial oxidative metabolism, lauric acid, a medium-chain saturated fatty acids did not impair mitochondrial membrane potential or promote mitochondrial fission in the primary myotubes of human subjects as opposed to the long-chain saturated fatty acid palmitic acid[107]. Furthermore, in rodents, a high-fat diet enriched in medium, compared with long-chain fatty acids, induced a greater increase in skeletal muscle markers of mitochondrial metabolism with this effect being associated with an improvement in insulin sensitivity in skeletal muscle and adipose tissue[127]. Thus, it is tempting to hypothesise that the preserved mitochondrial function in response to medium-chain saturated fatty acids would favour mitochondrial oxidative metabolism, thereby preventing intracellular ceramide buildup. However, this effect was tissue-specific as medium-chain fatty acid supplementation led to hepatic steatosis and liver-selective insulin resistance in rodents while preserving skeletal muscle and adipose tissue insulin sensitivity[127]. These effects may also be related to the divergent effect of palmitic and lauric acid on metabolic inflammation, with the former, but not the latter, being able to trigger the activation of the pro-inflammatory NFκB signalling in primary myotubes of human subjects[107] (Figure 3), which, in turn, has been reported to play a key role in disrupting mitochondrial function in myotubes[128]. Always in line with the effect of medium-chain fatty acid on inflammation, they have been shown to inhibit the pro-inflammatory effect of lipotoxicity by activating the G-protein-coupled receptor 84, as demonstrated on hepatic macrophages[129]. Finally, medium chain fatty acids are also able to act as signalling molecules given by their ability to increase intracellular AMP[130] while lowering ATP levels[131] (Figure 3b). The drop in ATP/AMP ratio, in turn, activates AMP-activated protein kinase which is pivotal in switching off anabolic pathways, including ceramide biosynthesis[132], while stimulating ATP-generating pathways such as β-oxidation[133]. Thus, the activation of intracellular signalling pathways that boost oxidative metabolism while inhibiting anabolic pathways represents a further mechanisms by which medium-chain fatty acids counter ceramide accumulation.

Conclusions

Ceramides are at the nexus between the derangements in lipid metabolism underlaying metabolically unhealthy obesity and insulin resistance. Indeed, the intracellular accumulation of ceramides is a pivotal driver of insulin resistance and arises as a direct consequence of the mismatch between fatty acids supply, both from the adipose tissue and the diet, and their catabolism via mitochondrial β-oxidation. Remarkably, ceramides not only represent a pathogenetic factor able to impair insulin signal transduction, but the raise in circulating levels of total and specific dihydroceramide species such as Cer(d18:0/22:0). Cer(d18:0/24:0) may also predict the onset of overt T2DM. In keeping with this, the levels of these dihydroceramide species were reported to be up-regulate up to 9 years before the diagnosis of T2DM, suggesting that the defect in lipid metabolism that derange ceramide homoeostasis precede the onset of the disease. The overconsumption of dietary saturated fatty acids has been widely reported to hamper insulin sensitivity with this effect being potentially mediated, at least in part, by their ability to foster ceramide synthesis. However, this effect is chain length-specific as long-chain saturated fatty acids, and particularly palmitic acid, foster ceramide accumulation by providing the building blocks for its synthesis and by triggering

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inflammatory responses. On the contrary, medium-chain saturated fatty acids not only have not been associated with an increase in ceramide synthesis, but some reports also suggest their ability to counter lipotoxicity.

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Competing interests

The authors declare none.

Authorship

D.S. conceived and wrote the first draft of the review. E.Z., C.C., M.P. and G.Z. contributed to writing, editing and critically reviewed the manuscript. All authors have read and approved the final version of the manuscript.

References

1. Bluher M (2019) Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol* **15**, 288–298.
2. Brown WV, Fujioka K, Wilson PW *et al.* (2009) Obesity: why be concerned? *Am J Med* **122**, S4–11.
3. Zatterale F, Longo M, Naderi J *et al.* (2019) Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. *Front Physiol* **10**, 1607.
4. Koliaki C, Liatis S, Kokkinos A (2019) Obesity and cardiovascular disease: revisiting an old relationship. *Metabolism* **92**, 98–107.
5. Spengler EK & Loomba R (2015) Recommendations for diagnosis, referral for liver biopsy, and treatment of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Mayo Clin Proc* **90**, 1233–1246.
6. Avgerinos KI, Spyrou N, Mantzoros CS *et al.* (2019) Obesity and cancer risk: emerging biological mechanisms and perspectives. *Metabolism* **92**, 121–135.
7. Mazon JN, de Mello AH, Ferreira GK *et al.* (2017) The impact of obesity on neurodegenerative diseases. *Life Sci* **182**, 22–28.
8. Cordain L, Eaton SB, Sebastian A *et al.* (2005) Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* **81**, 341–354.
9. Kopp W (2019) How western diet and lifestyle drive the pandemic of obesity and civilization diseases. *Diabetes, metabolic syndrome and obesity: targets and therapy* **12**, 2221.
10. Pietilainen KH, Kaprio J, Borg P *et al.* (2008) Physical inactivity and obesity: a vicious circle. *Obesity (Silver Spring)* **16**, 409–414.
11. Hall KD, Ayuketah A, Brychta R *et al.* (2019) Ultra-processed diets cause excess calorie intake and weight gain: an inpatient randomized controlled trial of *ad libitum* food intake. *Cell Metab* **30**, 67–77 e63.
12. Cecchini M, Sassi F, Lauer JA *et al.* (2010) Tackling of unhealthy diets, physical inactivity, and obesity: health effects and cost-effectiveness. *Lancet* **376**, 1775–1784.
13. Rui L (2013) Brain regulation of energy balance and body weight. *Rev Endocr Metab Disord* **14**, 387–407.
14. Suzuki K, Simpson KA, Minnion JS *et al.* (2010) The role of gut hormones and the hypothalamus in appetite regulation. *Endocr J* **57**, 359–372.
15. Bauer PV, Hamr SC, Duca FA (2016) Regulation of energy balance by a gut–brain axis and involvement of the gut microbiota. *Cell Mol Life Sci* **73**, 737–755.
16. Cowley MA, Smart JL, Rubinstein M *et al.* (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* **411**, 480–484.
17. Williams LM (2012) Hypothalamic dysfunction in obesity. *Proc Nutr Soc* **71**, 521–533.
18. Sergi D & Williams LM (2020) Potential relationship between dietary long-chain saturated fatty acids and hypothalamic dysfunction in obesity. *Nutr Rev* **78**, 261–277.
19. Gao Y, Bielohuby M, Fleming T *et al.* (2017) Dietary sugars, not lipids, drive hypothalamic inflammation. *Mol Metab* **6**, 897–908.
20. Zechner R, Zimmermann R, Eichmann TO *et al.* (2012) FAT SIGNALS–lipases and lipolysis in lipid metabolism and signaling. *Cell Metab* **15**, 279–291.
21. Fucho R, Casals N, Serra D *et al.* (2017) Ceramides and mitochondrial fatty acid oxidation in obesity. *FASEB J* **31**, 1263–1272.
22. Serra D, Mera P, Malandrino MI *et al.* (2013) Mitochondrial fatty acid oxidation in obesity. *Antioxid Redox Signal* **19**, 269–284.
23. Arner P & Ryden M (2015) Fatty acids, obesity and insulin resistance. *Obes Facts* **8**, 147–155.
24. Kim J-Y, Hickner RC, Cortright RL *et al.* (2000) Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol-Endocrinol Metab.* **279**, E1039–E1044.
25. McGillicuddy FC, Reynolds CM, Finucane O *et al.* (2013) Long-term exposure to a high-fat diet results in the development of glucose intolerance and insulin resistance in interleukin-1 receptor I-deficient mice. *Am J Physiol Endocrinol Metab* **305**, E834–844.
26. Aas V, Kase ET, Solberg R *et al.* (2004) Chronic hyperglycaemia promotes lipogenesis and triacylglycerol accumulation in human skeletal muscle cells. *Diabetologia* **47**, 1452–1461.
27. Anstee QM, Goldin RD (2006) Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* **87**, 1–16.
28. Assimacopoulos-Jeannet F (2004) Fat storage in pancreas and in insulin-sensitive tissues in pathogenesis of type 2 diabetes. *Int J Obes Relat Metab Disord* **28**(Suppl 4), S53–57.
29. Zhou YT, Grayburn P, Karim A *et al.* (2000) Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci USA* **97**, 1784–1789.
30. Unger RH (2002) Lipotoxic diseases. *Ann Rev Med* **53**, 319–336.
31. Sezer H (2017) Insulin resistance, obesity and lipotoxicity. In *Obesity and Lipotoxicity*, pp. 277–304. Springer.
32. Bosma M, Hesselink MK, Sparks LM *et al.* (2012) Perilipin 2 improves insulin sensitivity in skeletal muscle despite elevated intramuscular lipid levels. *Diabetes* **61**, 2679–2690.
33. Metcalfe LK, Smith GC, Turner N (2018) Defining lipid mediators of insulin resistance - controversies and challenges. *J Mol Endocrinol* **62**, R65–R82.
34. Chavez JA, Knotts TA, Wang LP *et al.* (2003) A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. *J Biol Chem* **278**, 10297–10303.
35. Chavez JA & Summers SA (2003) Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. *Arch Biochem Biophys* **419**, 101–109.
36. Chavez JA & Summers SA (2012) A ceramide-centric view of insulin resistance. *Cell Metab* **15**, 585–594.

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https://doi.org/10.1017/S0954422424000179 Published online by Cambridge University Press

37. Chaurasia B & Summers SA (2015) Ceramides–lipotoxic inducers of metabolic disorders. *Trends Endocrinol Metab* **26**, 538–550.
38. Sears B & Perry M (2015) The role of fatty acids in insulin resistance. *Lipids Health Dis* **14**, 121.
39. Chaurasia B, Tippetts TS, Mayoral Monibas R *et al.* (2019) Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science* **365**, 386–392.
40. Chaurasia B, Talbot CL, Summers SA (2020) Adipocyte ceramides-the nexus of inflammation and metabolic disease. *Front Immunol* **11**, 576347.
41. Chaurasia B & Summers SA (2021) Ceramides in metabolism: key lipotoxic players. *Annu Rev Physiol* **83**, 303–330.
42. Turpin-Nolan SM & Bruning JC (2020) The role of ceramides in metabolic disorders: when size and localization matters. *Nat Rev Endocrinol* **16**, 224–233.
43. Gault CR, Obeid LM, Hannun YA (2010) An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv Exp Med Biol* **688**, 1–23.
44. Kitatani K, Idkowiak-Baldys J, Hannun YA (2008) The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal* **20**, 1010–1018.
45. Kitatani K, Sheldon K, Rajagopalan V *et al.* (2009) Involvement of acid beta-glucosidase 1 in the salvage pathway of ceramide formation. *J Biol Chem* **284**, 12972–12978.
46. Hannun YA, Obeid LM (2018) Sphingolipids and their metabolism in physiology and disease. *Nat Rev Mol Cell Biol* **19**, 175–191.
47. Holland WL, Miller RA, Wang ZV *et al.* (2011) Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med* **17**, 55–63.
48. Vasiliauskaite-Brooks I, Sounier R, Rochaix P *et al.* (2017) Structural insights into adiponectin receptors suggest ceramidase activity. *Nature* **544**, 120–123.
49. Sergi D, Morris AC, Kahn DE *et al.* (2018) Palmitic acid triggers inflammatory responses in N42 cultured hypothalamic cells partially via ceramide synthesis but not via TLR4. *Nutritional neuroscience*, 1–14.
50. Boon J, Hoy AJ, Stark R *et al.* (2013) Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes* **62**, 401–410.
51. Contreras C, Gonzalez-Garcia I, Martinez-Sanchez N *et al.* (2014) Central ceramide-induced hypothalamic lipotoxicity and ER stress regulate energy balance. *Cell Rep* **9**, 366–377.
52. Powell DJ, Hajduch E, Kular G *et al.* (2003) Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol Cell Biol* **23**, 7794–7808.
53. Bourbon NA, Yun J, Kester M (2000) Ceramide directly activates protein kinase C zeta to regulate a stress-activated protein kinase signaling complex. *J Biol Chem* **275**, 35617–35623.
54. Salinas M, Lopez-Valdaliso R, Martin D *et al.* (2000) Inhibition of PKB/Akt1 by C2-ceramide involves activation of ceramide-activated protein phosphatase in PC12 cells. *Mol Cell Neurosci* **15**, 156–169.
55. Hage Hassan R, Pacheco de Sousa AC, Mahfouz R *et al.* (2016) Sustained action of ceramide on the insulin signaling pathway in muscle cells: implication of the double-stranded RNA-activated protein kinase. *J Biol Chem* **291**, 3019–3029.
56. Cimmino I, Lorenzo V, Fiory F *et al.* (2017) A peptide antagonist of Prep1-p160 interaction improves ceramide-induced insulin resistance in skeletal muscle cells. *Oncotarget* **8**, 71845–71858.
57. Holland WL, Brozinick JT, Wang LP *et al.* (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab* **5**, 167–179.
58. Blachnio-Zabielska AU, Hady HR, Markowski AR *et al.* (2018) Inhibition of ceramide de novo synthesis affects adipocytokine secretion and improves systemic and adipose tissue insulin sensitivity. *Int J Mol Sci* **19**, 3995. https://doi.org/10.3390/ijms19123995.
59. Ye R, Onodera T, Scherer PE (2019) Lipotoxicity and beta cell maintenance in obesity and type 2 diabetes. *J Endocr Soc* **3**, 617–631.
60. Turpin-Nolan SM, Hammerschmidt P, Chen W *et al.* (2019) CerS1-derived C18:0 ceramide in skeletal muscle promotes obesity-induced insulin resistance. *Cell Rep* **26**, 1–10 e17.
61. Turpin SM, Nicholls HT, Willmes DM *et al.* (2014) Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab* **20**, 678–686.
62. Lemaitre RN, Yu C, Hoofnagle A *et al.* (2018) Circulating sphingolipids, insulin, HOMA-IR, and HOMA-B: the strong heart family study. *Diabetes* **67**, 1663–1672.
63. Kolak M, Westerbacka J, Velagapudi VR *et al.* (2007) Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes* **56**, 1960–1968.
64. Coen PM, Dube JJ, Amati F *et al.* (2010) Insulin resistance is associated with higher intramyocellular triglycerides in type I but not type II myocytes concomitant with higher ceramide content. *Diabetes* **59**, 80–88.
65. Coen PM, Hames KC, Leachman EM *et al.* (2013) Reduced skeletal muscle oxidative capacity and elevated ceramide but not diacylglycerol content in severe obesity. *Obesity (Silver Spring)* **21**, 2362–2371.
66. Dube JJ, Amati F, Toledo FG *et al.* (2011) Effects of weight loss and exercise on insulin resistance, and intramyocellular triacylglycerol, diacylglycerol and ceramide. *Diabetologia* **54**, 1147–1156.
67. Sharma A, Krick B, Li Y *et al.* (2022) The use of ceramides to predict metabolic response to metformin in women with PCOS. *J Endocr Soc* **6**, bvac131.
68. Poss AM, Krick B, Maschek JA *et al.* (2022) Following Roux-en-Y gastric bypass surgery, serum ceramides demarcate patients that will fail to achieve normoglycemia and diabetes remission. *Med* **3**, 452–467 e454.
69. Warshauer JT, Lopez X, Gordillo R *et al.* (2015) Effect of pioglitazone on plasma ceramides in adults with metabolic syndrome. *Diabetes Metab Res Rev* **31**, 734–744.
70. Raichur S, Wang ST, Chan PW *et al.* (2014) CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab* **20**, 687–695.
71. Chathoth S, Ismail MH, Alghamdi HM *et al.* (2022) Insulin resistance induced by de novo pathway-generated C16-ceramide is associated with type 2 diabetes in an obese population. *Lipids Health Dis* **21**, 24.
72. Borg ML, Omran SF, Weir J *et al.* (2012) Consumption of a high-fat diet, but not regular endurance exercise training, regulates hypothalamic lipid accumulation in mice. *J Physiol* **590**, 4377–4389.
73. Posey KA, Clegg DJ, Printz RL *et al.* (2009) Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *Am J Physiol Endocrinol Metab* **296**, E1003–1012.
74. Zhang X, Zhang G, Zhang H *et al.* (2008) Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. *Cell* **135**, 61–73.

https://doi.org/10.1017/S0954422424000179 Published online by Cambridge University Press

75. Ramirez S & Claret M (2015) Hypothalamic ER stress: a bridge between leptin resistance and obesity. *FEBS Lett* **589**, 1678–1687.
76. Ozcan L, Ergin AS, Lu A *et al.* (2009) Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metab* **9**, 35–51.
77. Campana M, Bellini L, Rouch C *et al.* (2018) Inhibition of central *de novo* ceramide synthesis restores insulin signaling in hypothalamus and enhances beta-cell function of obese Zucker rats. *Mol Metab* **8**, 23–36.
78. Han J & Kaufman RJ (2016) The role of ER stress in lipid metabolism and lipotoxicity. *J Lipid Res* **57**, 1329–1338.
79. Ajoolabady A, Lebeaupin C, Wu NN *et al.* (2023) ER stress and inflammation crosstalk in obesity. *Med Res Rev* **43**, 5–30.
80. Rieusset J, Chauvin MA, Durand A *et al.* (2012) Reduction of endoplasmic reticulum stress using chemical chaperones or Grp78 overexpression does not protect muscle cells from palmitate-induced insulin resistance. *Biochem Biophys Res Commun* **417**, 439–445.
81. Hage Hassan R, Hainault I, Vilquin JT *et al.* (2012) Endoplasmic reticulum stress does not mediate palmitate-induced insulin resistance in mouse and human muscle cells. *Diabetologia* **55**, 204–214.
82. Glaros EN, Kim WS, Quinn CM *et al.* (2008) Myriocin slows the progression of established atherosclerotic lesions in apolipoprotein E gene knockout mice. *J Lipid Res* **49**, 324–331.
83. Ji R, Akashi H, Drosatos K *et al.* (2017) Increased *de novo* ceramide synthesis and accumulation in failing myocardium. *JCI Insight* **2**, e96203.
84. Kasumov T, Li L, Li M *et al.* (2015) Ceramide as a mediator of non-alcoholic Fatty liver disease and associated atherosclerosis. *PLoS One* **10**, e0126910.
85. Lightle S, Tosheva R, Lee A *et al.* (2003) Elevation of ceramide in serum lipoproteins during acute phase response in humans and mice: role of serine-palmitoyl transferase. *Arch Biochem Biophys* **419**, 120–128.
86. Wiesner P, Leidl K, Boettcher A *et al.* (2009) Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry. *J Lipid Res* **50**, 574–585.
87. Neeland IJ, Singh S, McGuire DK *et al.* (2018) Relation of plasma ceramides to visceral adiposity, insulin resistance and the development of type 2 diabetes mellitus: the Dallas Heart Study. *Diabetologia* **61**, 2570–2579.
88. Haus JM, Kashyap SR, Kasumov T *et al.* (2009) Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* **58**, 337–343.
89. Wigger L, Cruciani-Guglielmacci C, Nicolas A *et al.* (2017) Plasma dihydroceramides are diabetes susceptibility biomarker candidates in mice and humans. *Cell Rep* **18**, 2269–2279.
90. Zarini S, Brozinick JT, Zemski Berry KA *et al.* (2022) Serum dihydroceramides correlate with insulin sensitivity in humans and decrease insulin sensitivity in vitro. *J Lipid Res* **63**, 100270.
91. Carlier A, Phan F, Szpigel A *et al.* (2020) Dihydroceramides in triglyceride-enriched VLDL are associated with nonalcoholic fatty liver disease severity in type 2 diabetes. *Cell Rep Med* **1**, 100154.
92. Summers SA, Chaurasia B, Holland WL (2019) Metabolic messengers: ceramides. *Nat Metab* **1**, 1051–1058.
93. Summers SA (2020) Ceramides: nutrient signals that drive hepatosteatosis. *J Lipid Atheroscler* **9**, 50–65.
94. Xia JY, Holland WL, Kusminski CM *et al.* (2015) Targeted induction of ceramide degradation leads to improved systemic metabolism and reduced hepatic steatosis. *Cell Metab* **22**, 266–278.
95. Jiang C, Xie C, Li F *et al.* (2015) Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Invest* **125**, 386–402.
96. Wang CN, O'Brien L, Brindley DN (1998) Effects of cell-permeable ceramides and tumor necrosis factor-alpha on insulin signaling and glucose uptake in 3T3-L1 adipocytes. *Diabetes* **47**, 24–31.
97. Guenther GG, Peralta ER, Rosales KR *et al.* (2008) Ceramide starves cells to death by downregulating nutrient transporter proteins. *Proc Natl Acad Sci U S A* **105**, 17402–17407.
98. Hyde R, Hajduch E, Powell DJ *et al.* (2005) Ceramide down-regulates system a amino acid transport and protein synthesis in rat skeletal muscle cells. *FASEB J* **19**, 461–463.
99. Zigdon H, Kogot-Levin A, Park JW *et al.* (2013) Ablation of ceramide synthase 2 causes chronic oxidative stress due to disruption of the mitochondrial respiratory chain. *J Biol Chem* **288**, 4947–4956.
100. Chaurasia B, Kaddai VA, Lancaster GI *et al.* (2016) Adipocyte ceramides regulate subcutaneous adipose browning, inflammation, and metabolism. *Cell Metab* **24**, 820–834.
101. Luukkonen PK, Sadevirta S, Zhou Y *et al.* (2018) Saturated fat is more metabolically harmful for the human liver than unsaturated fat or simple sugars. *Diabetes Care* **41**, 1732–1739.
102. Wanders AJ, Blom WAM, Zock PL *et al.* (2019) Plant-derived polyunsaturated fatty acids and markers of glucose metabolism and insulin resistance: a meta-analysis of randomized controlled feeding trials. *BMJ Open Diabetes Res Care* **7**, e000585.
103. Rosqvist F, Kullberg J, Stahlman M *et al.* (2019) Overeating saturated fat promotes fatty liver and ceramides compared with polyunsaturated fat: a randomized trial. *J Clin Endocrinol Metab* **104**, 6207–6219.
104. Tuccinardi D, Di Mauro A, Lattanzi G *et al.* (2021) An extra virgin olive oil-enriched chocolate spread positively modulates insulin-resistance markers compared with a palm oil-enriched one in healthy young adults: a double-blind, crossover, randomized controlled trial. *Diabetes Metab Res Rev* **38**, e3492.
105. Blachnio-Zabielska A, Baranowski M, Zabielski P *et al.* (2010) Effect of high fat diet enriched with unsaturated and diet rich in saturated fatty acids on sphingolipid metabolism in rat skeletal muscle. *J Cell Physiol* **225**, 786–791.
106. Sergi D, Luscombe-Marsh N, Heilbronn LK *et al.* (2021) The inhibition of metabolic inflammation by EPA is associated with enhanced mitochondrial fusion and insulin signaling in human primary myotubes. *J Nutr* **151**, 810–819
107. Sergi D, Luscombe-Marsh N, Naumovski N *et al.* (2021) Palmitic acid, but not lauric acid, induces metabolic inflammation, mitochondrial fragmentation, and a drop in mitochondrial membrane potential in human primary myotubes. *Front Nutr* **8**, 663838.
108. Zhou H, Urso CJ, Jadeja V (2020) Saturated fatty acids in obesity-associated inflammation. *J Inflamm Res* **13**, 1–14.
109. Rocha D, Lopes LL, da Silva A *et al.* (2017) Orange juice modulates proinflammatory cytokines after high-fat saturated meal consumption. *Food Funct* **8**, 4396–4403.
110. Berg J, Seyedsadjadi N, Grant R (2020) Saturated fatty acid intake is associated with increased inflammation, conversion of kynurenine to tryptophan, and delta-9 desaturase activity in healthy humans. *Int J Tryptophan Res* **13**, 1178646920981946.
111. Williams LM, Campbell FM, Drew JE *et al.* (2014) The development of diet-induced obesity and glucose intolerance in C57BL/6 mice on a high-fat diet consists of distinct phases. *PloS one* **9**, e106159.

https://doi.org/10.1017/S0954422424000179 Published online by Cambridge University Press

112. Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* **444**, 860–867.
113. Hotamisligil GS (2017) Inflammation, metaflammation and immunometabolic disorders. *Nature* **542**, 177–185.
114. Xu J, Yeh CH, Chen S *et al.* (1998) Involvement of *de novo* ceramide biosynthesis in tumor necrosis factor-alpha/cycloheximide-induced cerebral endothelial cell death. *J Biol Chem* **273**, 16521–16526.
115. Meyer SG & de Groot H (2003) Cycloserine and threo-dihydrosphingosine inhibit TNF-alpha-induced cytotoxicity: evidence for the importance of *de novo* ceramide synthesis in TNF-alpha signaling. *Biochim Biophys Acta* **1643**, 1–4.
116. Kolesnick RN, Haimovitz-Friedman A, Fuks Z (1994) The sphingomyelin signal transduction pathway mediates apoptosis for tumor necrosis factor, Fas, and ionizing radiation. *Biochem Cell Biol* **72**, 471–474.
117. Mallampalli RK, Peterson EJ, Carter AB *et al.* (1999) TNF-alpha increases ceramide without inducing apoptosis in alveolar type II epithelial cells. *Am J Physiol* **276**, L481–490.
118. Schilling JD, Machkovech HM, He L *et al.* (2013) Palmitate and lipopolysaccharide trigger synergistic ceramide production in primary macrophages. *J Biol Chem* **288**, 2923–2932.
119. McKenzie AI, Reidy PT, Nelson DS *et al.* (2020) Pharmacological inhibition of TLR4 ameliorates muscle and liver ceramide content after disuse in previously physically active mice. *Am J Physiol Regul Integr Comp Physiol* **318**, R503–R511.
120. Lancaster GI, Langley KG, Berglund NA *et al.* (2018) Evidence that TLR4 is not a receptor for saturated fatty acids but mediates lipid-induced inflammation by reprogramming macrophage metabolism. *Cell Metab* **27**, 1096–1110 e1095.
121. Sergi D, Morris AC, Kahn DE *et al.* (2018) Palmitic acid triggers inflammatory responses in N42 cultured hypothalamic cells partially via ceramide synthesis but not via TLR4. *Nutr Neurosci* **23**, 321–334. https://doi.org/10.1080/1028415X.2018.1501533.
122. Erridge C & Samani NJ (2009) Saturated fatty acids do not directly stimulate Toll-like receptor signaling. *Arterioscler Thromb Vasc Biol* **29**, 1944–1949.
123. Morselli E, Fuente-Martin E, Finan B *et al.* (2014) Hypothalamic PGC-1alpha protects against high-fat diet exposure by regulating ERalpha. *Cell Rep* **9**, 633–645.
124. McFadden JW, Aja S, Li Q *et al.* (2014) Increasing fatty acid oxidation remodels the hypothalamic neurometabolome to mitigate stress and inflammation. *PloS One* **9**, e115642.
125. Mourad S, Abdualkader AM, Li X *et al.* (2024) A high-fat diet supplemented with medium-chain triglycerides ameliorates hepatic steatosis by reducing ceramide and diacylglycerol accumulation in mice. *Exp Physiol* **109**, 350–364.
126. DeLany JP, Windhauser MM, Champagne CM *et al.* (2000) Differential oxidation of individual dietary fatty acids in humans. *Am J Clin Nutr* **72**, 905–911.
127. Turner N, Hariharan K, TidAng J *et al.* (2009) Enhancement of muscle mitochondrial oxidative capacity and alterations in insulin action are lipid species dependent: potent tissue-specific effects of medium-chain fatty acids. *Diabetes* **58**, 2547–2554.
128. Nisr RB, Shah DS, Ganley IG *et al.* (2019) Proinflammatory NFkB signalling promotes mitochondrial dysfunction in skeletal muscle in response to cellular fuel overloading. *Cell Mol Life Sci* **76**, 4887–4904.
129. Ohue-Kitano R, Nonaka H, Nishida A *et al.* (2023) Medium-chain fatty acids suppress lipotoxicity-induced hepatic fibrosis via the immunomodulating receptor GPR84. *JCI Insight* **8**, e165469.
130. Takikawa M, Kumagai A, Hirata H *et al.* (2013) 10-Hydroxy-2-decenoic acid, a unique medium-chain fatty acid, activates 5'-AMP-activated protein kinase in L6 myotubes and mice. *Mol Nutr Food Res* **57**, 1794–1802.
131. Debeer LJ, Mannaerts G, De Schepper PJ (1974) Effects of octanoate and oleate on energy metabolism in the perfused rat liver. *Eur J Biochem* **47**, 591–600.
132. Erickson KA, Smith ME, Anthonymuthu TS *et al.* (2012) AICAR inhibits ceramide biosynthesis in skeletal muscle. *Diabetol Metab Syndr* **4**, 45.
133. Herms A, Bosch M, Reddy BJ *et al.* (2015) AMPK activation promotes lipid droplet dispersion on detyrosinated microtubules to increase mitochondrial fatty acid oxidation. *Nat Commun* **6**, 7176.

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