Ceramides and mitochondrial fatty acid oxidation in obesity

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ABSTRACT: Obesity is an epidemic, complex disease that is characterized by increased glucose, lipids, and low-grade inflammation in the circulation, among other factors. It creates the perfect scenario for the production of ceramide, the building block of the sphingolipid family of lipids, which is involved in metabolic disorders such as obesity, diabetes, and cardiovascular disease. In addition, obesity causes a decrease in fatty acid oxidation (FAO), which contributes to lipid accumulation within the cells, conferring more susceptibility to cell dysfunction. C16:0 ceramide, a specific ceramide species, has been identified recently as the principal mediator of obesity-derived insulin resistance, impaired fatty acid oxidation, and hepatic steatosis. In this review, we have sought to cover the importance of the ceramide species and their metabolism, the main ceramide signaling pathways in obesity, and the link between C16:0 ceramide, FAO, and obesity.—Fucho, R., Casals, N., Serra, D., Herrero, L. Ceramides and mitochondrial fatty acid oxidation in obesity. FASEB J. 31, 1263–1272 (2017). www.fasebj.org

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“Globesity” is the word that the World Health Organization uses to refer to the global epidemic of overweight and obesity, which is currently a major public health problem in many parts of the world. Obesity is no longer mainly a problem of high-income, developed countries. Indeed, the largest increases in obesity since 1980 have occurred in low and middle-income countries, particularly in urban settings in Oceania, Latin America, and North Africa (1).

Obesity reflects an imbalance between energy intake and energy expenditure and is characterized by excessive fat accumulation in adipose tissue and other organs that has a negative impact on health. It has been established that obesity is a risk factor for other pathological conditions such as insulin resistance and type 2 diabetes mellitus (T2DM) (2), as well as nonalcoholic fatty liver disease (3), cardiovascular disease (4, 5), and cancer (6). During obesity, adipose tissue expands to cope with extra nutrients in circulation and avoid lipid deposition in other organs. Unfortunately, this expansion has its limits, and eventually, adipose tissue becomes dysfunctional (6).

Another mechanism that has been postulated to contribute to obesity-related metabolic disorders is defective fatty acid oxidation (FAO). Even though there is some controversy about this topic, mainly because of tissue variability and the obesity of the subjects, there is evidence of a decrease in FAO capacity in obese humans and rodents that contributes to lipid accumulation and lipotoxicity (7–14). Strategies that focus on enhancing FAO have been developed to treat obesity with positive results (15–23).

Among obesity-derived adipose tissue dysfunctions, there are 2 factors that are crucial for the generation of ceramides, which are key metabolites of sphingolipid (SPL) metabolism that contribute to obesity-related disorders (24, 25). First, the insulin resistance of obese adipose tissue maintains adipocyte lipolysis switched on. As a result, free fatty acids (FFAs) are constantly pumped into the circulation. One of the main pathways of ceramide synthesis, the de novo pathway, depends on the availability of saturated FFAs (26). Therefore, an increase in saturated FFAs in the circulation is a perfect scenario to promote de novo ceramide synthesis. Second, adipocyte cell death and dysfunction caused by an excess of nutrients generates

ABBREVIATIONS: ACC, acetyl-CoA carboxylase; BMI, body mass index; CerS, ceramide synthase; CPT1, carnitine palmitoyltransferase; DES, dihydrolipoyl dehydratase; ER, endoplasmic reticulum; ETC, electron transport chain; FA, fatty acid; FAO, fatty acid oxidation; FFA, free fatty acid; HFD, high-fat diet; KO, knockout; MCD, malonyl-CoA decarboxylase; PP2A, serine/threonine phosphatase 2A; ROS, reactive oxygen species; SM, sphingomyelin; SPL, sphingolipid; SPT, serine palmitoyltransferase; SPTLC, serine palmitoyltransferase long-chain base; T2DM, type 2 diabetes mellitus; TG, triglyceride; WAT, white adipose tissue

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local inflammation, which promotes immune cell infiltration in the tissue. Then, inflammation is amplified systemically to reach the rest of the body (6, 27). A second ceramide synthesis pathway, the catabolic conversion of another SPL, sphingomyelin (SM), into ceramide by the action of sphingomyelinases, can be activated by inflammatory signals, such as TNF-α (28). TNF-α is a classic cytokine that is elevated in circulation during obesity and is known to cause insulin resistance (29, 30). Thus, both elevated saturated FFAs and inflammation, which are key signatures of obesity, promote ceramide synthesis.

Ceramides have been linked to obesity, insulin resistance, and metabolic disorders (24, 25). However, most studies have focused on total ceramide levels, rather than the presence of a specific ceramide (31, 32). The lipidomics era has brought attention to individual ceramide molecular species that are produced via specific pathways and perform distinct functions. Therefore, it is not only a matter of the quantity, but also the quality of ceramide that is modulated in pathological states (33).

Two studies have demonstrated an increase in specific ceramide species (palmitoyl ceramide or C16:0 ceramide) in obese humans and mice that inhibit FAO and negatively regulate insulin signaling and energy expenditure (34, 35). These two independent studies provide a link between obesity, insulin resistance, and impaired FAO through ceramide action. In this review, we will cover the relevance of specific ceramide species, their metabolism, and the common obesity-related ceramide signaling that leads to insulin resistance. In addition, given recent studies that identify C16:0 ceramide as the species responsible for the metabolic phenotype of obesity through modulation of FAO (34, 35), we will discuss recent findings that link C16:0 ceramide, FAO, and obesity.

CERAMIDE METABOLISM

Ceramides are members of the SPL family and are composed of a long-chain sphingoid base, sphingosine, in N-linkage to a variety of acyl groups. There are 3 well-characterized pathways of ceramide production: 1) the de novo pathway, which takes place in the endoplasmic reticulum (ER); 2) the sphingomyelinase pathway, which converts SM into ceramides in several cellular compartments such as the plasma membrane, lysosomes, Golgi and mitochondria; and 3) the salvage pathway, which occurs in lysosomes and endosomes and converts complex SPLs into sphingosine, which is reused through reacylation to produce ceramides. In this review, we will focus on de novo synthesis (Fig. 1).

Key enzymes of de novo ceramide synthesis in the obese state

In the past decade, there have been great advances in knowledge of the key enzymes involved in the de novo ceramide biosynthetic pathway. More of the regulatory proteins and enzymes involved in this pathway have been cloned, and the generation of knockout (KO) mice showed the physiological functions of these enzymes. Furthermore, new spectroscopic techniques allow researchers to analyze and quantify multiple ceramide species, which yield insights into which species are the most relevant in pathological conditions, such as obesity and related diseases.

De novo synthesis starts in the ER by the action of the serine palmitoyl transferase (SPT), the rate-limiting enzyme of SPL synthesis (Fig. 1). This enzyme catalyzes the condensation of serine and palmitoyl-CoA to produce 3-ketosphinganine (36). The product of SPT, 3-ketosphinganine, is reduced by 3-ketosphinganine reductase (37), to generate sphinganine, the substrate for ceramide synthases (CerSs). CerSs attach ACCs of different chain lengths to sphinganine to form dihydroceramides, which are converted to ceramides by dihydroceramide desaturase (DES).

We next analyze the enzymes involved in de novo synthesis in obesity. The main enzymes involved in de novo synthesis are SPTs, CerSs, and DES.

SPTs

The hypothesis that high de novo ceramide biosynthesis contributes to the pathogenesis of obesity, and metabolic syndrome has been tested by several research groups (31, 32, 38). They showed that treatment of genetically obese (ob/ob) and high-fat-diet (HFD)-induced obese rodent models with myriocin, a specific inhibitor of SPT, decreased circulating ceramides, hepatic steatosis, and bodyweight and improved insulin resistance. Mammalian SPTs are trimeric proteins composed of 2 large subunits [serine palmitoyltransferase long-chain base subunit (SPTLC)-1 and -2 or SPTLC] and a small subunit (SPTssa or -ssb). Recently, it has been described that these small subunits play an important role in controlling SPT activity and substrate affinity, forming ceramides with different sphingoid bases (39). Although the consequences of the changes in sphingoid bases in obesity and diabetes are not yet known, the researchers demonstrated a relevant role in neurodegeneration. Thus, although blocking ceramide synthesis at the SPT level seems a promising strategy to ameliorate metabolic syndrome pathogenesis, the complete inhibition of ceramide synthesis may have deleterious effects on the cell, because of the crucial role of ceramides in the formation of other SPL derivatives that are essential to cell membrane function and for diverse intracellular signaling pathways.

CerSs

The discovery of dramatic increases in individual ceramide chain-length species present in the serum of obese mice has increased the interest in this enzyme family (40). Six mammalian CerSs (CerS1–6) have been identified. They are codified by 6 genes, also named lass (longevity assurance) because of their homology to the yeast longevity assurance gene LAG1 (41). Lass 1–6 genes are located in different chromosomes, and their
protein products are integral membrane proteins located in the ER. Interesting recent reviews (42–44) revealed that CerSs differ in their 1) amino acid composition, protein structure, and transmembrane topologies; 2) long-chain ACC specificities and sphingoid base stereospecificity; 3) tissue distribution; 4) transcriptional, posttranslational and activity regulation; and 5) biological function (Table 1). These enzymes have emerged as a critical node in phospholipid metabolism. Some data suggest that ceramide with a different acyl-chain length is associated with cell dysfunction in lipotoxic conditions. C16:0 and C18:0 ceramides are associated with insulin resistance in mice liver (45) and in myotubes from the skeletal muscle of patients with T2DM (46). The identification of putative ceramides at the onset of insulin resistance and in lipotoxicity motivated the research community to perform many new studies to discern which CerS is responsible for these events. Recent data obtained from knockdown of different CerSs showed a high degree of redundancy and interregulation between different CerSs (47). Furthermore, KO mice from CerS1–5 (48–52) highlight that these enzymes are not only modulators of chain length in ceramide production, but also control the levels of other bioactive SPLs that have different roles depending on the tissue. Data from these studies indicate that CerS5 and -6 may be the main CerSs involved in obesity development. New studies are necessary to understand the precise role of each CerS, to discern which ceramide species is toxic in pathological processes, such as obesity or insulin resistance, and to develop pharmacological inhibitors of specific CerSs to counteract ceramide negative actions.

**DES**

Recently, dihydroceramides have also been considered bioactive lipid species. In obesity, there is an imbalance between dihydroceramide and ceramide, and it has been reported that plasma dihydroceramide levels correlate better than ceramides with body mass index (BMI) in cohorts of obese subjects (53, 54). There are 2 DES enzymes, DES-1 and -2, localized on the cytosolic face of the ER. They show different tissue distribution and substrate preferences (37, 55). Studies derived from pharmacological DES-1 inhibitors, such as fenretinide indicate that inhibition

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**Figure 1.** SPL/ceramide biosynthetic and remodeling pathways. There are 3 main pathways of ceramide generation. 1) The *de novo* pathway takes place in the ER. Palmitoyl-CoA and serine are condensed by SPTs to form 3-ketodihydrosphingosine. In turn, 3-keto-dihydrosphingosine is reduced to dihydrosphingosine by 3-ketosphinganine reductase (3-KR) to generate sphinganine, the substrate for CerSs. CerSs attach ACCs of different chain lengths to sphinganine to form dihydroceramides, which are converted to ceramides by DES. 2) The sphingomyelinase pathway takes place in the plasma membrane, lysosomes, Golgi, and mitochondria and converts long-chain sphingoid bases into ceramides through the action of CerSs. SMase, sphingomyelinase; SMS, sphingomyelin synthase; CDase, ceramidase; SPPase, sphingosine phosphate phosphatase; SphK, sphingosine kinase.
of this enzyme could be a new strategy to prevent and reduce insulin resistance and obesity (56, 57).

**CERAMIDE SIGNALING IN OBESITY**

Increasing evidence supports a role for ceramides in the pathogenesis of obesity-induced metabolic disorders. Ceramides have been shown to participate through several mechanisms, such as inflammation, apoptosis, reactive oxygen species (ROS), ER stress, and autophagy.

Ceramides, together with other stimuli, such as fatty acids (FAs), various PKC isoforms, proinflammatory cytokines, and oxidative and ER stresses, activate JNK, NF-κB, receptor for advanced glycation endproducts (RAGE), and TLR pathways that trigger inflammation and insulin resistance in obesity (58–60). Increases in hepatic and muscle ceramide content have been associated with insulin resistance in obese Zucker rats (61). Ceramides and the sphingosine analog FTY720 can activate serine/threonine phosphatase 2A (PP2A) through direct binding to its inhibitor SET/I2PP2A, liberating and reactivating PP2 (62–67). It has been reported that ceramide-mediated activation of PP2A dephosphorylates Akt, blunting insulin signaling (68–72). In addition, ceramide can activate PKC-ζ, which phosphorylates the Pleckstrin homology domain of PKB/Akt on a Thr34/Ser34 residue, preventing PKB/Akt recruitment to the plasma membrane, where it is normally activated in response to insulin (73). Thus, ceramides can block the PKB/Akt pathway, leading to insulin resistance (68–74).

In contrast, the insulin-sensitizing hormone adiponectin activates PKB/Akt downstream mediators of ghrelin and leptin signaling. In the hypothalamus, PKB/Akt is activated in response to insulin (73, 74). Thus, ceramides can block the PKB/Akt pathway, leading to insulin resistance (68–74).

Ceramides are known to be downstream mediators of ghrelin and leptin signaling in the hypothalamus, and increased levels of ceramides promote feeding and body weight gain (83, 86).

Finally, several reports have shown that macroautophagy is induced by ceramides through the participation of CerS1 (87–89). This finding implicates C18:0 ceramide in targeting mitochondria for autophagic clearance. The depletion of mitochondria by mitophagy leads to a lower FAO capacity, and beyond a certain threshold, it can drive the cell to irreversible cellular atrophy (lethal mitophagy) (90).

**FAO AND OBESITY**

Obese individuals and those with T2DM are known to have lower FAO rates and lower ETC activity in muscle (7, 12, 13), together with higher glycolytic capacities and increased cellular FA uptake compared to nonobese and nondiabetic individuals (91). This indicates that any strategy that can burn off the excess lipids could be a good approach to treating obesity-induced metabolic disorders.

Several studies have demonstrated the effectiveness of increased FAO to fight against obesity and insulin resistance (15, 16, 18–23, 92–94). Although some have focused on indirect enhancement of FAO through acetyl-coA carboxylase (ACC) suppression or malonyl-CoA
Ceramide as a key ceramide that negatively regulates insulin sensitivity, FAO and energy expenditure in obesity. C16:0 ceramide is de novo synthesized by CerS6 in the ER. Turpin et al. identified increased CerS6 expression in obese human adipose tissue that positively correlated with BMI, body fat content, hyperglycemia, and insulin resistance. The same pattern was observed in WAT of HFD-fed mice. Accordingly, acyl-chain ceramide profiles in both obese humans and mice showed increased C16:0 and C18:0 ceramide. Conversely, CerS6−/− mice, which have reduced hepatic and adipose tissue C16:0 ceramide content, are protected from HFD-induced obesity and glucose intolerance, because of increased lipid utilization in brown adipose tissue and liver, which increases whole-body energy expenditure (34). At the same time, Raichur et al. (35) published a CerS2+/− mouse model, which is more susceptible to steatohepatitis and insulin resistance. CerS2 is the dominant hepatic CerS isoform and preferentially makes very long-chain ceramides (C22:0, C24:0, C24:1). CerS2+/− upregulates CerS5 and -6 expression, increases hepatic C16:0 and C18:0 ceramide, and decreases C24:0 and C24:1 ceramide. Moreover, overexpression of CerS6 in primary hepatocytes can reproduce the CerS2−/− phenotype that increases C16:0 ceramide, decreases insulin signaling, and promotes oleic acid-induced steatosis. Thus, the CerS2−/− model displays a phenotype similar to the obese human and mouse characteristics described by Turpin et al. (34) and the phenotype opposite that of the CerS6+/− mouse model. These results indicate that upregulation of CerS6 expression and subsequent increases in specific acyl-chain ceramides are a central mechanism that contribute to obesity. CerS6 emerges as a new target to treat this problem. However, a recently published article by Gosejacob et al. (49) demonstrates that CerS5 also contributes to C16:0 ceramide synthesis in WAT, skeletal muscle, liver, and spleen. In fact, CerS5-deficient mice show reduced weight gain, improved glucose tolerance, and reduced WAT inflammation after an HFD challenge. However, this protection is not related to changes in β oxidation. This approach confirms the role of C16:0 ceramide as a weight-gain promoter lipid and obesity-sensing lipid.

The studies by Turpin et al. (34) and Raichur et al. (35) agree that C16:0 ceramide negatively regulates FAO. However, whereas Raichur et al. and previous studies by this group demonstrate that C16:0 ceramide impairs β oxidation through inactivation of complex II and IV of the ETC in the CerS2−/− model (35, 97), Turpin et al. (34) claim that the observed increase in lipid utilization in their CerS6−/− mouse is related to enhanced FAO capacity, regardless of respiratory chain capacity. Ceramide action on ETC has been previously described (98). However, most of the studies were conducted with short-chain soluble ceramide (76, 78), which is not the most abundant ceramide species in tissues and can exert different actions on the more physiological ceramide species. Nonetheless, some studies focused on the effects of C16:0 ceramide on ETC and demonstrated that C16:0 ceramide inhibits complex IV, which contributes to ROS formation with no effects on mitochondrial membrane potential (78, 97). Oxidative stress is a hallmark of obesity that can inactivate a large number of enzymes. A metabolomics study on
HeLa cells revealed that CPT1 is one of the enzymes inhibited by oxidative stress. In this study, they looked at pairs of substrate product altered by H2O2 and other ROS. Among all metabolite changes, the most significant indicated that CPT1 was a major target for oxidative inactivation. Furthermore, CPT1 activity can be recovered by adding catalase to cells. Thus, ROS mediates reversible CPT1 inhibition (99). In summary, this study provides a unique link between oxidative stress and CPT1 inactivation. These are 2 scenarios present during obesity that can explain decreased FAO.

With all this information in mind, we outline a model in which obesity increases saturated FAs and CerS6, leading to C16:0 accumulation, which can cause ETC dysfunction and generate ROS. The ROS can then inactivate CPT1, decreasing FAO, and as a result, promoting lipid accumulation within the cells (Fig. 2). Some human data can be found in the literature to support this model. A study of endurance training in obese humans showed a decrease in C16:0 ceramide after training, coupled with an increase in CPT1 activity and FAO in muscle, all of which lead to improved glucose tolerance (98). Exercise training decreases C16:0 ceramide and increases CPT1 activity. Overall, it rescues FAO in human obese skeletal muscle and whole-body glucose metabolism.

Unfortunately, it is widely known that lifestyle interventions fail as a treatment for obesity, because they entail patients’ long-term commitment. One strategy that could mimic exercise training is to enhance FAO through CPT1 overexpression. Several animal and cellular models have been developed to increase FAO to treat obesity successfully, and some of them showed lower total ceramide content as part of the improved phenotype (16, 18, 93). However, no specific data on ceramide species were available in most of the studies. Only a few studies showed changes in ceramide species after FAO modulation. In an in vitro study, enhanced FAO in skeletal muscle cells protected them from palmitate-induced lipotoxicity and insulin resistance, which correlated with a decrease in total ceramide and specifically C16:0 ceramide (17). That study

Figure 2. C16:0 ceramide regulates FAO, steatosis, and insulin resistance during obesity. Obesity increase levels of saturated Fas, such as palmitic acid, the limiting substrate of de novo ceramide synthesis in the ER. Obesity also increases CerS6, which is responsible for C16:0 ceramide formation, which also depends on palmitic acid availability. C16:0 ceramide can inhibit FAO in an ETC in an independent or dependent manner, leading to cellular steatosis. ETC dysfunction generates ROS, which can inhibit CPT1 activity and decrease the entry of FA into mitochondria for oxidation. Again, this leads to cellular steatosis. Finally, C16:0 ceramide can inhibit the insulin-signaling pathway, which contributes to obesity-derived insulin resistance.
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positively correlates with a reduction in plasma TNF-α (101, 102). In addition, a decrease in plasma C16:0 ceramide after gastric bypass. After surgery, obese patients had lower circulating levels of C16:0 ceramide may become a metabolic marker of obesity and associated metabolic dysfunctions. An example can be given the recent findings, research on metabolic diseases should include the role of C16:0 ceramide in them. Obesity increases C16:0 ceramide (34), and circulating levels of C16:0 ceramide may be a metabolic marker of obesity and associated metabolic dysfunctions. An example can be found in human studies of obese subjects who underwent gastric bypass. After surgery, obese patients had lower body weight and decreased plasma C16:0 ceramide levels (101, 102). In addition, a decrease in plasma C16:0 ceramide positively correlates with a reduction in plasma TNF-α, an inflammatory cytokine that is involved in insulin resistance (102). In animal models, genetic obese ob/ob mice display increased levels of plasma ceramide. Specifically, C16:0 and C18:0 ceramide are higher than in lean mice (103). Altogether, these data suggest that C16:0 ceramide could be used as a metabolic marker of obesity and associated diseases.

Obesity dysfunctions depend on individual susceptibility. Genetic background differs from individual to individual, predisposing to or protecting from pathological conditions. C16:0 ceramide could mediate the transition from the obese to the insulin-resistant phenotype, and gene variants of CerS2, -5, or -6 could have an impact on C16:0 ceramide levels. We could find only 1 article on CerS gene variants and metabolic diseases. In that study, a human gene variant of CerS2 was associated with an increase in albuminuria in patients with diabetes, a common condition that indicates progression of the disease (102). No data were provided on the activity of this CerS2 variant or on levels of C16:0 ceramide, but it would be interesting to investigate how many gene variants of CerS2, -5, and -6 exist in humans, their effects on enzyme activity, and whether they can modulate C16:0 levels and have an impact on metabolic diseases.

As it is known that C16:0 ceramide has a negative impact on metabolism, it is crucial to develop specific CerS5 and -6 inhibitors to treat obesity and associated comorbidities. This task is difficult, because of the high homology between ceramide synthases. To the best of our knowledge, only 1 study has been conducted to develop specific CerS competitive inhibitors derived from the immunosuppressant fingolimod (FTY720). Compound ST1072 can inhibit CerS4 and CerS6 (105), but there are no data yet on in vivo effects under an HFD challenge. The new data on regulation of CerS activity by phosphorylation or deacetylation (106, 107) open up novel therapeutic options to control C16:0 ceramide production and its negative effects on health.

The strategy that we, and other laboratories, have to treat obesity is to enhance FAO. Enhancing FAO through CPT overexpression forces FFAs to enter into the mitochondria for oxidation. Ceramide de novo synthesis relies on saturated FFA availability. In obesity in particular, palmitic acid is essential for C16:0 ceramide formation. By enhancing FAO, it is possible to 1) reduce overall ceramide formation and 2) capture the palmitoyl-CoA necessary for C16:0 ceramide generation. These effects could reduce the deleterious effects associated with this obesity-related ceramide species. More studies on enhancing FAO with lipidomic data are needed to prove this concept.

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AUTHOR CONTRIBUTIONS

R. Fucho, N. Casals, D. Serra, and L. Herrero wrote the manuscript; and all authors contributed to the conception and design of the study and revised and approved the final manuscript.

REFERENCES

1. Ahima, R. S. (2011) Digging deeper into obesity. J. Clin. Invest. 121, 2070–2079
2. Kahn, S. E., Hull, R. L., and Utzschneider, K. M. (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444, 840–846
3. Cusi, K. (2012) Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology 142, 711–725.e6
4. Van Gaal, L. F., Mertens, I. L., and De Block, C. E. (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444, 875–880
5. Sun, B., and Karin, M. (2012) Obesity, inflammation, and liver cancer. J. Hepatol. 56, 704–713
6. Virtue, S., and Vidal-Puig, A. (2010) Adipose tissue expandability, lipotoxicity and the metabolic syndrome: an allostatic perspective. Biochem. Biophys. Acta 1801, 338–349
7. Houmard, J. A. (2008) Intramuscular lipid oxidation and obesity. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1111–R1116
18. Monséngo, J., Mansouri, A., Akkaoui, M., Lenoir, V., Esnous, C., Chavez, J. A., and Summers, S. A. (2012) A ceramide-centric view of mitochondria and adipocytes in type 2 diabetic mice. *Diabetologia* **55**, 784–791.

Fromenty, B., and Pessayre, D. (1995) Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacol. Ther.* **67**, 101–154.

11. Petersen, K. F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G. I. (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* **350**, 664–671.

28. Smith, E. L., and Schuchman, E. H. (2008) The unexpected role of acute sphingomyelinase in cell death and the pathophysiology of common diseases. *FASEB J.* **22**, 3419–3431.

29. Peraldi, P., Hotamisligil, G. S., Buurman, W. A., White, M. F., and Spiegelman, B. M. (1996) Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *J. Biol. Chem.* **271**, 13018–13022.

K. T., Wiesbrock, S. M., Marino, M. W., and Hotamisligil, G. S. (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* **389**, 610–614.

31. Petersen, K. F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G. I. (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* **350**, 664–671.

28. Smith, E. L., and Schuchman, E. H. (2008) The unexpected role of acute sphingomyelinase in cell death and the pathophysiology of common diseases. *FASEB J.* **22**, 3419–3431.

12. Ritov, V. B., Menshikova, E. V., He, J., Ferrell, R. E., Goodpaster, B. H., and Kelley, D. E. (2005) Deficiency of subsarcomerrial mitochondria in obesity and type 2 diabetes. *Diabetes* **54**, 8–14.

Kelley, D. E., He, J., Menshikova, E. V., and Ritov, V. B. (2002) Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* **51**, 2944–2950.

Adams, S. H., Hoppel, C. L., Lok, K. H., Zhao, L., Wong, S. W., Minkler, P. E., Hwang, D. H., Newman, J. W., and Garvey, W. T. (2009) Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. *J. Nutr.* **139**, 1073–1081.

15. Herrero, L., Rubi, B., Sebastián, D., Serra, D., Asins, G., Macchler, P., Pretuki, M., and Hegardt, F. G. (2005) Alteration of the malonyl-CoA/HSL pathway and palmitoyltransferase 1 interaction in the beta-cell impairs glucose-mediated insulin secretion. *Diabetes* **54**, 462–471.

19. Orellana-Gavaldà, J. M., Herrera, L., Malandrino, M. I., Paredes, A., Sol Rodríguez-Peña, M., Petry, H., Asins, G., Van Deventer, S., Hegardt, F. G., and Serra, D. (2011) Molecular therapy for obesity and diabetes based on a long-term increase in hepatic fatty acid oxidation. *Hepatology* **53**, 821–832.

Choi, X., Li, K., Kwon, C., Sweeney, G., Wang, Y., Xu, A., Teng, M., Liu, P., and Wu, D. (2011) Carnitine palmitoyltransferase 1A prevents fatty acid-induced adipocyte dysfunction through suppression of c-Jun N-terminal kinase. *Biochem. J.* **435**, 723–732.

Namgaladze, D., Lips, S., Leiker, T. J., Murphy, R. C., Ekroos, K., Ferreiros, N., Geisslinger, G., and Brüne, B. (2014) Inhibition of macrophage fatty acid beta-oxidation exaggerates palmitate-induced inflammatory and endoplasmic reticulum stress responses. *Diabetologia* **57**, 1067–1077.

Malandrin, M. I., Uysal, K. T., Wiesbrock, S. M., Marino, M. W., and Hotamisligil, G. S. (2009) Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. *J. Nutr.* **139**, 1073–1081.

33. Hand, W. T., Smith, S. R., Rustan, A. C., and Ravussin, E. (2014) Lipid in novo synthesis of long-chain ceramides. *FASEB J.* **28**, E765–E769.

23. Serra, D., Mera, P., Malandrino, M. I., Mir, J. F., and Herrero, L. (2013) Mitochondrial fatty acid oxidation in obesity. *Antioxid. Redox Signal.* **19**, 269–284.

24. Chavez, J. A., and Summers, S. A. (2012) A ceramide-centric view of insulin resistance. *Cell Metab.* **15**, 385–394.

25. Bakman, B. T., and Summers, S. A. (2011) Ceramides as modulators of cellular and whole-body metabolism. *J. Clin. Invest.* **121**, 4222–4230.

26. Bartke, N., and Hannun, Y. A. (2009) Bioactive sphingolipids: metabolism and function. *J. Lipid Res.* **50**(Suppl.), S91–S96.

27. Mathis, D. (2013) Immunological going-on in visceral adipose tissue. *Cell Metab.* **17**, 851–859.

28. Smith, E. L., and Schuchman, E. H. (2008) The unexpected role of acute sphingomyelinase in cell death and the pathophysiology of common diseases. *FASEB J.* **22**, 3419–3431.
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63. Chalfant, C. E., Kishikawa, K., Mumby, M. C., Kamibayashi, C., Turinsky, J., O...
61. Turinsky, J., O...
60. Chaurasia, B., and Summers, S. A. (2015) Ceramides: lipotoxic inducers of metabolic disorders. Trends Endocrinol. Metab. 26, 238–239
65. Saddoughi, S. A., Gencer, S., Peterson, Y. K., Ward, K. E., Mukhopadhyay, A., Oaks, J., Bielawski, J., Szule, Z. M., Thomas, R. J., Schwan, S. P., Senkal, C. E., Garrett-Mayer, E., De Palma, R. M., Fedarovich, D., Liu, A., Habib, A. A., Stahelin, R. V., Perrotti, D., and Ogemten, B. (2013) Sphingosine analogue drug FIT270 targets I2PP2A/SET and mediates lung tumour suppression via activation of PP2A-RIPK1-dependent necroptosis. EMBO Mol. Med. 5, 105–121
64. Zinda, M. J., Vlahos, C. J., and Lai, M. T. (2001) Ceramide induces the dephosphorylation and inhibition of constitutively activated Akt in PTEN negative U87mg cells. Biochem. Biophys. Res. Commun. 280, 1107–1115
66. Powell, D. J., Hajducz, K., Kulag, G., and Huland, H. S. (2003) Ceramide 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
67. Hölttä, T. J., Huttunen, T. O., Katainen, T., and Himberg, M. (2005) Regulation of phosphoinositide production from pleckstrin homology domain translocation. Biochem. J. 384, 359–368
68. Veluthakal, R., Palanivel, R., Zhao, Y., McDonald, P., Gruber, S., and Kowaluk, A. (2005) Ceramide induces mitochondrial abnormalities in insulin-secreting INS-1 cells: potential mechanisms underlying ceramide-mediated metabolic dysfunction of the beta cell. Apoptosis 10, 841–850
69. Costello, J. F., Tershak, M. F., and Hoppel, C. L. (1997) Direct inhibition of mitochondrial respiratory chain complex III by cell-permeable ceramide. J. Biol. Chem. 272, 24134–24138
70. Di Paolo, M., Cocco, T., and Lorussi, M. (2000) Ceramide interaction with the respiratory chain heart mitochondria. Biochemistry 39, 6660–6668
71. Boslem, E., Macintosh, G., Preston, A. M., Bartley, C., Busch, A. K., Fuller, M., Laybutt, D. R., Meikle, P., and Böken, T. J. (2011) A lipidomic screen of palmitate-treated MIN6 β-cells links sphingolipid metabolites with endoplasmic reticulum (ER) stress and impaired protein trafficking. Biochem. J. 435, 267–276
72. Lei, X., Zhang, S., Emani, B., Barbour, S. E., and Ramanadham, S. (2010) A link between endoplasmic reticulum stress-induced β-cell apoptosis and the group Ia Ca2+-independent phospholipase A2 (iPLA2). Diabetes Obes. Metab. 12(Suppl 2), 93–98
73. Contreras, C., Gonzalez-Garcia, I., Martinez-Sanchez, N., Seoane-Colhano, F., Jhac, J., Morgan, D. A., Serra, D., Gallego, R., Nogales, C., Casas, N., Reg´er, R., Rabanou, N., Díez, G., and López, M. (2014) Central ceramide-induced hypothalamic...
lipotoxicity and ER stress regulate energy balance. *Cell Rep.* **9**, 366–377
83. Gao, S., Zhuo, G., Gao, X., Wu, D., Carrasco, P., Casals, N., Hegardt, F. G., Moran, T. H., and Laposchuk, G. D. (2011) Important roles of brain-specific carnitine palmitoyltransferase and ceramide metabolism in leptin hypothyroidic control of feeding. *Proc. Natl. Acad. Sci. USA* **108**, 9691–9696
84. Brown, N. F., Hill, J. K., Esser, V., Kirkland, J. L., Corkey, B. E., Foster, D. W., and Garry, J. D. M. C. (1997) Mouse white adipocytes and 3T3-L1 cells display an anomalous pattern of carnitine palmitoyltransferase (CPT I) isoform expression during differentiation. *Biochem. J.* **351**, 229–231
85. Price, N., van der Leij, F., Jackson, V., Corstorphine, C., Thomson, R., Sorensen, A., and Zammit, V. (2002) A novel brain-expressed protein related to carnitine palmitoyltransferase I. *Genomics* **80**, 433–442
86. Ramírez, S., Martín, L., Jacas, J., Carrasco, P., Pozo, M., Clotet, J., Serra, D., Hegardt, F. G., Diéguez, C., López, M., and Casals, N. (2013) Hypothalamic ceramide levels regulated by CPT1C: mediate the orexigenic effect of ghrelin. *Diabetes* **62**, 2329–2337
87. Sims, K., Haynes, C. A., Kelly, S., Allegood, J. C., Wang, E., Momin, A., Leipelt, M., Reichart, D., Glass, C. K., Sullards, M. C., and Merrill, A. H., Jr. (2010) Kdo2-lipid A, a TLR4-specific agonist, induces de novo sphingolipid biosynthesis in RAW264.7 macrophages, which is essential for induction of autophagy. *J. Biol. Chem.* **285**, 38568–38579
88. Scarlatti, F., Basny, C., Ventruti, A., Sala, G., Ciucazeau, F., Vanelvaule, A., Ghidoni, R., and Codogno, P. (2004) Ceramide-mediated macroautophagy involves inhibition of protein kinase B and up-regulation of beclin 1. *J. Biol. Chem.* **279**, 18384–18391
89. Sentelle, R. D., Senkal, C. E., Jiang, W., Ponusamy, S., Gencer, S., Selvak, S. P., Ranshak, V. K., Peterson, Y. K., Lemasters, J. J., Slik, Z. M., Bielawski, J., and Ogretmen, B. (2012) Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy. *Nat. Chem. Biol.* **8**, 831–838
90. Jiang, W., and Ogretmen, B. (2014) Autophagy paradox and ceramide. *Biochim. Biophys. Acta* **1841**, 783–792
91. Simoneau, J. A., Veerkamp, J. H., Turcotte, L. P., and Kelley, D. E. (1999) Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *faSEB J.* **13**, 2051–2060
92. Stefaniic-Racic, M., Perdomo, G., Mantell, B. S., Sipula, I. J., Brown, N. F., and O’Doherty, R. M. (2008) A moderate increase in carnitine palmitoyltransferase Ia activity is sufficient to substantially reduce hepatic triglyceride levels. *Am. J. Physiol. Endocrinol. Metab.* **294**, E960–E977
93. Bruce, C. R., Hoy, A. J., Turner, N., Watt, M. J., Allen, T. L., Carpenter, K., Cooney, G. J., Febbraro, M. A., and Kraegen, E. W. (2009) Overexpression of carnitine palmitoyltransferase-I in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet-induced insulin resistance. *Diabetes* **58**, 550–558
94. Perdomo, G., Commerford, S. R., Richard, A.-M. T., Adams, S. H., Corkey, B. E., O’Doherty, R. M., and Brown, N. F. (2004) Increased beta-oxidation in muscle cells enhances insulin-stimulated glucose metabolism and protects against fatty acid-induced insulin resistance despite intramyocellular lipid accumulation. *J. Biol. Chem.* **279**, 27177–27186
95. Blachnio-Zabielska, A. U., Koutsari, C., Tchkonia, T., and Jensen, M. D. (2012) Sphingolipid content of human adipose tissue: relationship to adiponectin and insulin resistance. *Obesity (Silver Spring)* **20**, 2341–2347
96. Blachnio-Zabielska, A. U., Baranowski, M., Hirnle, T., Zabielski, P., Lewczuk, A., Dmitruk, I., and Góski, J. (2012) Increased bioactive lipids content in human subcutaneous and epicardial fat tissue correlates with insulin resistance. *Lipids* **47**, 1131–1141
97. Zigdon, H., Kogot-Levin, A., Park, J. W., Goldschmidt, R., Kelly, S., Merrill, A. H., Jr., Scherez, A., Pezner-Jun, Y., Saada, A., and Futterman, A. H. (2013) Ablation of ceramide synthase 2 causes chronic oxidative stress due to disruption of the mitochondrial respiratory chain. *J. Biol. Chem.* **288**, 4947–4956
98. Kogot-Levin, A., and Saada, A. (2014) Ceramide and the mitochondrial respiratory chain. *Biochimie* **100**, 88–94
99. Setoyma, D., Fujimura, Y., and Miura, D. (2013) Metabolomics reveals that carnitine palmitoyltransferase-I is a novel target for oxidative inactivation in human cells. *Genes Cells* **18**, 1107–1119
100. Bruce, C. R., Thrush, A. B., Merz, V. A., Bezaire, V., Chabowski, A., Heijenhuus, G. J., and Dyck, D. J. (2006) Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am. J. Physiol. Endocrinol. Metab.* **291**, E99–E107
101. Heneghan, H. M., Huang, H., Kashyap, S. R., Gornik, H. L., McCullough, A. J., Schauer, P. R., Brethauer, S. A., Kirwan, J. P., and Kasumov, T. (2013) Reduced cardiovascular risk after bariatric surgery is linked to plasma ceramides, apolipoprotein-B100, and ApoB100/Al ratio. *Surg. Oves. Relat. Dis.* **9**, 100–107
102. Huang, H., Kasumov, T., Gattamian, P., Heneghan, H. M., Kashyap, S. R., Schauer, P. R., Brethauer, S. A., and Kirwan, J. P. (2011) Gastric bypass surgery reduces plasma ceramide subspecies and improves insulin sensitivity in severely obese patients. *Obesity (Silver Spring)* **19**, 2235–2240
103. Samad, F., Hester, K. D., Yang, G., Hannah, Y. A., and Bielawski, J. (2006) Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. *Diabetes* **55**, 2579–2587
104. Shiffman, D., Pare, G., Oberbauer, R., Louie, J. Z., Rowland, C. M., Selvam, S. P., Ramshesh, V. K., Peterson, Y. K., Lemasters, J. J., Slik, Z. M., Bielawski, J., and Ogretmen, B. (2011) Overexpression of carnitine palmitoyltransferase-I in skeletal muscle is sufficient to substantially reduce hepatic triglyceride levels. *Am. J. Physiol. Endocrinol. Metab.* **294**, E960–E977
105. Schiffmann, S., Hartmann, D., Fuchs, S., Bäred, K., Merz, V. A., Schröter, Y., Ziejkovic, A., Geisslinger, G., Grösch, S., and Stark, H. (2012) Inhibitors of specific ceramide synthases. *Biochimie* **94**, 558–565
106. Sasa, T., Hirayama, T., and Kihara, A. (2016) Enzyme activities of the ceramide synthases CERS2-6 are regulated by phosphorylation in the C-terminal region. *J. Biol. Chem.* **291**, 7477–7487
107. Novgorodov, S. A., Riley, C. L., Kefllner, J. A., Yu, J., Kindy, M. S., Macklin, W. B., Lombard, D. B., and Gutz, T. I. (2016) SIRT3 deacylates ceramide synthases: implications for mitochondrial dysfunction and brain injury. *J. Biol. Chem.* **291**, 1957–1973

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