

# Perspectives in Diabetes

## A Role for Sphingolipids in Producing the Common Features of Type 2 Diabetes, Metabolic Syndrome X, and Cushing's Syndrome

Scott A. Summers and Don H. Nelson

**Metabolic syndrome X and type 2 diabetes share many metabolic and morphological similarities with Cushing's syndrome, a rare disorder caused by systemic glucocorticoid excess. Pathologies frequently associated with these diseases include insulin resistance, atherosclerosis, susceptibility to infection, poor wound healing, and hypertension. The similarity of the clinical profiles associated with these disorders suggests the influence of a common molecular mechanism for disease onset. Interestingly, numerous studies identify ceramides and other sphingolipids as potential contributors to these sequelae. Herein we review studies demonstrating that aberrant ceramide accumulation contributes to the development of the deleterious clinical manifestations associated with these diseases. *Diabetes* 54:591–602, 2005**

**M**etabolic syndrome X (1), type 2 diabetes (2), and Cushing's syndrome (3) share a common set of pathogenic characteristics. A hallmark of these diseases is insulin resistance, i.e., when a maximal dose of the hormone is incapable of optimally eliciting its pleiotropic biological effects (e.g., stimulation of glucose uptake and glycogen, protein, and lipid synthesis in skeletal muscle). Other common disorders associated with these diseases include atherosclerosis, susceptibility to infection, poor wound healing, and hypertension. An enigma plaguing scientists has been to understand how these syndromes with markedly different etiologies share such a common clinical profile.

Obesity predisposes individuals to both type 2 diabetes and metabolic syndrome X, but the mechanism by which

increased adiposity induces defects in tissues other than adipose has remained elusive. A hypothesis gaining credibility is that increased deposition of lipid molecules in tissues not suited for fat storage, because of increased exposure to free fatty acids (4), induces these health complications (5,6). An alternative explanation is that fat-derived circulating factors initiate the various deleterious manifestations of these diseases (7–9). For example, adipose tissue secretes factors that regulate insulin sensitivity in peripheral tissues (e.g., tumor necrosis factor [TNF]- $\alpha$ , interleukin 6, resistin, adiponectin, and leptin), produce an inflammatory response (e.g., TNF- $\alpha$  and interleukin 6), induce vasoconstriction (i.e., angiotensinogen, the precursor for the vasoactive peptide angiotensin 2), and inhibit fibrinolysis (i.e., plasminogen activator inhibitor 1).

Cushing's syndrome results from excessive secretion of glucocorticoids that produce the sequelae listed above. While individuals with this disease generally display increased central adiposity and elevated circulating free fatty acid (FFA) levels, these conditions are likely to be a consequence of insulin resistance, rather than the primary cause. First, glucocorticoids induce insulin resistance within hours of administration and can do so independently of circulating FFAs (10,11). Second, dexamethasone directly antagonizes the effects of insulin when added to isolated cells or tissues (12–15).

Interestingly, FFAs, TNF- $\alpha$ , and glucocorticoids, through different mechanisms, stimulate the accumulation of ceramide and various ceramide metabolites (16–24), and these sphingolipids have been shown to amass in tissues from insulin-resistant rodents (25,26) and humans (27,28). Moreover, studies in isolated cells or tissues reveal putative roles for ceramide or its derivatives in either the onset or the progression of many of the pathologies associated with these diseases. Herein we review literature suggesting that aberrant sphingolipid accumulation accounts for some of the common clinical findings in type 2 diabetes, metabolic syndrome X, and Cushing's syndrome.

### INCREASED SPHINGOLIPID PRODUCTION IN TYPE 2 DIABETES, METABOLIC SYNDROME X, AND CUSHING'S SYNDROME

To understand how FFAs, TNF- $\alpha$ , and glucocorticoids regulate sphingolipid production, one needs to understand the biochemical pathways underlying ceramide synthesis and

From the Division of Endocrinology, Metabolism and Diabetes, University of Utah School of Medicine, Salt Lake City, Utah.

Address correspondence and reprint requests to Scott A. Summers, Division of Endocrinology, Metabolism and Diabetes, Department of Internal Medicine, University of Utah, Salt Lake City, UT 84132. E-mail: scott.summers@hsc.utah.edu.

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FFA, free fatty acid; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PPAR, peroxisome proliferator-activated receptor; S1P, sphingosine 1-phosphate; SPT, serine palmitoyltransferase; TNF, tumor necrosis factor; TZD, thiazolidinedione.

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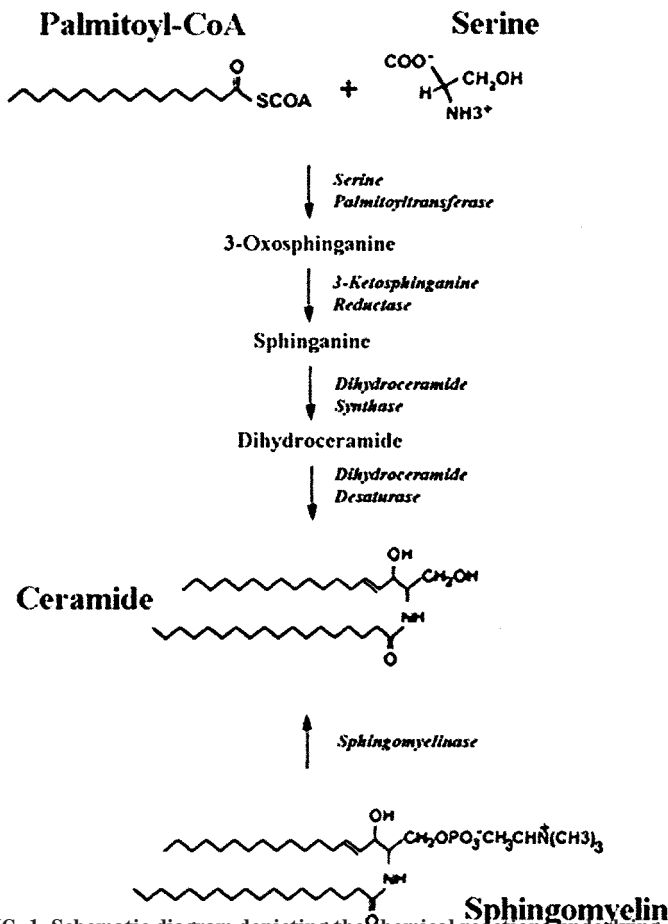


FIG. 1. Schematic diagram depicting the chemical reactions underlying de novo ceramide synthesis.

degradation. Although sphingolipids represent a significant dietary component, they are largely degraded in the mammalian intestine (29), and their production in animal tissues is primarily dependent on a widespread biosynthetic pathway (30). The initial, rate-limiting reaction is the condensation of palmitoyl CoA and serine, a reaction catalyzed by serine palmitoyltransferase (SPT), to produce 3-oxosphinganine (Fig. 1). The mechanisms underlying the regulation of this enzyme are largely unknown, but the availability of palmitoyl-CoA and serine strongly influences the rate of this reaction. Three reactions follow, resulting in sphinganine  $\rightarrow$  dihydroceramide  $\rightarrow$  ceramide production. Once generated, ceramide is a basic building block for numerous additional sphingolipid derivatives

including sphingomyelin, sphingosine 1-phosphate (S1P), ceramide 1-phosphate, and a large family of glucosylceramides (Fig. 2).

**Saturated FFAs and ceramide synthesis.** Intramyocellular lipid concentrations correlate more tightly with the severity of insulin resistance than other known risk factors, including BMI, percent body fat, waist-to-hip ratio, or age (6). In particular, numerous dietary and epidemiological studies in human populations indicate that saturated fats markedly decrease insulin responsiveness in peripheral tissues, while unsaturated fats have weaker or in some cases insulin-sensitizing effects (rev. in 31). These observations have prompted researchers to search for metabolites of saturated FFAs that inhibit insulin signaling or action. The most prevalent saturated FFA in both the circulation and in muscle is palmitate (32), which stimulates de novo ceramide synthesis by fueling the pathway with substrate (i.e., palmitoyl-CoA) (22–24) and, in at least one cell type examined, by inducing expression of SPT (33). Using C2C12 myotubes, the saturated FFAs stearate (18:0), arachidate (20:0), and lignocerate (24:0), which all have hydrocarbon tails longer than that of palmitate, were also shown to induce ceramide accrual (24). By contrast, neither unsaturated FFAs nor saturated FFAs with hydrocarbon chains shorter than that of palmitate (i.e., laurate [12:0] and myristate [14:0]) stimulated ceramide accumulation. The mechanism by which longer FFAs might induce ceramide synthesis is unclear, as SPT has a great deal of specificity for FFAs with  $16 \pm 1$  carbon atoms (30). We hypothesize that long-chain saturated FFAs selectively induce ceramide accumulation by increasing the intracellular pool of palmitoyl-CoA, either by being oxidized themselves to the shorter C16 form or by protecting the endogenous palmitoyl-CoA from further metabolism.

**TNF- $\alpha$  and ceramide synthesis.** The observation that ceramide plays a role in TNF- $\alpha$  signaling initially derived from studies investigating the cytokine's ability to regulate differentiation of HL60 promyelocytic leukemia cells. Briefly, the ability of TNF- $\alpha$  to induce HL60 cell differentiation corresponded with its ability to hydrolyze sphingomyelin, and the addition of C2-ceramide, a short-chain ceramide analog, was sufficient to induce cell differentiation in the absence of exogenous TNF- $\alpha$  (18). More recent studies have identified ceramide as a requisite intermediate for many of the cytokine's biological effects (34,35). TNF- $\alpha$  acutely produces ceramide by activating neutral and acidic forms of sphingomyelinase (19,36–38). More-

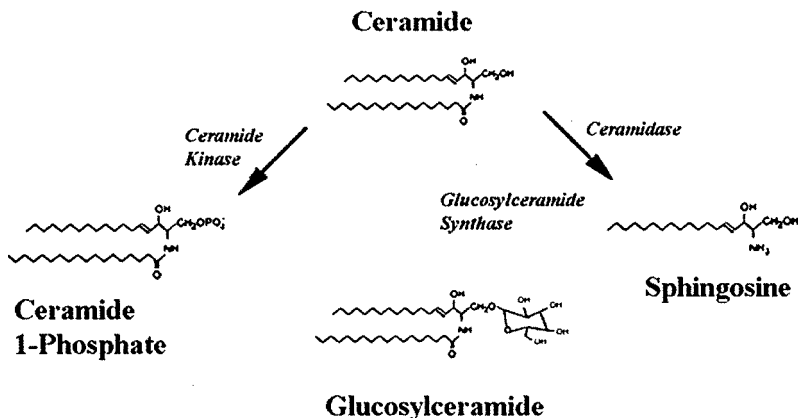


FIG. 2. Schematic diagram depicting the metabolic pathways regulating ceramide degradation and metabolism.

over, TNF- $\alpha$  also stimulates de novo ceramide synthesis, although the mechanism underlying this effect is unclear (20,21).

**Glucocorticoids and ceramide synthesis.** The discovery that glucocorticoids have a large and specific effect on sphingolipids derived from studies addressing the theory that the broad scope of corticosteroid action was due to the ability of different hormones to directly modify membrane lipids (39). Specifically, the well-recognized importance of corticosteroids in protection against stress was considered to possibly relate to membrane fluidity, which is involved in the adaptation of bacteria, fish, and hibernating animals to extremes of heat and cold (40). To investigate this possibility, investigators quantified the fatty acid, phospholipid, and sphingolipid composition of membranes in various cell types incubated with dexamethasone. Notably, the glucocorticoid increased membrane sphingomyelin in rat epididymal fat cells (41). Dexamethasone was subsequently shown to increase sphingomyelin levels in HeLa cells (42) and human polymorphonuclear leukocytes (17), ceramide levels in a murine B lymphoma cell line (16), and sphingosine levels in 3T3-L1 preadipocytes (43). In vivo, epididymal fat cell ghosts isolated from adrenalectomized rats demonstrated decreased sphingomyelin levels, which could be restored by the administration of dexamethasone (44). By contrast, these researchers detected no change in either the levels or fatty acid composition of phospholipids, nor did they detect a change in cellular cholesterol levels.

Recent studies have investigated the mechanism by which glucocorticoids regulate sphingolipid production. Dexamethasone increases the expression and/or activity of SPT (45) and neutral and acidic forms of sphingomyelinase (17,43,46,47). These observations are consistent with the established roles of glucocorticoid receptors as transcription factors whose entry into the nucleus is regulated by ligand binding. Interestingly, Cifone et al. (47) demonstrated that dexamethasone also acutely stimulates ceramide accumulation (i.e., within 15 min of dexamethasone addition) in thymocytes by activating acid sphingomyelinase.

#### ROLE OF CERAMIDES IN INSULIN RESISTANCE

Since the majority of glucose disposal occurs in skeletal muscle, insulin resistance in this tissue is generally thought to contribute most significantly to the glucose intolerance associated with nutrient or glucocorticoid oversupply. Thus far, ceramide has been shown to accumulate in insulin-resistant muscles in both rodents (25,26) and humans (27,28). Moreover, Strackowski et al. (28) described a negative correlation between ceramide content of muscles and insulin sensitivity in 13 human subjects and further demonstrated that lipid infusion markedly elevated ceramide levels while decreasing insulin sensitivity. Exercise training, which improves insulin sensitivity, markedly decreases muscle ceramide levels in both rats and humans (48–50). Although the increase in ceramide seen in these samples is modest, recent studies in cultured myotubes indicate that inducing a comparable increase (~1.5- to 2-fold) in endogenous ceramide is sufficient to inhibit insulin signaling (23,24).

**Inhibition of insulin signaling and action by ceramides.** Insulin accelerates glucose entry into skeletal muscle and

adipose tissue by evoking the translocation of GLUT4 glucose transporters from intracellular stores to the plasma membrane. Simultaneously, the hormone regulates numerous metabolic enzymes (e.g., glycogen synthase or pp70 S6-kinase) to promote storage of the incoming glucose as glycogen, triglyceride, or protein. Insulin initiates these responses through its receptor, a tyrosine kinase, which subsequently phosphorylates a family of insulin receptor substrates (IRSs) (IRS-1, IRS-2, etc.). The phosphorylated IRS proteins activate a signaling pathway involving the sequential activation of phosphatidylinositol 3-kinase (PI3K) and Akt/protein kinase B (PKB), which are obligate intermediates in insulin's metabolic, antiapoptotic, and mitogenic effects (51).

Ceramide acutely inhibits insulin-stimulated glucose uptake, GLUT4 translocation, and/or glycogen synthesis in cultured adipocytes and/or muscle (52–55). These effects appear to result from the sphingolipid's ability to block activation of either IRS-1 or Akt/PKB (Fig. 3). Specifically, in 1996, two independent laboratories found that treating cultured cells with short-chain ceramide analogs or bacterial sphingomyelinases, which hydrolyze sphingomyelin to form choline and ceramide (Fig. 1), blocked insulin-stimulated tyrosine phosphorylation of IRS-1 and its subsequent recruitment and activation of PI3K (56,57). A third group found that ceramide directly inhibited PI3K isolated from serum-stimulated cells (58). However, the effect of ceramide on IRS and/or PI3K appears to be specific to certain cells or treatment conditions, as a number of different laboratories have shown that ceramide has no effect on PI3K or the production of its lipid products (23,53–55,59–60). In all cell types tested, however, ceramide has been shown to block activation of Akt/PKB “downstream” of PI3K by either inhibiting its translocation to the cell membranes (61–63) and/or by promoting its dephosphorylation via protein phosphatase 2A (23,60,62,64–66). While acute treatment with ceramides has these effects on insulin signaling, prolonged treatment of 3T3-L1 adipocytes with the sphingolipid was shown to downregulate GLUT4 expression (67).

**Role of ceramides in FFA-induced insulin resistance.** Skeletal muscles exposed to excess lipid demonstrate decreased sensitivity to insulin. For example, incubating isolated muscle strips or cultured muscle cells with FFAs (22,68–71), infusing lipid emulsions into rodents or humans (72–75), or expressing lipoprotein lipase in skeletal muscle of transgenic mice (76,77) promotes intramyocellular lipid accumulation and compromises insulin-stimulated glucose uptake. To evaluate the role of ceramides in the insulin resistance associated with lipid oversupply, scientists have investigated the lipid's role in FFA-induced insulin resistance using cultured myotubes. Schmitz-Pfeiffer et al. (22) first observed that treating C2C12 myotubes with concentrations of saturated FFAs within the physiological serum range increased the intracellular pool of ceramide, while simultaneously inhibiting activation of Akt/PKB, but not PI3K. In this cell type, short-chain ceramide analogs recapitulated this pattern of effects on insulin signal transduction. The authors thus speculated that ceramide was the primary intermediate linking saturated fats to the inhibition of insulin signaling. To definitively identify a role for ceramide as an intermediate in these effects of saturated FFAs in C2C12 myotubes, Chavez et al.

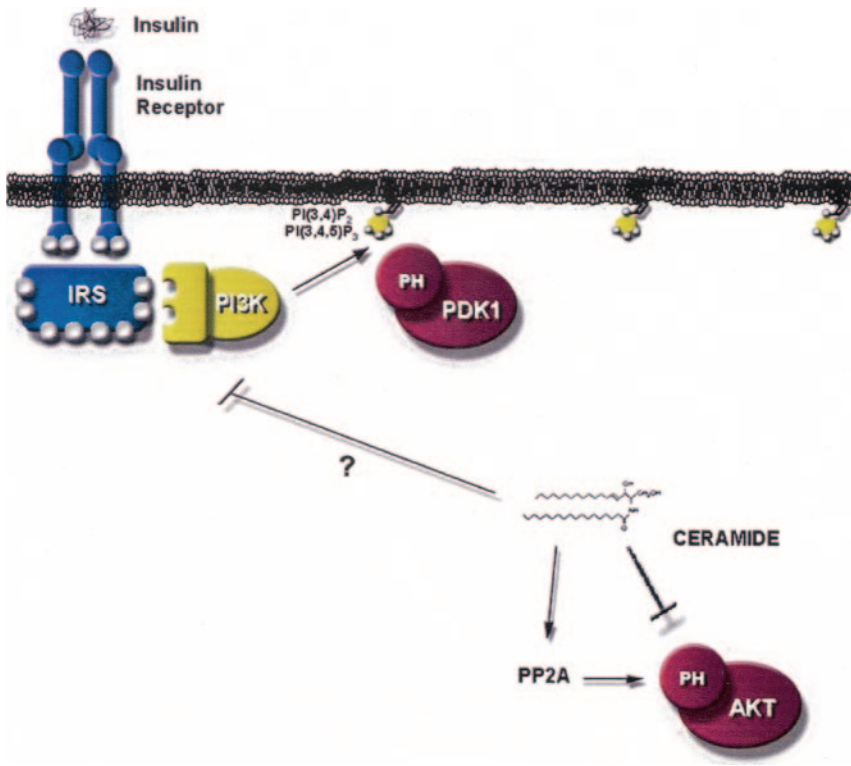


FIG. 3. Schematic diagram depicting the pathways linking ceramides to the inhibition of IRS-1 and Akt/PKB. As described in the text, ceramides have been shown to independently promote the serine/threonine phosphorylation and inhibition of IRS-1, block the translocation of Akt/PKB to the plasma membrane, and catalyze the dephosphorylation of Akt/PKB by protein phosphatase 2A.

(23) demonstrated that inhibitors of the biosynthetic enzymes SPT or dihydroceramide synthase prevented the effects of palmitate on both ceramide accumulation and Akt/PKB. Moreover, inhibitors of ceramide degradation (i.e., its glycosylation or deacylation) were shown to both mimic and exacerbate the palmitate effect on ceramide accrual and insulin signaling (23). Collectively these studies strongly indicate that ceramide is required for the inhibitory effects of saturated FFAs in cultured myotubes.

Of note, researchers recently demonstrated that infusing a lipid mixture enriched in unsaturated fatty acids into rodents may induce insulin resistance through a ceramide-independent mechanism. Specifically, infusion of Liposyn II (Abbott, North Chicago, IL), a triglyceride emulsion that is predominantly comprised of the fatty acid linoleate (18:2), induced insulin resistance by inhibiting insulin signaling to IRS-1 and PI3K (78). This lipid cocktail did not affect Akt2/PKB- $\beta$  or glycogen synthase kinase 3- $\beta$  (79), nor did it induce ceramide accumulation (75,78). In a subsequent study, a lipid emulsion of comparable composition was shown to increase muscle ceramide levels in humans, and the authors speculated that different methods of muscle preparation may account for the opposite findings (28). Nonetheless, the relative absence of saturated fats in this cocktail makes it unlikely to markedly induce ceramide synthesis, which is dependent on the availability of

palmitate. Quite possibly, different types of fat induce insulin resistance through distinct mechanisms, with saturated FFAs inducing insulin resistance through a ceramide-dependent pathway and unsaturated ones doing so through another lipid intermediate (e.g., diacylglycerol). Indeed, some evidence supports the hypothesis that diacylglycerol comprised of predominantly unsaturated FFAs is a potent activator of certain intracellular substrates (e.g., protein kinase C), while that composed of saturated fatty acids is a relatively poor agonist (80,81). An important future step will be to determine whether ameliorating ceramide accumulation, using either pharmacological or genetic manipulation strategies, quantitatively improves insulin sensitivity in intact organisms, such as insulin-resistant rodents.

**Role of ceramides in TNF- $\alpha$ -induced insulin resistance.** TNF- $\alpha$  is a proinflammatory cytokine that inhibits insulin-stimulated glucose uptake and/or hepatic glucose production when administered to either cultured cells (82) or animals (83). Obesity increases expression of TNF- $\alpha$  in white adipose tissue, prompting speculation that the cytokine induces insulin resistance in cases of increased adiposity. Although concentrations found in the circulation of obese patients are typically low, even in obese individuals, researchers have speculated that TNF- $\alpha$  functions in an autocrine or paracrine manner and is secreted

from fat cells pervading muscle tissue in obese animals (84) or directly from muscle (85). Neutralization of TNF- $\alpha$  activity, either by infusing *fa/fa* Zucker rats with a soluble TNF receptor IgG fusion protein or by crossing insulin-resistant mice with knockout mice lacking either TNF- $\alpha$  or TNF- $\alpha$  receptors, was shown previously to increase peripheral insulin sensitivity (86,87). Although the systemic administration of a TNF- $\alpha$ -neutralizing antibody failed to improve insulin sensitivity in humans (88), the effectiveness of this strategy at negating TNF- $\alpha$  activity could not be confirmed directly, and scientists speculate that increased TNF- $\alpha$  levels could exacerbate the insulin-resistant condition.

Under conditions whereby TNF- $\alpha$  inhibits insulin signaling, the cytokine promotes ceramide accumulation in brown adipocytes (60), 3T3-L1 adipocytes, and C2C12 myotubes (A. Chavez, S.A.S., unpublished observation). Moreover, like ceramides, TNF- $\alpha$  has been shown to block insulin signaling at the level of IRS-1 and Akt/PKB, depending on the cell type being examined (57,60,89,90), and to decrease the expression of IRS-1 and GLUT4 (91). Could ceramide mediate the inhibitory effects of TNF- $\alpha$  on insulin signaling? In myeloid 32D progenitor cells and 3T3-L1 adipocytes, the effects of TNF- $\alpha$  on IRS-1 were recapitulated by the addition of exogenous bacterial sphingomyelinase or ceramides (57). In brown adipocytes, TNF- $\alpha$  was shown to promote the dephosphorylation of Akt/PKB by activating PP2A (60). In this cell type, exogenous ceramides again recapitulated these TNF- $\alpha$  effects, and the PP2A inhibitor okadaic acid prevented the effects of both antagonists. The authors concluded that ceramide was the principle mediator of the signaling pathway linking TNF- $\alpha$  to the inhibition of insulin signaling.

**Role of ceramides in glucocorticoid-induced insulin resistance.** When added to cultured cells or isolated tissues, glucocorticoids block glucose uptake (12–15,92–95) and glycogen and protein (96–98) synthesis, but the mechanisms underlying these inhibitory effects remain unclear. Studies both *in vitro* and *in vivo* have demonstrated that dexamethasone decreases expression and/or activation of insulin receptors, IRS-1, or PI3K (99–101), but others have failed to see inhibition by dexamethasone at these early signaling steps (102–105). The inconsistencies between studies done *in vivo* may be explained by differing degrees of hyperinsulinemia under the different treatment regimes. When the compensatory increase in insulin levels was prevented, the effects on insulin receptor levels or binding affinity were abolished (106). Further downstream, corticosteroids have been shown to blunt the phosphorylation of Akt/PKB, 4E-BP1, p70S6K, and glycogen synthase (97,98,100,107–109) and to inhibit the translocation and expression of GLUT4 (14,15). Elucidating the mechanisms underlying these antagonistic actions of glucocorticoids is not only important for comprehending the pathogenesis of Cushing's syndrome, but is also extremely relevant for understanding the complications associated with exogenous glucocorticoid therapy.

As described above, glucocorticoids activate synthetic pathways promoting sphingolipid formation in a wide variety of tissues, including insulin-responsive ones. In adipocytes, for example, low doses of dexamethasone, which antagonize insulin-stimulated glucose uptake (110), selectively increase sphingolipid levels (41). By contrast, adre-

nalectomy, which increases insulin sensitivity in adipocytes (111), markedly decreases adipocyte sphingolipid levels (44). An important aspect of these studies is the remarkable size and specificity of the glucocorticoid effect. In 3T3-L1 preadipocytes, for example, glucocorticoids induce a 50% increase in membrane sphingomyelin levels within 3 h after their addition, without affecting phospholipids or cholesterol (112). Based on studies in cultured cells (23,24), one would predict that increasing ceramide levels by this amount would be likely to block insulin action.

Though sphingolipids have been shown to be obligate intermediates in the pathways linking glucocorticoids to the regulation of various biological processes (e.g., thymocyte apoptosis [46]), researchers have yet to perform analogous studies determining whether ameliorating ceramide accumulation abates the glucocorticoid effect on insulin signaling or action. Nonetheless, two studies suggest that dexamethasone and ceramide inhibit insulin signaling and action using a common pathway. In L6 myoblasts, the effects of dexamethasone on distal constituents of the PI3K/Akt signaling pathway [i.e., ribosomal protein S6 kinase [p70(S6k)] and the cap-dependent translational repressor, eukaryotic initiation factor 4E (eIF4E)] were blocked by okadaic acid and calyculin A (109), which, as described above, have been shown to reverse the effects of ceramide on insulin signaling (24,60,66). Moreover, in 3T3-L1 cells, the inhibitory effects of sphingosine, sphinganine, or dexamethasone on glucose transport were not additive (113). Collectively, these studies suggest the involvement of redundant intracellular mechanisms linking both sphingolipids and glucocorticoids to the regulation of insulin signaling or action.

#### ROLES OF SPHINGOLIPIDS IN OTHER FEATURES OF CUSHING'S SYNDROME, TYPE 2 DIABETES, AND THE METABOLIC SYNDROME

In addition to having marked effects on insulin sensitivity, ceramide and its metabolites are implicated in a wide array of different biological processes. Interestingly, many of the complications evident in individuals with Cushing's syndrome, type 2 diabetes, and metabolic syndrome X may be significantly influenced by increased sphingolipid deposition in other tissues. Herein we discuss evidence supporting a role for sphingolipids in the cardiovascular and immune systems.

**Atherosclerosis.** Aggregation of LDLs within the arterial wall is a critical step in the initiation of atherosclerosis. These circulating entities consist of a core of cholesterol esters and triacylglycerols surrounded by a surface film containing apolipoprotein B-100, phosphatidylcholine, sphingomyelin, and cholesterol. LDLs extracted from atherosclerotic lesions are either aggregated or prone to aggregate (114,115). Interestingly, the ceramide content of these aggregated LDLs is 10- to 50-fold higher than that of plasma LDL (116). In fact, nonaggregated LDLs isolated from the same lesions are not enriched in ceramide, suggesting that ceramides could drive associations between LDLs. Support for this hypothesis derives from studies using isolated LDLs or artificial lipid vesicles, as exposing LDL particles to a small amount of bacterial sphingomyelinase was shown to promote LDL aggregation (116), and biophysical studies with artificial vesicles reveal that cer-

amides have a pronounced tendency for self-aggregation (117,118). Ceramide present in these plaques may be produced by secreted forms of neutral sphingomyelinase, which associate with the LDL particle but not with VLDLs or HDLs (119).

Scientists have also proposed roles for ceramide or its metabolites in other steps of atherogenesis (120,121). First, ceramide and sphingosine have been shown to induce apoptosis of certain cells lining the vascular wall, a process implicated in plaque erosion and thrombosis (122,123). In fact, increased myocardial levels of ceramide have been linked to cardiac myocyte apoptosis and the resulting cardiac dysfunction associated with obesity (124). Second, the ceramide metabolite S1P stimulates the proliferation of endothelial and smooth muscle cells in vascular walls (125,126), which would promote thickening and favor plaque stabilization. Third, ceramide, lactosyl ceramide, or S1P mediate an inflammatory response initiated by cytokines or oxidized LDL, which upregulates adhesion molecule expression and induces adhesion and migration of monocytes, important events in initiation and progression of atherogenesis (127). Fourth, various sphingolipids, by modulating platelet activation and aggregation (128–130), may favor thrombosis.

**Hypertension.** Hypertension results from increased peripheral resistance, which maintains elevated levels of arterial blood pressure. The increase in peripheral resistance results, in part, from abnormal constrictor and dilator responses and vascular remodeling. Though a clear picture has not yet emerged, accumulating evidence suggests that ceramide or its metabolites play important roles in regulating vascular tone (131).

One mechanism by which sphingolipids might contribute to hypertension is by altering membrane fluidity, which has been shown to be decreased in hypertensive rats. Because of the extensive intermolecular hydrogen bonding between ceramides, they have been shown to markedly decrease membrane fluidity (132). Interestingly, Dorrance et al. (133) observed that membrane sphingomyelin levels were elevated in erythrocyte membranes isolated from stroke-prone, spontaneously hypertensive rats.

Alternatively, several studies reveal potent vasoactive roles for various sphingolipid metabolites. For example, treating arterial rings with ceramide or sphingomyelinase has been shown to induce an endothelium-independent, sustained contraction (134). Moreover, sphingosine has been shown to impair endothelium-dependent relaxation (135), and S1P and sphingophosphorylcholine have been reported to induce contraction of mesenteric and intrarenal microvessels (136) and coronary arterial strips (137) and to inhibit renal blood flow when given to rats (138). Interestingly, Bolz et al. (139) found that overexpressing sphingosine kinase type 1 in vascular smooth muscle cells of resistance arteries increased both resting tone and myogenic responses, while overexpression of a dominant-negative sphingosine kinase mutant completely inhibited these processes. Paradoxically, TNF- $\alpha$  has been shown to have strong vasodilatory powers, and ceramide has been shown to mediate its endothelium-independent vasodilatory effects on aortic segments (140) and its endothelium dependent effects in coronary arteries (141). Moreover, the

lipid has been shown to attenuate the contractile responses of phenylephrine (142).

The development of hypertension in individuals with the metabolic disorders discussed herein might relate to the regulation of nitric oxide (NO), which has strong vasodilatory functions. Corticosteroids have been shown to inhibit NO production in endothelial cells (143) by both inhibiting NO synthase (144) and increasing production of superoxides (145), which are NO scavengers. In diabetic patients, vascular superoxide production is similarly increased due to dysfunctional endothelial NO synthase (146). Ceramides have been shown to attenuate the vasodilatory effects of bradykinin in coronary arteries by increasing superoxide production and thus lowering NO accumulation (147). Moreover, the ability of TNF- $\alpha$  to inhibit endothelium-dependent relaxation of coronary arteries is blocked by the addition of either superoxide scavengers or acid sphingomyelinase inhibitors (141). Further complexity in the vessel wall response, however, is due to the fact that whereas sphingolipids can induce NO production, NO can promote ceramide generation (148–150). **Sphingolipids, the immune response, and susceptibility to infection.** Patients with either diabetes or Cushing's syndrome are particularly susceptible to infection. Interestingly, certain sphingolipids have been speculated to render one susceptible to infection by either facilitating the entry of viruses or bacteria into host cells or by decreasing an individual's resistance to these pathogens.

Due to their long, largely saturated acyl chains, sphingolipids tend to pack together in microdomains that exclude phospholipids. In the presence of cholesterol, these sphingolipids organize themselves in raft structures that can be isolated from other membrane fractions due to their insolubility to some nonionic detergents. Ceramide, because of its tendency to self-associate, induces the coalescence of microscopic rafts into large-membrane macrodomains. Raft domains have been shown to recruit certain types of cellular proteins, while excluding others, and are important for processes such as signal transduction, sorting, and endocytosis. Interestingly, an abundance of evidence indicates that these raft structures facilitate entry of various pathogens (151–155). The importance of ceramides in pathogen entry is underscored in studies investigating *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Sindbis virus*, which have been shown to activate acid sphingomyelinase to rapidly induce ceramide formation (156–159). A strength of these studies was the observation that inactivation of acid sphingomyelinase greatly hindered pathogen internalization. In addition, the protozoan, *Leishmania donovani*, was shown to induce ceramide generation through both de novo synthesis and activation of sphingomyelinase, and the elevated ceramide was shown to facilitate the survival of leishmanial parasites in the intramacrophageal milieu (160). Rafts, in addition to playing a potential role in pathogen entry, have been shown to serve as platforms for viral assembly or budding (154).

The susceptibility to infection for individuals with these diseases additionally involves an altered immune response that renders them susceptible to opportunistic pathogens. For example, uncontrolled diabetes demonstrates defective migration of polymorphonuclear leukocytes, which ingest

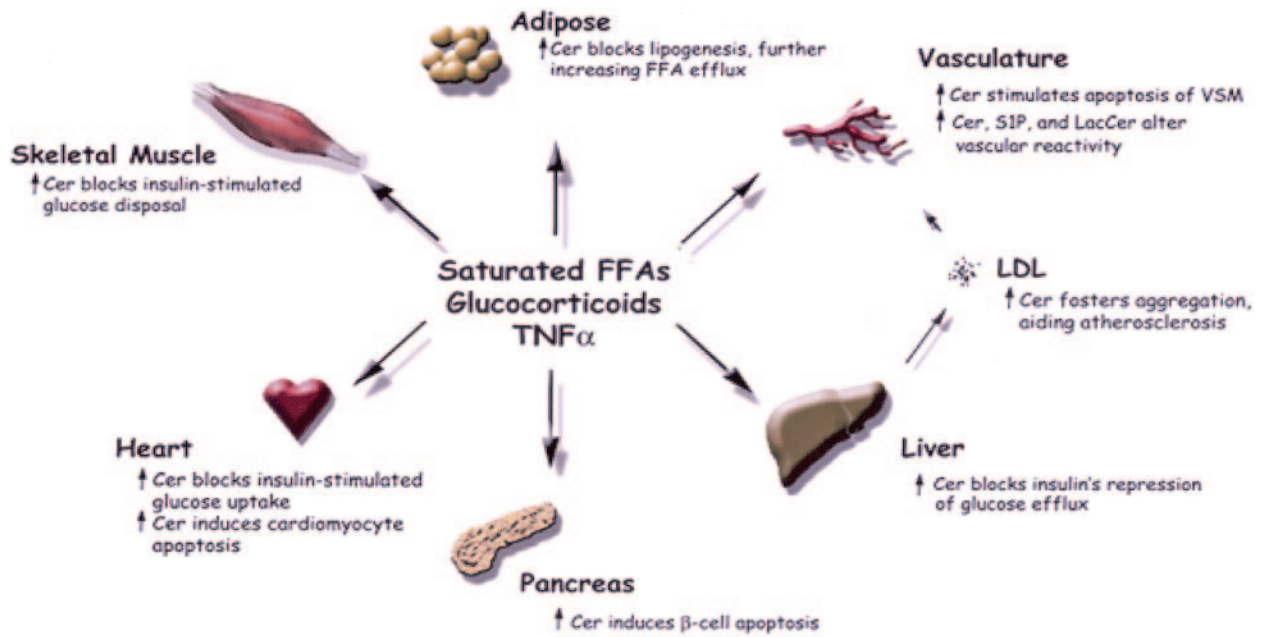


FIG. 4. Schematic diagram depicting the multifactorial effects of ceramides in the regulation of insulin sensitivity, vascular reactivity, and cardiovascular function.

and destroy microbes, as well as impaired phagocytosis of the invading pathogen (161). Similarly, glucocorticoids have been shown to inhibit superoxide production, which is important for the destruction of the invading agent, both in vitro and in vivo (162,163). Interestingly, ceramides have been shown to mimic these effects as well. For example, increasing endogenous ceramide levels to a maximal level terminates functional responses in polymorphonuclear leukocytes, as ceramide inhibits phagocytosis and blocks superoxide release (164–167). Diabetic individuals additionally exhibit an exaggerated inflammatory response to microbial products, which further compromises healing (161), and ceramides or sphingosine have been shown to augment the inflammatory response of TNF- $\alpha$  or other proinflammatory cytokines (168–170). In addition, ceramides and other sphingolipids have been shown to positively or negatively affect the function of mononuclear phagocytes, mast cells, dendritic cells, natural killer cells, cytotoxic T-cells, B-cells, and others (rev. in 171). While a complete understanding of the role of these sphingolipids in the immune response is beyond the scope of this article, one can easily envision how globally altering sphingolipid levels could interfere with the development of an appropriate defense against invading pathogens.

#### THIAZOLIDINEDIONES, GLUCOSE TRANSPORT, CORTICOSTEROIDS, AND SPHINGOLIPIDS

Thiazolidinediones (TZDs), which serve as ligands for the peroxisome proliferator-activated receptor (PPAR)- $\gamma$  transcription factors, have been shown to reduce insulin resistance induced by nutrient (172,173) or corticosteroid (174) oversupply. Additional studies demonstrate protective effects toward atherosclerosis and hypertension (175–177). The mechanism by which PPAR- $\gamma$  improves insulin sensitivity in skeletal muscles is unclear, although many models have been proposed. In adipose tissue, which has the highest expression of PPAR- $\gamma$ , these drugs have been shown to inhibit expression or secretion of TNF- $\alpha$ , resis-

tin, and interleukin-6 and to stimulate expression of the insulin-sensitizing adiponectin (rev. in 178). Moreover, TZDs decrease circulating FFA levels by increasing the number of adipocytes and stimulating the expression of genes promoting the incorporation of FFAs into triglycerol. Lastly, TZDs have been shown to downregulate 11- $\beta$  hydroxysteroid dehydrogenase-1, an enzyme that generates the active cortisol from the inactive precursor cortisone, and thus could influence adipocyte secretion of glucocorticoids. Interestingly, studies also reveal a direct role in skeletal muscle, as rodents lacking the PPAR- $\gamma$  in muscle demonstrate muscle and hepatic insulin resistance (179,180).

By decreasing circulating TNF- $\alpha$  and FFA levels, TZDs might be expected to lower tissue ceramide levels in insulin-resistant rodents. Indeed, TZDs have been shown to reduce ceramide levels in cardiac muscle of obese Zucker rats (124). However, additional studies imply a direct relationship between sphingolipids and the actions of PPAR- $\gamma$ . TZDs prevent the inhibitory effects of TNF- $\alpha$  (181–183), glucocorticoids (13), and C2-ceramide (183) on insulin signaling when added to isolated cells or tissues. Conversely, sphingolipids have been shown to antagonize the effects of PPAR- $\gamma$ . Specifically, sphingomyelin downregulates PPAR- $\gamma$  gene expression in 3T3-F442 adipocytes and is an independent predictor of insulin resistance in obese women (184,185). Ceramide has similarly been shown to inhibit PPAR- $\gamma$  expression (186). Could ceramides themselves serve as antagonists of PPAR- $\gamma$ , whose agonists include other lipid moieties such as certain FFAs and prostaglandins? Sphinganine, but not ceramide, has been shown to bind directly to PPAR- $\alpha$  (187). However, to this author's knowledge, no reports have shown a direct interaction between sphingolipids and PPAR- $\gamma$ .

#### OUTSTANDING QUESTIONS AND FUTURE DIRECTIONS

Type 2 diabetes, metabolic syndrome X, and Cushing's disease share a variety of common metabolic and hormonal abnormalities that likely contribute to the progression of

the disease, creating a cyclical and amplifying process underlying these health abnormalities. In searching for common molecular events that account for the long list of clinical complications, we considered the possibility that aberrant sphingolipid accumulation in peripheral tissues could contribute to the pathogenesis of these diseases. Review of the literature reveals that elevated sphingolipid production in peripheral tissues could contribute to the development of insulin resistance, atherosclerosis, hypertension, and susceptibility to infection (Fig. 4). Important hurdles remain, however. An important future step will be to determine whether ameliorating ceramide accumulation in rodent models of these diseases, using either pharmacological or genetic manipulation strategies, improves insulin sensitivity and prevents the host of cardiovascular and immunological abnormalities that develop. Moreover, the existing literature investigating the mechanisms by which FFAs, TNF- $\alpha$ , glucocorticoids, and ceramides induce their biological effects have shown considerable variability, with little consensus between groups. An explanation for these marked differences in effect is lacking and is clearly a major challenge for researchers. Lastly, although sphingoid bases and their related compounds are the subject of >5,000 publications in the past decade (188), the mechanisms underlying their effects have remained both elusive and controversial (189). In addition to defining a role for sphingolipids in the pathogenesis of disease, studies investigating the mechanism by which ceramides participate in this diverse array of biological responses have the opportunity to shed needed light on the role of this complicated family of molecules as regulators of basic cell function.

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#### NOTE ADDED IN PROOF

After acceptance of this manuscript, Hojjati et al. (*J Biol Chem*; in press) reported that treating the apoE-deficient mouse, a well-known model of atherosclerosis, with an inhibitor of ceramide biosynthesis decreased the appearance of atherosclerotic lesions. These findings further support a role for the sphingolipid or its metabolites in the formation of atherosclerotic plaques.

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