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Ceramides with Different Acyl Chain Length in the Pathogenesis of Insulin Resistance

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Abstract: *The review analyzes the literature data that forms the basis for the "ceramide-centric" view of insulin resistance pathogenesis and obesity-associated diabetes mellitus type 2. The results of lipid analysis of adipose tissue, skeletal muscles and liver in rodents with experimental obesity, as well as biopsic specimens of diabetics indicate the high pathogenicity of specific ceramide family memebers. The degree of pathogenicity is determined by the length of the acyl chain included in de novo synthesized ceramide with the involvement of one of the six ceramide synthase isoenzymes. Selective inhibition of the ceramide synthase-6 isoenzyme to reduce the tissue level of the most pathogenic C16:0-ceramide may be a promising approach for correcting insulin resistance.*

Keywords: *ceramide, insulin resistance, obesity, diabetes mellitus type 2*

Abbreviations: *aSMase - acid sphingomyelinase, BMI - body mass index, CAT-I - carnitine acyltransferase I, CerS - ceramide synthase and its six isoenzymes (CerS1 – CerS6), DES - dihydroceramide Δ4-desaturase, ER - endoplasmic reticulum, FADD - Fas/Apo-1-associated death domain, FAN - factor associated with neutral sphingomyelinase activation, HDL - high-density lipoproteins, IL-6 - interleukin-6, IRS - insulin receptor substrate, LPS – lipopolysaccharides, MCP-1- monocyte chemoattractant protein-1, MIP-1 - macrophage inflammatory protein-1, NADPH - nicotinamide adenine dinucleotide phosphate, reduced, nSMase - neutral sphingomyelinase, PAI-1 - plasminogen activator inhibitor-1, PDK1 - 3-phosphoinositide dependent protein kinase-1, PI3K - phosphatidylinositide 3-kinase, PKB - protein kinase B, PKC - protein kinase C zeta, PP2A - phosphoprotein phosphatase 2A, PtdIns(3,4,5)P3 - phosphatidylinositide (3,4,5) trisphosphate, PtdIns(4,5)P2 - phosphatidylinositide(4,5) bisphosphate, RBP4 - retinol binding protein-4, ROS - reactive oxygen species, SAA - serum amyloid A protein, Ser – serine, SPT - serine C-palmitoyltransferase, TAG – triacylglyceride, TGF - transforming growth factor- , Thr– threonine, TLR4 - toll-like receptor 4, TNFR - tumor necrosis factor-alpha receptor, TNFα - tumor necrosis factor alpha , TRADD - TNF receptor-associated death domain, Tyr – tyrosine, VLDL - very-low-density lipoproteins.*

1. Introduction

George Turinsky and his team were the first to demonstrate an increase in the level of ceramides in the skeletal muscles of insulin-resistant rats [1]. Thanks to their discovery the role of metabolic change in sphingolipids in the pathogenesis of obesity-associated metabolic disorders and diabetes mellitus type 2 has become clear. The results of recent independent studies have shown that normal sphingolipid metabolism is one of the most important conditions for maintaining glucose homeostasis in the body [2, 3]. These findings provided the basis for a gradual transition from the traditional "glucocentric" paradigm of insulin resistance to the "ceramid-centric" view [4, 5, 6, 7, 8]. During *de novo* ceramide biosynthesis, the sphinganine acylation reaction is catalyzed by six isoenzymes of ceramide synthase (CerS), each of which exhibits specificity to a particular acyl-CoA. This reaction determines the appearance of the ceramide molecules differing from the length of the acyl chain [9, 10, 11]. It has been established that C16:0-ceramide (palmitoyl ceramide) has the most pathogenic properties, and its synthesis is catalysd by the CerS-6 isoenzyme [12, 13, 14, 15].

2. Leading Links in the Pathogenesis of Obesity-Associated Adipose Tissue Dysfunction

Diet-induced obesity in an initially healthy person is a direct result of a positive imbalance between the amount of energy entering the body

with food and the intensity of its expenditure [16]. In case of obesity the body mass index (BMI) exceeds 30 kg/m^2 [17] and its value is positively correlated with the percentage of adipose tissue in relation to body weight [18]. Not only is the mass of subcutaneous and visceral fat growing (greater and lesser omentum of the abdominal cavity, bowel mesentery) [19], but there is also ectopic fat deposition in organs that not supposed to be used for such deposition. For example, in interfibrillar spaces of skeletal muscles and myocardium, as well as in the liver. This leads to fatty degeneration of organs [20]. Ectopic fat plays a crucial role in the development of systemic insulin resistance [20]. Visceral fat is a leading and independent risk factor for type 2 diabetes, cardiovascular diseases and diabetic cardiomyopathies [21, 22]. Adipose tissue of different localization has significant metabolic features that determine a higher pathogenic potential of visceral fat as compared to subcutaneous fat [23, 24, 25, 26]. The dysfunction of adipose tissue is underpinned by the following phenomena.

2.1. Adipocyte Hypertrophy

Hypertrophy is the leading mechanism of fat mass growth primarily, visceral fat. Due to the growth of the lipid droplet, the cell size can increase by 3-4 times [27, 28, 29]. Adipocyte hypertrophy is the leading determinant of the cardinal changes in the biochemical, endocrine and immunological activities of adipose tissue [30, 31]. The secretion of monocyte chemo attractant protein-1 (MCP-1) [25, 26, 32, 33] is increasing in the adipocytes even in the initial phases of adipocyte hypertrophy. The protein attracts monocytes from the bloodstream into adipose tissue [34], especially into visceral fat [35, 36, 37]. The degree of macrophage infiltration is proportional to the increase in BMI, fat mass and the degree of adipocyte hypertrophy [36].

2.2. Intensifying the Synthesis and Secretion of Proinflammatory Cytokines

Macrophages infiltrating adipose tissue become the main source of pro-inflammatory cytokines, and primarily, tumor necrosis factor-□ (TNF-□□ and interleukin-6 (IL-6) [26, 38]. The pro-inflammatory cytokins are involved in the induction of chronic low-intensity inflammation of adipose tissue from where they enter the bloodstram in increasd levels. At the same time, the secretion of leptin [39], resistin [40] and retinol-binding protein (RBP4) [41] increases in adipose tissue, while adiponectin secretion decreases [42]. Adipocyte hypertrophy leads to adipocyte death and macrophages group around such cells, forming crown-like structures [43]. Fatty mass growth leads to hypoxia of whole clusters of adipocytes that move away from the capillary bed [44]. The diameter of the hypertrophied adipocyte can reach 150 microns, which exceeds the distance of oxygen molecule diffusion inside the tissue. [45] In other words, increasing the distance between the adipocyte and capillary is the leading factor of the hypoxia of growing adipose tissue, which is exacerbated by its inadequate vascularization. Hypoxia is shown in the obesity model [46] and in obese patients [47]. Hypoxic microregions of adipose tissue become the sites of increased local expression of chemoattractant factors, which in its turn promotes the attraction and retention of additional macrophages into adipose tissue [48, 49].

2.3. Chronic Inflammation

Hypoxia stimulates the secretion of many adipokines, including pro-inflammatory cytokines, which, acting paracrinely, produce chronic inflammation [50]. Along with the above-mentioned pro-inflammatory cytokines (TNF-□ and IL-6), the growing adipose tissue increases the secretion of other inflammatory mediators: interferon-γ, C-reactive protein, IL-1, IL-8, fibrinogen, haptoglobin, metallothionein, progranulin, transforming growth factor-□, plasminogen activator inhibitor-1 (plasminogen activator inhibitor-1: PAI-1), serum amyloid A (SAA protein) and macrophage inflammatory protein-1 [25, 26, 33, 51]. The growth of fat mass, its hypoxia and inflammation are highly integrated processes that play the primary role not only in the pathogenesis of local insulin resistance (in the adipose tissue itself), but also in systemic insulin resistance (liver, skeletal muscles and myocardium) [16, 26].

2.4. Oxidative Stress

The growth of fat mass leads to the formation of oxidative stress [52, 53, 54, 55, 56, 57, 58]. It was found that the mass of visceral fat positively correlates with the tissue content of oxidative stress markers [59]. Stress disrupting cellular redox homeostasis is one of the significant factors of pathogenesis and aggravation of obesity-associated insulin resistance in other body tissues [60, 61]. The leading causes of oxidative stress in hypertrophied adipocytes are increased activity of NADPH oxidase, increased production of hydrogen peroxide by mitochondria respiratory chain, and weakening

of the antioxidant defense system [62, 63, 64, 65]. Pathogenetic links of oxidative stress and inflammation in adipose tissue form a vicious cycle and mutually reinforce each other. Excessive reactive oxygen species (ROS), active forms of nitric oxide and lipid hydroperoxide together with inflammatory mediators leave the adipose tissue and enter the bloodstream, thereby participating in the formation of systemic chronic inflammation and oxidative stress in the body of an obese patient [66].

2.5. Permanent Activation of Deposited Triacylglycerol Lipolysis

Hydrolysis of triacylglycerol (TAG) accumulated in adipocytes is catalyzed by hormone-sensitive lipase. Insulin inhibits the lipase by suppressing its phosphorylation. In the case of obesity, the relative deficiency of insulin contributes to the stabilization of the hormone-sensitive lipase in phosphorylated, that is, in the active form [67]. Another mechanism for enhancing lipolysis in adipose tissue is associated with an increase in the concentration of glucocorticoids in the blood and an increase in the expression of β_1 and β_2-adrenergic receptors in adipocytes [68, 69, 70]. For these reasons, one of the characteristic manifestations of abnormal fat metabolism in case of obesity is the permanent activation of lipolysis, due to which persistent hyperlipidemia is formed and large quantities of nonesterified fatty acids are delivered to skeletal muscles, the liver and the myocardium [71, 72]. Along with hyperlipidemia, dyslipidemia is produced. It is characterized by an increase in the amount of very low-density lipoproteins (VLDL), a decrease in high density lipoproteins (HDL) [73], and a substantial increase in the level of ceramides and other sphingolipids in the bloodstream [74]. This is facilitated by a significant increase in the amount of ceramides in hypertrophied adipocytes [54, 58]. Thus, dysfunction of adipose tissue provides the tissue with a high pathogenic potential and gives reason to consider it as a primary site for localization of insulin resistance in the body, followed by the formation of systemic resistance to hormone [31].

3. Mechanisms of Ceramide Accumulation in Adipocytes in the Case of Obesity

Each of the leading metabolic events that cause dysfunction of adipose tissue increases the amount of ceramides in adipocytes.

Ceramide accumulation within the cells ensures two main processes. One of them is an activation of *de novo* ceramide synthesis and enhancement of the hydrolysis of membrane sphingomyelin with the release of ceramide [6, 75]. Thus, the increase in the concentration of saturated nonesterified fatty acid and primarily palmitic acid provides an increased inflow of one of the substrates for the first rate-limiting reaction of the *de novo* pathway for the ceramide synthesis catalyzed by serine palmitoyltransferase (SPT) [76, 77]. In addition, palmitic acid, being one of the naturally occurring ligands of toll-like receptor 4 (TLR4) macrophages [78], is capable of stimulating *de novo* ceramide synthesis, presumably as a result of TLR4-mediated activation of genes encoding SPT [58]. These data confirm the involvement of long chain saturated fatty acid in the activation of macrophages of adipose tissue and are consistent with the results [79], indicating the essential role of TLR4 in the formation of obesity-associated insulin resistance in skeletal muscles and the liver. Confirmation of TLR4 involvement was obtained in studies on mutant mice with inactivated toll-receptors. It was discovered that the animals were resistant to the development of obesity while following a high-calorie diet [80].

Experiments on mice of C57BL / 6J show that administration of TNF-α increased both SPT activity in adipose tissue, and the activity of neutral and acidic isoforms of sphingomyelinase (nSMase and aSMase) that catalyze the hydrolysis of membrane sphingomyelin with the release of ceramide, helping to increase its intracellular concentration [54]. The effect mechanism can be explained by the fact that TNF-α activates the tumor necrosis factor receptor (TNFR). TNFR has two cytoplasmic domains, one of which is associated with the adapter protein factor associated with neutral sphingomyelinase activation (FAN). Due to this, there is a functional interface between TNFR-TNF-α and nSMase associated with the plasma membrane, which ensures its activation. Another TNFR domain, the deth domain, is associated with TNF receptor-associated death domain (TRADD) pro-apoptotic adapter proteins and Fas / Apo-1-associated death domain (FADD). They provide activation of aSMase, which is translocated to the plasma membrane from the lysosome compartment [81, 82, 83]. It is also shown that incubation of adipocyte culture 3T3-L1 of mouse with ceramides and sphingosine induced gene expression induction encoding proinflammatory cytokines (PAI-1, TNF-α, MCP-1 and IL-6). This emphasizes the role of sphingolipids in the

pathogenesis of adipose tissue inflammation [54]. These observations are consistent with the results of studies [84], which found out that TNF-α and other pro-inflammatory cytokines are involved in the activation of lipolysis deposited TAG by affecting adipocytes with paracrine mechanisms.

In obese patients there is a significant decrease in the expression of the gene coding the antioxidant enzyme of adipose tissue NADPH: quinone oxidase reductase-1. It was established that there is a direct correlation between the degree of suppression of gene expression and the severity of obesity and the degree of insulin resistance [85]. This information is consistent with the data of other authors who previously demonstrated the ability of ceramides to inhibit electron transportation at levels I [86] and III [87] of the mitochondrial respiratory chain complexes, which led to a significant increase in the formation of ROS in organelles [88]. Elevated concentrations of ROS are capable of inducing reversible inhibition of carnitinacyltransferase-I (CAT-I) [89]. The decrease in the activity of CAT-I disrupts the transportation of long-chain acylcarnitins to the mitochondrial matrix, suppressing their oxidation, which, along with the permanent activation of lipolysis, promotes the growth of the concentration of long chain saturated fatty acids in the cell and increases their availability for SPT.

Ultimately, due to the increase in the concentration of saturated fatty acids, ceramides and other sphingolipids in adipocytes, they enter the bloodstream from adipose tissue and, together with pro-inflammatory cytokines, are delivered to the liver, skeletal muscles, myocardium and other organs, which triggers the formation of systemic resistance to insulin [76, 90, 91, 92, 93].

4. Adipose Tissue as a Source of Substrates for the Activation of Sphingolipid Synthesis in the Liver and Skeletal Muscles

The accumulation of ceramides and other sphingolipids in skeletal muscle, liver and myocardium cells as a result of ectopic lipid deposition in obesity plays an important role in the pathogenesis of systemic insulin resistance. This is indicated by the results of both experimental studies [1, 94, 95, 96, 97, 98], as well as data from a survey of obese patients [99, 100, 101]. Thus, it was shown that the intracellular content of ceramides and other sphingolipids in obese patients with insulin resistance is always significantly higher than normal. On average, their content in liver was higher by 26-150% and in skeletal muscles by 22-94% [6].

Only long-chain saturated fatty fcids are involved into the *de novo* ceramide synthesis: palmitic (C16:0), stearic (C18:0), arachidonic (C20:0) and linoceric (C24:0) fatty acids. [97, 102, 103]. This information formed the basis for the assumption that the excessive intake of saturated fatty acids in the cells, combined with the inhibition of their oxidation, can promote the activation of ceramide and other sphingolipid synthesis, as well as their accumulation in cells [104, 105]. Evidence for this was obtained by the rodent obesity model. The model helped to establish the direct relationship between the content of ceramides in hepatocytes and the degree of manifestation of fatty liver dystrophy [106].

The possibility of a significant weakening of obesity-associated insulin resistance in rats by inhibiting the synthesis of ceramides is shown [92]. However, the administration of a balanced medication to intact mice with the myriocin, an SPT inhibitor, was conducted to suppress the *de novo* ceramide synthesis, to impair glucose tolerance and to form insulin resistance [3]. This indicates that a certain level of ceramide is necessary in cells for the stabilization of glucose homeostasis.

Experimental observations and results of lipid analysis of biomaterial (adipose tissue and skeletal muscle biopsy from obese patients) indicate that activation of synthesis and intracellular accumulation of nonesterified fatty acid and sphingolipids in hypertrophied adipocytes ensure their excessive intake through the bloodstream into the liver, skeletal muscles and myocardium of various lipids and substrates for the *de novo* ceramid synthesis. Thus, at least in the initial phase of the disease, the flow of precursors from hypertrophied adipose tissue becomes an important factor in enhancing the synthesis of ceramides and other sphingolipids in the liver and muscles by participating in ectopic accumulation of lipids.

5. Violation Mechanisms of Insulin Signaling Under the Influence of Ceramides

The results of experiments and examinations of obese patients allow the consideration of ceramide as an intermediate, which provides a connection between all known links in the

pathogenesis of obesity-associated insulin resistance [107].

Protein kinase B (PKB) is the central mediator of multiple anabolic insulin effects. The ability of ceramide to inhibit PKB is confirmed by the following data. It was shown that, in response to infusion of rodent emulsions of animal fat (predominantly saturated lipids) or soybean oil (predominantly unsaturated lipids), inhibition of PKB activity and inhibition of glucose uptake by muscles and adipose tissue were observed. However, the increase in ceramide tissue content occurred only with the introduction of animal fat emulsions [2, 92]. Similar results were obtained when examining obese patients with insulin resistance, which showed more than 2-fold excess of tissue content of ceramide compared to healthy individuals [99]. Infusion of the lipid emulsion to humans caused an increase in the level of ceramide in skeletal muscles, which was accompanied by a violation of their sensitivity to insulin [100]. An inverse correlation was established between intracellular ceramide content and PKB activity [108]. In the experimental obesity model, it was shown that the tissue level of ceramides was always combined with a decrease in the activity of PKB [109], while the administration of ceramide synthesis inhibitors, myriocin and cycloserine (inhibitors of SPT) or fumonisin B1, CerS inhibitor, led to the restoration of PKB activity [97]. Incubation of adipocyte culture in 3T3-L1 [110] and other cell types with palmitic acid was accompanied by ceramide accumulation and PKB inhibition. In the culture of 3T3-L1 cells, it was shown that ceramide analogues reproduced the effect of palmitic acid [111]. Cell cultures also demonstrated that inhibition of ceramide glycosylation exacerbated PKB inactivation induced by palmitic acid [112]. This effect turned out to be possible under the conditions of increased ceramidase activity, which contributed to a decrease in ceramide content [113].

It has been shown that ceramide introduced into the culture medium of hepatocytes and myocytes causes a decrease in glycogen synthesis, as well as glucose uptake in adipocytes and muscle cells, which is based on PKB inhibition activity [91, 92].

On many types of cells, including adipocytes, the mechanism of PKB inactivation by ceramide is well recorded, which is realized in two ways. First, ceramide is a direct activator of phosphoprotein phosphatase 2A (PP2A) [96, 97, 117] under the influence of which the dephosphorylation of serine-473 (Ser-473) and threonine-308 (Thr-308) residues, which are necessary for the stabilization of the active kinase conformation, occurs in the PKB molecule [96, 114, 115, 116]. Okadaic acid, an inhibitor of PP2A, reverses the effect of ceramide on PKB [117]. Secondly, ceramide is able to prevent the binding of PKB to the membrane complex, which includes phosphatidylinositol-3,4,5-trisphosphate (PtdIns (3,4,5) P3) and 3-phosphoinositide-dependent protein kinase-1 (PDK1) [118, 119]. Ceramide acts indirectly. It activates PKC□ as a result of interaction with its domain, which is rich in cysteine [120, 113, 121, 122]. PKC□ □phosphorylates the Ser and Thr residues in the PH domain of the PKB, which deprives it of its ability to bind to the PtdIns (3,4,5) P3-PDK1 complex [120]. The experiment showed that PKC□ inhibitors can improve insulin sensitivity and reverse ceramide-induced inhibition of PKB in animal tissues [120, 123]. Both mechanisms of PKB inactivation by a ceramide (PP2A- and PKC□-mediated mechanisms) operate in adipocyte (culture 3L3-L1) [124, 125]. The reality of ceramide effects was confirmed in the paper by D.J. Powell and the coauthrs [113], whose results showed that the endogenous increase in the intracellular content of ceramide (approximately 50% of the initial value) was sufficient for activation of PP2A and PKC□-mediated mechanisms of PKB inhibition.

According to the literature, it is possible that there are other mechanisms for insulin signaling violation under the action of ceramides, but not all researchers have confirmed their existence. Thus, there is evidence that ceramide is able to prevent the phosphorylation of tyrosine residues (Tyr) in the molecule of the insulin receptor substrate (IRS), which catalyzes the tyrosine protein kinase of the cytoplasmic domains of the insulin receptor [126]. A possible mechanism for the effect of ceramide is its ability to activate mixed-line kinase-3 [127], which then activates kinase of the c-Jun N-terminal kinase (JNK) [128]. JNK phosphorylates the Ser-307 residue in the IRS molecule [129, 130], counteracting this phosphorylation of Tyr residues there [131]. In favor of the reality of such a mechanism, the work [130] can be seen in which it is shown that in mice with experimental obesity JNK activity is significantly increased, and in the IRS molecule the Ser-307 residue is phosphorylated. It is also shown that in humans, a gene mutation that encodes a protein that binds JNK and causes inhibition of this kinase is accompanied

by type 2 diabetes [132]. The result of excessive phosphorylation of the Ser residues in the IRS composition, deprives this molecule of the ability to interact further with phosphatidylinositol-3-kinase (PI3K) and activate it. In turn, inhibition of PI3K activity cancels the phosphorylation of membrane phosphatidylinositol-4,5-bisphosphate (PtdIns (4,5) P2) at position 3 of its inositol ring, which excludes the formation of PtdIns (3,4,5) P3. The absence of the PtdIns (3, 4, 5) P3 in the inner layer of a cell membrane deprives PDK1 of a specific binding site, which ultimately prevents the formation of the PtdIns (3, 4, 5) P3-PDK1 complex. Activation of PKB occurs only as a result of its accession to this complex. The absence of the PtdIns (3, 4, 5) P3-PDK1 complex excludes the transfer of the PKB to the active conformation.

6. The Ceramidocentric View of the Insulin Resistance Pathogenesis

This view emerged due to the great progress achieved over the past decade in studying the diversity of functions of sphingolipids and, in particular, ceramides. This was made possible by the improvement in lipidomics methods (the use of mass spectroscopy to study the qualitative composition of ceramides and other sphingolipids) and genomics (cloning of genes encoding most known enzymes of sphingolipid metabolism and obtaining lines of knockout mice according to corresponding genes) [4, 5, 6]. The first work that drew attention to the role of sphingolipids in the pathogenesis of metabolic dysfunction in obesity was the article by J. Turinsky and co-authors [1], which reported a substantial increase in the content of ceramide in skeletal muscle of insulin resistant rats. The chronology of the key publications that confirmed the role of ceramides and the pathogenesis of insulin resistance is given in the review [133].

Modern research approaches and model systems, including the lines of mice with knockout genes encoding the enzymes of ceramide synthesis, and the use of their specific inhibitors, allow initiation of endogenous ceramide accumulation in the cells of the tissues studied. With their help, it was proven that palmitic acid is able to increase the intracellular content of ceramide, which, unlike other sphingolipids, is responsible for inhibition of PKB [92, 94, 96, 97, 113, 120, 134]. These facts were confirmed on rodent preparations in the experiment [91], as well as on biopsy specimens of skeletal muscles and subcutaneous fat from obese patients [135].

In the mammalian body, ceramide is a family of closely related molecules numbering more than one hundred representatives, which is proved by mass spectrometric analysis [136, 137, 138]. This set of ceramides is synthesized due to the combined action of several enzymes (combinatorial biosynthesis) that determine the specificity of each structural modification of the ceramide molecule [4, 139]. Ceramide (N-acylsfingosine) consists of sphingosine (a "structural base"), to which a variable length acyl chain is attached to the C-2 atom of which an amide bond is attached. Five positions are distinguished in the ceramide molecule, according to which its modification can occur in vivo. This explains the existence of approximately 150 individual members of the ceramide family that have been identified in human and animal tissues using mass-spectrometry [4, 139].

De novo ceramide synthesis begins with the condensation reaction of Ser and palmitoyl-CoA catalyzed by SPT. The availability of palmitic acid limits the rate of biosynthesis of ceramide. The reaction product, 3-ketosfinguanine, is reduced with the participation of 3-ketosfinguan reductase to form sphinganine. Further, the sphinganin is acylated to form N-acylsfiganine (dihydroceramide): acyl is added to the amino group at position 2 of the sphinganin. The reaction catalyzes CerS, or sphinganin-N-acyltransferase. Finally, N-acylsfiganine (dihydroceramide), with the participation of dihydroceramide desaturase (DES), is converted to ceramide [140]. The length of the acyl chain in the individual ceramide determines its biological activity [4, 139]. Six isoforms of the CerS (CerS-1 - CerS-6), which have substrate specificity with respect to the length of the acyl-CoA to be joined, are identified. Obviously, specific CerS isoforms determine the appearance of ceramides with an individual acyl chain length that can contain from 14 to 30 carbon atoms [9, 10, 11].

The introduction of lipid analysis allowed to assess the tissue balance in ceramides, which differ in length of the acyl chain. It was proved that the qualitative composition of the tissue pool of ceramides plays a decisive role, which regularly changes under obesity conditions [138]. It was found that in mice with genetic obesity (ob/ob line), compared to wild-type mice, the activity of SPT was significantly increased in adipose tissue. In the adipocytes of these animals, the content of $C_{14:0}$-ceramide was statistically significantly higher and the content

of $C_{18:1}$-, $C_{24:0}$- and $C_{24:1}$-ceramides was reduced. In the blood serum, the content of $C_{16:0}$-ceramide significantly increased in the presence of a high content of $C_{18:1}$-, $C_{24:0}$- and $C_{24:1}$-ceramides. When the culture of adipocytes was incubated in the presence of exogenous ceramide and other sphingolipids, the synthesis of TNF-α and other pro-inflammatory cytokines were significantly increased [54].

When examining the composition of subcutaneous fat in obese women (BMI 30-40 kg/m^2) with varying degrees of hepatic steatosis, it was found that $C_{18:0}$-, $C_{18:1}$-, $C_{22:0}$-, and $C_{24:1}$-ceramides, and the expression of genes encoding aSMase was increased, compared to those in a subgroup of patients with a normal lipid content in the liver [141].

A significant increase in the content of ceramides was shown in abdominal subcutaneous fat in obese individuals (BMI > 30 kg/m^2): 31% in women and 34% in men compared with healthy individuals (BMI < 25 kg/m^2). Regardless of the sex, the content of $C_{14:0}$-, $C_{16:0}$- and $C_{24:0}$-ceramides was increased in adipose tissue. In addition to this, $C_{18:1}$-ceramide predominated in men, and in women - $C_{24:1}$-ceramide. The activity of SPT and nSMase in subcutaneous fat was significantly higher in obese persons than in the comparison group. Reliable correlations were found between the content of total ceramide in adipose tissue and the activity of SPT (r = 0.72) and nSMase (r = 0.59). According to the authors, the increase in the content of ceramides in subcutaneous fat is caused by an increase in the activity of SPT and nSMase [142].

The suppression of *de novo* ceramide synthesis with the help of myriocin preparation (specific inhibitor of SPT - rate-limiting enzyme *de novo* synthesis of ceramide [143]) contributed to the leveling of insulin resistance of skeletal muscle in rats with experimental (dexamethasone) diabetes, among other things by eliminating the inhibitory effect of ceramide on PKB activity. Knockout for the genes encoding the enzyme DES (homozygous null mice) was accompanied by a complete lack of its activity and an extremely low content of ceramide in the liver, muscles, myocardium, and white mice in comparison with not only wild type mice, but also heterozygous animals with DES knockout, and heterozygotes showed poor resistance to the induction of experimental diabetes. Homozygous null mice differed from heterozygotes with low survival after birth. In the rats of the Zucker line at the age of 10-11 weeks the diabetes mellitus is spontaneously formed. Course administration to rats of this line of myriocin, starting from the 8th week after birth, had a pronounced antidiabetic effect, including preventing the accumulation of ceramides in liver, muscles and blood serum. In rats of the Zucker line with already formed diabetes, the course administration of myriocin significantly increased the sensitivity of tissues to insulin. These data suggest that selective suppression of the synthesis of the most pathogenic representatives of the ceramide family can be used as a basis for new approaches to drug correction of insulin resistance in alimentary obesity [2, 133].

In visceral fat of overweight people, as well as in white fat and liver of mice with obesity caused by a lipid diet, similar shifts in sphingolipid metabolism were observed: increased expression of CerS-6 and increased content of $C_{16:0}$-ceramide. Experimental obesity intensified the expression of CerS-6 also in the liver. In people and mice, the increase in CerS-6 expression was positively correlated with the degree of resistance to insulin. Knockout of the gene encoding CerS-6 in mice was accompanied by a decrease in the tissue content of $C_{14:0}$- and $C_{16:0}$-ceramides. Mouse mutants showed resistance to obesity induced by a lipid diet, they had difficulty developing insulin resistance. Deficiency of activity of CerS-6 promoted more active β-oxidation of fatty acids, which increased the energy expenditure of the organism of these animals [13].

It is known that the dominant isoform of ceramide synthase in the liver of mice is CerS-2, which catalyzes the synthesis of ceramides with a very long acyl chain: $C_{22:0}$-, $C_{24:0}$- and $C_{24:1}$-ceramides [11]. In this regard, the results of studies with knockout of the gene encoding CerS-2 are of interest. It was shown [12] that knockout resulted in a decrease in the liver content of $C_{24:0}$- and $C_{24:1}$-ceramide mice in mice without significant effect on the content of $C_{16:0}$-ceramide. However, it was important that a diet high in lipids multiply increased the $C_{16:0}$-ceramide content in the liver of mutant mice compared to that of normal animals. This was consistent with the fact that obesity-induced diet in knockout mice significantly increased the activity of CerS-6 in the liver and decreased the activity of CerS-2 compared to their activity in normal animals [12]. These data were confirmed by the authors in separate studies on hepatocyte

culture. It turned out that the overexpression of CerS-2 did not lead to a significant shift in the spectrum of ceramides, while overexpression of CerS-6 significantly increased the content of $C_{16:0}$-ceramides in cells, while decreasing ceramides with a very long acyl chain [12].

7. CONCLUSION

The physiological level of specific ceramides and other sphingolipids in cells is necessary to preserve glucose homeostasis in the body. Both quantitative and qualitative changes in the spectrum of tissue ceramides are an important factor in the pathogenesis of obesity-associated insulin resistance.

Ceramide is represented by a vast family of closely related molecules, which in particular have specificic length of the acyl chain in its molecule. CerS is one of the four enzymes of the *de novo* pathway for the ceramide synthesis, which is responsible for the inclusion of a strictly defined length in the molecule to be synthesized. Analysis of the clinical material and the results of model experiments showed that increased expression of the isoenzyme CerS-6 positively correlated with the degree of resistance to insulin. These patterns are confirmed by studies on the lines of mice with knockout of genes encoding CerS-6. CerS-6 shows a high specificity for palmitic acid and catalyzes the synthesis of $C_{16:0}$-ceramide (palmitoylceramide). $C_{16:0}$-ceramide has the greatest pathogenic potential: it not only suppresses the activity of PKB, which underlies the molecular mechanism of systemic resistance to insulin, but also integrates all links of its pathogenesis with alimentary obesity.

The creation of a specific inhibitor of CerS-6 will allow selectively to reduce the tissue content of $C_{16:0}$-ceramide, which, apparently, may contribute to the development of a new direction of pathogenetically grounded pharmacological correction of obesity-associated insulin resistance.

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