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Research Article

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Increased Level of Serum Ceramides Correlate with Liver Steatosis, Hba1c and Cholesterol in Obese Patients

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Abstract

Objective: *Sphingolipids including ceramides are biological active components of all cell membranes. They play a great role in cell interconnections in the process of proliferation, maturation, cell apoptosis and any fluctuations of their level can lead to development of metabolic diseases such as type 2 diabetes (T2D) and nonalcoholic fatty liver disease. Nevertheless, there is lack of information about what type of ceramides play a role in aforementioned diseases. Here we investigated the relationship between the serum level of some type of ceramides and parameters of metabolic syndrome that is commonly present in obese patients.*

Design: *We performed cross-sectional study in two groups. One of them was control group – lean healthy people (n=10, body mass index, BMI 21, 2±1, 89) and the second group included patients with the obesity (n=24, BMI 33, 9±3, 1). We quantified the levels of serum ceramide by liquid chromatography-mass spectrometry, analyzed the parameters for insulin resistance, liver function and lipid metabolism by biochemical blood test.*

Results: *The subjects with obesity had elevated level of ceramide C16:0, C18:0, C24:0 comparing with control group (p<0,001). As results of our study, we found correlation of the level ceramide C16:0, C18:0, C24:0 with the results of steatometry and some metabolic parameters (glycosylated hemoglobin (Hb A1C), cholesterol).*

Conclusion: *These results demonstrate that obese subjects had increased level of ceramide C16:0, C18:0, C24:0 that correlated with some metabolic parameters supposedly recognizing them as new biomarkers of metabolic syndrome.*

Keywords: Obesity, Ceramide, Mass-Spectrometry, Metabolic Syndrome

Introduction

Many experimental and clinical studies have described the role of sphingolipids in the pathogenesis of lifestyle diseases such as myocardial infarction, hypertension, stroke and diabetes mellitus [1-5]. One of sphingolipids that has potential role to become the new early marker of these diseases is ceramide.

Ceramides are fats of the sphingolipid family consisting of saturated, monounsaturated and unsaturated fatty acid chains linked to the amino group, sphingosine. The synthesis of ceramides is carried out by three mechanisms: de novo, using sphingomyelinase (SMase) and by converting sphingosine (salvage path) under the action of ceramide synthase (CerS) [6].

In addition to the barrier function they perform, ceramides are also involved in the processes of proliferation, differentiation and maintenance of cellular homeostasis [7]. Depending on the length of the fatty acids, there are short-, long- and very long-chain ceramide fractions. According to many studies, different ceramide fractions, plays a significant role in the development of insulin resistance and T2D by affecting the PI3K / Akt signaling pathway, inhibiting the expression of insulin receptors [8]. Nevertheless, there is not definite information concerning specific fractions of ceramide that could be responsible in development of insulin resistance or type 2 diabetes (T2D). Also, we have very obscure understanding of the role ceramides in non-alcoholic fatty liver disease [9]. Therefore, the objective of our study is to compare the level of different ceramide fractions between lean subjects and the subjects with the

obesity and find correlation link between ceramide and other metabolic parameters.

Materials and Methods

Our metabolic study took place in the Kyiv, Ukraine, between January 2019 to May 2019. The current analysis comprised 24 obese (BMI 33±3, 1 kg/m2) patients and 10 healthy lean subjects (BMI 21±1, 89 kg/m2) of the same age group. Weight was stable (±3 lb) in all subjects for the 3 months before study, and no subject participated in an excessively heavy exercise program. Written informed consent for enrollment and participation in this study was obtained from all subjects before any testing procedures. Inclusion criteria were body mass index (BMI) of 20 or 25 kg/m2 for healthy lean subjects and 28 or 40 kg/m2 for obese. None of the subjects was on any medication or was smokers and was free of liver, kidney, thyroid, or lung disease. Detailed history including AUDIT (Alcohol Use Disorders Inventory Test), CAGE (cut-annoyed-guilty- eye) questionnaires and laboratory markers (serum γ-glutamyl transpeptidase and mean corpuscular volume of erythrocytes) assessed alcohol consumption. The patient's alcohol intake was less than 140 g per week. The primary outcome of this study was to compare the level of ceramides C16:0, C18:0, C24:0 in obese patients and in healthy lean persons. The second outcome was to find out could elevated level of ceramides C16:0, C18:0, C24:0 influence on their correlation link with metabolic parameters (liver steatosis, HbA1c and cholesterol) in obese patients.

Blood Examination

A blood specimen was obtained from all subjects after an 8–12 h overnight fast. The blood was used for an assessment of liver function and measurement of the level of fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c) and lipids. The insulin resistance index was calculated from the homeostasis model assessment of insulin resistance (HOMA-R) using the following equation: (fasting insulin in μU l−1) × (FBG in mmol l−1)/22.5.

Body Composition Measurement

We used bioelectrical impedance in all subjects as a noninvasive and accurate method for evaluating body composition [9].

Ultrasonography

We used steatometry (Ultrasign, Soneus P7) of the liver in all the subjects in the supine position to evaluate the stage liver steatosis (ultrasound attenuation coefficient). Liver steatosis was diagnosed when there was an increase in echogenicity of the liver compared with the kidney. Then in the supine position, with calm breathing, without sensor compression, steatometry were conducted. During steatometry study we performed 5 measurements in every part of the liver to obtain recurring quantities of stiffness and ultrasound attenuation coefficient. The steatosis was graded: S0-steatosis is absent - <2, 19 dB/cm, S1-mild - 2, 2-2, 29 dB/cm, S2-moderate - 2, 3-2, 9 dB/cm, S3-severe - >2, 9 dB/cm.

Ceramide Measurement

Quantitative analyses of plasma ceramide subspecies were performed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) [10]. Lipids were extracted according to the protocol of Bligh and Dryer.

Statistical Analysis.

The results are presented as mean ± SD and as median with lower quartile and upper quartile. To perform the test for normal distribution we used the D'Agostino-Pearson test. For indicators that do not show differences in the distribution of values from normal, we used the Student's t-test for independent samples to compare the averages. For indicators that differ from the normal distribution of values when comparing central trends for two independent samples, we used the Wilcoxon W-test. Correlations were analyzed using Spearman's rank correlation. In all the tests, a P-value of <0.05 was considered significant. All statistical analyses were performed using Med Stat version 5.2 (NM University, Kyiv, Ukraine).

The study protocol was approved by the institutional review board at the Bogomolets National Medical University (NMU) Kyiv, Ukraine and was conducted in accord with the principles of Good Clinical Practice and the principles of the Declaration of Helsinki. All patients gave written informed consent prior to enrollment.

Results

The demographic and metabolic characteristics are shown in Table 1 & 2. The attenuation coefficient of steatometry in the obese group was 2.5 (2.4-2.6), which corresponded to the mild degree of steatosis (p<0.001).

Table 1: Subject characteristics

	Control (n=10)	Obese patients (n=24)	p-Value
Sex	5 female/5 male	10 female/14 male	-
Age	55 (49-59)	55 (50-59)	0,825
BMI	21,2±1,89	33,9±3,1	0,001.
Ultrasonography (Steatometry dB/cm)	2,13 (2,1-2,17)	2,5 (2,4-2,6)	0,001.
C16:0 μmol/L	0,21±0,05	0,42±0,04	0,001
C18:0 μmol/L	0,02 (0,02-0,03)	0,08 (0,07-0,09)	0,001
C24:0 μmol/L	1,12 (1,09-1,15)	3,24 (3,15-3,32)	0,001
Hb A1c, %	3,3 (3-3,9)	5,1 (4,9-5,2)	0,001
CH mmol/L	4,1 (3,9-4,6)	5,9 (5,7-6,1)	0,001
HOMA-IR	1,6	2,1	0,720
FBG mmol/L	4,8±0,2	5,1±0,2	0,001
AST	25 (21-26)	45 (40-49)	0,825
ALT	26 (22-28)	55 (50-58)	0,001

Table 2: Correlation between plasma ceramide subspecies and metabolic parameters

	C16:0 µmol/L	C18:0 µmol/L	C24:0 µmol/L
Age	-	-	-
BMI	-	-	-
Steatometry dB/cm	-	0,309	-
C16:0 µmol/l	-	-	0,717
C18:0 µmol/l	0,304	-	-
C24:0 µmol/l	0,717		-
Hb A1c, %	0,303	0,323	-
CH mmol/L	-	0,394	-
HOMA-IR	-	0,288	-
FBG mmol/L	-	-	-
AST	-	-	-
ALT	-	-	-

Concentrations of ceramide C16:0 in the plasma were elevated in the obese group 0.42 ± 0.04 vs 0.21 ± 0.05 in controls (p<0.001) (Figure 1).

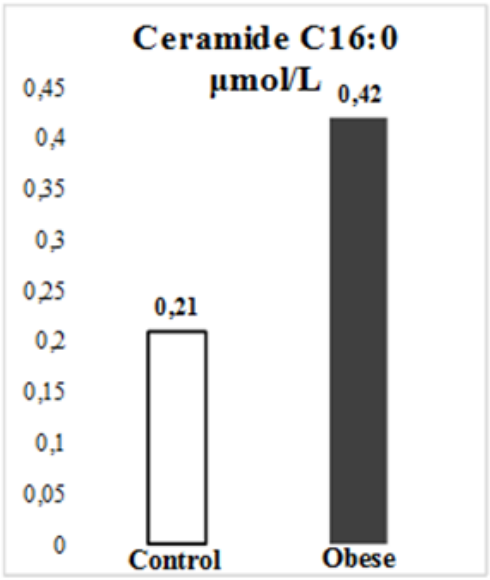

Figure 1: Elevated plasma ceramides C16:0 in obese vs. control subjects.

The concentrations of C18:0, C24:0 ceramide fractions were also increased in the obese group 0.08 (0.07-0.09) vs 0.21 (0.02-0.03) in the controls (p<0.001), and 3.24 (3.15-3.32) vs 1.12 (1.09-1.15) in controls (p<0.001), respectively (Figure 2).

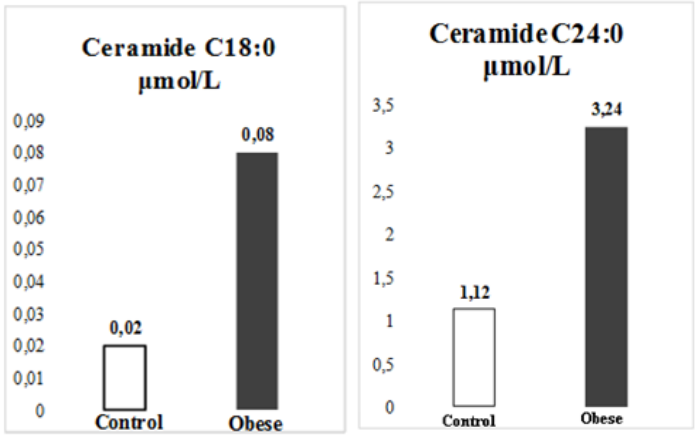

Figure 2: Elevated plasma ceramides C18:0 and C24:0 in obese vs. control subjects.

Spearman rank correlations were used to determine potential relationships between individual ceramide subspecies and some metabolic parameters.

Ceramides C16:0 had a positive correlation as with ceramide C18:0 (r=0,304, p=0, 03) as with C24:0 (r=0,717, p=0, 03). Also, there is a correlation between Hb a 1c and ceramide C16:0 (r=0,303, p=0, 03). Ceramide C18:0 had a positive correlation with the results of steatometry (r=0,309, p=0, 03). Hb A1C correlated with ceramide C18:0 (r=0,323, p=0, 02). Cholesterol had a positive correlation with ceramide C18:0 (r=0,394, p<0, 01). Ceramide C18:0 correlated with HOMA-IR (r=0,288, p=0, 04).

Discussion

Insulin resistance is the main reason for the development of NAFLD. Reduced insulin sensitivity leads to lipolysis and excess free fatty acids in the liver. Lipids in hepatocytes are represented by triglycerides, which in an excess amount can contribute to the progression of NAFLD [11-18]. This is primarily due to the precursors and intermediates of triglycerides. Of particular importance is ceramides.

The first data on the role of ceramides in the development of insulin resistance appeared in the study of their effect on isolated myocytes and adipocytes. The results of which revealed that insulin-activated glucose uptake and glycogen synthesis were reduced under the action of ceramides [9]. The development of insulin resistance is due to inhibition of protein kinase B (PKB / Akt) phosphorylation by activation of protein phosphatase 2A (PKC) and ceramides. As a result, Akt translocation from the membrane to the cytosol is blocked and GLUT-4 receptor expression is reduced [9].

The role of ceramides in the development of NAFLD and IR was identified in a study by Maria Apostolopoulou et al (2017). The purpose was to identify a link between sphingolipids and NAFLD.

The study involved 21 patients with obesity and insulin resistance, which were divided into three groups: patients without NAFLD, NAFLD and NASH. The study found that the total number of ceramides was higher in patients with NASH and NAFLD by 50% and 33%, respectively, compared with patients without NAFLD. Among the ceramides that were elevated in patients with NASH were dehydroceramides: 16:0, 22:0, and 24:1 [12-19]. The following study examined 13 obese and type 2 diabetes patients. The results showed that all patients had an increased total concentration of ceramides with a predominance of the C18: 0, C20: 0 and C24: 1 fractions.

The results of our study revealed that the levels of ceramides C16:0, C18:0, C24:0 were higher in the obese group. In addition, we found a correlation between ceramides C16:0 with HbA1c and ceramide C18:0 with cholesterol level and with the results of steatometry. However, we did not find a correlation between ceramides C16:0, C18:0, C24:0 and age, anthropometric parameters (BMI), glucose levels, and hepatic transaminases activity. In our opinion, the lack of correlation between these indicators may be due to the absence of severe metabolic disorders (increased glucose level, increase in the HOMA-IR index etc.), which may be in case of type 2 diabetes. The increase in ceramide levels in obese patients is explained by the results of studies that found a link between a diet rich in fatty acids (palmitate and oleate) and an increase in ceramide levels in blood plasma and skeletal muscle. This may explain that in patients with obesity, the route of formation of ceramides de novo predominates, the substrate of which is fatty acids (palmitate and oleate).

Increasing the levels of ceramides C16:0, C18:0, C24:0 can lead to inhibition of mitochondrial ion transport, contributing to the reduction of β-oxidation of fats and the accumulation of triglycerides in the liver. Objectively, this is reflected in an increase in the attenuation coefficient during steatometry.

The role of ceramides are highly described in various researches. However, our study has been firstly performed in Ukraine. We understand that these are the first results of our work but we hope that o.

Conclusion

According to the results of our study, we can conclude that patients who are overweight have a tendency to increase the levels of ceramides C16:0, C18:0, C24:0. In addition, at the stage where classic insulin resistance markers are within normal range in obese patients, their ceramide levels are increased. Also, an elevated level of C18:0 ceramides influences the degree of liver steatosis by steatometry. Given this data, further study of this topic will allow us to use elevated plasma ceramide levels as an early marker of insulin resistance, atherosclerotic risk, and/or obesity induced inflammation.

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Patient consent for publication not required

Data availability statement all data relevant to the study are included in the article or uploaded as supplementary information

References

1. Reijo Laaksonen, Kim Ekroos, Marko Sysi-Aho, Mika Hilvo, Terhi Vihervaara, et al. (2016) Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. European Heart Journal Advance 37: 1967-1976.
2. Leonardo P de Carvalho, Sock Hwee Tan, Ow GS, Tang Z, Ching J, et al. (2018) Plasma Ceramides as Prognostic Biomarkers and Their Arterial and Myocardial Tissue Correlates in Acute Myocardial Infarction JACC Basic Transl Sci 3: 163-175.
3. Spijkers LJ, van den Akker RF, Janssen BJ, Debets JJ, De Mey JG, et al. (2011) Hypertension is associated with marked alterations in sphingolipid biology: a potential role for ceramide. PLoS One 6: e21817.
4. Na Sun, Richard F Keep, Ya Hua, Guohua Xi (2016) Critical role of the sphingolipid pathway in stroke: a review of current utility and potential therapeutic targets. Transl Stroke Res 7: 420-438.
5. Sehamuddin Galadari, Anees Rahman, Siraj Pallichankandy, Alaa Galadari, Faisal Thayyullathil (2013) Role of ceramide in diabetes mellitus: evidence and mechanisms. Galadari. Lipids in Health and Disease 8: 12-98.
6. Hanin Aburasayn, Rami Al Batran, John R Ussher1 (2016) Targeting ceramide metabolism in obesity. Am J Physiol Endocrinol Metab 311: E423-435.
7. Nikolova-Karakashian MN, Rozenova KA (2010) Ceramide in stress response. Adv. Exp. Med. Biol 688: 86-108.
8. Gault CR, LM Obeid, YA Hannun (2010) An overview of sphingolipid metabolism: from synthesis to breakdown. Adv. Exp. Med Biol 688: 1-23.
9. Galadari S, Rahman A, Pallichankandy S, Galadari A, Thayyullathil F (2013) Role of ceramide in diabetes mellitus: Evidence and mechanisms. Lipids in Health and Disease 8: 12-98.
10. Takhar Kasumov, Hazel Huang, Yoon-MiChung, Renliang Zhang, Arthur J.McCullough, et al (2010) «Quantification of ceramide species in biological samples by liquid chromatography electrospray ionization tandem mass spectrometry», Analytical Biochemistry 401: 154-161.
11. Naoki Tanaka, Takefumi Kimura, Naoyuki Fujimori, Tadanobu Nagaya, Michiharu Komatsu, et al. (2019) Current status, problems, and perspectives of non-alcoholic fatty liver disease research. World J Gastroenterol. Jan 25: 163-177.
12. Maria Apostolopoulou, Ruth Gordillo, Chrysi Koliaki, Sofia Gancheva, Tomas Jelenik (2018) Specific Hepatic Sphingolipids Relate to Insulin Resistance, Oxidative Stress, and Inflammation in Nonalcoholic Steatohepatitis Diabetes Care 41:

1235-1243
13. C16:0-Ceramide Signals Insulin Resistance. Timothy Hla, Richard Kolesnick 20: 703-705.
14. Lorenzo AD, Andreoli A (2003) Segmental bioelectrical impedance analysis. Curr Opin Clin Nutr Metab Care 6: 551-555.
15. Am J Clin Nutr (2014) 99: 436-445. Published online 2014 Jan 15. doi: 10.3945/ajcn.113.070557
16. Rozenn N Lemaitre, Chaoyu Yu, Andrew Hoofnagle, Nair Hari, Paul Jensen et al (2018) Circulating Sphingolipids, Insulin, HOMA-IR, and HOMA-B: The Strong Heart Family Study Diabetes y 67: 1663-1672.
17. Drobnik W, Liebisch G, Audebert FX, Frohlich D, Gluck T, et al. (2003) Schmitz G: Plasma ceramide and lysophosphatidylcholine inversely correlate with mortality in sepsis patients. J Lipid Res 44: 754-761.
18. Ichi I, Nakahara K, Miyashita Y, Hidaka A, Kutsukake S, et al (2006) Association of ceramides in human plasma with risk factors of atherosclerosis. Lipid 41: 859-863.
19. Holland WL, Summers SA (2008) Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. Endocr. Rev 29: 381-402.