Mol Cell Biochem (2010) 340:125–131
DOI 10.1007/s11010-010-0409-6

High fat diet induces ceramide and sphingomyelin formation in rat's liver nuclei

Grzegorz Chocian · Adrian Chabowski · Małgorzata Żendzian-Piotrowska · Ewa Harasim · Bartłomiej Łukaszuk · Jan Górski

Received: 14 September 2009 / Accepted: 10 February 2010 / Published online: 20 February 2010

Abstract Obesity increases the risk for hepatic steatosis. Recent studies have demonstrated that high fat diet (HFD) may affect sphingolipid formation in skeletal muscles, heart, and other tissues. In this work we sought to investigate whether HFD feeding provokes changes in content and fatty acids (FAs) composition of sphingomyelin and ceramide at the level of liver and hepatic nuclei. Furthermore, we investigated whether the ceramide formation is related to the activity of either neutral sphingomyelinase (N-SMase) or acidic sphingomyelinase (A-SMase). Three weeks of HFD provision induced pronounced ceramide and sphingomyelin accumulation in both liver and hepatic nuclei, accompanied by increased activity of N-SMase but not A-SMase. Furthermore, a shift toward greater FAs saturation status in these sphingolipids was also observed. These findings support the conclusion that HFD has a major impact on sphingolipid metabolism not only in the liver, but also in hepatic nuclei.

Keywords Ceramide · Sphingomyelin · Liver · Nuclei · High fat diet · Sphingomyelinase

Introduction

Sphingomyelin (SM) is one of the most important membrane sphingolipids [1, 2] and its hydrolysis by specific sphingomyelinases, generates ceramide (CER). It is widely accepted that sphingomyelin and ceramide have numerous biological roles and the sphingomyelin–ceramide signaling pathway is present in the nuclei in different tissues [3, 4]. Electron microscopy analysis on rat liver section revealed that N-SMase is present in nuclear membrane [5–7] and further studies showed significant involvement of this N-SMase in the hydrolysis of the nuclear sphingomyelin [8]. As far, there is no data providing strong evidence for the presence of other sphingomyelinases (i.e., acid sphingomyelinase, A-SMase) in the nuclei. Interestingly, A-SMase was shown to be present in endosomal/lysosomal compartments and based on this finding the endosomal compartment was implicated as an additional site for the ceramide formation [9]. Whether, ceramide accumulation in these endosomal vesicles is able to interact with other ceramide-related molecular targets in nuclei is still unclear.

Recently, both SM and CER have been implicated in the development of atherosclerosis, insulin resistance, diabetes, and obesity [10]. Recent studies have demonstrated that high fat diet (HFD) may provoke ceramide and sphingomyelin formation in skeletal muscles [11, 12], heart [13], and other tissues [14]. However, there is still ongoing debate regarding the effects of high fat feeding on the changes in intracellular lipids in the liver [15, 16]. Recent studies indicated that HFD provision results in an excess accumulation of phospholipids, triacylglycerols, diacylglycerols, and free fatty acids (FAs) in hepatocytes [15, 16].

Far less is known regarding the HFD effects on more biologically active lipid fractions such as sphingomyelin and ceramide in the liver. Additionally, there are evidences indicating that blood-borne FAs rapidly enter the nuclear lipid pool in hepatocytes [17], and one would expect the changes in the content and composition of nuclear lipids are related to non-esterified fatty acid (NEFA) availability in blood [17, 18]. Therefore, it is of particular interest to

G. Chocian · A. Chabowski (✉) · M. Żendzian-Piotrowska · E. Harasim · B. Łukaszuk · J. Górski
Department of Physiology, Medical University of Białystok, ul. Mickiewicza 2C, 15-222 Białystok, Poland
e-mail: adrian@umwb.edu.pl

examine whether HFD feeding provokes changes in sphingomyelin and ceramide (i) content and (ii) FA composition at the level of: (1) liver and (2) hepatic nuclei. Furthermore, we prompted to investigate whether the ceramide formation is related to the activity of either neutral sphingomyelinase (N-SMase) or acidic sphingomyelinase (A-SMase) in both the liver homogenate and hepatocellular nuclei.

Materials and methods

Male Wistar rats (200–250 g) were housed in approved animal holding facilities (at 22 ± 2°C, on a 12 h/12 h light–dark cycle, with unrestricted access to water and to a commercial chow). Animal maintenance and treatment were approved by the Ethical Committee for Animal Experiments at the Medical University of Białystok.

Rats were randomly divided into two groups: (1) control (receiving standard chow diet), (2) HFD receiving chow enriched in saturated long chain FAs for 3 weeks as described previously [16]. HFD was administrated daily in the same amount of calories as in control rats fed on a standard chow (82 kcal per animal per day) to minimize differences in body mass gain. After 3 weeks of treatment, rats were overnight fasted and anaesthetized with an intraperitoneal injection of pentobarbital in a dose of 80 mg/kg of body weight. Samples of the liver were excised, cleaned of blood and immediately freeze-clamped with aluminum tongs pre-cooled in liquid nitrogen and then stored at −80°C until further analysis.

Nuclei isolation

Nuclei were isolated from a defrosted and homogenized liver samples by centrifugation in a sucrose gradient [19]. Purity and integrity of the nuclear fraction were evaluated by determining the activities of 5′-nucleotidase and lactic dehydrogenase (BioMerieux, enzymatic kit) [20] and by electron microscopy.

The extraction and identification of the liver nuclear ceramides and sphingomyelins

The rat liver samples or nuclear fraction pellets were pulverized in an aluminum mortar with a stainless steel pestle pre-cooled in liquid nitrogen. The powder was then transferred to clean glass tubes containing methanol at a temperature of −20°C. Butylated hydroxytoluene (Sigma) was added, as an antioxidant, to methanol in a dose of 30 mg/100 ml. Lipids were extracted by the method of Van der Vusse et al. [21]. Ceramide was isolated using the modified method of Previati et al. [22]. The samples were spotted on thin-layer chromatography silica plates (Kieselgel 60, 0.22 mm, Merck) and developed to one-third of the total length of the plate in chloroform–methanol–25% NH_3 (20:5:0.2, vol/vol/vol). Then, the plates were dried and rechromatographed in hexane–isopropyl ether–acetic acid (60:40:3, vol/vol/vol). To isolate sphingomyelin, lipids were fractionated on silica plates (as above) using chloroform–methanol–acetic acid–water (50:37.5:3.5:2, vol/vol/vol/vol) as the developing solvent [23]. Standards of ceramide (Sigma) and sphingomyelin (Sigma) were run along with the samples. Lipid bands were visualized under ultraviolet light after spraying with a 0.5% solution of 3′7′-dichlorofluorescein in absolute methanol. The gel bands corresponding to ceramide and sphingomyelin were scraped off the plate and transferred into screw tubes containing methylpentadecanoic acid (Sigma) as an internal standard. FAs were then *trans*-methylated with l ml of 14% boron fluoride. The methyl esters were dissolved in hexane and analyzed by gas–liquid chromatography. A Hewlett-Packard 5890 Series II and a fused Hp-INNOWax (50 m) capillary column were used. The gas chromatograph was equipped with a double flame ionization detector. Injector and detector temperatures were set at 250°C. The oven temperature was increased linearly from 160 to 230°C at a rate of 5°C/min. Individual FA methyl esters were quantified: myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidonic (20:4), docosahexaenoic (22:6), and nervonic (24:1). We have also calculated the total amount of ceramide and sphingomyelin fractions as a sum of FA indices present in examined fraction.

Sphingomyelinase assay

The activities of the neutral sphingomyelinase (N-SMase, Mg^{2+}-dependent) and acidic sphingomyelinase (A-SMase) were determined as described previously [24, 25] with a 0.5 mM [*N*-methyl-^{14}C]-sphingomyelin, as a substrate. The enzyme activity was calculated by measuring the amount of released [^{14}C]-phosphocholine with the use of liquid scintillation counter. The activity of sphingomyelinases was expressed in nanomole of released phosphocholine/mg of protein/h. Protein content was determined with bicinchonic acid method with BSA serving as a protein standard.

Statistical analyses

All data are expressed as mean ± SEM. Statistical difference between groups was tested with analyses of variance and appropriate post-hoc tests, or with Student's *t*-test. Statistical significance was set at $P \leq 0.05$.

Results

General features of the experimental groups

No significant differences were observed in the whole body mass and the weight of the liver in between animals from control and HFD groups. However, there was a significant increase in the NEFA serum levels (+50%, $P < 0.05$) in HFD fed rats compared to control (data not shown).

Nuclear fraction purity

The activity of lactic dehydrogenase (cytoplasmic marker) in the nuclei fraction was below 1% comparing to the whole liver homogenates. The activity of 5′-nucleotidase (plasma membrane marker) was undetectable (data not shown). The electron microscope estimations showed intact nuclei with no visible contaminations of plasma membranes or mitochondria (Fig. 1).

Effects of HFD on the sphingomyelin and ceramide content

HFD induced significant increases in the content of sphingomyelin in both liver (+43%, $P < 0.05$) and liver nuclei (+38%, $P < 0.05$) (Fig 2a), similarly, but to a much higher extent, HFD increased the content of ceramide in the liver (3.0-fold, $P < 0.001$) with a parallel increase in ceramide in liver nuclei (3.8-fold, $P < 0.001$) (Fig. 2b).

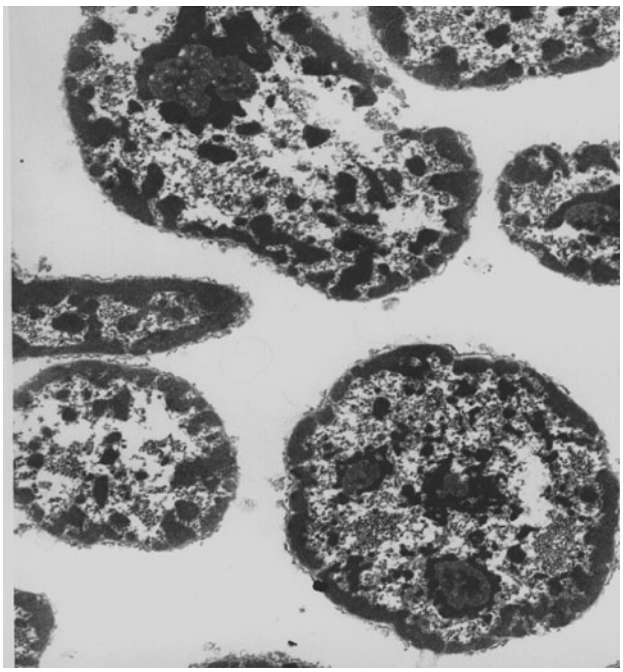

Fig. 1 Electron micrograph of the isolated hepatic nuclei. Magnification ×3000

Effects of HFD on the FA composition in ceramide and sphingomyelin fractions

In both preparations examined, in the liver and hepatic nuclei, the most pronounced changes were observed as significant increases in FA saturation status (especially in 14:0, 16:0, 18:0, FA species) after HFD feeding, in either sphingomyelin or ceramide fractions (Fig. 3).

Effects of HFD feeding on the sphingomyelinase activity

HFD also induced significant elevation of the N-SMase activity in either nuclear fraction or liver homogenates (2.0-fold, $P < 0.001$ and 3.2-fold, $P < 0.001$, respectively) (Fig. 4a). Interestingly, the activity of A-SMase was reduced in liver homogenates in HFD group (−15%, $P < 0.05$) and in the nuclear fraction the activity of A-SMase was not detected in both groups (Fig. 4b).

Discussion

This study revealed excessive sphingomyelin and ceramide accumulation in the liver and hepatic nuclei followed HFD feeding. HFD protocol used in our study, although had no significant effect on the whole body mass or liver weight, resulted in a significant increase in NEFA serum content, which is consistent with other reports using pair-fed rodents [26, 27]. It is well accepted that NEFA enter hepatocytes (via passive diffusion or protein-mediated transport) and subsequently undergo mitochondrial oxidation or esterification into different intrahepatic lipids. However, upon increased plasma FA availability, excessive hepatic FA esterification occurs, which has been shown as the abundant presence of large (macrovesicular) or small (microvesicular) intracytoplasmic fat droplets [28]. Recent studies confirmed that HFD provision results in an excess accumulation of specific lipid fractions, namely: phospholipids, triacylglycerols, diacylglycerols, and free FAs in hepatocytes [16]. In this study, we also observed a significant increase in hepatic content of sphingolipids, namely, sphingomyelin and ceramide. It is widely accepted that dietary factors, including exogenous FAs, promote hepatic lipogenesis, especially when FAs flux to the liver overrides hepatic capacity for FA oxidation [29]. However, this effect of HFD strongly depends on diet composition (saturated versus poly- and mono-unsaturated FAs), calorie restriction or time frame of the diet [29, 30]. In this regard, it was shown that serine palmitoyltransferase (SPT) is highly specific for saturated FAs and so ceramide does not accumulate following a polyunsaturated FAs challenge [31]. Secondly, poly- and mono-unsaturated FAs are more

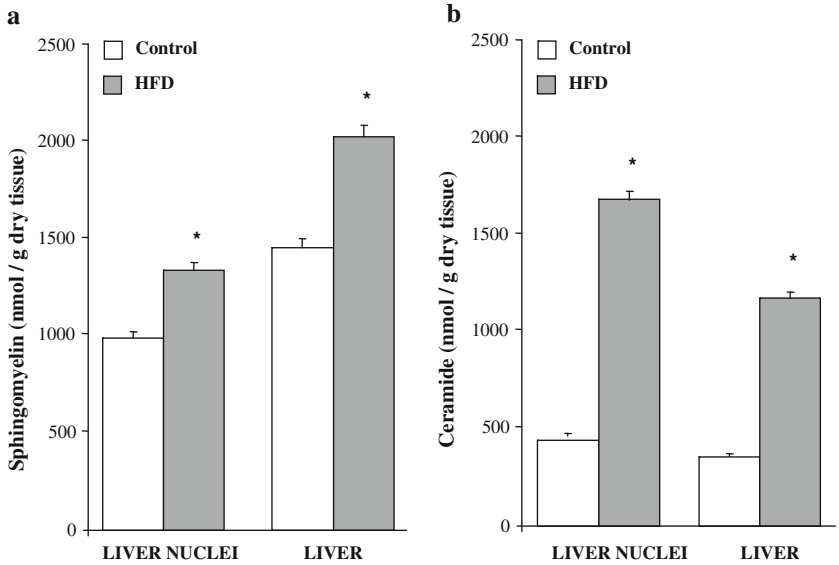

Fig. 2 Effects of high fat (HFD) feeding on the content of **a** sphingomyelin and **b** ceramide in the liver nuclei and liver ($n = 10$). * $P < 0.05$ HFD versus control

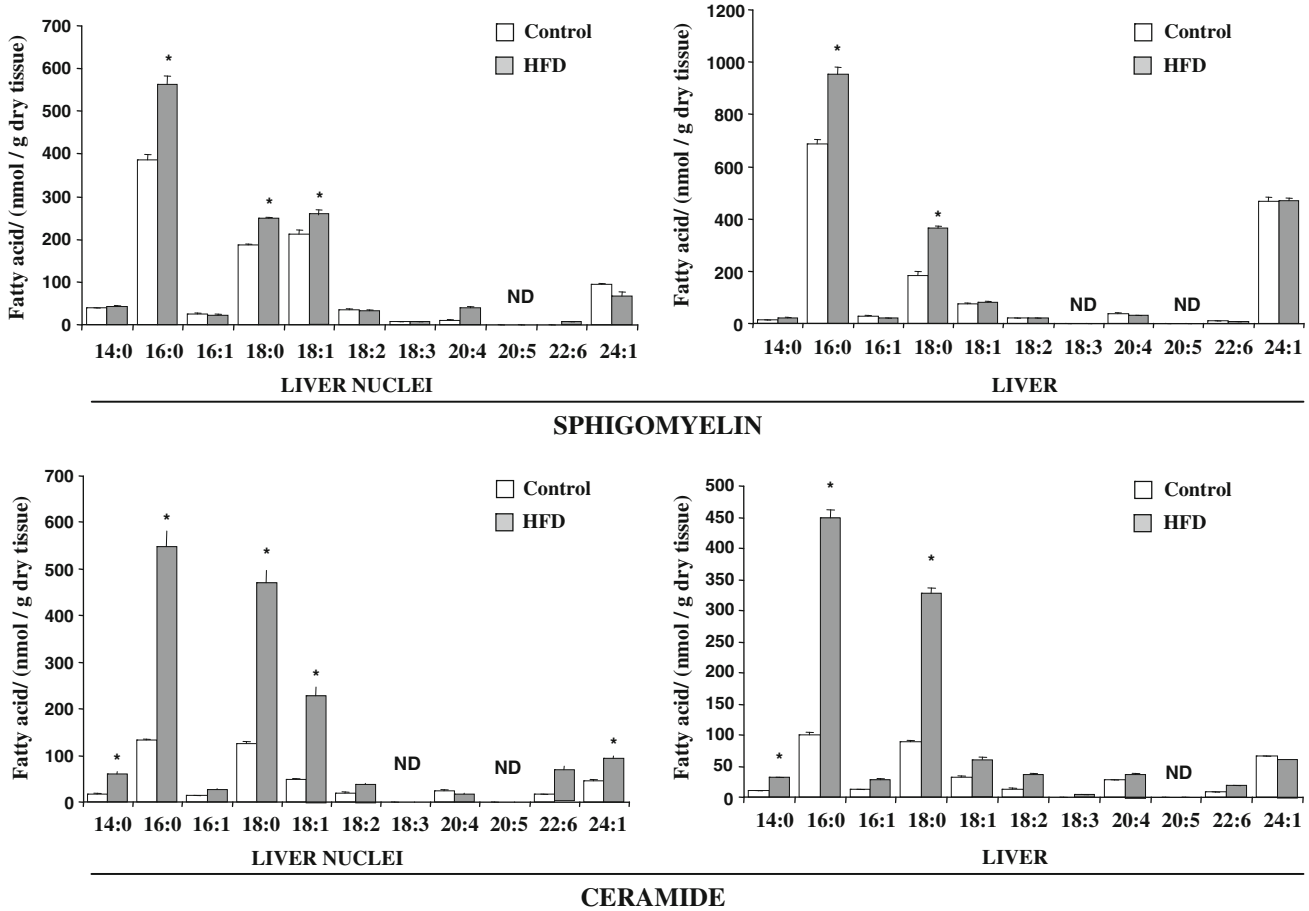

Fig. 3 Effects of high fat (HFD) feeding on FA's composition in ceramide and sphingomyelin in the liver nuclei and liver ($n = 10$). The following fatty acids were assayed: myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidonic (20:4), docosahexaenoic (22:6), and nervonic (24:1). * $P < 0.05$ HFD versus control. *ND* not detected

readily oxidized than saturated FAs [32, 33] thus preventing excessive accumulation of ceramide and/or other lipid fractions [34]. In line with abovementioned evidences are also studies showing increased expression of genes of enzymes mediating ceramide generation in response to the palmitate exposure [35, 36]. However, under these

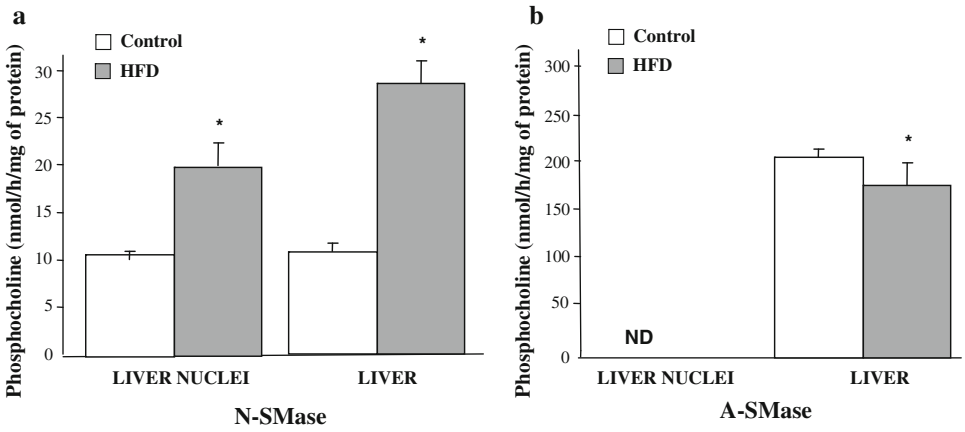

Fig. 4 Effects of high fat (HFD) feeding on the activity of N-SMase (**a**) and A-SMase (**b**) in the liver nuclei and liver ($n = 10$). * $P < 0.05$ versus control

circumstances, much less is known regarding pathways that are predominantly involved in ceramide generation. Ceramide production may occur through either the novo pathway (e.g., SPT) or via the hydrolysis of sphingomyelin (A-SMase or N-SMase) [37] or through the breakdown of glycosphingolipids [38]. Whichever is the case, our HFD enriched in saturated FA species promoted sphingolipids accumulation rather than FA oxidation. Beside well documented, de novo synthesis of ceramide following HFD via increased activity of SPT, here we present that ceramide may accumulate through enhanced sphingomyelin hydrolysis (Fig. 4). In the later situation, in parallel to increased activity of N-SMase we should observe a decrease in SM content, which is not the case in our study. Likely explanation would be that HFD modulates the activity of many of rate-limiting enzymes in sphingolipid metabolism, and ceramide might be converted back to SM by the sphingomyelin synthase [39]. However, convincing data to support this hypothesis will require studies in which the activity of each purported enzyme can be manipulated independently.

Importantly, this is the first study to report the excessive accumulation of sphingomyelin/ceramide lipid fractions at the level of hepatic nuclei. This is an important finding based on the multiple nuclear sphingomyelin and ceramide actions. There is still very little data available regarding specific effects of ceramide in the nuclei, although it is precursor sphingomyelin, has been implicated in the regulation of the transcription [5, 40] and chromatin stabilization [7]. It was shown that increased sphingomyelin presence leads to RNA protection from RNA-ase digestion. Interestingly, this effect was reversed by enhanced activity of sphingomyelinase and as a consequence RNA was hydrolyzed [41]. Currently there is no sufficient data to confirm that ceramide derived from sphingomyelin exerts similar effects in nuclei. Whether there are specific nuclear targets for ceramide is still unclear, although by analogy to cytosolic signaling, it has been proposed that ceramide and protein kinase C (especially PKCδ) activity are interrelated [42]. It is of particular interest that, certain extracellular factors, such as HFD used in our study, are able to induce lipid changes at the nuclear level. We have to point that, our previous studies suggested such a possibility by indication that blood-borne FAs rapidly enter the nuclear lipid pool in hepatocytes [17, 18]. More recently, it has been shown that liver nuclei possess necessary enzymes to incorporate exogenous FAs into nuclear lipid pool [43]. To further support this possibility, engagement of cytoplasmic lipid binding proteins in transferring lipids into the nuclei was presented [44]. Based on these studies it may be speculated that increased provision of long chain FAs in the diet not only leads to the accumulation of lipids intracellularly, but also severs as a potent reservoir of palmitylo-CoA for nuclear sphingomyelin and ceramide formation.

It is widely accepted that SM is present in almost all subcellular membranes with the highest concentration in the outer layer of plasma membrane [45] and accordingly the plasma membrane is thought to be a major site for ceramide generation [46, 47]. However, other sites for ceramide formation have been proposed, including endosomal/lysosomal compartments and the nucleus [5, 7, 8, 40]. It is important to acknowledge the presence of nuclear sphingomyelinase, sphingomyelin synthase or reverse sphingomyelin synthase as this suggests that sphingolipids are directly metabolized in the nucleus [5]. Regarding this possible involvement of the nuclei in ceramide/sphingomyelin metabolism, we observed increased N-SMase activity, but not A-SMase in the hepatic nuclei. Likely, nuclear sphingolipid metabolism, at least in part, depends on the actions of enzymes in situ, rather than its translocation from endosomal/lysosomal compartments or plasma membranes to the nuclei. It seems important to acknowledge this phenomenon, since so far only cytosolic elevation of ceramide has been carefully investigated, but one can envision that, molecular targets of ceramide may also depend on the subcellular localization. However, one cannot exclude possibility of ceramide translocation to the nucleus since the discovery of sphingolipid transfer proteins (e.g., CERT and FAPP2) [48].

To the best of our knowledge this is also the first report showing particular changes in FA profile in ceramide and sphingomyelin fractions in rat liver as well as in hepatic nuclei induced by HFD. Our HFD was enriched in saturated FAs and we may speculate that increased availability of saturated FA leads to enhanced accumulation of these FA species in ceramide and sphingomyelin, since saturated FAs are less readily oxidized [32–34]. Very few studies have assessed the FA species composition of ceramide and sphingomyelin pools, however, some evidence suggest that the saturation of various lipid species is also important to consider in relation to their metabolic actions. In accordance with this hypothesis are studies showing that enhancement of saturated FA's in ceramide might be responsible for a decrease in insulin sensitivity due to inhibition of PKB pathway [34, 40].

In summary, this study revealed that a HFD provision induced pronounced ceramide and sphingomyelin accumulation in the rat liver and hepatic nuclei. Importantly, there was a shift toward greater FA saturation status in both lipid fractions in either preparation. Moreover, these effects were accompanied by increased activity of N-SMase and not A-SMase.

Acknowledgment This study was supported by Medical University of Bialystok (Grant No. 3-18787 and 3-18717).

References

1. Jayadev S, Liu B, Bielawska AE, Lee JY, Nazaire F, Pushkareva MYu, Obeid LM, Hannun YA (1995) Role for ceramide in cell cycle arrest. J Biol Chem 270(5):2047–2052
2. Hannun YA (1994) The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 269(5):3125–3128
3. Neitcheva T, Peeva D (1995) Phospholipid composition, phospholipase A2 and sphingomyelinase activities in rat liver nuclear membrane and matrix. Int J Biochem Cell Biol 27:995–1001
4. James JL, Clawson GA, Chan CH, Smuckler EA (1981) Analysis of the phospholipid of the nuclear envelope and endoplasmic reticulum of liver cells by high pressure liquid chromatography. Lipids 16:541–545
5. Albi E, Lazzarini R, Viola Magni M (2008) Phosphatidylcholine/sphingomyelin metabolism crosstalk inside the nucleus. Biochem J 410(2):381–389
6. Rossi G, Viola Magni M, Albi E (2007) Signal transducer and activator of transcription 3 and sphingomyelin metabolism in intranuclear complex during cell proliferation. Arch Biochem Biophys 464:138–143
7. Albi E, Viola Magni MP (1997) Chromatin neutral sphingomyelinase and its role in hepatic regeneration. Biochem Biophys Res Commun 236:29–33
8. Albi E, Peloso I, Viola Magni MP (1999) Nuclear membrane sphingomyelin-cholesterol changes in rat liver after hepatectomy. Biochem Biophys Res Commun 262:692–695
9. Schutze S, Pottho K, Machleidt T, Berkovic D, Wiegmann K, Kronke M (1992) TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. Cell 71:765–776
10. Alewijnse AE, Peters SL (2008) Sphingolipid signalling in the cardiovascular system: good, bad or both? Eur J Pharmacol 585 (2–3):292–302
11. Straczkowski M, Kowalska I, Nikolajuk A, Dzienis-Straczkowska S, Kinalska I, Baranowski M, Zendzian-Piotrowska M, Brzezinska Z, Gorski J (2004) Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. Diabetes 53(5):1215–1221
12. Smith AC, Mullen KL, Junkin KA, Nickerson J, Chabowski A, Bonen A, Dyck DJ (2007) Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced hyperglycemia. Am J Physiol Endocrinol Metab 293:E172–E181
13. Baranowski M, Zabielski P, Blachnio A, Gorski J (2008) Effect of exercise duration on ceramide metabolism in the rat heart. Acta Physiol 192(4):519–529
14. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J (2006) Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. Diabetes 55(9):2579–2587
15. Oosterveer MH, van Dijk TH, Tietge UJ, Boer T, Havinga R, Stellaard F, Groen AK, Kuipers F, Reijngoud DJ (2009) High fat feeding induces hepatic fatty acid elongation in mice. PLoS One 4(6):e6066
16. Wierzbicki M, Chabowski A, Żendzian-Piotrowska M, Harasim E, Górski J (2009) Chronic, in vivo, PPARα activation prevents lipid overload in rat liver induced by high fat feeding. Adv Med Sci 54(1):59–65
17. Bucki R, Żendzian-Piotrowska M, Nawrocki A, Górski J (1997) Effect of increased uptake of plasma fatty acids by the liver on lipid metabolism in the hepatocellular nuclei. Prostaglandins Leukot Essent Fatty Acids 57(1):27–31
18. Górski J, Elsing C, Bucki R, Żendzian-Piotrowska M, Stremmel W (1996) The plasma borne free fatty acids rapidly enter the hepatocellular nuclei. Life Sci 59:2209–2215
19. Lamar C, Munger WL, Pitot HC (1967) Studies on a 32s component of nuclear RNA. Arch Biochem Biophys 119:98–104
20. Bosser R, Aligué R, Guerini D, Agell N, Carafoli E, Bachs OJ (1993) Calmodulin can modulate protein phosphorylation in rat liver cells nuclei. J Biol Chem 268(21):15477–15483
21. Van der Vusse GJ, Roemen TH, Reneman RS (1980) Assessment of fatty acids in dog left ventricular myocardium. Biochim Biophys Acta 617(2):347–349
22. Previati M, Bertolaso L, Tramarin M, Bertagnolo V, Capitani S (1996) Low nanogram range quantitation of diglycerides and ceramide by high-performance liquid chromatography. Anal Biochem 233:108–114
23. Mahadevappa VG, Holub BJJ (1987) Chromatographic analysis of phosphoinositydes and their breakdown products in activated blood platelets/neutrophils. J Chromatogr Libr 37:225
24. Liu B, Hannun YA (2000) Sphingomyelinase assay using radiolabeled substrate. Meth Enzymol 311:164–167
25. Zabielski P, Baranowski M, Zendzian-Piotrowska M, Błachnio-Zabielska A, Górski J (2008) Bezafibrate decreases growth stimulatory action of the Sphingomyelin signaling pathway in regenerating rat liver. Prostaglandins Other Lipid Mediat 85(1–2):17–25
26. Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, Smith SR (2005) A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes 54(7):1926–1933
27. Oakes ND, Kennedy CJ, Jenkins AB, Laybutt DR, Chisholm DJ, Kraegen EW (1994) A new antidiabetic agent, BRL 49653, reduces lipid availability and improves insulin action and glucoregulation in the rat. Diabetes 43(10):1203–1210
28. Adams LA, Angulo P, Lindor KD (2005) Nonalcoholic fatty liver disease. CMAJ 172:899–905

29. Weiss R (2007) Fat distribution and storage: how much, where, and how? Eur J Endocrinol 157(1):S39–S45
30. Ichi I, Nakahara K, Kiso K, Kojo S (2007) Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Nutrition 23(7–8):570–574
31. Itani SI, Ruderman NB, Schmieder F, Boden G (2002) Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes 51:2005–2011
32. Gaster M, Rustan AC, Beck-Nielsen H (2005) Differential utilization of saturated palmitate and unsaturated oleate: evidence from cultured myotubes. Diabetes 54:648–656
33. Leyton J, Drury PJ, Crawford MA (1987) Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. Br J Nutr 57:383–393
34. Chavez JA, Knotts TA, Wang LP, Li G, Dobrowsky RT, Florant GL, Summers SA (2003) A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. J Biol Chem 278:10297–10303
35. Hu W, Bielawski J, Samad F, Merrill AH Jr, Cowart LA (2009) Palmitate increases sphingosine-1-phosphate in C2C12 myotubes via upregulation of sphingosine kinase message and activity. J Lipid Res 50(9):1852–1862
36. Chavez JA, Holland WL, Bär J, Sandhoff K, Summers SA (2005) Acid ceramidase overexpression prevents the inhibitory effects of saturated fatty acids on insulin signaling. J Biol Chem 280(20): 20148–20153
37. Shah C, Yang G, Lee I, Bielawski J, Hannun YA, Samad F (2008) Protection from high fat diet-induced increase in ceramide in mice lacking plasminogen activator inhibitor 1. J Biol Chem 283(20):13538–13548
38. Tettamanti G (2004) Ganglioside/glycosphingolipid turnover: new concepts. Glycoconj J 20:301–317
39. Tafesse FG, Ternes P, Holthuis JC (2006) The multigenic sphingomyelin synthase family. J Biol Chem 281(40):29421–29425
40. Albi E, Viola-Magni MP (2003) Chromatin-associated sphingomyelin: metabolism in relation to cell function. Cell Biochem Funct 21(3):211–215
41. Gao J, Zhang RL, Zhou CQ, Ma Y, Zhuang GL (2009) RNA interference targeting of sphingomyelin phosphodiesterase 1 protects human granulosa cells from apoptosis. J Obstet Gynaecol Res 35(3):421–428
42. Abboushi N, El-Hed A, El-Assaad W, Kozhaya L, El-Sabban ME, Bazarbachi A, Badreddine R, Bielawska A, Usta J, Dbaibo GS (2004) Ceramide inhibits IL-2 production by preventing protein kinase C-dependent NF-kappaB activation: possible role in protein kinase Ctheta regulation. Immunology 173(5):3193–3200
43. Ves-Losada A, Maté SM, Brenner RR (2001) Incorporation and distribution of saturated and unsaturated fatty acids into nuclear lipids of hepatic cells. Lipids 36(3):273–282
44. Schroeder F, Petrescu AD, Huang H, Atshaves BP, McIntosh AL, Martin GG, Hostetler HA, Vespa A, Landrock D, Landrock KK, Payne HR, Kier AB (2008) Role of fatty acid binding proteins and long chain fatty acids in modulating nuclear receptors and gene transcription. Lipids 43(1):1–17
45. Koval M, Pagano RE (1991) Intracellular transport and metabolism of sphingomyelin. Biochim Biophys Acta 1082(2):113–125
46. Haimovitz-Friedman A, Kan CC, Ehleiter D, Persaud RS, McLoughlin M, Fuks Z, Kolesnick RM (1994) Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. J Exp Med 180:525–535
47. Mathias S, Younes A, Kan CC, Orlow I, Joseph C, Kolesnick RN (1993) Activation of the sphingomyelin signaling pathway in intact EL4 cells and in a cell-free system by IL-1 beta. Science 259:519–522
48. Yamaji T, Kumagai K, Tomishige N, Hanada K (2008) Two sphingolipid transfer proteins, CERT and FAPP2: their roles in sphingolipid metabolism. IUBMB Life 60(8):511–518