HHS Public Access

Author manuscript

Obesity (Silver Spring). Author manuscript; available in PMC 2013 June 01.

Published in final edited form as:

Obesity (Silver Spring). 2012 December ; 20(12): 2341–2347. doi:10.1038/oby.2012.126.

Sphingolipid content of human adipose tissue: relationship to adiponectin and insulin resistance

Agnieszka U. Blachnio-Zabielska[a], **Christina Koutsari**[a], **Tamara Tchkonia**[b], and **Michael D. Jensen**[a]

[a]Endocrine Research Unit, Mayo Clinic, 200 1st St SW, Rm 5-194 Joseph, Rochester, MN 55905

[b]Robert and Arlene Kogod Center on Aging, Mayo Clinic, 200 1st Street SW, Rochester, Minnesota 55905

Abstract

Ceramides (Cer) are implicated in obesity-associated skeletal muscle and perhaps adipocyte insulin resistance. We examined whether the sphingolipid content of human subcutaneous adipose tissue and plasma varies by obesity and sex as well as the relationship between ceramide content and metabolic indices. Abdominal subcutaneous adipose biopsies were performed on 12 lean adults (males = 6), 12 obese adults (males =6) for measurement of sphingolipid content and activity of the main ceramide metabolism enzymes. Blood was sampled for glucose, insulin (to calculate HOMA-IR) adiponectin and IL-6 concentrations.

Results—Compared to lean controls, total ceramide content (pg/adipocyte) was increased by 31 % ($p<0.05$) and 34 % ($p<0.05$) in obese females and males, respectively. In adipocytes from obese adults sphingosine, sphinganine, sphingosine-1-phosphate, C14-Cer, C16-Cer and C24-Cer were all increased. C18:1-Cer was increased in obese males and C24:1-Cer in obese females. For women only, there was a negative correlation between C16-Cer ceramide and plasma adiponectin ($r=-0.77$, $p= 0.003$) and a positive correlation between total ceramide content and HOMA-IR ($r=0.74$, $p= 0.006$). For men only there were significant (at least $P < 0.05$), positive correlations between adipocyte ceramides containing saturated FA and plasma IL-6 concentration. We conclude that the sexual dimorphism in adipose tissue behavior in humans extends to adipose tissue sphingolipid content its association with adiponectin, IL-6 and insulin resistance.

Keywords

weight regulation and obesity; insulin sensitivity and resistance; human; cytokines

Correspondence: Michael D. Jensen, MD, Endocrine Research Unit, 200 1st St SW, Rm 5-194 Joseph, Rochester, MN 55905, 507-255-6515 (tel), 507-255-4828 (fax), jensen@mayo.edu.

DISCLOSURE

The authors have no conflict of interest to disclose.

Contribution Statement: AUB-Z – Designed study, collected research data, edited manuscript; CK - Collected research data, edited manuscript; TT - Collected research data, edited manuscript; MDJ - Designed study, edited manuscript.

Duality of Interest: None.

INTRODUCTION

Upper body/abdominal obesity is a risk factor for insulin resistance, type two diabetes (T2DM) and metabolic syndrome (1,2). The mechanism(s) by which abdominal obesity induces metabolic disorders is poorly understood. We know that white adipose tissue (WAT) has both energy storage and important endocrine functions, secreting number of biologically active molecules, that affect glucose homeostasis, energy metabolism, food intake, body weigh regulation and insulin sensitivity (3,4). Among these molecules are TNF-α, adiponectin, IL-6.

Ceramides, central molecules on the cross-roads of sphingolipid metabolism, act as second messengers by altering the activity of a variety of kinases, phosphatases and transcription factors (5,6). In turn, these transcription factors influence cellular functions ranging from proliferation and differentiation to growth arrest and apoptosis (7–10). The cellular content of ceramides depends on the balance between their rates of generation and degradation. Ceramides may be produced from the hydrolysis of sphingomyelin by the action of neutral and acid sphingomyelinases (nSMase and aSMase), or they can be synthesized de novo. The first, rate-limiting step in this pathway is catalyzed by the enzyme serine palmitoyltransferase (SPT). The main way of ceramide degradation is its deacylation, catalyzed by ceramidase (CDase).

Most work in the field of sphingolipids, obesity and insulin resistance has focused on sphingolipid metabolism in skeletal muscle (11–15) because excess ceramides have been implicated in skeletal muscle insulin resistance (16–18). Increased intracellular ceramide content can impair skeletal muscle insulin sensitivity at the level of Akt/PKB and ceramides (C2) reduce GLUT4 translocation and glucose uptake in L6 myotubes (19).

Recent studies confirm the presence of ceramides in adipose tissue (20–23). In 3T3-L1 adipocytes and in brown adipocytes, increased ceramide can impair insulin stimulated GLUT4 expression and glucose uptake (24). It has also been shown that ceramide mediates the effect of TNF-alpha on GLUT4 mRNA content in these cells (25,26). In brown adipocytes the effects of TNF-alpha on insulin action are mediated by de novo ceramide biosynthesis, whereas in 3T3-L1 adipocytes the source of ceramides to inhibit GLUT4 expression is sphingomyelin hydrolysis (24). In ob/ob mice the hyperinsulinemia and elevated TNF-α associated with obesity may increase the expression of three major ceramide production enzymes: nSMase, aSMase and serine palmitoyltransferase (SPT) in adipose tissue. Despite greater mRNA of above mentioned enzymes, the ceramide and sphingomyelin content per gram of adipose tissue protein in ob/ob mice is reduced compared to their lean counterparts (23). However, diet-induced obesity in mice is associated with increased adipose tissue ceramide content (27).

We found only two reports on the ceramide content of human subcutaneous adipose tissue. Ceramide concentrations were greater in adipose tissue of obese women with fatty liver than in BMI-matched obese individuals with no hepatic steatosis (22). However, another group found total adipose tissue ceramide content was less in adipose tissue from obese than lean adults despite greater mRNA levels of SMases, SPT and CDases (28). The aim of the

present study was to examine the effect of obesity and sex on sphingolipid content in human fat subcutaneous abdominal tissue and to understand whether relationships exist between adipokine concentrations, insulin sensitivity and adipose tissue ceramides.

RESEARCH DESIGN AND METHODS

Twenty-four adults participated in this study. The volunteers included 6 lean (body mass index - BMI<25) males, 6 lean females (n=6), 6 obese (BMI>30) males and 6 obese females. All participants were healthy, non-smokers, and were taking no medications, including oral contraceptives. All participants gave written, informed consent before entering the study.

Body composition

Total body and regional fat and lean body mass (LBM) were assessed using dual-energy x-ray absorptiometry (DXA) (Lunar Radiation, Madison, WI) combined with a single slice abdominal CT scan at the L_2–L_3 interspace (29).

Adipose tissue biopsies

Subcutaneous abdominal adipose tissue was obtained from just lateral to the umbilicus using a needle liposuction technique with sterile conditions and local anesthesia. Samples were rinsed with saline through Nitex Nylon Fiber 250/50 and frozen at −70°C before analysis. The volunteers were in the postabsorptive state after an overnight fast.

Blood samples

Arterialized venous blood was obtained using the heated hand vein technique within 30 minutes of the adipose tissue biopsies. We measured glucose, insulin, TNF-α, IL-6, adiponectin, FFA, triacylglycerols, total cholesterol and HDL-cholesterol. The plasma samples were stored at −80°C before analysis.

Adipose tissue and plasma sphingolipids

The content of sphingolipids was measured using a UPLC/MS/MS approach. Briefly, the adipose tissue samples (40mg) were homogenized in a solution composed of 0.25 M sucrose, 25 mM KCl, 50 mM Tris and 0.5 mM EDTA, pH 7.4. Immediately afterwards 10μl of the internal standard solution (17C-sphingosine and 17C-S1P, and 17C16-Cer Avanti Polar Lipids) as well as 1.5 ml of an extraction mixture (isopropanol:water:ethyl acetate, 30:10:60; v:v:v) were added to each homogenate. The mixture was vortexed, sonicated and then centrifuged for 10 min at 4000 rpm (Sorvall Legend RT). The supernatant was transferred to new tube and pellet was re-extracted. After centrifugation supernatants were combined and evaporated under nitrogen. The dried sample was reconstituted in 100μl of LC Solvent A (2 mM ammonium formate, 0.15% formic acid in methanol) for UPLC/MS/MS analysis. Plasma sphingolipids were measured using a similar approach requiring 100μl of plasma.

Enzyme activity assays

The activities of nSMase and aSMase were determined according to Liu and Hannun (30) using [N-methyl-14C]- sphingomyelin (Perkin-Elmer Life Sciences, Waltham, MS) as the radiolabeled substrate. The product of reaction - ^{14}C-choline phosphate – was extracted with $CHCl_3$/methanol (2:1, v/v), transferred to scintillation vials and counted using a Packard TRI-CARB 1900 TR scintillation counter. The activity of neutral (nCDase), and acid (aCDase) ceramidase was measured by the method of Nikolova-Karakashian and Merrill (31) using radiolabeled [N-palmitoyl-1-^{14}C] – sphingosine (Moravek Biochemicals, Brea, CA) as a substrate. Unreacted Cer and liberated ^{14}C-palmitate were separated with basic Dole solution (isopropanol/heptane/1M NaOH, 40:10:1, v/v/v). Radioactivity of the ^{14}C-palmitate was measured by scintillation counting. SPT activity was measured as described by Merrill (32) using [^{3}H]-L-serine (Moravek Biochemicals) as the labeled substrate. Briefly, microsomal fraction was obtained by ultracentrifugation at 150,000g for 40 min. Microsomes were incubated for 10 min at 37°C in the reaction buffer (100mM HEPES (pH 8.3), 5mM DTT (dithiothreitol), 2.5mM EDTA (pH 7.0), 50mM pyridoxal phosphate, 200mM palmitoyl-CoA, and 2mM L-serine, 44,000 dpm/nmol). The labeled lipid product 3-ketosphinganine was extracted with $CHCl_3$/methanol (1:2, v/v), and the radioactivity was measured by scintillation counting.

Adipocyte size

Adipocyte size (μg lipid/cell) was assessed using the approach of Di Girolamo et al (33) which involves collagenase digestion of the adipose tissue sample, separation of adipocytes by centrifugation, methylene blue staining to identify nuclei, and measurement of cell diameter (33).

Adipose tissue total cell number

The total number of cells per gram of tissue was assessed using the DNA content of tissue, assuming that 1 ng of DNA corresponds to 150 cells. Frozen, powdered fat tissue was homogenized in 800 μl ice cold radioimmunoprecipitation assay buffer (RIPA) composed of 25 mM Tris-HCl, ph 7,6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0,1% SDS. The homogenate was centrifuged for 5 min at 10000rpm/min and lipid layer was removed by aspiration. DNA concentration was measured in homogenate using Quant-it Pico-Green (Invitrogene, Carlsbad, CA) according manufacturer's instructions. DNA concentrations were calculated using a standard curve of fluorescence from known concentration of the bacteriophage lambda DNA.

Number of adipocyte and non-adipocyte cells

The number of adipocytes was calculated by dividing the fat content per gram adipose tissue by the mean adipocyte lipid content (μg/cell). The number of non-adipocytes was calculated by subtracting the number of adipocytes from the total cell number.

Plasma metabolites

Plasma glucose concentrations were measured using a glucose analyzer (Beckman Instruments, Fullerton, CA, USA). Insulin concentrations were measured using a

chemiluminescence method (Access Ultrasensitive Immunoenzymatic; Beckman, Chaska, MN, USA). Total cholesterol, HDL cholesterol, and TG concentrations were measured with enzymatic colorimetric methods (Roche Diagnostics Corporation, Indianapolis, IN). Plasma adiponectin concentrations were measured using the human adiponectin double antibody radioimmunoassay kit (Linco Research, Inc. St. Louis, MO 63304). Plasma interleukin 6 and TNF-α concentrations were measured using a quantitative two-site enzyme immunoassays from R & D Systems (Minneapolis, MN).

Statistical analysis

The statistics were performed with PASW Statistics 17.0 software (SPSS Inc, Chicago, IL, USA). All data are presented as means ± SD. Data were analyzed by one-way analysis of variance - ANOVA, followed by Tukey-Kramer post hoc test or Games-Howell post hoc test (in the case of uneven variances). P values < 0.05 were taken to indicate statistical significance.

RESULTS

Subject characteristics (Table 1)

The characteristics of the participants are provided in Table 1. As expected, percent body fat, regional fat masses, and fat cell size were greater in obese female and male groups compared to the corresponding lean groups. We also saw the well-known sex differences in body composition with respect to regional fat masses. In addition, abdominal adipocytes were significantly larger in lean women than lean men. Plasma insulin concentrations were significantly greater in obese than lean females and males. There were no sex differences in fasting plasma insulin concentrations and no statistically significant differences in plasma glucose or total plasma cholesterol concentrations between any of the groups. HDL-cholesterol and adiponectin concentration were reduced whereas plasma TG and IL-6 concentrations were significantly greater in the obese compared to lean groups. Adiponectin was significantly less and plasma IL-6 was significantly greater in men than women (lean or obese). There were no significant differences in plasma TNF-α concentration among groups. Lean men and women were more insulin sensitive than the corresponding obese groups as assessed by HOMA.

Adipose sphingolipids (Table 2)

Adipocyte sphinganine, sphingosine and sphingosine-1-phosphate (S1P) content was greater ($p<0.001$) in obese than lean volunteers. The C14-Cer and C16-Cer content was significantly greater in obese than lean males and females counterparts. The adipocyte C18:1-Cer content was greater in obese than lean males ($p<0.01$), but it was lower in obese females than obese males ($p<0.001$). The C18-Cer content of adipocytes did not differ significantly between obese and lean groups, however, it was significantly lower in obese females than obese males. Adipocyte C20-Cer content was not significantly different between any of the groups, whereas C24:1-Cer was greater ($p<0.01$) in obese than lean females. C24:1-Cer was greater ($p<0.05$) in lean females than lean males and greater ($p<0.001$) in obese females than obese males. The content of C24-Cer increased in obese males ($p<0.05$) and females ($p<0.01$) compared to lean men and women. As expected from

the analysis of individual ceramides, the total ceramide content was greater in obese females ($p<0.05$) and males ($p<0.05$) than their lean counterparts.

Plasma sphingolipids (Table 3)

Plasma sphingosine-1-phosphate content was greater in obese males and females (both $P<0.01$) compared to their lean counterparts. Total plasma ceramide content was greater in both obese males and females (all $p<0.001$) groups compared to the lean groups. Specifically, the concentrations of C14-Cer ($p<0.001$), C16-Cer, C18-Cer, C18:1-Cer (all $p<0.01$), and C24:1-Cer ($p<0.001$) in plasma were significantly greater in obese men and women (all $p<0.05$) than in their lean counterparts.

Ceramide metabolism enzyme activity (Table 4)

SPT activity (males $p<0.05$, females $p<0.02$) and nSMase activity (both sexes $p<0.05$) were significantly greater in both obese groups as compared to the same sex lean group. There were no significant differences in aSMase activity between lean and obese groups. The activity of nCDase increased in the obese females ($p<0.05$) compared to lean females. There were no significant differences in aCDase activity between lean and obese groups.

In general, the activities of adipose tissue SPT, nSMase, nCDase and aCDase were correlated (all $r > 0.6$, $P < 0.005$). We found a strong positive correlation between total ceramide content and both SPT ($r=0.72$, $p<0.0002$) and nSMase ($r=0.59$, $p<0.004$) activities. Surprisingly, similar positive relationships were observed between total adipose tissue ceramide and nCDase ($r=0.72$, $p<0.0002$) and aCDase ($r=0.56$, $p<0.006$) activities. When tested separately by sex, the correlations between total ceramide content and enzyme activities were stronger in females than males.

Associations between sphingolipids and obesity markers

We found a negative correlation ($r=-0.73$, $p= 0.007$) between total adipocyte ceramide and plasma adiponectin concentration in females (Figure 1 upper panel). The strongest correlation was noticed with C16-Cer ($r = -0.77$, $p= 0.004$). There was also a negative correlation between adipocyte total ceramide content and fasting plasma insulin concentrations ($r=0.85$, $p= 0.0005$) as well as ceramide and HOMA-IR ($r=0.74$, $p= 0.006$) in females (Figure 1 lower panel). No such associations were detected adipocyte ceramide content in men. There were positive correlations between ceramides containing saturated FA (C16:0-Cer, C18:0-Cer and C20:0-Cer) and plasma IL-6 concentration ($r=0.57$, $p=0.05$; $r=0.65$, $p=0.02$ and $r=0.62$, $p=0.03$ respectively) in males, but not females.

There was a positive correlation between plasma total ceramide and IL-6 concentrations ($r=0.68$, $p=0.02$) and between total ceramide content in plasma and insulin concentration ($r=0.65$, $p=0.03$) in females. For men, there was a significant correlation between plasma total ceramide and TNFα concentrations ($r=0.59$, $p=0.05$) and total plasma ceramide concentration and HOMA-IR ($r=0.61$, $p=0.04$).

DISCUSSION

Our goal was to understand whether adipocyte ceramide content is altered in human obesity and, if so, whether there was any association between adipocyte ceramides and adipokines or insulin resistance. We collected abdominal subcutaneous adipose tissue from 24 lean and obese adults for measurement of fat cell size and number, total cell number, as well as ceramide content. Ceramide content was significantly greater in obese than lean men and women. In women adipocyte ceramide content correlated with hyperinsulinemia and adiponectin, whereas in men it correlated with IL-6. It is possible that accumulation of ceramides in adipocytes in obese humans contributes to adipocyte dysfunction, perhaps in a sex-specific manner. An association between adiponectin and ceramide metabolism has been reported (34,35), but the directionality is not entirely clear. For example, progestin and adipoQ receptors (PAQRs) are responsible for production of sphingoid bases through the activation of ceramidase in yeast (34) and transfection with cDNAs encoding adiponectin receptors into HEK-293-T enhanced ceramidase activity. Moreover, adiponectin reduces ceramide and glucosylceramide content in the liver of mice and a negative correlation between plasma and cardiomyocyte ceramide concentrations and adiponectin concentration has been reported (35). In these experiments we found a negative relationship between adipocyte ceramide content and plasma adiponectin concentrations in woman. The apparent co-regulation of enzymes involved in both ceramide synthesis and degradation makes it difficult to speculate on a causal relationship between adiponectin production and action as regards ceramide metabolism in human adipocytes.

There are few reports on the sphingolipid content of human adipose tissue (22). It was reported that ceramide (C22:0-Cer and C24:1-Cer) concentration per g of subcutaneous adipose tissue in women with fatty liver was greater than in BMI-matched women with no liver steatosis (22). Another study indicated that obesity with and without diabetes is associated with decreased adipose tissue ceramide content (28). Moreover a positive correlation between ceramide content and plasma adiponectin concentration and negative correlation between total ceramide content and HOMA-IR has been demonstrated (28). In ob/ob mice total adipose tissue ceramide content was reduced compared to the lean animals (23). However, the means of data expression may well explain the discrepancy between our findings and those mentioned above. Previous investigators express adipose tissue sphingolipid content per mg of adipose tissue protein, whereas we expressed it per adipocyte. It may be that, because the protein content of adipose tissue is an imperfect reflection of the number of adipocytes, previous investigators underestimated the ceramide burden of adipocytes. Our findings are consistent with those of Wu et al (36), who reported total adipocyte ceramide (expressed pmol/μg DNA) was increased in old, insulin resistant mice compared to young insulin sensitive animals. We suggest that expressing ceramide content and enzyme activity per adipocyte may be the most appropriate, because numbers and size of adipocytes differs in obese and lean subjects. Because obese adults typically have fewer but larger adipocytes per mg tissue (or per mg of tissue protein), the ceramide content from these lesser number of cells would be diluted even though the content per cell was greater.

The greater ceramide content in adipocytes from obese volunteers that we observed is likely the result of increased activity of SPT and nSMase (both enzymes are responsible for ceramide production). The finding that SPA (the main intermediate in de novo ceramide biosynthesis) was greater in adipocytes from obese adults appears to confirm the accelerated de novo ceramide biosynthesis. However, the greater activity of adipose tissue nCDases in obese females suggests a compensatory mechanism is in place to dampen the accumulation of ceramides in adipocytes.

Not all ceramides species were increased in our obese volunteers. In both obese men and women the ceramides species that were most increased were those containing saturated fatty acids: C14-Cer, C16-Cer and C24-Cer. Of interest, there were a number of sub-species of ceramides that displayed sex differences as regards the difference between obese and lean participants. It is possible that the greater C18:1-Cer concentrations offset potential down-regulation of adiponectin by other species (obese men) or greater C24:1-Cer is uniquely disadvantageous (obese women). We could find no data regarding specificity of the various species of ceramides with regards to regulation of adipocyte metabolism or the association between adipocyte ceramides and adiponectin. However, the relationship between adipocyte ceramides and IL-6 in men is consistent with the observation that 3T3-L1 adipocytes treated with ceramides show significant induction of IL-6 expression (23). Our observations raise the possibility that the secretion of adipokines is regulated differently in men and women, and that these processes may be related to adipose tissue saturated FA ceramide content.

Greater total ceramide content in plasma and adipose tissue of obese mice has been reported (27), although the predominant species that were increased were the long chain ceramides. Similarly, we noticed the greatest elevation in C14-Cer, C16-Cer and C24-Cer in adipocytes and C14-Cer, C16-Cer, C18:1-Cer, C18-Cer and C24:1-Cer in plasma.

Obesity is associated with a state of chronic low-level inflammation (37,38), which likely contributes to ceramide accumulation. Our findings that plasma ceramides are elevated in obese adults (Table 3) is consistent with previous studies (23,39). There is some evidence for causal, not mere correlational links, between ceramides and obesity-related disorders. The administration of myriocin (an inhibitor of de novo ceramide biosynthesis) decreased plasma ceramides, body fat mass and improved metabolic and inflammatory parameters in obese mice (40).

In conclusion, this is the first report on ceramide metabolism in human subcutaneous adipose tissue of lean and obese males and females. We found elevated adipocyte content of particular ceramide species, sphinganine, sphingosine, and sphingosine-1-phosphate. In females, but not males, there was a strong negative correlation between total adipocyte ceramide content and plasma adiponectin concentrations and a positive correlation between total adipocyte ceramide content and HOMA-IR. Taken together elevated adipose tissue sphingolipid content is associated with hypo-adiponectinemia and insulin resistance in women. However, it remains to be shown whether the increased ceramide content in adipocyte from obese adults are responsible for adipose tissue dysfunction. The ability to specifically modulate ceramide content, and especially those of various sub-species, would allow investigators to address this question.

ACKNOWLEDGMENTS

This work was supported by 1 UL1 RR024150 from the National Center for Research Resources (NCRR), by grants DK40484, DK45343, DK50456, and AG013925 from the U.S. Public Health Service, 7-07-DCS-03 from the American Diabetes Association, and by the Mayo Foundation. Dr. Blachnio-Zabielska was supported by an educational grant from sanofi-aventis.

REFERENCES

1. Despres J-P, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006; 444:881–887. [PubMed: 17167477]
2. Lapidus L, Bengtsson C, Bjorntorp P. The quantitative relationship between"the metabolic syndrome " and abdominal obesity in women. Obes Res. 1994; 2:372–377. [PubMed: 16353584]
3. Axelsson J, Heimburger O, Lindholm B, Stenvinkel P. Adipose tissue and its relation to inflammation: the role of adipokines. J Ren Nutr. 2005; 15:131–136. [PubMed: 15648022]
4. Hotamisligil GS. Molecular mechanisms of insulin resistance and the role of the adipocyte. Int J Obes Relat Metab Disord. 2000; 24(Suppl 4):S23–S27. [PubMed: 11126235]
5. Verheij M, Bose R, Lin XH, et al. Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. Nature. 1996; 380:75–79. [PubMed: 8598911]
6. Wang YM, Seibenhener ML, Vandenplas ML, Wooten MW. Atypical PKC zeta is activated by ceramide, resulting in coactivation of NF-kappaB/JNK kinase and cell survival. J Neurosci Res. 1999; 55:293–302. [PubMed: 10348660]
7. Kolesnick R, Fuks Z. Radiation and ceramide-induced apoptosis. Oncogene. 2003; 22:5897–5906. [PubMed: 12947396]
8. MacRae VE, Burdon T, Ahmed SF, Farquharson C. Ceramide inhibition of chondrocyte proliferation and bone growth is IGF-I dependent. J Endocrinol. 2006; 191:369–377. [PubMed: 17088406]
9. Oh HL, Seok JY, Kwon CH, Kang SK, Kim YK. Role of MAPK in ceramide-induced cell death in primary cultured astrocytes from mouse embryonic brain. Neurotoxicology. 2006; 27:31–38. [PubMed: 16143399]
10. Ohanian J, Ohanian V. Sphingolipids in mammalian cell signalling. Cell Mol Life Sci. 2001; 58:2053–2068. [PubMed: 11814056]
11. Adams JM 2nd, Pratipanawatr T, Berria R, et al. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes. 2004; 53:25–31. [PubMed: 14693694]
12. Gorski J, Dobrzyn A, Zendzian-Piotrowska M. The sphingomyelin-signaling pathway in skeletal muscles and its role in regulation of glucose uptake. Ann N Y Acad Sci. 2002; 967:236–248. [PubMed: 12079851]
13. Straczkowski M, Kowalska I, Baranowski M, et al. Increased skeletal muscle ceramide level in men at risk of developing type 2 diabetes. Diabetologia. 2007; 50:2366–2373. [PubMed: 17724577]
14. Straczkowski M, Kowalska I, Nikolajuk A, et al. Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. Diabetes. 2004; 53:1215–1221. [PubMed: 15111489]
15. Turinsky J, O'Sullivan DM, Bayly BP. 1,2-Diacylglycerol and ceramide levels in insulin-resistant tissues of the rat in vivo. J Biol Chem. 1990; 265:16880–16885. [PubMed: 2211599]
16. Chavez JA, Knotts TA, Wang L-P, et al. A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. J Biol Chem. 2003; 278:10297–10303. [PubMed: 12525490]
17. Chavez JA, Summers SA. Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. Arch Biochem Biophys. 2003; 419:101–109. [PubMed: 14592453]
18. Hajduch E, Balendran A, Batty IH, et al. Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. Diabetologia. 2001; 44:173–183. [PubMed: 11270673]

19. JeBailey L, Wanono O, Niu W, Roessler J, Rudich A, Klip A. Ceramide- and oxidant-induced insulin resistance involve loss of insulin-dependent Rac-activation and actin remodeling in muscle cells. Diabetes. 2007; 56:394–403. [PubMed: 17259384]
20. Bonzon-Kulichenko E, Schwudke D, Gallardo N, et al. Central leptin regulates total ceramide content and sterol regulatory element binding protein-1C proteolytic maturation in rat white adipose tissue. Endocrinology. 2009; 150:169–178. [PubMed: 18801905]
21. Fernandez-Veledo S, Hernandez R, Teruel T, Mas JA, Ros M, Lorenzo M. Ceramide mediates TNF-alpha-induced insulin resistance on GLUT4 gene expression in brown adipocytes. Arch Physiol Biochem. 2006; 112:13–22. [PubMed: 16754199]
22. Kolak M, Westerbacka J, Velagapudi VR, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. Diabetes. 2007; 56:1960–1968. [PubMed: 17620421]
23. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. Diabetes. 2006; 55:2579–2587. [PubMed: 16936207]
24. Long SD, Pekala PH. Lipid mediators of insulin resistance: ceramide signalling down-regulates GLUT4 gene transcription in 3T3-L1 adipocytes. Biochem J. 1996; 319:179–184. [PubMed: 8870666]
25. Stephens JM, Pekala PH. Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-α. J.Biol.Chem. 1991; 266:21839–21845. [PubMed: 1939208]
26. Stephens JM, Pekala PH. Transcriptional repression of the C/EBP-alpha and GLUT4 genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. Regulations is coordinate and independent of protein synthesis. J Biol Chem. 1992; 267:13580–13584. [PubMed: 1618860]
27. Shah C, Yang G, Lee I, Bielawski J, Hannun YA, Samad F. Protection from high fat diet-induced increase in ceramide in mice lacking plasminogen activator inhibitor 1. J Biol Chem. 2008; 283:13538–13548. [PubMed: 18359942]
28. Blachnio-Zabielska AU, Pulka M, Baranowski M, et al. Ceramide metabolism is affected by obesity and diabetes in human adipose tissue. J Cell Physiol. 2012; 227:550–557. [PubMed: 21437908]
29. Jensen MD, Kanaley JA, Reed JE, Sheedy PF. Measurement of abdominal and visceral fat with computed tomography and dual-energy x-ray absorptiometry. Am J Clin Nutr. 1995; 61:274–278. [PubMed: 7840063]
30. Liu B, Hannun YA. Sphingomyelinase assay using radiolabeled substrate. Methods Enzymol. 2000; 311:164–167. [PubMed: 10563321]
31. Nikolova-Karakashian M, Merrill AH Jr. Ceramidases. Methods Enzymol. 2000; 311:194–201. [PubMed: 10563325]
32. Merrill AH Jr. Characterization of serine palmitoyltransferase activity in Chinese hamster ovary cells. Biochim Biophys Acta. 1983; 754:284–291. [PubMed: 6652105]
33. Tchoukalova YD, Harteneck DA, Karwoski RA, Tarara J, Jensen MD. A quick, reliable, and automated method for fat cell sizing. J Lipid Res. 2003; 44:1795–1801. [PubMed: 12777477]
34. Villa NY, Kupchak BR, Garitaonandia I, et al. Sphingolipids function as downstream effectors of a fungal PAQR. Mol Pharmacol. 2009; 75:866–875. [PubMed: 19066337]
35. Holland WL, Miller RA, Wang ZV, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. Nat Med. 2011; 17:55–63. [PubMed: 21186369]
36. Wu D, Ren Z, Pae M, et al. Aging up-regulates expression of inflammatory mediators in mouse adipose tissue. J Immunol. 2007; 179:4829–4839. [PubMed: 17878382]
37. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005; 115:1111–1119. [PubMed: 15864338]
38. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol. 2004; 25:4–7. [PubMed: 14698276]
39. Haus JM, Kashyap SR, Kasumov T, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. Diabetes. 2009; 58:337–343. [PubMed: 19008343]

40. Yang G, Badeanlou L, Bielawski J, Roberts AJ, Hannun YA, Samad F. Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. American journal of physiology. 2009; 297:E211–E224. [PubMed: 19435851]

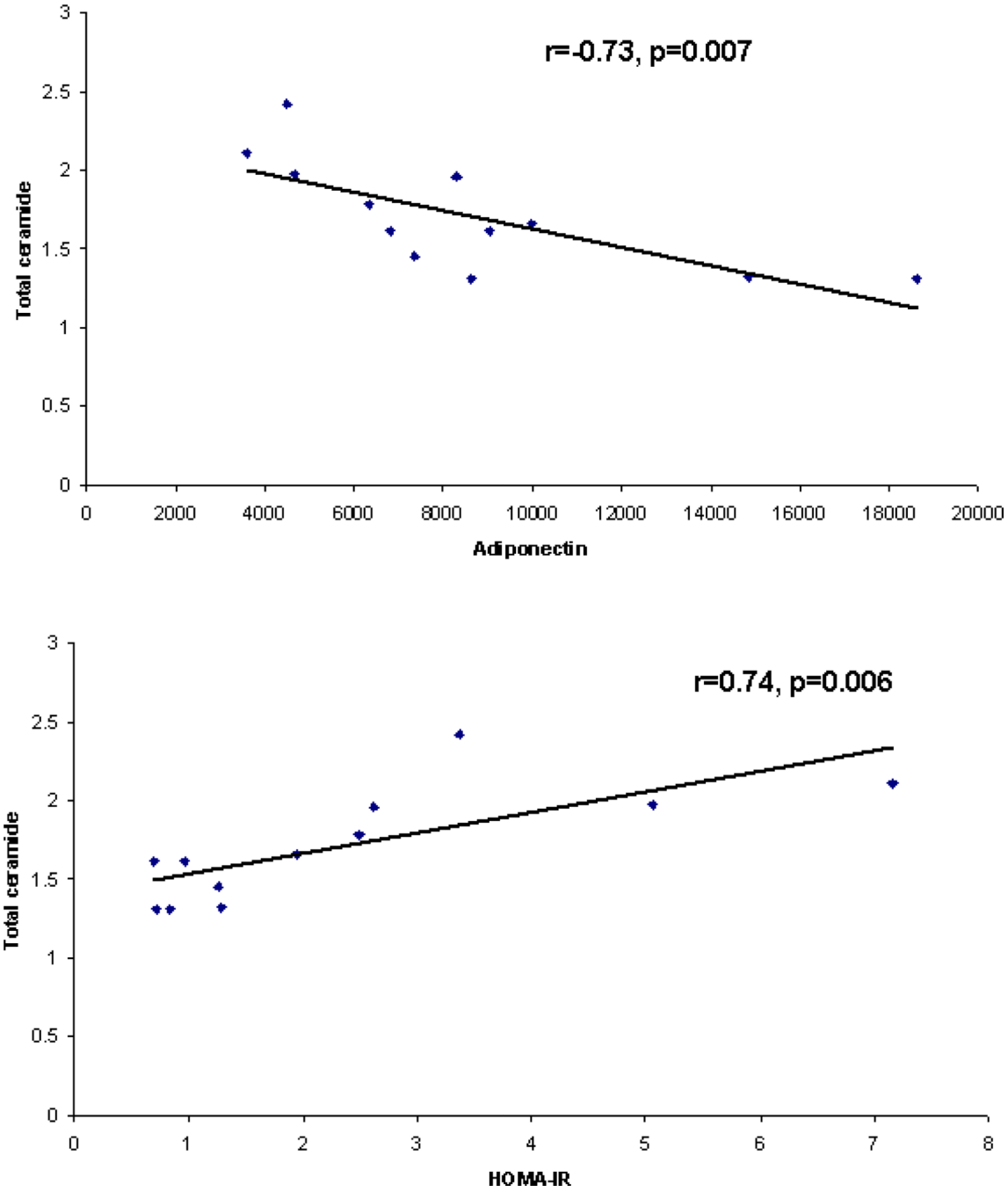

Figure 1.

Relationship between plasma adiponectin concentration and total ceramide content in white adipose tissue (upper panel) and HOMA-IR and total ceramide content in white adipose tissue (lower panel) in females.

Table 1

Anthropometric and biochemical parameters in the studied groups

	Lean Females	Lean Males	Obese Females	Obese Males
Number	6	6	6	6
Age	37 ± 11	31 ± 9	41 ± 4	37 ± 7
Body mass (kg)	66.4 ± 8.5	77.7 ± 12.7	89.5 ± 12,2	111.2 ± 14.2
BMI (kg/m^2)	23 ± 1.7	24 ± 1.7	33 ± 3.7^{d}	34 ± 2.4^{d}
Body fat %	32 ± 5	19 ± 6***	45 ± 6^{d}	33 ± 5$^{d\ *}$
UBSQ fat (kg)	11.3 ± 2.2	7.2 ± 3.3*	19.4 ± 3.8^{d}	19.8 ± 3.6^{d}
Visc fat (kg)	1.1 ± 1.0	1.7 ± 0.4**	4.3 ± 2.8^{d}	5.6 ± 2.0$^{d\ *}$
FFM (kg)	44.5 ± 6.6	62.5 ± 7.8*	48.3 ± 8.8	72.8 ± 9.2*
FCS (μg/cell)	0.44 ± 0.23	0.35 ± 0.16*	0.62 ± 0.26^{c}	0.69 ± 0.18^{d}
Insulin (μU/ml)	3.6 ± 1.8	3.6 ± 1.6	9.7 ± 3.5^{c}	10.5 ± 7.1^{c}
Glucose (mg/dl)	89 ± 5	87 ± 6	93 ± 7	100 ± 9
Cholesterol (mg/dl)	164 ± 15	163 ± 21	175 ± 42	172 ± 41
HDL-cholesterol (mg/dl)	70 ± 13	54 ± 6*	56 ± 18^{a}	43 ± 15^{a*}
TG (mg/dl)	67 ± 12	59 ± 6	133 ± 70^{d}	139 ± 42^{d}
FFA (μmol/L)	414 ± 90	389 ± 151	495 ± 137^{a}	429 ± 134
TNF-α (pg/ml)	1.06 ± 0.50	0.82 ± 0.3	0.74 ± 0.08	0.85 ± 0.15
ADIP (ng/ml)	10443 ± 5078	8319 ± 3628**	6684 ± 2736^{c}	3297 ± 1509^{d**}
IL-6 (pg/ml)	1.17 ± 0.17	1.92 ± 1.25***	1.62 ± 0.28^{c}	2.2 ± 1.56^{a**}
HOMA-IR	1.26 ± 0.72	0.81 ± 0.40	3.48 ± 2.32	2.96 ± 1.68

Values are expressed in pg per adipocyte (mean ± SD);

^{a} p<0.05;

^{b} p<0.02;

^{c} p<0.01;

^{d} p<0.001 vs the same sex lean group;

* p<0.05;

** p<0.01;

*** p<0.001 vs. lean or obese group but opposite sex.

UBSQ – upper body subcutaneous fat mass; FFM – fat free mass; FCS – fat cell size; TG – plasma triglyceride concentrations; ADIP – adiponectin;

Table 2

Sphingolipids content in white adipose tissue in lean and obese males and females groups.

	Sph	SPA	S1P	C14-Cer	C16-Cer	C18:1-Cer	C18-Cer	C20-Cer	C24:1-Cer	C24-Cer	Total Cer
LM	0.02±0.009	0.01±0.002	0.01±0.001	0.06±0.009	0.55±0.07	0.05±0.008	0.06±0.012	0.14±0.04	0.22±0.09	0.29±0.08	1.37±0.3
LF	0.02±0.006	0.01±0.002	0.01±0.002	0.05±0.007	0.52±0.04	0.04±0.024	0.05±0.008	0.14±0.06	0.32±0.04*	0.34±0.05	1.46±0.2
OM	0.09±0.013^{d}	0.03±0.008^{d}	0.03±0.006^{d}	0.09±0.014^{c}	0.71±0.09^{b}	0.09±0.017^{c}	0.07±0.010	0.19±0.04	0.24±0.05	0.40±0.08^{a}	1.79±0.2^{a}
OF	0.08±0.016^{d}	0.03±0.009^{d}	0.09±0.020^{d**}	0.09±0.020^{d}	0.62±0.09^{a}	0.05±0.007***	0.05±0.010**	0.17±0.04	0.47±0.09^{c***}	0.51±0.09^{c}	1.95±0.3^{a}

Values are expressed in pg per adipocyte (mean ± SD);

^{a}p<0.05;

^{b}p<0.02;

^{c}p<0.01;

^{d}p<0.001 vs the same sex lean group;

*p<0.05;

**p<0.01;

***p<0.001 vs. lean or obese group but opposite sex.

LM-lean males, LF-lean females, OM-obese males, OF-obese female.

Obesity (Silver Spring). Author manuscript; available in PMC 2013 June 01.

Table 3

Plasma sphingolipids content in lean and obese males and females groups.

	Sph	SPA	S1P	C14-Cer	C16-Cer	C18:1-Cer	C18-Cer	C20-Cer	C22-Cer	C24:1-Cer	C24-Cer	Total
LM	0.80±0.07	0.19±0.02	145±10	123±11	138±19	5.4±0.6	4.6±0.5	6.6±0.8	135±10	196±28	592±76	1200±94
LF	0.87±0.13	0.19±0.03	143±16	151±19	145±32	5.4±1.1	4.6±0.3	6.8±0.8	132±21	199±15	564±59	1207±72
OM	0.73±0.08	0.21±0.02	182±18^{c}	197±28^{d}	216±36^{c}	7.3±0.8^{c}	6.2±0.8^{c}	6.8±0.8	183±42^{a}	307±37^{d}	632±80	1555±81^{d}
OF	0.74±0.13	0.22±0.03	173±12^{c}	198±26^{c}	203±35^{a}	7.4±0.9^{b}	6.5±0.9^{c}	7.4±1.0	164±25	287±27^{d}	634±44	1508±43^{d}

Values are expressed in ng per 100μl of plasma (mean ± SD);

^{a}p<0.05;

^{b}p<0.02;

^{c}p<0.01;

^{d}p<0.001 vs the same sex lean group;

LM-lean males, LF-lean females, OM-obese males, OF-obese female.

Table 4

Activities of enzymes of ceramide metabolism in lean and obese males and females groups.

	SPT	nSMase	aSMase	nCDase	aCDase
LM	0.03±0.006	0.0020±0.00018	0.010±0.0025	0.0007±0.00017	0.0013±0.00019
OM	0.04±0.007[a]	0.0025±0.00020[a]	0.008±0.0016	0.0009±0.00014	0.0015±0.00012
LF	0.028±0.004	0.0025±0.00021	0.012±0.0033	0.0009±0.00025	0.0016±0.00016
OF	0.060±0.021[b]	0.0038±0.0011[a]	0.013±0.0036	0.0014±0.00038[a]	0.0023±0.00083

SPT – serine palmitoyltransferase activity (pmol serine × min^{-1} × 1000 $cells^{-1}$; nSMase and aSMase (neutral and acidic sphingomyelinase respectively) activity (nmol ceramides × h^{-1} × 1000 cells $^{-1}$); nCDase and aCDase (neutral and acidic ceramidase respectively) activity (nmol sphingosine × h^{-1} × 1000 $cells^{-1}$).

Values are expressed as mean ± SD;

[a] $p<0.05$;

[b] $p<0.02$; vs the same sex lean group;

LM-lean males, LF-lean females, OM-obese males, OF-obese female.