Serum sphingolipids: relationships to insulin sensitivity and changes with exercise in humans

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Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui H, Sanders P, Siddall P, Kuo MS, Perreault L. Serum sphingolipids: relationships to insulin sensitivity and changes with exercise in humans. Am J Physiol Endocrinol Metab 309: E398–E408, 2015.—Ceramides and sphingolipids are a family of lipid molecules that circulate in serum and accumulate in skeletal muscle, promoting insulin resistance. Plasma ceramide and dihydroceramide are related to insulin resistance, yet less is known regarding other ceramide and sphingolipid species. Despite its association with insulin sensitivity, chronic endurance exercise training does not change plasma ceramide and sphingolipid content, with little known regarding a single bout of exercise. We measured basal relationships and the effect of acute exercise (1.5 h at 50% \( \dot{V}_{\text{O}_2 \text{max}} \)) and recovery on serum ceramide and sphingolipid content in sedentary obese individuals, endurance-trained athletes, and individuals with type 2 diabetes (T2D). Basal serum C18:0, C20:0, and C24:1 ceramide and C18:0 and total dihydroceramide were significantly higher in T2D and, along with C16:0 ceramide and C18:0 sphingomyelin, correlated positively with insulin resistance. Acute exercise significantly increased serum ceramide, glucosylceramide, and GM3 gangliosides, which largely decreased to basal values in recovery. Sphingosine 1-phosphate and sphingomyelin did not change during exercise but decreased below basal values in recovery. Serum C16:0 and C18:0 ceramide and C18:0 sphingomyelin, but not the total concentrations of either of them, were positively correlated with markers of muscle NF-kB activation, suggesting that specific species activate intracellular inflammation. Interestingly, a subset of sphingomyelin species, notably C14:0, C22:3, and C24:4 species, was positively associated with insulin secretion and glucose tolerance. Together, these data show that unique ceramide and sphingolipid species associate with either protective or deleterious features for diabetes and could provide novel therapeutic targets for the future.

insulin sensitivity; athlete’s paradox; lipid composition; plasma biomarkers

THE GLOBAL BURDEN OF DIABETES continues to rise, fueled by the even greater epidemic of prediabetes (9). Nevertheless, not all of those with prediabetes will develop diabetes (27), highlighting the need to identify nonglycemic markers of disease progression that may also prove useful as new targets for the treatment of diabetes itself. Advances in lipidomics have provided new tools to investigate processes that govern the development of diabetes and its complications beyond that of glucose itself. Serum ceramides, a family of sphingolipids involved in diverse and relevant metabolic processes, have received considerable attention in relation to diabetes, making them prime candidates for further examination. Unlike other biomarkers, discrete serum ceramide profiles are rendered across the spectrum of diabetogenesis, creating speculation that ceramides not only mark but contribute to the disease process (8, 17).

Much of this speculation is derived from data generated from in vitro and animal experimentation. Human data to date are limited largely to associations between serum ceramide concentration and risk for both diabetes (15) and related complications (37). Intervention studies in humans showing commensurate change in insulin sensitivity or secretion with change in serum ceramides are lacking. Despite the known insulin-sensitizing effects of exercise, chronic endurance exercise has been shown to have no impact on plasma ceramide and sphingolipid content (2). However, it should be pointed out that most would contend that it is the acute, rather than chronic, effect of exercise that exerts its most potent influence on insulin action (30). Exploitation of this latter point would allow for proof of concept that change in serum ceramides and/or sphingolipids modifies insulin action and possibly diabetes risk.

Toward this pursuit, we sought to perform the most complete evaluation to date of molecular species of ceramides and sphingolipids in serum in a population of individuals spanning a broad range of insulin sensitivity. This allowed us to determine which of these species were positively and negatively related to insulin sensitivity and how they changed immediately following an acute exercise bout as well as after 2 h of recovery. Athletes were studied to gain insight into how chronic endurance exercise training changes serum ceramides and sphingolipids. Understanding how and which serum lipids are related to insulin sensitivity would significantly advance our knowledge and may provide insight into potential therapeutic targets for insulin sensitization.

METHODS

Subjects. Fourteen obese sedentary controls (Ob), 15 individuals with type 2 diabetes (T2D), and 15 endurance-trained athletes (Ath) were recruited for this study. Subjects gave written informed consent and were excluded if they had a body mass index (BMI) of <20 or >25 kg/m² for Ath and <28 or >40 kg/m² for Ob and T2D or had fasting triglycerides of >150 mg/dl or liver, kidney, thyroid, or lung disease. Sedentary subjects were engaged in planned physical activity <2 h/wk. Endurance-trained subjects were competitive cyclists, triathletes, and runners with average lactate thresholds of 78 ± 1.4% \( \dot{V}_{\text{O}_2 \text{max}} \) and were training on average 12.3 ± 0.8 h/wk for the past 9.7 ± 2.1 yr for the purpose of competition. Individuals with T2D were excluded from the study if they used insulin and/or thiazolidinediones. All other medications were permissible but washed out for 2 wk prior to metabolic testing. Obese subjects and athletes were not taking medications. Subjects were weight stable in the 6 mo prior to...
All women were premenopausal and were studied in the midfollicular phase of their menstrual cycle. This study was approved by the Colorado Multiple Institution Review Board at the University of Colorado Denver.

Preliminary testing. Subjects reported to the Clinical Translational Research Center (CTRC) for screening procedures following a 12-h overnight fast, where they were given a health and physical examination, followed by a fasting blood draw. Body composition was determined using dual-energy X-ray absorptiometry analysis (Lunar DPX-IQ; Lunar, Madison, WI).

Insulin sensitivity. After preliminary testing, insulin sensitivity was determined via an intravenous glucose tolerance test (IVGTT) using standard methods after an overnight fast (7). Briefly, after baseline samples, intravenous glucose (0.3 g/kg) was infused over 1 min, followed by insulin at 0.03 U/kg, 20 min after glucose administration. Blood was then frequently sampled and used to calculate whole body insulin sensitivity (SI) and insulin secretion [acute insulin response (AIR) and disposition index (DI)] using the Bergman minimal model (7) (Millennium version; MINMOD, Los Angeles, CA).

Diet and exercise control. All subjects were given a prescribed diet for 3 days prior to admission to the CTRC. Daily caloric requirement was estimated as described previously (4). Composition of this diet was 55% carbohydrate, 30% fat, and 15% protein. The fat content of the diet was controlled with the composition of saturated, monounsaturated, and polyunsaturated fat in a 1:1:1 ratio. Subjects were asked to refrain from planned physical activity for 48 h before the metabolic study.

Metabolic study. After a 12-h overnight fast an antecubital vein in one arm was cannulated for isotope infusion, and a retrograde dorsal

<table>
<thead>
<tr>
<th>Variable</th>
<th>Athletes (n = 15)</th>
<th>T2D (n = 15)</th>
<th>Obese (n = 14)</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>41.3 ± 0.86</td>
<td>42.5 ± 1.1</td>
<td>39.7 ± 1.6</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>11/4</td>
<td>11/4</td>
<td>9/5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.2 ± 0.65*</td>
<td>29.9 ± 2.6</td>
<td>33.2 ± 0.8</td>
</tr>
<tr>
<td>%Fat</td>
<td>17.0 ± 1.65*</td>
<td>27.2 ± 4.0</td>
<td>33.2 ± 2.0</td>
</tr>
<tr>
<td>$V_{O_2_\text{max}}$, mL·kg$^{-1}$·min$^{-1}$</td>
<td>47.8 ± 3.8</td>
<td>18.7 ± 2.7</td>
<td>23.8 ± 2.5</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.4 ± 0.1</td>
<td>7.9 ± 0.6</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>85 ± 2</td>
<td>136 ± 15#§</td>
<td>89 ± 1</td>
</tr>
<tr>
<td>Fasting insulin, mg/dl</td>
<td>3.5 ± 0.2</td>
<td>15.2 ± 2.6#§</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td>2-h OGTT, mg/dl</td>
<td>75 ± 7.3</td>
<td>268 ± 18#§</td>
<td>87 ± 6</td>
</tr>
<tr>
<td>S$_{I}$, mU·1$^{-1}$·min$^{-1}$</td>
<td>9.4 ± 1.06*</td>
<td>2.0 ± 0.3</td>
<td>2.93 ± 0.2</td>
</tr>
<tr>
<td>AIR$_{g}$</td>
<td>202 ± 258*</td>
<td>43 ± 15#§</td>
<td>472 ± 69#*</td>
</tr>
</tbody>
</table>

Values are means ± SE. T2D, type 2 diabetes; BMI, body mass index; $V_{O_2_\text{max}}$, maximum oxygen uptake; OGTT, oral glucose tolerance test; S$_{I}$, insulin sensitivity; AIR$_{g}$, acute insulin response to glucose. #Significantly different from athletes; *significantly different from T2D; §significantly different from obese.
hand vein in the contralateral side was catheterized for blood sampling via the heated hand technique. [U\(^{13}\)C]\(_2\) palmitate was infused at 0.0174 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\) and [6,6-\(^2\)H\(_2\)]glucose at 0.03 mg·kg\(^{-1}\)·min\(^{-1}\) and reported separately. After 3.5 h of rest, blood sampling was performed every 10 min for 30 min for metabolic and hormonal analysis. A percutaneous needle biopsy (~150 mg) was then taken from midway between the greater trochanter of the femur and the patella.

After the resting muscle biopsy subjects exercised on a cycle ergometer for 1.5 h at 50% of \(\dot{V}O_2\) max. Exercise intensity was determined using indirect calorimetry and exercise workload adjusted to maintain the relative intensity. Blood sampling was performed during the last 30 min of exercise. A biopsy muscle was taken within 1 min after exercise was stopped from the same site used during rest. Subjects then remained supine during 2 h of recovery after the exercise bout. Blood sampling was performed during the last 30 min of the recovery period. Only water consumption was allowed, as subjects remained fasted throughout the duration of the study.

**Substrate and hormone analyses.** Standard enzymatic assays were used to measure glucose and triglycerides (Olympus AU400e Chemistry Analyzer; Olympus America, Center Valley, PA), lactate (Sigma Kit no. 826; Sigma, St. Louis, MO), glycerol (r-Biopharm, Darmstadt, Germany), and free fatty acids [nonesterified fatty acid (NEFA) Kit; Wako Diagnostics, Richmond, VA]. Plasma insulin and glucagon were measured using a radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX).

**Lipidomics analysis.** LC/ESI/MS/MS analysis of ceramide and sphingolipids was performed using a TSQ Quantum Ultra-triple quadrupole mass spectrometer (Thermo Fisher, San Jose CA) interfaced with an Agilent 1100 HPLC (Agilent Technologies, Wilmington, DE) and an Xbridge C8 column (2.1 \(\times\) 30 mm; Waters, Milford, MA). Lipids from serum were extracted using one phase extraction (methanol-dichloromethane) with internal standards. Quantification was performed using the ratio of analyte to internal standards relative to sphingolipid calibration curves.

**Western blotting.** To determine whether plasma concentration of the various ceramides (their precursors, metabolites, and subspecies) had any association with end organ effects, measurements of skeletal muscle inflammation were made. Twenty micrograms of sample protein was run on an SDS-PAGE 8% Bis-Tris gel (Invitrogen, Carlsbad, CA), transferred to a polyvinylidene fluoride membrane, and blocked with 5% BSA for 1 h at room temperature. Primary antibodies were from Cell Signaling Technology (Danvers, MA), and incubations were performed in 5% BSA overnight at 4°C. Horseradish peroxidase-conjugated secondary antibody was incubated for 1 h at room temperature. Enhanced chemiluminescence was used to visualize protein bands of interest. Intensity of protein bands was captured using an AlphaImager 3300 and quantified using FluorChem software (Alpha Innotech Corp, San Leandro, CA).

**Statistical analysis.** Data are presented as means \(\pm\) SE. Differences in normally distributed basal data between groups were analyzed by 10.220.33.6 on November 16, 2017 http://ajpendo.physiology.org/ Downloaded from
using a one-way ANOVA (SPSS, Chicago, IL). Nonnormally distributed data were log transformed prior to analysis. When significant differences were detected, individual means were compared using Student’s t-tests to determine differences between groups. Differences with exercise and recovery were determined using repeated-measures ANOVA with group × time interactions to evaluate potential differences between groups. When the effect of time was significant, paired t-tests were performed to determine which time points were different from one another. Differences in ceramide and sphingolipid species between men and women were determined using a one-way ANOVA and adjusted for multiple comparisons using a Bonferroni test. The relationship between serum ceramides and sphingolipids and parameters of glucose homeostasis were corrected for multiple comparisons against BMI, insulin sensitivity, fasting and 2-h glucose, AIR, and disposition index but not the number of sphingomyelin species. This resulted in a P value for significance of <0.008. In all other comparisons, an α-level of 0.05 was used for statistical significance. Relationships between measurements were determined using Pearson’s correlation coefficient.

RESULTS

Demographics. Demographic information for participants is shown in Table 1. There were no significant differences in age or sex between groups. As expected, Ob and T2D had greater BMI and percent body fat, and Ath had roughly twice the maximal oxygen consumption compared with Ob and T2D. The Hb A1c, fasting glucose, and 2-h glucose were significantly higher in T2D compared with the other two groups (P < 0.0001). The AIR to glucose during the IVGTT was highest in T2D compared with the other two groups (P < 0.0004), whereas C24:1 dihydroceramide content was lower in T2D compared with the other two groups (P = 0.005). Differences between groups persisted largely during exercise and recovery, as there was no change in dihydroceramide concentration (Fig. 2B). Similarly to total concentration, C24:1 dihydroceramide (P = 0.009) was inversely related to insulin sensitivity.

We found no significant differences in serum glucosylceramide between groups and no significant relationships of total or individual glucosylceramide species to insulin sensitivity (Fig. 4A). Acute exercise increased total glucosylceramide concentration, which decreased back to resting values in recovery (Fig. 4B). The increase during exercise was driven by significant increases in every species in athletes compared with rest (Fig. 4C).

Individual species (Fig. 2A). T2D had significantly greater serum concentrations of C18:0 (P ≤ 0.02), C20:0 (P ≤ 0.008), and C24:1 ceramide (P ≤ 0.02) compared with the other groups. Total serum ceramide content was not significantly related to insulin sensitivity (P = 0.14), whereas inverse relationships were found for C16:0 (P = 0.03), C18:0 (P = 0.0004), C20:0 (P = 0.007), and C24:1 ceramide (P = 0.03). Serum ceramide concentration increased during exercise and recovery in all groups combined (Fig. 2B), which was explained mostly by the change in T2D and Ath (Fig. 2, C and D). C18:0 and C24:1 ceramide remained significantly elevated in T2D compared with the other two groups during exercise and recovery.

At rest, total serum dihydroceramide concentration was significantly greater in T2D compared with the other groups (P = 0.003; Fig. 3A) and was inversely related to insulin sensitivity (P = 0.02). The concentration of C18:0 dihydroceramide was higher in T2D compared with the other two groups (P ≤ 0.006), whereas C24:1 dihydroceramide content was lower in Ath compared with the other two groups (P = 0.005). Differences between groups persisted largely during exercise and recovery, as there was no change in dihydroceramide concentration (Fig. 3B). Similarly to total concentration, C24:1 dihydroceramide (P = 0.009) was inversely related to insulin sensitivity.

Resting serum dihydroceramide species

![Graph A](https://example.com/graphA.png)

**Graph A:** Concentration of serum dihydroceramide (DHCER) species between groups at rest (A) and total concentration during rest, exercise, and recovery (B). Values are means ± SE. §Significantly different from athletes; $Significantly different from obese.

The total concentration of serum ceramides was not different between groups (P = 0.28) despite significant differences in the number of species detected. There was a positive relationship between sphingosine and insulin sensitivity (r = 0.39, P = 0.009). Sphingosine and sphingosine 1-phosphate were not altered by acute exercise, but the latter decreased significantly during recovery (Fig. 1B).

The increase during exercise was explained by increases in T2D and athletes only. In T2D, total GM3 content was significantly increased...
after recovery compared with rest, which was attributed largely to increases in C24:0.

The concentrations of resting sphingomyelin species are shown in Fig. 6A. Obese subjects had greater concentrations of C14:0 sphingomyelin compared with T2D, 18:2 compared with Ath, and 20:3, 22:3, 24:3, and 24:4 compared with T2D and Ath. Individuals with T2D had greater concentrations of C18:0 and C18:1 sphingomyelin compared with athletes and lower 22:1 sphingomyelin compared with Ob and Ath. After adjusting for multiple comparisons using Bonferroni, serum sphingomyelin 20:1 content was significantly greater in women compared with men (women: 3.5 ± 0.13, men: 2.9 ± 0.08; P = 0.0002). There were no significant changes in serum sphingomyelin with exercise, but there was a significant decrease in recovery in all groups combined (Fig. 6B). Although most species in all groups were decreased in recovery, the most significant decreases were found in T2D (Fig. 6C).

The relationship of serum ceramides and sphingolipids to parameters related to glucose homeostasis is shown in Table 2. Two general categories were evident with ceramides and sphingolipids positively related to diabetes risk and sphingomyelin species negatively related to diabetes risk. A number of serum sphingomyelin species were associated with higher insulin secretion and glucose tolerance, which was not a compensatory phenomenon since there were no relationships between these serum sphingomyelin species and BMI, SI, or DI. A completely different group of serum ceramides and sphingolipids was related to insulin resistance, glucose intolerance, and fasting hyperglycemia and hyperinsulinemia.

Relationship between serum NEFA and serum ceramides. We found no significant relationships between total NEFA content and serum ceramide and sphingolipids. There was a significant positive relationship between serum palmitate and serum dihydroceramide concentration (r = 0.33, P = 0.03).

Muscle protein expression. No significant differences were found between groups for skeletal muscle IKKα Ser176/180 phosphorylation, a marker of NF-κB activation (Fig. 7A). However, significant positive relationships were found between IKKα phosphorylation and serum C16:0 and C18:0 ceramides (P = 0.0003 and P = 0.007; Fig. 7, B and C, respectively), and C18:0 sphingomyelin (P = 0.03; Fig. 7D),
with no relationship observed for total serum ceramides or sphingomyelin.

**DISCUSSION**

Discrete serum ceramide profiles are rendered across the spectrum of diabetes development, creating speculation that ceramides not only mark but contribute to the disease process (8, 17). Nevertheless, a paucity of interventional studies in humans has made this speculation difficult to confirm. To fill this knowledge gap, we sought to perform the most complete evaluation to date of molecular species of serum ceramides and sphingolipids in a population of individuals spanning a broad range of insulin sensitivity before and after an insulin-sensitizing bout of exercise. Major findings from the current study demonstrate that basal serum C18:0, C20:0, and C24:1 ceramide and total dihydroceramide were significantly higher in T2D and along with C16:0 ceramide and C18:0 sphingomyelin correlated with whole body insulin resistance. Serum C16:0 and C18:0 ceramide and C18:0 sphingomyelin, but not total serum concentration of either of these, correlated with markers of muscle NF-κB activation, suggesting that specific species activate intracellular inflammation. Interestingly, a subset of sphingomyelin species was positively associated with insulin secretion and glucose tolerance. Together, these data show that unique ceramide and sphingolipid species associate with either protective or deleterious features for diabetes and could prove to be novel therapeutic targets in the future.

In healthy humans, synthesis of ceramide-related lipids is tightly regulated. Under fasting conditions, sphingomyelin is the most abundant serum sphingolipid, representing 88% of ceramides and sphingolipids, and circulates bound predominately to HDL2 (12). Serum sphingomyelin has been reported to increase or decrease in the postprandial state (12, 34), whereas serum ceramides (C14:0–20:0) increase, preferentially carried by circulating LDL (12). Observed changes from the fasted to fed state represent the effect of dietary fats to stimulate the de novo pathway for ceramide synthesis (12). Interplay between the different pathways yields a myriad of ceramide species, each with their own roles in maintaining health or promoting disease.

Dietary lipid composition has a well-known and profound effect on de novo ceramide synthesis. Simple enrichment of either isocaloric (21, 23) or hypercaloric (16, 33) diets with saturated fats leads to a robust increase in serum ceramide
concentration and commensurate fall in insulin sensitivity that can be reversed with weight loss (17, 31). Data from the current study would also contend that dietary fat consumption may influence serum dihydroceramide composition. Serum NEFAs reflect habitual intake of lipid since NEFAs are derived from adipocyte lipid storage. The positive relationship between serum palmitate and dihydroceramide suggests that palmitate may upregulate hepatic ceramide synthesis. Ceramide synthase acylates sphingosine to dihydroceramide, and each of the four isoforms in the liver have acyl chain specificity (24). Ceramide synthase 5 and 6 preferentially incorporate palmitate into dihydroceramide, and upregulation of these isoforms in T2D could promote dihydroceramide synthesis and lead to insulin resistance in times of dietary palmitate oversupply.

Lifestyle interventions are one of the most potent treatments to reverse insulin resistance. However, the contribution of changes in serum ceramides to exercise-induced insulin sensitization is relatively unknown. One study found that chronic exercise training increased serum sphingosine 1-phosphate concentration only, whereas serum ceramide and sphingolipids were stable during acute exercise (2). Another study reported decreased plasma ceramide content after weight loss and exercise training in obese subjects with and without type 2 diabetes (19). Similarly to the former report, we found no differences in serum ceramides and sphingolipids in endurance-trained athletes compared with obese individuals, suggesting minimal changes with chronic exercise training. Contrarily, serum ceramides, glucosylceramides, and gangliosides increased during acute exercise and largely decreased in recovery, with no changes observed for dihydroceramide. Moreover, these changes were seen almost exclusively in athletes and individual with type 2 diabetes. The relevance of the observed lipid changes during and after an acute bout of exercise to insulin action is not easily explained. Most noteworthy, however, was the observation that athletes had significantly less basal serum C18:0 sphingomyelin than T2D. There is reason to believe that C18:0 sphingomyelin is particularly deleterious for insulin sensitivity in humans (14, 22) and may serve as novel biomarker in this regard. Furthermore, serum sphingomyelin decreased in recovery from exercise with a similar response in most species. Total serum sphingomyelin relates to risk of cardiovascular disease (CVD) (18, 29), so the decrease in recovery could reflect a common pathway by which exercise is cardioprotective and promotes insulin sensitivity.

Serum ceramides are increased in obesity and type 2 diabetes (13, 17) and are associated with the development of ath-

Fig. 6. Concentration of serum sphingomyelin (SPM) species between groups at rest (A), total concentration during rest, exercise, and recovery (B), and change from rest to recovery by group (C). Values are means ± SE. #Significantly different from athletes; *significantly different from T2D; §significantly different from obese; ¥significantly different from rest; ‡significantly different from exercise.
Table 2. Serum sphingolipid relationships to glucose homeostasis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sphingolipid</th>
<th>r Value</th>
<th>Effect Size</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positively related to diabetes risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2-h OGTT glucose</td>
<td>C18:0 sphingomyelin</td>
<td>-0.46</td>
<td>-1.547</td>
<td>0.002</td>
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<tr>
<td>Fasting glucose</td>
<td>C18:0 dihydroceramide</td>
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<td>4.665</td>
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<td>Fasting glucose</td>
<td>C18:0 glucosylceramide</td>
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<td>0.753</td>
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<td>Fasting glucose</td>
<td>C24:1 dihydroceramide</td>
<td>0.50</td>
<td>0.673</td>
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<tr>
<td>Fasting glucose</td>
<td>C23:0 ceramide</td>
<td>0.44</td>
<td>0.170</td>
<td>0.003</td>
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<td>0.218</td>
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<tr>
<td>Fasting insulin</td>
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<td>0.130</td>
<td>&lt;0.0001</td>
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<td>AIR_g C14:0 sphingomyelin</td>
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<td>211.279</td>
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</table>

Serum sphingomyelins are generated by virtue of both salvage and de novo ceramide pathways (37), which may be relevant to their debated role in health and disease. Epidemiologic data demonstrate a positive correlation between total serum sphingomyelin concentration and CVD (18, 29), yet total serum sphingomyelin has been reported as lower in CVD-prone states such as obesity, type 1 diabetes, and type 2 diabetes (35, 39). Metabolic profiling has helped clarify this discrepancy by associating certain sphingomyelin species, rather than total concentration, with metabolic parameters. For example, sphingomyelin 18:0, 20:0, 22:0, and 24:0 were associated with higher BMI and lower insulin sensitivity (14, 22), whereas serum sphingomyelin 16:1 was related to enhanced insulin sensitivity and lower risk for diabetes (10). Thus, like most lipids, sphingomyelins are heterogeneous and have different effects related to acyl chain composition. In our data set a number of sphingomyelins were associated with higher insulin secretion, particularly species 14:0, 22:3, and 24:4. These species had the largest effect size, suggesting that small changes in sphingomyelins were associated with large changes in insulin secretion. Notably, the effect size for the positive relationship between sphingomyelins and insulin secretion was much larger than the negative relationship between ceramides and insulin resistance. The higher insulin secretion does not appear to be a compensatory phenomenon, as there were no relationships between these sphingomyelin species and BMI, SI, or DI. Furthermore, sphingomyelin 16:0 and 22:1 were related to lower circulating fasting glucose and/or 2-h glucose concentration. Our data suggest that not all sphingomyelins are created equal, with some positive and others negatively related to insulin sensitivity and glucose intolerance. Together, the association of specific serum sphingomyelin species (14:0, 16:0, 16:1, 20:1, 22:1, 22:3, 24:3, and 24:4) with greater insulin secretion compared with controls as well as mitochondrial dysfunction and increased reactive oxygen species. It is intriguing to hypothesize that certain serum sphingomyelin species may exchange with β-cell membranes, which may influence β-cell function and insulin secretion.

The athlete’s paradox was first suggested by Goodpaster et al. (11) and described similar intramuscular triglyceride content in endurance-trained athletes and individuals with diabetes despite vastly different sensitivities to insulin. Prior to this report, the content of intramuscular triglyceride was considered to be negatively related to insulin sensitivity, hence the paradox in insulin-sensitive athletes. The field has now moved past total triglycerides to more closely scrutinize differences in lipid intermediates between groups that are known to promote insulin resistance, such as diacylglycerol and ceramide. But even within those classes of lipids, specific species of diacylglycerol and ceramide associate with insulin sensitivity in endurance-trained athletes (1, 4, 5). The current data suggest that a similar phenomenon exists in the serum lipidome, where specific ceramide and sphingolipid species may influence muscle insulin sensitivity. Therefore, it is possible that alterations in specific serum ceramides and sphingolipids in endurance-trained athletes influence insulin sensitivity and contribute to the athlete’s paradox.

There are several limitations in this study that are important to address. There is no lean control group in this study, which...
would allow for a more thorough evaluation of the influence of body weight on our outcomes. The relationships between serum ceramide and sphingolipids and insulin sensitivity, insulin secretion, and muscle inflammation are correlations only and cannot be used to imply causation. We do not have information regarding the lipoprotein distribution of plasma ceramides. As a result, we may have missed relationships between serum ceramides and insulin sensitivity that may be more powerful when only certain lipoprotein-bound ceramide species are accounted for. We did not correct for changes in plasma volume during this study, which likely influenced the concentration of ceramides and sphingolipids during both exercise and recovery. Measurements of insulin sensitivity by the hyperinsulinemic euglycemic clamp would have been more powerful and could have uncovered more relationships between serum lipids and insulin resistance. Finally, we do not have measurements of insulin sensitivity after exercise to parallel changes in serum ceramides.

In summary, findings from the current study demonstrate that basal serum C18:0, C20:0, and C24:1 ceramide and total dihydroceramide were significantly higher in T2D. These species along with C16:0 ceramide and C18:0 sphingomyelin correlated with whole body insulin resistance. Serum C16:0 and C18:0 ceramide and C18:0 sphingomyelin, but not total serum concentration of either of these, correlated with markers of muscle NF-κB activation, suggesting that specific species activate intracellular inflammation. Acute exercise increased serum ceramides and sphingolipids, which largely decreased with recovery. The exception is sphingomyelin, which was decreased in recovery compared with rest. Interestingly, a subset of sphingomyelin species was positively associated with insulin secretion and glucose tolerance. Combined, these data show that different ceramide and sphingolipid species associate with either protective or harmful features of glucose tolerance and insulin sensitivity and could prove to be novel therapeutic targets in the future.

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Fig. 7. Skeletal muscle protein expression for obese sedentary individuals, individuals with T2D, and Ath for IKKα Ser176/180 phosphorylation (A) as well as the relationship of IKKα Ser176/180 phosphorylation to serum C16:0 ceramide (B), C18:0 ceramide (C), and C18:0 sphingomyelin (D).
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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