Effect of gender on lipid-induced insulin resistance in obese subjects

Bodil Vistisen, Lars I Hellgren, Torill Vadset, Celena Scheede-Bergdahl, Jørn Wulff Helge, Flemming Dela and Bente Stallknecht

Department of Biomedical Sciences, The Panum Institute, The Copenhagen Muscle Research Centre, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark and 1BioCentrum-DTU and The Centre for Advanced Food Studies, Technical University of Denmark, Lyngby, Denmark

(Correspondence should be addressed to B Stallknecht; Email: b.stallknecht@mfi.ku.dk)

Abstract

Objective: In obese subjects, chronically elevated plasma concentrations of non-esterified fatty acids (NEFAs) exert a marked risk to contract insulin resistance and subsequently type 2 diabetes. When NEFA is acutely increased due to i.v. infusion of lipid, glucose disposal during a hyperinsulinemic–euglycemic clamp is reduced. This effect has been explained by a NEFA-induced decrease in skeletal muscle insulin sensitivity caused by accumulation of the lipid intermediates such as ceramide and diacylglycerol in the myocytes. However, neither the lipid-induced reduction of glucose disposal nor the intramyocellular lipid deposition has been compared directly in obese females and males.

Design: We studied eight obese females and eight obese males (body mass index (BMI): 32.6 ± 1.4 and 32.8 ± 0.8 respectively, non significant (NS)) matched for cardiorespiratory fitness relative to lean body mass (43.7 ± 1.6 and 47.6 ± 1.3 ml/kg min respectively, NS).

Methods: Each subject underwent two hyperinsulinemic–euglycemic clamps with infusion of lipid or saline respectively. Furthermore, the subjects exercised during the last half an hour of each clamp.

Results: The lipid-induced reduction in glucose disposal during the clamp was similar in females and males (46 ± 10 and 60 ± 4% respectively, NS). However, whole-body insulin sensitivity as well as non-oxidative glucose disposal was higher in obese females compared with obese males both during lipid and saline infusion (P < 0.001 and P = 0.01 respectively). Muscle ceramide, triacylglycerol (TAG), diacylglycerol (DAG), and glycogen content were similar between sexes and remained unchanged during the clamp and when exercise was superimposed.

Conclusions: The lipid-induced inhibition of glucose disposal is similar in obese females and males. However, obese females are more insulin sensitive compared with obese males (both during saline and lipid infusion), which is not due to differences in the concentration of the muscle lipid intermediates such as ceramide and DAG.

Introduction

Elevated plasma concentration of non-esterified fatty acids (NEFAs) is an independent risk factor for type 2 diabetes (T2D) (1–3), and NEFA exerts an inhibitory effect on insulin-stimulated glucose uptake in skeletal muscle (4). In humans, infusion of lipid acutely increases plasma NEFA concentrations and insulin-mediated glucose uptake is subsequently reduced in young healthy subjects (5, 6), relatives of type 2 diabetic patients (7, 8), and in healthy obese subjects (9).

The effect of NEFA on insulin action may differ between females and males. Some studies of females have shown a lipid-induced reduction in insulin-mediated glucose uptake (10–12) as also seen in males (5, 6), while other studies of females have failed to demonstrate an effect of NEFA on insulin action (13–15). The discrepancy may partly be explained by different study designs and characteristics of the subjects (e.g., body weight and age). However, to our knowledge, the effect of lipid infusion (and thus elevated NEEA concentrations) on insulin-mediated glucose uptake rates has never been directly compared in obese female and male subjects. Interestingly, a study of lean subjects showed a striking gender-dependent difference, where lipid infusion did not inhibit insulin-stimulated glucose disposal in lean females, whereas this was the case in the lean males (13). The discrepancy was partly due to an inhibition of the non-oxidative glucose disposal, that is, glycogen deposition, during lipid infusion in the lean males, which was not observed in the lean females.

In obese individuals, a chronic elevation of NEFA markedly increases the risk of developing the metabolic syndrome and T2D (16). It has been suggested, that the chronic NEFA elevation is linked to the decreased insulin sensitivity through increased accumulation of
diacylglycerol (DAG) and/or ceramide in the myocytes, which leads to an attenuation of the phosphoinositide 3-kinase (PI-3) kinase signaling pathway through the activation of novel and atypical protein kinase C respectively (17, 18). However, to the best of our knowledge, gender differences in NEFA-induced myocellular lipid accumulation and/or in obesity-induced ectopic lipid deposition have not been studied before. In order to clarify the importance of gender on the inhibitory effect of NEFA on insulin action, we have now studied the response to a manifest increase in NEFA in high-risk individuals by measuring whole-body glucose uptake rates during insulin infusion and superimposed exercise, with and without concomitant lipid infusion. By adding exercise, we wanted to address whether the exercise-induced glucose uptake would add on to the insulin-induced glucose uptake. Furthermore, multiple muscle biopsies were obtained to elucidate differences in intramyocellular lipid and glycogen deposition, which could explain gender differences in the metabolic response to the treatments.

We hypothesized that an acute increase in plasma NEFA would reduce the insulin-stimulated glucose disposal in both obese females and males, and that this would be due to NEFA-induced changes in muscle ceramide and DAG content.

Subjects and methods

Subjects

Eight females and eight males participated in the study and all gave their written consent after full oral and written information. The study was approved by the Copenhagen Ethics Committee (KF/01 259270) and conducted according to the principles of the Declaration of Helsinki. Inclusion criteria were healthy (no medication), middle aged (30- to 50-year old) and obese (Table 1). No subjects participated in any regular leisure sports activities. One female and one male subject were smokers. All experiments with female subjects were performed during the first 10 days after the onset of their menstruation.

Table 1 Description of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42 ± 2</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>89.9 ± 3.9</td>
<td>107.9 ± 1.5 *</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.6 ± 1.4</td>
<td>32.8 ± 0.8 *</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>47.2 ± 1.8</td>
<td>34.1 ± 2.0 *</td>
</tr>
<tr>
<td>Upper body fat (%)</td>
<td>55.7 ± 1.4</td>
<td>64.4 ± 1.3 *</td>
</tr>
<tr>
<td>Lower body fat (%)</td>
<td>41.1 ± 1.3</td>
<td>30.9 ± 1.3 *</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>47.2 ± 1.5</td>
<td>70.9 ± 1.5 *</td>
</tr>
<tr>
<td>VO₂max (l/min)</td>
<td>2.06 ± 0.08</td>
<td>3.38 ± 0.14 *</td>
</tr>
<tr>
<td>VO₂max/LBM (ml/kg min)</td>
<td>43.7 ± 1.6</td>
<td>47.6 ± 1.3 *</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.8 ± 0.2</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>OGGT (2 h), blood glucose (mmol/l)</td>
<td>7.8 ± 0.4</td>
<td>6.7 ± 0.4</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M. *Differences between females and males (P<0.001): LBM, lean body mass; OGGT, oral glucose tolerance test.

Pre-screening test protocol

All subjects were required to partake in an oral glucose tolerance test (75 g glucose diluted in 300 ml water). Body composition was determined by dual-energy X-ray absorptiometry (DPX-IQ scanner, Lunar, Madison, WI, USA). Finally, maximal oxygen uptake (VO₂max) was determined by an incremental exercise test on a bicycle ergometer (Ergoline, Ergonometrics 800, Jaeger, Hoechberg, Germany). Respiratory measurements were carried out with an Oxycon Pro (Jaeger). Those females and males who had the best match with respect to maximal oxygen consumption relating to lean body mass (LBM; NS, Table 1) were included in the study. Three days before the experimental day, subjects ate a carbohydrate-rich diet (~ 65% and a total of more than 250 g carbohydrate/day) to ensure full glycogen stores. This was done to minimize intra- and inter-subject differences in food intake and composition before the experimental days and had only little effect on the habitual diet and caloric intake.

Experimental days

Subjects completed both an intervention day with i.v. lipid infusion and a control day with saline infusion separated by at least 2 weeks and performed in randomized order. After an overnight fast (12 h), subjects arrived at 0800 h to the laboratory by car or bus. A catheter was inserted in the retrograde direction in a hand vein for later sampling of arterialized venous blood (using a heating pad). Catheters for infusion of glucose, insulin, and lipid (intervention trial) or saline (control trial) were inserted in two forearm veins. Then basal blood and muscle samples were obtained. The hyperinsulinemic–euglycemic clamp was initialized with a priming dose of 260 mU/m² insulin (see below), and the lipid or saline infusion was started with the onset of the clamp (refer to (19) for further details). After 4 h, a muscle biopsy was obtained and then the subject was moved to a supine bike (Ergoline, Ergonometrics 900, Jaeger) to cycle at a low work load (42 ± 1% of VO₂max) during the final 30 min of the clamp. Heart rate, O₂ uptake, and CO₂ excretion were continuously measured during exercise. At the end of the exercise period, a final arterialized blood sample and a muscle biopsy were obtained.

Muscle biopsies

Muscle biopsies were obtained from M. vastus lateralis under local anesthesia using sterile technique. Visible fat and connective tissue were removed, and the biopsy was frozen in liquid nitrogen and stored at −80 °C for subsequent analysis.

Infusates

Insulin (actrapid 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 40 mU/min per m².
The glucose (182 g/l) infusion rate was varied in order to maintain a plasma glucose concentration of 5.5 mmol/l. Lipid (intralipid 200 g/l, Fresenius Kabi, Bad Homburg v.d. H., Germany) was mixed with heparin (14 μl heparin/ml intralipid, Heparin-Leo, Leo-Pharma, Ballerup, Denmark) and infused at a rate of 0.014 ml/min per kg. During the control trial, isotonic saline was infused with the same infusion rate as the lipid/heparin mixture.

**Plasma concentrations of insulin, NEFA, and TAG**

Plasma insulin concentrations were determined by sandwich ELISA performed according to manufacturer’s instructions (DakoCytomations, Glostrup, Denmark). Plasma NEFA was determined using an NEFA C kit (Wako, Neuss, Germany) on an automatic analyzer (Cobas Fara, Roche). Plasma concentrations of triacylglycerol (TAG) were measured enzymatically on the automatic analyzer (Cobas Fara).

**Muscle ceramide and DAG analysis**

Lipids were analyzed essentially according to the Folch procedure. The dissected freeze-dried tissue was homogenized on ice in methanol using a Polytron PT-2100 (Kinematica, Littau-Lucerne, Switzerland). Finally, internal standard (C6-ceramide, Avanti Polar Lipids, Al, USA) and 0.2 volume of distilled water (dH2O) were added to obtain the final ratio of chloroform:methanol:dH2O in 2:1:0.6 (v:v:v) as described by Folch (20). After phase separation at 4 °C, the organic solution was transferred to a new glass and dried down under N2. Ceramide and DAG were quantified using the DAG-kinase assay as described by Bielawska (21). Radioactivity in phosphatidic acid and ceramide-1-phosphate was determined using a PhosphoI-mager (Bio-Rad). A ceramide standard curve was prepared from ceramide derived from bovine brain sphingomyelin (Sigma).

**Muscle glycogen and TG analysis**

Muscle glycogen concentration was determined as glucose residues after hydrolysis of dissected freeze-dried muscle sample in 1 M HCl at 100 °C for 2 h (22). Muscle TAG content was in principle analyzed as described by Kiens & Richter (23). In the final part of the analysis, the glycerol concentration was analyzed in triplicate on a cylindrical mirror analyzer (CMA) analyzer (CMA 600 microdialysis analyzer, CMA/Microdialysis AB, Stockholm, Sweden).

**Calculations and statistics**

Glucose and fat oxidation rates were calculated by the non-protein respiratory quotient (24). Non-oxidative glucose disposal was calculated as the difference between glucose infusion rate and glucose oxidation rate.

Plasma insulin, plasma NEFA, plasma TAG, glucose infusion rate, percentage inhibition of glucose infusion, and muscle data were tested for effects of gender and trial by two-way ANOVA with repeated measurements and Tukey’s post hoc test.

Comparison of the respiratory exchange ratio (RER), fat and glucose oxidation, non-oxidative glucose disposal, and fasting plasma glucose between sexes and trials was performed by either three- or two-way ANOVA and by Tukey’s post hoc test.

A probability of 0.05 was chosen a priori as the level of significance. The statistical analysis was performed using SigmaStat (version 2.03, Gotteborg, Sweden).

**Results**

**Glucose disposal**

Glucose infusion rates were lower (P<0.001) during lipid infusion compared with saline in both females (Fig. 1A) and males (Fig. 1B). Furthermore, during lipid as well as saline infusion, the glucose infusion rates were lower (P<0.001) during lipid infusion compared with saline in both males and females.
were higher (P<0.001) in females (Fig. 1A) compared with males (Fig. 1B) both at rest and during exercise.

After 4 h of lipid infusion, the glucose infusion rate was inhibited by 46±10 and 60±4% in females and males respectively (NS). During exercise, the lipid-induced inhibition of the glucose infusion decreased to 38±8 and 44±7% in females and males respectively (NS).

**Plasma glucose, insulin, NEFA, and TAG**

Basal plasma glucose as well as basal plasma insulin (46±7 and 67±6 pmol/l in females and males respectively) did not differ significantly between sexes (NS, Table 1). Infusion of insulin increased (P<0.001) plasma insulin to similar concentrations in both females and males in the lipid infusion trial at rest (after 4-h clamp: 440±24 and 459±24 pmol/l respectively) as well as during exercise (after 4 1/2-h clamp: 476±17 and 506±25 pmol/l respectively) without differences between sexes (NS). Likewise in the control trial, plasma insulin concentrations increased (P<0.001) similarly in females and males and reached 429±31 and 429±37 pmol/l respectively, after 4 h of clamp and 502±31 and 464±33 pmol/l respectively, during exercise (after 4 1/2-h clamp) with no differences between sexes (NS). No significant differences in plasma insulin concentrations were observed between the trials.

Basal plasma NEFA did not differ between sexes (0.78±0.11 and 0.78±0.15 mmol/l in females and males respectively, NS). Infusion of lipid increased (P<0.001) plasma NEFA compared with fasting values to similar levels in both sexes at rest (2.35±0.47 and 3.68±0.76 mmol/l in females and males respectively, NS) as well as during exercise (2.11±0.41 and 3.73±0.76 mmol/l in females and males respectively, NS). Plasma NEFA during saline infusion was 0.19±0.05 and 0.23±0.07 mmol/l in females and males respectively (NS) and during exercise 0.17±0.05 and 0.22±0.06 mmol/l in females and males respectively (NS). Plasma NEFA was higher (P<0.001) during the lipid compared with the saline trial.

Fasting plasma TAG did not differ between sexes (0.87±0.15 and 1.38±0.14 mmol/l in females and males respectively, NS). Infusion of lipid increased (P<0.001) plasma TAG compared with fasting values with no gender-dependent differences both during rest (3.47±0.78 and 4.59±0.33 mmol/l in females and males respectively, NS) and exercise (2.79±0.59 and 3.83±0.33 mmol/l in females and males respectively, NS). During saline infusion, the plasma TAG did not change from the basal level (NS), but was higher (P<0.001) during the lipid compared with the saline trial with no difference between sexes (NS).

**Muscle glycogen, ceramide, TAG, and DAG**

The basal glycogen content of the muscle ranged from 349±22 to 421±57 mmol/dw muscle with no difference between trials or sexes (Fig. 2A and B). After 4 h (at rest) and 4 1/2 h (rest + 1/2 h of exercise) of clamps, the muscle glycogen was not significantly changed and ranged from 352±27 to 386±35 and from 356±36 to 381±37 mmol/dw muscle respectively.

Likewise, muscle ceramide during basal conditions were similar between sexes and before each trial (ranges: 51±5 to 63±7 pmol/mg per dw muscle: Fig. 2C and D). No significant changes in muscle ceramide were observed between basal and 4 and 4 1/2 h of clamp, with concentrations ranging from 55±6 to 72±9 and from 54±6 to 70±5 mmol/mg per dw muscle respectively.

At basal, the muscle TAG content ranged from 137±32 to 190±42 mmol/mg per dw muscle (Fig. 2E and F, NS) and did not change during the clamp (133±39 to 188±39 and 80±19 to 130±55 mmol/mg per dw muscle after 4 and 4 1/2 h respectively).

Likewise, the muscle DAG concentration was similar in the basal samples and ranged from 55±15 to 111±24 mmol/mg per dw muscle (Fig. 2G and H, NS). Muscle DAG was unchanged after 4 and 4 1/2 h of clamp and ranged from 133±39 to 188±39 and 73±10 to 91±25 mmol/mg per dw muscle respectively (NS).

**RER, fat and glucose oxidation, and non-oxidative glucose disposal**

RER was lower (P=0.002) after 4 h of lipid compared with saline infusion in both females and males (females: 0.85±0.04 vs 0.95±0.03 and males: 0.84±0.03 vs 0.95±0.02) and, accordingly, the fat oxidation was higher (P=0.002; Fig. 3A). Correspondingly, the glucose oxidation in both females and males was lower (P=0.006) during lipid compared with saline infusion during the fourth hour of the clamp (Fig. 3C). Likewise, the non-oxidative glucose disposal was lower (P<0.001) during lipid compared with saline infusion during the fourth hour of the clamp (Fig. 3E). No significant gender-dependent differences in substrate oxidation were revealed during the fourth hour of the clamp (NS). However, the non-oxidative glucose disposal was higher (P=0.02) in females compared with males.

During exercise, the RER was lower (P<0.001) during lipid compared with saline infusion in both females and males (females: 0.91±0.01 and 0.98±0.01; males: 0.90±0.02 and 0.96±0.01) and, accordingly, the fat oxidation was higher (P<0.001; Fig. 3B). Consequently, during exercise, the glucose oxidation in both females and males was lower (P<0.001) during lipid compared with saline infusion (Fig. 3D). Furthermore, the non-oxidative glucose disposal rate was lower (P<0.001) during lipid compared with saline infusion (Fig. 3F). There were no significant gender differences in RER and substrate oxidation during the exercise period, but the non-oxidative glucose disposal was higher (P=0.01) in females compared with males. In fact, deposition of glucose was observed in females, whereas in males glucose deposition was not observed (Fig. 3F).
Figure 2 (A and B) Muscle glycogen, (C and D) ceramide, (E and F) TAG, and (G and H) DAG at basal and during hyperinsulinemic–euglycemic clamp without and with exercise in eight obese females and eight obese males. Values are means ± S.E.M.
Discussion

The main finding of the present study was strikingly higher whole-body insulin sensitivity in obese females compared with obese males during lipid as well as during saline infusion. It is also noteworthy that despite the higher whole-body insulin sensitivity, the lipid-induced insulin resistance, measured as lipid-induced reduction in glucose disposal during a hyperinsulinemic–euglycemic clamp, was not affected by gender in obese subjects. Finally, ceramide, TAG, and DAG contents in skeletal muscle were similar in females and males, and did not change with hyperinsulinemia or exercise of low intensity.

Our finding of a gender-independent lipid-induced insulin resistance contrasts with the findings of a previous study, where a 23% inhibition in insulin-stimulated glucose disposal was observed in males, but no inhibition was observed in females (13). However, the subjects in that study were lean, while the subjects in our study were obese. Furthermore, the subjects of the previous study were only matched in regards to BMI (13), whereas in addition to BMI we also matched females and males with regard to maximal oxygen consumption per kilogram of LBM, because fitness level is an important determinant of insulin-stimulated glucose disposal.

In the present study, the inhibition of the insulin-stimulated glucose disposal peaked with ~45 and 60% in the females and males respectively after 4 h of lipid infusion, which is similar to the range of inhibition observed in most studies. Lipid infusion studies performed in females showed an inhibition of 30–40% (10–12), and Boden et al. (25) found an inhibition of glucose disposal of ~50% in young healthy male subjects after 6 h of lipid infusion. Likewise, an inhibition of 40–50% was observed in overweight T2D patients (a mixed group of females and males) after 4 h of lipid infusion (7). Kruszynska et al. (9) found that lipid infusion for 8 h decreased the insulin-stimulated glucose uptake by 17–24% in both lean and obese subjects (4 females and 11 males). These studies together with the present study indicate that the lipid-induced inhibition of the glucose disposal is similar in lean and obese subjects. This implies that subjects with permanent high plasma NEFA do not adapt to this condition with a lower inhibition response, when exposed to very high plasma NEFA concentrations. In other words, it seems as if very high plasma NEFA always has a
detrimental effect on insulin sensitivity independent of the subject’s basal level of plasma NEFA. Interestingly, the lipid-induced inhibition of glucose disposal was less during the 30 min of low-intensity exercise (38–44%) compared with rest (45–60%) in both sexes in the present study. This indicates that the contraction-stimulated glucose disposal is not inhibited by high plasma NEFA.

One of the major findings of the present study was higher whole-body insulin sensitivity in obese females compared with obese males during lipid as well as during saline infusion. This has previously been observed in lean subjects, where a higher insulin-stimulated glucose disposal per kilogram LBM was observed in females compared with males (26, 27). Despite the higher insulin sensitivity in females compared with males as seen in the present and the aforementioned studies, the mechanism(s) involved in this gender-dependent difference has not been fully elucidated. As expected, we found a significantly higher fat percentage in the upper body in males compared with females (Table 1). The amount of abdominal adipose tissue is known to affect insulin sensitivity negatively, so this might partly explain the higher insulin sensitivity in females compared with males. Obviously, there are differences in the level of sex hormones between females and males, and females treated for hyperandrogenism develop increased insulin sensitivity as androgen level decreases (28).

Another potential mechanism behind the gender-dependent difference in insulin sensitivity may be located in skeletal muscle, where most of the insulin-induced glucose uptake occurs. Nuutila et al. (29) showed with the use of positron emission tomography a 50% higher insulin-stimulated glucose uptake in skeletal muscle of young lean females compared with males, who were matched in regard to BMI and maximal oxygen uptake capacity. Likewise, we observed that females had a higher non-oxidative glucose disposal compared with males (most pronounced during exercise) at the same plasma insulin concentration (Fig. 3E and F), although this did not result in a higher total muscle glycogen concentration in females compared with males. This was due to non-oxidative glucose disposal rate being very low compared with the total content of glycogen in the muscle. Frias et al. (13) also found a higher glucose disposal in lean females compared with lean males during infusion of lipid and consequently high plasma NEFA. Therefore, the permanent high plasma NEFA in the obese subjects of the present study could inhibit the glucose disposal more in the males compared with the females and thus partly explain the gender difference in insulin-stimulated glucose disposal and also during saline infusion.

In the present study, we did not observe any gender-related differences in muscle DAG and ceramide concentrations either at baseline or after insulin infusion. This implies that the observed gender difference in whole-body insulin sensitivity during insulin stimulation cannot be explained by gender-specific accumulation of muscle lipid intermediates. Furthermore, the lipid infusion also did not influence intramyocellular ceramide and DAG concentrations, which is consistent with the observed similar lipid-induced inhibition of glucose disposal in females and males in the present study. A recent study by Serlie et al. (30) also found that manipulation of plasma NEFA did not influence muscle ceramide concentration in middle-aged lean and obese subjects, which is in contrast to the findings by Straczkowski et al. (31), showing that lipid infusion induced a 48% increase in muscle ceramide content. The discrepancy is not easily explained, but the subjects in the study of Straczkowski et al. were lean young subjects (31), while our study population was obese and middle aged. This indicates that obesity and age-related metabolic changes attenuate the NEFA-induced ceramide and DAG accumulation.

In the present study, muscle TAG remained unchanged by the intervention and was not different between sexes. Interestingly, prior studies (32, 33) have found a higher muscle TAG concentration in lean females compared with lean males. Lean females also have a higher gene and protein expression of components involved in fatty acid uptake in muscle cells (e.g., CD36, FABPpm) (34) and a higher ratio of types 1–2 muscle fibers than lean males (28, 32). However, the gender differences apparently do not persist when somewhat older obese females and males are compared.

In conclusion, the present study demonstrated for the first time that an acute increase in plasma NEFA induces the same degree of insulin resistance in obese females and males matched in regard to fitness level and BMI. Obese females, however, have a significantly higher whole-body insulin sensitivity compared with obese males (both during lipid and saline infusion), which is not explained by differences in the concentration of the muscle lipid intermediates ceramide and DAG.

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