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Gender-Related Differences in the Metabolic Response to Fasting

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Context: Free fatty acids (FFA) may induce insulin resistance via synthesis of intramyocellular ceramide. During fasting, women have lower plasma glucose levels than men despite higher plasma FFA, suggesting protection from FFA-induced insulin resistance.

Objective: We studied whether the relative protection from FFA-induced insulin resistance during fasting in women is associated with lower muscle ceramide concentrations compared with men.

Main Outcome Measures and Design: After a 38-h fast, measurements of glucose and lipid fluxes and muscle ceramide and fatty acid translocase/CD36 were performed before and after a hyperinsulinemic euglycemic clamp.

Results: Plasma glucose levels were significantly lower in women than men with a trend for a lower endogenous glucose production in women, whereas FFA and lipolysis were significantly higher. Insulin-mediated peripheral glucose uptake was not different between sexes. There was no gender difference in muscle ceramide in the basal state, and ceramide did not correlate with peripheral glucose uptake. Muscle fatty acid translocase/CD36 was not different between sexes in the basal state and during the clamp.

Conclusion: After 38 h of fasting, plasma FFA were higher and plasma glucose was lower in women compared with men. The higher plasma FFA did not result in differences in peripheral insulin sensitivity, possibly because of similar muscle ceramide and fatty acid translocase/CD36 levels in men and women. We suggest that during fasting, women are relatively protected from FFA-induced insulin resistance by preventing myocellular accumulation of ceramide. **(*J Clin Endocrinol Metab* 92: 3646–3652, 2007)**

THE ADAPTIVE METABOLIC response to short-term fasting consists of integrated metabolic alterations that guarantee substrate availability for energy production and prevent hypoglycemia. During fasting, plasma insulin levels are low and plasma concentrations of catecholamines, glucagon, and GH are increased, resulting in increased lipolysis and thus high plasma free fatty acid (FFA) concentrations (1–3).

It has been known for a long time that women have lower plasma glucose and higher plasma FFA concentrations than men after short-term fasting (1, 4–6). However, despite extensive research on gender-related distinctions in glucose and lipid metabolism, these differences in plasma glucose and FFA concentrations have not been explained in full detail so far (7–16).

In women, the combination of lower plasma glucose levels on one hand and higher plasma FFA levels on the other hand is intriguing, because it is generally accepted that high plasma FFA levels increase endogenous glucose production (EGP) and decrease peripheral glucose uptake (17, 18). Consistently, it has been shown that women are relatively protected from FFA-induced insulin resistance (12, 16).

The exact underlying mechanisms by which FFA interfere with insulin signaling have not yet been unraveled completely. One potential mechanism may involve the *de novo* synthesis of ceramide from palmitate, because intramyocellular ceramide was found to be increased in obese insulin-resistant patients and correlated with whole-body insulin sensitivity (19, 20). Moreover, *in vitro* studies showed that intracellular ceramide synthesis from palmitate was found to be one of the mechanisms by which palmitate interferes negatively with insulin-stimulated phosphorylation of protein kinase B (21, 22). Furthermore, metabolites from ceramide (*i.e.* glycosphingolipids such as glucosylceramide) might be involved in the induction of insulin resistance (23, 24). Because intramyocellular ceramide concentration correlates positively with plasma FFA levels, it might be expected that the increased levels of plasma FFA in women result in higher muscle ceramide levels (20). However, this would contradict the reported relative protection from FFA-induced insulin resistance in women.

Two mechanisms may explain this relative insensitivity to increased plasma FFA levels: first, lower myocellular uptake of plasma FFA, and second, differences in muscle fatty acid handling. Cellular uptake of plasma FFA occurs by protein-mediated transport and via flip-flop of protonated fatty acids (25–27), depending on transmembrane concentration gradients and intracellular fatty acid metabolism (25, 27–29). Fatty

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Abbreviations: BMI, Body mass index; EGP, endogenous glucose production; FAT, fatty acid translocase; FFA, free fatty acids; MCR, metabolic clearance rate; Rd, rate of disappearance; REE, resting energy expenditure.

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acid translocase (FAT)/CD36 is the main protein involved in muscle fatty acid uptake (26). Fatty acid handling involves storage into complex lipids or oxidation of fatty acyl-CoAs. To the best of our knowledge, gender differences in muscle (glyco)sphingolipids and the fatty acid transporter CD36 content after short-term fasting in relation to glucose and lipid metabolism have not been studied before.

In this study, we measured glucose and lipid fluxes after short-term fasting in healthy lean men and women in the basal state and during a hyperinsulinemic euglycemic clamp (stable isotope technique). Furthermore, we assessed total muscle content of ceramide, glucosylceramide, and the lipid-binding protein FAT/CD36 in the basal state and during the clamp. We hypothesized that the relative protection from FFA-induced insulin resistance during fasting in women results from lower muscle ceramide or glucosylceramide levels due to lower muscle FFA uptake, subsequently resulting in higher muscle glucose uptake with lower plasma glucose concentrations.

Subjects and Methods

Subjects

Ten male and 10 female subjects were recruited via advertisements in local magazines. Criteria for inclusion were 1) absence of a family history of diabetes; 2) age 18–35 yr; 3) Caucasian race; 4) body mass index (BMI) 20–25 kg/m^2; 5) no excessive sport activities, *i.e.* fewer than three times per week; and 6) no medication. Women were studied during the follicular phase of the menstrual cycle. Subjects were in self-reported good health, confirmed by medical history and physical examination. Written informed consent was obtained from all subjects after explanation of purposes, nature, and potential risks of the study. The study was approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam.

Experimental protocol

For 3 d before the fasting period, all volunteers consumed a weight-maintaining diet containing at least 250 g carbohydrates per day. Then, the subjects were fasting from 2000 h 2 d before the start of the study until the end of the study. Volunteers were admitted to the metabolic unit of the Academic Medical Center at 0730 h. Subjects were studied in the supine position and were allowed to drink water only.

A catheter was inserted into an antecubital vein for infusion of stable isotope tracers, insulin, and glucose. Another catheter was inserted retrogradely into a contralateral hand vein and kept in a thermoregulated (60 C) Plexiglas box for sampling of arterialized venous blood. In all studies, saline was infused as NaCl 0.9% at a rate of 50 ml/h to keep the catheters patent. [6,6-$^2H_2$]Glucose and [1,1,2,3,3-$^2H_5$]glycerol were used as tracers (>99% enriched; Cambridge Isotopes, Andover, MA).

To study total triglyceride hydrolysis, we used [1,1,2,3,3-$^2H_5$]glycerol. This may result in underestimation of FFA release (in contrast to a fatty acid tracer). However, curves of glycerol and fatty acid tracers are very similar during fasting (2), although the latter is preferred to quantify adipose tissue lipolysis (30).

At time (T) = 0 h (0800 h), blood samples were drawn for determination of background enrichments and a primed continuous infusion of both isotopes was started: [6,6-$^2H_2$]glucose (prime, 8.8 μmol/kg; continuous, 0.11 μmol/kg·min) and [1,1,2,3,3-$^2H_5$]glycerol (prime, 1.6 μmol/kg; continuous, 0.11 μmol/kg·min) and continued until the end of the study. After an equilibration period of 2 h (38 h of fasting), three blood samples were drawn for glucose and glycerol enrichments and one for glucoregulatory hormones, FFA, and adiponectin. Thereafter (T = 3 h), infusions of insulin (60mU/m^2·min) (Actrapid 100 IU/ml; Novo Nordisk Farma B.V., Alphen aan den Rijn, The Netherlands) and glucose 20% (to maintain a plasma glucose level of 5 mmol/liter) were started. [6,6-$^2H_2$]Glucose was added to the 20% glucose solution to achieve glucose enrichments of 1% to approximate the values for enrichment reached in plasma and thereby minimizing changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose. Plasma glucose levels were measured every 5 min at the bedside. At T = 8 h, five blood samples were drawn at 5-min intervals for determination of glucose and glycerol enrichments. Another blood sample was drawn for determination of glucoregulatory hormones, FFA, and adiponectin.

Body composition and indirect calorimetry

Body composition was measured with bioelectrical impedance analysis (Maltron BF906; Maltron, Rayleigh, UK). Oxygen consumption (VO_2) and CO_2 production (VCO_2) were measured continuously during the final 20 min of both the basal state and the hyperinsulinemic euglycemic clamp by indirect calorimetry using a ventilated hood system (Sensormedics model 2900; Sensormedics, Anaheim, CA).

Muscle biopsy

Muscle biopsies were performed to assess muscle content of ceramide, glucosylceramide, and FAT/CD36 at the end of both the basal state and the hyperinsulinemic euglycemic clamp. The muscle biopsy was performed under local anesthesia (lidocaine 20 mg/ml; Fresenius Kabi, Den Bosch, The Netherlands) using a Pro-Mag I biopsy needle (MDTECH, Gainesville, FL). Biopsy specimens were quickly washed in a buffer (0.9% NaCl/28.3g/liter HEPES) to remove blood, inspected for fat or fascia content, dried on gauze swabs, and subsequently stored in liquid nitrogen until analysis.

Glucose and lipid metabolism measurements

Plasma glucose concentrations were measured with the glucose oxidase method using a Beckman Glucose Analyzer 2 (Beckman, Palo Alto, CA) with intraassay variation of 2–3%. Plasma FFA concentrations were determined with an enzymatic colorimetric method (NEFA-C test kit; Wako Chemicals GmbH, Neuss, Germany) with intraassay variation of 1%, interassay variation of 4–15%, and detection limit of 0.02 mmol/liter. [6,6-$^2H_2$]Glucose enrichment was measured as described earlier (31). [6,6-$^2H_2$]Glucose enrichment (tracer/tracee ratio) intraassay variation was 0.5–1% with an interassay variation of 1% and detection limit of 0.04%. [1,1,2,3,3-$^2H_5$]Glycerol enrichment was determined as described earlier (32). Intraassay variation for glycerol was 1–3% and for [1,1,2,3,3-$^2H_5$]glycerol was 4%; interassay variation for glycerol was 2–3% and for [1,1,2,3,3-$^2H_5$]glycerol was 7%.

Glucoregulatory hormones and adiponectin

Insulin and cortisol were determined on an Immulite 2000 system (Diagnostic Products Corp., Los Angeles, CA). Insulin was measured with a chemiluminescent immunometric assay with intraassay variation of 3–6%, interassay variation of 4–6%, and detection limit of 15 pmol/liter. Cortisol was measured with a chemiluminescent immunoassay with intraassay variation of 7–8%, interassay variation of 7–8%, and detection limit of 50 nmol/liter. Glucagon was determined with the Linco ^{125}I RIA (Linco Research, St. Charles, MO) with an intraassay variation of 9–10%, interassay variation of 5–7%, and detection limit of 15 ng/liter. Norepinephrine and epinephrine were determined with an in-house HPLC method. Intraassay variation for norepinephrine: 2%; epinephrine 9%; interassay variation norepinephrine was 10% and for epinephrine was 14–18%; detection limit was 0.05 nmol/liter. Adiponectin was determined by a RIA (Linco) with intraassay variation of 2–7%, interassay variation of 16–17%, and detection limit of 1 ng/ml.

Ceramide and glucosylceramide measurements

Ceramide and glucosylceramide in muscle biopsies were measured with an HPLC method as described (33). Muscle biopsies were weighed and homogenized in 300 μl water by sonification, and 50 μl muscle homogenates was used. All samples were run in duplicate, and in every run, two reference samples were included. Interassay variation was 4%, and intraassay variation was less than 14%.

FAT/CD36 measurements

Muscle biopsies were homogenized four times for 5 sec each (Ultra-Turrax; Ika-Werke, Staufen, Germany) in 10 vol cold (4 C) buffer containing 250 mM sucrose, 2 mM Na-EDTA, and 10 mM Tris (pH 7.4), followed by four 5-sec ultrasonic treatments (ultrasonic processor; Hielsher GmbH, Teltow, Germany). Total protein was determined with the bicinchoninic acid method (Pierce, Rockford IL). All samples were diluted to the same protein concentration and mixed with sample buffer (4:1, vol/vol) before being subjected to SDS-PAGE and immunoblotting exactly as described before (34).

The CD36 antigen-antibody band at 88 kDa was visualized with a chemiluminescence substrate (ECL; Amersham Biosciences UK Ltd., Buckinghamshire, UK) and quantified using Quantity One software (Bio-Rad, Hercules, CA). Rat heart and liver whole homogenates were used as positive and negative controls, respectively.

Calculations and statistics

EGP and peripheral glucose uptake [rate of disappearance (Rd)] were calculated using the modified forms of the Steele equations as described previously (35, 36). EGP and Rd were expressed as micromoles per kilogram per minute. Glucose metabolic clearance rates (MCR) were calculated as MCR = Ra/[glucose]. Lipolysis (glycerol turnover) was calculated by using formulas for steady-state kinetics adapted for stable isotopes (32). Lipolysis was expressed as micromoles per kilogram per minute and as micromoles per kilocalorie as proposed by Koutsari and Jensen (30). Resting energy expenditure (REE) and glucose and fat oxidation rates were calculated from O_2 consumption and CO_2 production as reported previously (37).

All data were analyzed with nonparametric tests. Comparisons between groups (at T = 2 and 8 h) were performed using the Mann-Whitney *U* test. Comparisons within groups (between T = 2 and 8 h) were performed with the Wilcoxon signed rank test. Correlations were expressed as Spearman's rank correlation coefficient (ρ). The SPSS statistical software program version 12.0.1 (SPSS Inc., Chicago, IL) was used for statistical analysis. Data are presented as median (minimum–maximum).

Results

Anthropometric characteristics

Men and women did not differ in age or BMI (Table 1). Weight and percent lean body mass were higher and percent fat mass was lower in men (Table 1).

REE and glucose and lipid kinetics

Total REE was higher in men than in women, both in the basal state and during the hyperinsulinemic clamp (Table 2). Rates of glucose and fat oxidation did not differ between men and women in the basal state and during the clamp (Table 2). In the female group, plasma glucose concentrations were significantly lower after 38 h of fasting compared with the male group (Table 3). Basal EGP (and Rd) tended to be lower in women (Table 3). Insulin-mediated peripheral glucose uptake during the clamp (Rd) did not differ significantly between men and women (Table 3). MCR in the basal state was not different between women and men [2.0 (1.8–3.2) ml/kg·min *vs.* 2.1 (1.7–2.4) ml/kg·min, respectively; $P = 1$]. Basal plasma FFA were significantly higher in women compared with men but were equally suppressed during the hyperinsulinemic euglycemic clamp in both groups (Table 3). Lipolysis expressed as micromoles per kilogram per minute was not different between men and women in the basal state and during the clamp, but when expressed in micromoles per kilocalorie, it was shown that women had significantly higher lipolysis rates in the basal state but not during the clamp (Table 3).

Glucoregulatory hormones and adiponectin

Plasma insulin, cortisol, glucagon, and norepinephrine levels were not different between sexes in the basal state and during the clamp (Table 4). Plasma epinephrine was significantly lower in females than males during the hyperinsulinemic euglycemic clamp (Table 4).

Adiponectin was significantly higher in females in both the basal state and during the clamp (Table 4). Adiponectin decreased significantly from baseline during the clamp, although no gender difference in relative decrease was observed (data not shown).

Ceramide and glucosylceramide measurements

Muscle ceramide concentrations in the basal state were not different between sexes (Fig. 1). There was a trend to lower muscle ceramide levels in women compared with men during the hyperinsulinemic euglycemic clamp (Fig. 1). However, the change in muscle ceramide content during the clamp from baseline did not differ between women and men (data not shown). There were no differences in muscle glucosylceramide levels between women and men [basal state, 1.9 (1.0–4.9) pmol/mg wet weight *vs.* 1.4 (1.2–3.0) pmol/mg wet weight, respectively ($P = 0.16$), and during the clamp, 1.8 (1.3–6.7) pmol/mg wet weight *vs.* 2.2 (1.5–3.8) pmol/mg wet weight, respectively ($P = 0.5$)]. In the basal state, muscle ceramide and glucosylceramide levels did not correlate with plasma FFA in women and men [ceramide (females), $\rho = 0.26$ ($P = 0.47$); ceramide (males), $\rho = 0.35$ ($P = 0.33$); glucosylceramide (females); $\rho = 0.41$ ($P = 0.26$); glucosylceramide (males), $\rho = 0.53$ ($P = 0.12$)]. No correlation was found between insulin-mediated peripheral glucose uptake and muscle ceramide or glucosylceramide levels in women and men [ceramide (females), $\rho = 0.14$ ($P = 0.69$); ceramide (males), $\rho = -0.37$ ($P = 0.29$); glucosylceramide (females), $\rho = -0.05$ ($P = 0.89$); glucosylceramide (males), $\rho = 0.48$ ($P = 0.16$)].

TABLE 1. Clinical characteristics of male and female subjects

	Men (n = 10)	Women (n = 10)	*P*
Age (yr)	21.3 (18.9–25.1)	22.2 (19.1–28.9)	0.3
Weight (kg)	74.6 (65.5–89.0)	63.5 (54.5–72.0)	0.001
Height (cm)	187 (179–195)	169 (160–177)	0.4
BMI (kg/m^2)	21.5 (19.2–24.7)	22.9 (18.7–24.2)	0.4
Lean body mass (%)	89 (77–91)	75 (70–89)	0.002
Fat mass (%)	11 (9–23)	25 (11–30)	0.002

Data are presented as median (minimum–maximum).

TABLE 2. REE and oxidation rates for glucose and fat

	Basal state			Hyperinsulinemic euglycemic clamp		
	Men (n = 10)	Women (n = 10)	*P*	Men (n = 10)	Women (n = 10)	*P*
REE (kcal/d)	1995 (1840–2356)	1511 (884–1833)	0.001	1992 (1685–2177)	1467 (1150–1842)	0.001
Glucose oxidation (μmol/kg·min)	5.0 (0–18.3)	3.9 (0–7.8)	0.5	20 (8.9–33.3)	16.1 (11.7–28.3)	0.4
Glucose oxidation (μmol/kg LBM·min)	5.6 (0–20.6)	5.0 (0–10.6)	0.8	22.2 (11.1–37.8)	21.7 (13.9–38.3)	0.9
Fat oxidation (μmol/kg·min)	2.0 (0.9–2.8)	1.7 (1.3–2.1)	0.3	0.7 (0–1.5)	0.5 (0–1.0)	0.4
Fat oxidation (μmol/kg LBM·min)	2.3 (1.0–3.0)	2.2 (1.9–2.9)	0.9	0.8 (0–1.7)	0.6 (0–1.3)	0.7

Data are presented as median (minimum–maximum). LBM, Lean body mass.

FAT/CD36 measurements

There were no differences in muscle FAT/CD36 content between women and men during both the basal state and the hyperinsulinemic euglycemic clamp (Fig. 2). Also, there was no significant change in muscle FAT/CD36 content between the basal state and the end of the hyperinsulinemic euglycemic clamp in both sexes (data not shown).

Discussion

We studied the metabolic adaptation to 38 h of fasting in healthy lean women and men to elucidate the mechanism behind the well-established finding of lower plasma glucose levels and higher plasma FFA during fasting in women.

We confirmed earlier reports on lower plasma glucose levels in women during fasting (1, 4, 6). The lower basal plasma glucose concentrations in women are probably attributed to the trend toward a lower EGP. Our findings are not in complete agreement with previous reports. Two studies showed equal EGP between women and men between 16 and 22 h of fasting (11, 13). In another study, the decline in EGP between 16 and 64 h of fasting was greater in women than men, but absolute EGP did not differ at both time points between sexes (4). The discrepancy with these studies in finding a trend toward a lower EGP might be explained by the fact that we studied more subjects (4, 11, 13). Moreover, EGP is the major determinant of basal plasma glucose concentration (38), and the MCR was not different between men and women in the basal state. This suggests that lower EGP plays a causal role in the lower plasma glucose levels in women. Notably, gender differences have been described in circulating gluconeogenic substrates during short-term fasting (1). The difference in EGP between men and women may be caused by differences in plasma concentrations of ovarian steroids because it was shown that in female ovariectomized mice, gluconeogenesis was higher compared with intact control mice (39). To our knowledge, there have been no other reports on gender differences in EGP after 38 h of fasting.

A remarkable finding in our study was the absence of a difference in peripheral insulin sensitivity, despite significantly higher plasma FFA in females after fasting for 38 h. So far, there have been no reports on gender differences regarding insulin sensitivity after short-term fasting. However, equal or higher insulin sensitivity in women after an overnight fast has been found (10, 15, 16, 40).

The finding of higher plasma FFA probably results from a higher lipolytic flux. Lipolysis per kilogram body weight did not differ between sexes. However, if expressed as flux per REE, women displayed a higher rate of lipolysis (30, 41). The most appropriate way to express lipolysis is still under discussion (30). The FFA flux can be seen not only as a function of adipose tissue mass but also as a function of fatty acid-consuming tissue requirements. Koutsari and Jensen (30) argue that expressing lipolysis per REE is a better alternative, because resting energy requirements are a major factor determining the rate of FFA release in resting humans.

Despite higher plasma FFA levels in the basal state, women were equally insulin sensitive compared with men, suggesting a relative protection from FFA-induced insulin resistance. This finding confirms other reports. Perseghin *et al.* (16) found no differences in insulin sensitivity between sexes, despite higher levels of plasma as well as intramyocellular triglycerides in women after an overnight fast. Frias *et al.* (12) demonstrated that women were less sensitive to FFA-induced insulin resistance during a lipid infusion. The higher plasma FFA levels during short-term fasting in the women we studied may result from a decreased FFA uptake in skeletal muscle, caused by differences in skeletal muscle fatty acid transporter proteins. The uptake of FFA is thought to depend on, besides transmembrane concentrations, intracellular fatty acid metabolism, *i.e.* storage or oxidation (28, 29). We did not find differences in fat oxidation (whole body or per kilogram lean body mass), which makes increased fatty acid oxidation in skeletal muscle in our female subjects less likely.

TABLE 3. Glucose and lipid metabolism measurements

	Basal state			Hyperinsulinemic euglycemic clamp		
	Men (n = 10)	Women (n = 10)	*P*	Men (n = 10)	Women (n = 10)	*P*
Glucose (mmol/liter)	4.4 (4.0–4.8)	3.9 (2.7–4.5)	0.023	4.9 (4.7–5.2)	5.1 (4.9–5.3)	0.14
EGP (μmol/kg·min)	9.0 (7.3–10.3)	8.0 (7.1–10.1)	0.07	[a]	[a]	
Rd (μmol/kg·min)				46.8 (41.4–66.5)	48.5 (40.8–72.2)	0.9
FFA (mmol/liter)	0.96 (0.72–1.18)	1.26 (0.93–1.54)	0.015	<0.02	<0.02	
Lipolysis (μmol/kg·min)	4.1 (2.4–5.3)	4.1 (3.6–9.8)	0.3	0.7 (0.1–1.1)	0.9 (0.5–1.5)	0.16
Lipolysis (μmol/kcal)	194 (147–269)	259 (207–526)	0.004	39 (5–61)	50 (29–99)	0.11

Data are presented as median (minimum–maximum).
[a] During the clamp, EGP was completely suppressed in both men and women.

TABLE 4. Glucoregulatory hormones and adiponectin

	Basal state			Hyperinsulinemic euglycemic clamp		
	Men (n = 10)	Women (n = 10)	*P*	Men (n = 10)	Women (n = 10)	*P*
Insulin (pmol/liter)	18 (15–39)	19 (15–29)	0.6	519 (477–624)	551 (433–644)	0.7
Glucagon (ng/liter)	75 (44–108)	66 (34–87)	0.6	35 (26–86)	36 (15–51)	0.13
Cortisol (nmol/liter)	285 (207–467)	259 (177–357)	0.9	202 (78–311)	207 (95–278)	0.9
Epinephrine (nmol/liter)	0.25 (0.11–0.53)	0.17 (0.08–0.30)[a]	0.11	0.33 (0.15–0.90)	0.17 (0.05–0.26)	0.006
Norepinephrine (nmol/liter)	0.68 (0.34–1.52)	0.87 (0.54–4.45)[a]	0.17	1.01 (0.44–3.10)	0.82 (0.56–2.12)	0.4
Adiponectin (μg/ml)	7.7 (3.7–16.0)	16.0 (10.1–21.5)	0.005	6.9 (3.4–15.5)	14.5 (9.3–19.7)	0.005

Data are presented as median (minimum–maximum).
[a] n = 9.

To detect potential differences in FFA uptake and fatty acid handling, we assessed muscle concentrations of ceramide and glucosylceramide. Ceramide and glucosylceramide levels in skeletal muscle did not differ between females and males in the basal state, suggesting that these are not causally involved in the finding of lower fasting plasma glucose in women. During the clamp, muscle ceramide levels (but not glucosylceramide) tended to be lower in women compared with men, despite similar Rd. Accordingly, we found no correlations between muscle ceramide or glucosylceramide and FFA or Rd.

There were no differences between women and men in total muscle FAT/CD36 content in the basal state and during the clamp, which is remarkable because it has been described earlier that women have higher levels of this lipid-binding protein after an overnight fast (8), which is in concordance with the findings that the intramyocellular lipid content during the postabsorptive period in women is increased compared with men (16). Our data suggest that the initial differences in intramyocellular lipids (16) and fatty acid transporters after an overnight fast (8) are abolished after 38 h of fasting.

Intracellular stored FAT/CD36 is translocated to the plasma membrane, thereby promoting sarcolemmal long-chain fatty acid uptake, and this effect is stimulated by insulin (26). Whether a difference in FAT/CD36 activity (translocation or up-regulation) during fasting exists between males and females is not known, but our data imply that such differences exist. Unfortunately, we did not measure the intracellular localization or activity of the FAT/CD36 protein, which could have clarified higher plasma FFA levels despite similar total levels of this transporter in skeletal muscle (25–27, 29).

Because we did find higher lipolysis rates in women without an increase in whole-body fat oxidation, the key question remains how the increased plasma FFA in women are disposed. Uptake of plasma FFA predominantly occurs in liver, adipocytes, and muscle. Shadid *et al.* (42) showed that FFA recycling occurs in the postabsorptive state and that women display greater efficiency in direct FFA uptake in sc tissue (femoral area). The exact role of the adipocyte in FFA uptake under fasting conditions is currently unknown. The liver may be another site of increased nonoxidative FFA disposal. Although livers of females do not contain more fat than male livers (43), it is known that female livers contain more FAT/CD36 and have a greater capacity for FFA uptake and synthesis of ketone bodies and very-low-density lipoproteins-triglycerides (1, 14, 44). Despite our data on muscle ceramide and FAT/CD36, we cannot completely rule out increased FFA disposal in muscle of our female subjects because of earlier mentioned arguments on possible differences in FAT/CD36 location and activity.

It is unlikely that the difference in plasma epinephrine levels during the clamp can explain our findings, because the concentration threshold for an effect of epinephrine on glycemia has been reported to be between 0.55 and 1.0 nmol/liter (45–47).

Finally, women had higher plasma adiponectin levels than

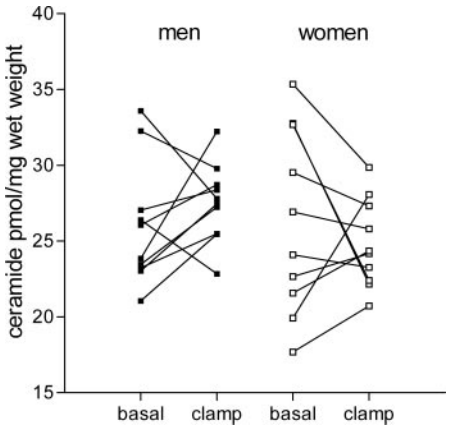

FIG. 1. Muscle ceramide content in men (■) and women (□) in the basal state (men *vs.* women, $P = 1.0$) and during the hyperinsulinemic euglycemic clamp (men *vs.* women, $P = 0.059$).

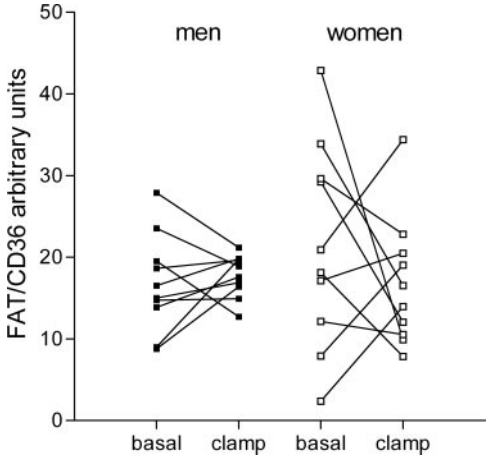

FIG. 2. Protein levels of muscle FAT/CD36 content in men (■) and women (□) in the basal state (men *vs.* women, $P = 0.4$) and during the hyperinsulinemic euglycemic clamp (men *vs.* women, $P = 0.4$).

men, which may be another mechanism by which women are relatively protected from FFA-induced insulin resistance. It has been shown *in vitro* that adiponectin can stimulate muscle fatty acid oxidation via stimulation of AMP kinase, which hypothetically may result in lower intramyocellular lipid content with beneficial effects on peripheral insulin sensitivity (48). However, whole-body fat oxidation did not differ between sexes. This challenges the hypothesis of an important role for adiponectin as protecting factor.

In conclusion, women display lower plasma glucose concentrations and higher plasma FFA concentrations after 38 h of fasting. The former is at least in part explained by lower EGP and the latter by higher rates of lipolysis. Moreover, women are relatively protected from FFA-induced insulin resistance. This protection does seem to result neither from differences in fat oxidation rates or muscle concentrations of ceramide and glucosylceramide, nor differences in total amount of FAT/CD36 in skeletal muscle. However, a difference in activity of this fatty acid-binding protein or intracellular localization between fasting men and women may result in lower plasma FFA uptake in women, thereby increasing plasma FFA and decreasing deleterious effects of long-chain fatty acids on insulin signaling.

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References

1. **Haymond MW, Karl IE, Clarke WL, Pagliara AS, Santiago JV** 1982 Differences in circulating gluconeogenic substrates during short-term fasting in men, women, and children. Metabolism 31:33–42
2. **Klein S, Sakurai Y, Romijn JA, Carroll RM** 1993 Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. Am J Physiol Endocrinol Metab 265:E801–E806
3. **Norrelund H, Nielsen S, Christiansen JS, Jorgensen JO, Moller N** 2004 Modulation of basal glucose metabolism and insulin sensitivity by growth hormone and free fatty acids during short-term fasting. Eur J Endocrinol 150:779–787
4. **Clore JN, Glickman PS, Helm ST, Nestler JE, Blackard WG** 1989 Accelerated decline in hepatic glucose production during fasting in normal women compared with men. Metabolism 38:1103–1107
5. **Hojlund K, Wildner-Christensen M, Eshoj O, Skjaerbaek C, Holst JJ, Koldkjaer O, Moller Jensen D, Beck-Nielsen H** 2001 Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting. Am J Physiol Endocrinol Metab 280:E50–E58
6. **Merimee TJ, Fineberg SE** 1973 Homeostasis during fasting. II. Hormone substrate differences between men and women. J Clin Endocrinol Metab 37: 698–702
7. **Binnert C, Koistinen HA, Martin G, Andreelli F, Ebeling P, Koivisto VA, Laville M, Auwerx J, Vidal H** 2000 Fatty acid transport protein-1 mRNA expression in skeletal muscle and in adipose tissue in humans. Am J Physiol Endocrinol Metab 279:E1072–E1079
8. **Kiens B, Roepstorff C, Glatz JF, Bonen A, Schjerling P, Knudsen J, Nielsen JN** 2004 Lipid-binding proteins and lipoprotein lipase activity in human skeletal muscle: influence of physical activity and gender. J Appl Physiol 97:1209–1218
9. **Blaak E** 2001 Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care 4:499–502
10. **Borissova AM, Tankova T, Kirilov G, Koev D** 2005 Gender-dependent effect of ageing on peripheral insulin action. Int J Clin Pract 59:422–426
11. **Corssmit EP, Stouthard JM, Romijn JA, Endert E, Sauerwein HP** 1994 Sex differences in the adaptation of glucose metabolism to short-term fasting: effects of oral contraceptives. Metabolism 43:1503–1508
12. **Frias JP, Macaraeg GB, Ofrecio J, Yu JG, Olefsky JM, Kruszynska YT** 2001 Decreased susceptibility to fatty acid-induced peripheral tissue insulin resistance in women. Diabetes 50:1344–1350
13. **Mittendorfer B, Horowitz JF, Klein S** 2001 Gender differences in lipid and glucose kinetics during short-term fasting. Am J Physiol Endocrinol Metab 281:E1333–E1339
14. **Mittendorfer B** 2005 Insulin resistance: sex matters. Curr Opin Clin Nutr Metab Care 8:367–372
15. **Paula FJ, Pimenta WP, Saad MJ, Paccola GM, Piccinato CE, Foss MC** 1990 Sex-related differences in peripheral glucose metabolism in normal subjects. Diabetes Metab 16:234–239
16. **Perseghin G, Scifo P, Pagliato E, Battezzati A, Benedini S, Soldini L, Testolin G, Del Maschio A, Luzi L** 2001 Gender factors affect fatty acids-induced insulin resistance in nonobese humans: effects of oral steroidal contraception. J Clin Endocrinol Metab 86:3188–3196
17. **Boden G** 2002 Interaction between free fatty acids and glucose metabolism. Curr Opin Clin Nutr Metab Care 5:545–549
18. **Boden G, Chen X, Ruiz J, White JV, Rossetti L** 1994 Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 93:2438–2446
19. **Straczkowski M, Kowalska I, Nikolajuk A, Dzienis-Straczkowska S, Kinalska I, Baranowski M, Zendzian-Piotrowska M, Brzezinska Z, Gorski J** 2004 Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. Diabetes 53:1215–1221
20. **Adams 2nd JM, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, Mandarino LJ** 2004 Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes 53:25–31
21. **Powell DJ, Turban S, Gray A, Hajduch E, Hundal HS** 2004 Intracellular ceramide synthesis and protein kinase Cζ activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. Biochem J 382(Pt 2):619–629
22. **Chavez JA, Knotts TA, Wang LP, Li G, Dobrowsky RT, Florant GL, Summers SA** 2003 A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. J Biol Chem 278:10297–10303
23. **Tagami S, Inokuchi Ji J, Kabayama K, Yoshimura H, Kitamura F, Uemura S, Ogawa C, Ishii A, Saito M, Ohtsuka Y, Sakaue S, Igarashi Y** 2002 Ganglioside GM3 participates in the pathological conditions of insulin resistance. J Biol Chem 277:3085–3092
24. **Aerts JM, Ottenhoff R, Powlson AS, Grefhorst A, van Eijk M, Dubbelhuis PF, Aten Kuipers F, Serlie MJ, Wennekes T, Sethi JK, O'Rahilly S, Overkleeft HS** 2007 Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. Diabetes 56:1341–1349
25. **Hamilton JA** 2003 Fast flip-flop of cholesterol and fatty acids in membranes: implications for membrane transport proteins. Curr Opin Lipidol 14:263–271
26. **Koonen DP, Glatz JF, Bonen A, Luiken JJ** 2005 Long-chain fatty acid uptake and FAT/CD36 translocation in heart and skeletal muscle. Biochim Biophys Acta 1736:163–180
27. **Schaffer JE** 2002 Fatty acid transport: the roads taken. Am J Physiol Endocrinol Metab 282:E239–E246
28. **Mashek DG, Coleman RA** 2006 Cellular fatty acid uptake: the contribution of metabolism. Curr Opin Lipidol 17:274–278
29. **Stremmel W, Pohl L, Ring A, Herrmann T** 2001 A new concept of cellular uptake and intracellular trafficking of long-chain fatty acids. Lipids 36:981–989
30. **Koutsari C, Jensen MD** 2006 Thematic review series: patient-oriented research. Free fatty acid metabolism in human obesity. J Lipid Res 47:1643–1650
31. **Ackermans MT, Pereira Arias AM, Bisschop PH, Endert E, Sauerwein HP, Romijn JA** 2001 The quantification of gluconeogenesis in healthy men by $^{2}H_2O$ and [2-^{13}C]glycerol yields different results: rates of gluconeogenesis in healthy men measured with $^{2}H_2O$ are higher than those measured with [2-^{13}C]glycerol. J Clin Endocrinol Metab 86:2220–2226
32. **Ackermans MT, Ruiter AF, Endert E** 1998 Determination of glycerol concentrations and glycerol isotopic enrichments in human plasma by gas chromatography/mass spectrometry. Anal Biochem 258:80–86
33. **Groener JEM, Poorthuis BJHM, Kuiper S, Helmond MTJ, Hollak CEM, Aerts JMFG** 2007 HPLC for simultaneous quantification of total ceramide, glucosylceramide, and ceramide trihexoside concentrations in plasma. Clin Chem 53:742–747
34. **Bonen A, Luiken JJFP, Arumugam Y, Glatz JFC, Tandon NN** 2000 Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. J Biol Chem 275:14501–14508
35. **Finegood DT, Bergman RN, Vranic M** 1987 Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. Diabetes 36:914–924
36. **Steele R** 1959 Influences of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420–430

37. **Frayn KN** 1983 Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 55:628–634
38. **Fery F** 1994 Role of hepatic glucose production and glucose uptake in the pathogenesis of fasting hyperglycemia in type 2 diabetes: normalization of glucose kinetics by short-term fasting. J Clin Endocrinol Metab 78:536–542
39. **Ahmed-Sorour H, Bailey CJ** 1981 Role of ovarian hormones in the long-term control of glucose homeostasis, glycogen formation and gluconeogenesis. Ann Nutr Metab 25:208–212
40. **Yki-Jarvinen H** 1984 Sex and insulin sensitivity. Metabolism 33:1011–1015
41. **Nielsen S, Guo Z, Albu JB, Klein S, O'Brien PC, Jensen MD** 2003 Energy expenditure, sex, and endogenous fuel availability in humans. J Clin Invest 111:981–988
42. **Shadid S, Koutsari C, Jensen MD** 2007 Direct free fatty acid uptake into human adipocytes in vivo: relation to body fat distribution. Diabetes 56:1369–1375
43. **Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, Fredriksson J, Yki-Jarvinen H** 2004 Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. Diabetologia 47:1360–1369
44. **Magkos F, Patterson BW, Mohammed BS, Klein S, Mittendorfer B** 2007 Women produce fewer but triglyceride-richer very low-density lipoproteins than men. J Clin Endocrinol Metab 92:1311–1318
45. **Chu CA, Sindelar DK, Neal DW, Cherrington AD** 1996 Direct effects of catecholamines on hepatic glucose production in conscious dog are due to glycogenolysis. Am J Physiol 271(1 Pt 1):E127–E137
46. **Clutter WE, Bier DM, Shah SD, Cryer PE** 1980 Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. J Clin Invest 66:94–101
47. **Cryer PE** 1993 Adrenaline: a physiological metabolic regulatory hormone in humans? Int J Obes Relat Metab Disord 17(Suppl 3):S43–S46; discussion S68
48. **Yoon MJ, Lee GY, Chung JJ, Ahn YH, Hong SH, Kim JB** 2006 Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor α. Diabetes 55:2562–2570

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