Gender differences in lipid and glucose kinetics during short-term fasting

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Mittendorfer, Bettina, Jeffrey F. Horowitz, and Samuel Klein. Gender differences in lipid and glucose kinetics during short-term fasting. Am J Physiol Endocrinol Metab 281: E1333–E1339, 2001.—Data obtained from studies conducted in animal models and humans suggest that gender affects the metabolic response to fasting. However, differences in body composition between males and females confound the interpretation of these studies, because increased adiposity itself alters the metabolic response to short-term fasting. We evaluated whole body lipid and glucose kinetics during basal (14-h fast) and short-term fasting (22-h fast) conditions in six women and six men who were matched for adiposity (24 ± 2 and 23 ± 2% body wt as fat, respectively). Substrate kinetics were measured by infusing stable isotope labeled tracers of glucose ([2H2]glucose) and glycerol ([2H5]glycerol). Basal glycerol rate of appearance (Ra) in plasma, an indicator of whole body lipolytic rate, was greater in women than in men (2.1 ± 0.2 vs. 1.5 ± 0.1 µmol·kg body wt⁻¹·min⁻¹; P < 0.05). However, the relative increase in glycerol Ra with continued fasting was blunted in women compared with men (40 ± 7 vs. 80 ± 4% increase; P < 0.05), resulting in similar lipolytic rates in both genders at 22 h (2.8 ± 0.2 and 2.6 ± 0.1 µmol·kg body wt⁻¹·min⁻¹ for women and men, respectively). In contrast, glucose Ra was similar in men and women at 14 h (11 ± 0.6 vs. 12 ± 0.7 µmol·kg body wt⁻¹·min⁻¹) and 22 h of fasting (9 ± 0.6 vs. 10 ± 0.6 µmol·kg body wt⁻¹·min⁻¹). These data demonstrate the presence of sexual dimorphism in lipid, but not glucose, metabolism during both basal and short-term fasting conditions.

The adaptive response to fasting involves a carefully integrated series of metabolic alterations that are critical for survival. The initial events are characterized by an increase in the mobilization of adipose tissue triacylglycerides and a decrease in the production and oxidation of glucose. These processes provide fatty acids and ketone bodies as fuels to body tissues and reduce the need for muscle protein breakdown to release gluconeogenic amino acid precursors.

Data obtained from studies conducted in animals demonstrate that gender influences the metabolic responses to energy restriction (20, 21, 46). Female rats use more fat and preserve lean body mass better than male rats during short-term fasting (20, 21) and during chronic undernutrition (20, 21, 46). The higher rate of protein catabolism in male than in female animals is responsible for the marked differences in survival observed between genders during low-energy feeding or total starvation. For example, 87% of female pigs, compared with only 22% of male pigs, survive after 1 yr of severe energy restriction (46).

Gender differences in the metabolic response to fasting have also been observed in humans. The increase in plasma fatty acid and ketone body concentrations (17, 34, 35) and the decrease in plasma glucose concentration (17, 34) that normally occur during short-term fasting (60–86 h) are greater in women than in men. Although these findings suggest that the mobilization of fatty acids from adipose tissue and the decline in hepatic glucose production are greater in women than in men during fasting, few studies have evaluated the physiological processes that are responsible for these differences. We are not aware of any studies that compared lipid kinetics in men and women during fasting, and we know of only two studies that have compared glucose kinetics (5, 6), with conflicting results. In one study (5), the decline in glucose rate of appearance (Ra) into plasma during short-term fasting (from 16 to 64 h of fasting) was greater in women than in men, whereas in the other study, the decline in glucose Ra (from 16 to 22 h of fasting) was similar in both genders (6). However, these studies are difficult to interpret because of the inherent differences in body composition between men and women. Females generally have more body fat than males (5, 20, 21), which can independently influence the metabolic response to fasting. Within the same gender, studies performed in humans (22, 23) and in rats (12, 19) have found that increased adiposity itself alters the metabolic response to short-term fasting.

The purpose of this study was to evaluate the effect of gender on lipid and glucose kinetics during basal and short-term fasting conditions. We measured whole body glycerol and glucose kinetics by using stable isotope labeled tracers at 14 and 22 h of fasting in men and women. Our men and women were matched on percent body fat to eliminate the potential influence of body composition.
gender-related differences in body composition on substrate metabolism. We hypothesized that the increase in lipolytic activity and the decrease in glucose production that occur during early fasting would be greater in women than in men when both groups were matched on adiposity.

METHODS

Subjects

Six men (32 ± 4 yr old) and six premenopausal women (27 ± 2 yr old), who were matched on percent body fat, participated in this study (Table 1). All subjects were considered to be in good health after completing a comprehensive medical evaluation that included a history and physical examination, an electrocardiogram, and standard blood and urine tests. None of the subjects was taking regular medications or smoked tobacco. All subjects had a stable body weight for at least 6 mo and had been sedentary (regular exercise <1 h/wk) for ≥6 mo before the study. In female subjects, the study was performed during the first 2 wk of the follicular phase of their menstrual cycle. Each subject’s fat mass (FM) and fat-free mass (FFM) were determined by dual-energy X-ray absorptiometry (Hologic QDR 1000/w, Waltham, MA). The truncal region was defined as the body segment between 1) lines passing through the humeral head and the apex of the axilla to separate the arms, 2) a line passing from the iliac crest to the perineum to separate the legs, and 3) a horizontal line passing just below the mandible to eliminate the head. Written informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Subjects Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine in St. Louis, MO.

Experimental Protocol

The evening before the isotope infusion study, subjects were admitted to the GCRC at Washington University School of Medicine. At 1800, subjects consumed a standard meal containing 12 kcal/kg body wt (55% of total energy as carbohydrate, 30% as fat, and 15% as protein). At 2000, the subjects ingested a liquid formula (Ensure; Ross Laboratories, Columbus, OH), containing 250 kcal (40 g carbohydrate, 6.1 g fat, and 8.8 g protein) and then fasted until completion of the study the following day.

The following morning, an isotope infusion study was performed to measure whole body glycerol and glucose kinetics at 14 and 22 h of fasting. At 0530, catheters were inserted into a forearm vein for isotope infusion and into a radial artery for blood sampling. At 0630, a constant infusion of [6,6-²H₆]glucose (priming dose: 18 µmol/kg; infusion rate: 0.22 µmol·kg body wt⁻¹·min⁻¹; Cambridge Isotope Laboratories, Andover, MA) was started and maintained for 210 min. At 0830, a constant infusion of [1,1,2,3,3-²H₅]glycerol (priming dose: 1.8 µmol/kg; infusion rate: 0.12 µmol·kg body wt⁻¹·min⁻¹; Cambridge Isotope Laboratories) was started and maintained for 90 min. All isotopes were infused via calibrated syringe pumps (Harvard Apparatus, South Natick, MA). Blood samples were obtained before the isotope infusions were started to determine background glucose and glycerol tracer-to-tracee ratios (TTR) and every 10 min between 0930 and 1000 (for the 14 h of fasting) to determine plasma glucose and glycerol TTR and plasma substrate and hormone concentrations. At 1000, all isotope infusions were stopped, and catheters were kept patent by infusing 0.9% saline. At 1430, the isotope infusion protocol was repeated. Blood samples were obtained before restarting the isotope infusions to determine background glucose and glycerol TTR and every 10 min between 1730 and 1800 (for the 22 h of fasting) to determine plasma glucose and glycerol TTR and plasma substrate and hormone concentrations. Subjects remained in bed for the entire duration of the study.

Sample Collection

Blood samples were collected in 1) chilled tubes containing EDTA to determine plasma fatty acid, glycerol, and glucose concentrations and glycerol and glucose enrichments; 2) chilled tubes containing EDTA and Trasylol to measure insulin and glucagon concentrations; and 3) chilled tubes containing reduced glutathione and EGTA to determine plasma catecholamine concentrations. Blood samples were placed in ice, and plasma was separated by centrifugation within 30 min of collection. Plasma was stored at −70°C until final analyses were performed.

Sample Analyses

Plasma insulin (13) and glucagon (15) concentrations were measured by radioimmunoassay. Plasma catecholamine concentrations were determined by a single isotope derivative radioenzymatic method (45). Plasma glucose concentration was determined by using an automated glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma glycerol concentration was determined by gas chromatography-mass spectrometry (GC-MS) after [2-¹³C]glycerol was added to plasma as an internal standard. Plasma fatty acid concentrations were quantified by gas chromatography (Hewlett-Packard 5890-II, Palo Alto, CA) after adding heptadecanoic acid to plasma as an internal standard (32).

Plasma glycerol and glucose TTR were determined by GC-MS (MSD 5973 system with capillary column; Hewlett-Packard) as previously described (22, 23, 40). Briefly, plasma proteins were precipitated with ice-cold acetone, and hexane was used to extract plasma lipids. The aqueous phase, containing glycerol and glucose, was dried by speed-vac centrifugation (Savant Instruments, Farmingdale, NY). Heptafluorobutyric (HFB) anhydride was used to form an HFB derivative of glycerol and glucose, and ions were produced by EI ionization. Glycerol TTR were determined by selectively monitoring ions at mass-to-charge ratios (m/z) 253, 254, and 257, and glucose TTR were determined by selectively monitoring ions at m/z 519 and 521.
A physiological and isotopic steady state was achieved at 14 and 22 h of fasting. Therefore, substrate (glycerol and glucose) rate of appearance (Ra) and rate of disappearance (Rd) from plasma were calculated by using Steele’s equation for steady-state conditions (45). Glycerol Ra represents the rate of glycerol released into plasma from hormone-sensitive lipase hydrolysis of adipose tissue and intramuscular triglycerides and the rate of glycerol that is released into plasma during lipoprotein lipase hydrolysis of very low-density lipoprotein triglyceride. Glycerol released during lipolysis of intra-abdominal adipose tissue triglycerides that are cleared by the liver are not detected by systemic tracer infusion.

Statistical Analysis

A two-way analysis of variance (ANOVA), with gender and time as factors, was used to test for significant differences in substrate kinetics between men and women during fasting. Significant F-ratios were followed by post hoc comparisons with the use of Student’s t-test for multiple comparisons. Statistical analyses were performed by using JMP statistical software version 3.2 (SAS Institute, Carey, NC). A value of P ≤ 0.05 was considered to be statistically significant. All data are expressed as means ± SE.

RESULTS

Plasma Hormone Concentrations

Mean basal (14 h of fasting) plasma insulin concentration tended to be lower in women than in men, but the difference was not statistically significant (P = 0.09; Table 2). The failure to demonstrate statistically significant differences in basal plasma insulin concentrations between groups may reflect a type II statistical error because of the small number of study subjects. With continued fasting, plasma insulin concentration decreased in both groups (P < 0.05), but the relative decline was greater in women (~40% decrease) than in men (~20% decrease) (P = 0.07), resulting in a significantly lower mean plasma insulin concentration in women than in men at 22 h of fasting (P < 0.05). Plasma glucagon concentrations were similar in both groups at 14 and 22 h of fasting. At 14 h of fasting, mean plasma epinephrine concentration was twice as high in women than in men (P < 0.05). Plasma epinephrine concentration increased significantly in men but not in women from 14 to 22 h of fasting, resulting in similar plasma epinephrine concentrations with continued fasting in men and women. Mean basal plasma norepinephrine concentration was similar in both groups and was not affected by continued fasting.

Plasma Substrate Concentrations

At 14 h of fasting, mean plasma fatty acid and glycerol concentrations were higher in women than in men; they increased with continued fasting in both groups (P < 0.05). Plasma glucose concentration was similar in both men and women at 14 h and 22 h of fasting (Table 2).

Substrate Kinetics

Basal conditions (14-h fast). At 14 h of fasting, glycerol Ra, expressed per kilogram of body weight, per kilogram of FFM, or per kilogram of FM, was greater in women than in men (Table 3 and Fig. 1). In contrast, mean basal glucose Ra (and Rd) was similar in men and women (Table 3 and Fig. 1).

Continued fasting (22 h fast). Glycerol Ra increased with continued fasting in both groups. However, the relative increase in glycerol Ra between 14 and 22 h of fasting was greater (P < 0.05) in men (80 ± 4%) than in women (40 ± 7%) (Fig. 2). The absolute increase in glycerol Ra between 14 and 22 h, however, was similar in men (1.1 ± 0.2 μmol·kg body wt·min⁻¹) and women (0.8 ± 0.1 μmol·kg body wt·min⁻¹) (Fig. 2). In contrast to lipid kinetics, glucose Ra decreased (P < 0.05) with continued fasting in all subjects. The relative and absolute decreases in glucose Ra were similar in men and women (Fig. 2).

DISCUSSION

In this study, we examined the effect of gender, independent of adiposity, on whole body substrate ki-
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in lipolysis that occurs during exercise (1, 14) or after burn injury (47). Therefore, it is possible that the lower plasma insulin concentrations present in our female compared with our male subjects contributed to the higher basal glycerol Ra values in women. Although basal plasma epinephrine concentration was greater in our women than in men, it is unlikely to have influenced basal glycerol Ra, because the epinephrine concentrations in both groups were below the threshold needed to increase lipolysis (11).

Although several studies have evaluated lipolytic activity in men and women, the effect of gender itself on basal lipolytic rate remains unclear because of conflicting data between studies and differences in body composition between the male and female subjects. Earlier studies that used isotope tracer methods found that whole body fatty acid Ra, expressed per kilogram of FFM, was either similar (24) or greater (26, 29) in women than in men. The interpretation of data from these studies is confounded by differences in body composition and fat distribution between female and male subjects, which can independently influence lipolytic rate (22, 23). In the present study, basal glycerol release into plasma was greater in women than in men, who were matched on percent body fat. These results are particularly striking, because our men had greater
amounts of abdominal fat than our women, and abdominal adiposity is associated with increased lipolytic rates (28).

The techniques that are available to measure whole body lipid kinetics have some limitations. The tracer infusion method that we used to evaluate glycerol Ra underestimates true whole body lipolytic rates. Systemically infused glycerol tracers are unable to detect glycerol released from visceral adipose tissue that is taken up by the liver and does not enter the systemic circulation. However, it is unlikely that this methodological limitation affected our conclusions because undetected glycerol release from visceral adipose tissue triglycerides probably accounts for <5% of whole body lipid flux (16). Therefore, the lower rate of basal glycerol kinetics observed in our male compared with our female subjects was due to the effect of gender itself rather than undetected lipolysis of visceral fat associated with gender-related differences fat distribution.

In contrast to lipid kinetics, basal glucose Ra was similar in men and women. Insulin is the major circulating hormone that regulates both basal rates of lipolysis (4, 33, 38) and glucose production (3, 42). In the present study, basal plasma insulin concentration tended to be lower in women than in men, which is consistent with previous studies that evaluated plasma insulin concentration in men and women (39, 49). The presence of similar glucose Ra values despite lower plasma insulin concentrations suggests that hepatic glucose production was more sensitive to insulin in women than in men. Although several studies have reported that insulin sensitivity with respect to glucose metabolism was similar in men and women (2, 10), other investigators have found that insulin sensitivity, assessed by an oral glucose tolerance test (7, 9) or a hyperinsulinemic euglycemic clamp (8, 39, 41, 49), was greater in women than in men after adjustment for body fat. Our results also suggest that the enhanced effect of insulin action on hepatic glucose production in women is not a result of insulin’s indirect effect on glucose metabolism through alterations in lipolysis (36, 37), because basal lipolytic rates and plasma fatty acid concentrations were greater in our women than in our men.

To our knowledge, this is the first study that has evaluated the effect of gender on lipid kinetics during short-term fasting. Several earlier studies have evaluated the effect of gender on plasma substrate concentrations during fasting. However, plasma substrate concentration represents the balance between substrate delivery into plasma and substrate tissue uptake but does not provide information regarding the dynamic metabolic events responsible for the observed concentrations. Moreover, the relationship between plasma substrate concentration and substrate metabolism can vary markedly depending on the physiological state (30). Therefore, direct assessment of substrate kinetics is necessary for understanding the metabolic adaptations that occur in response to fasting.

The results of the present study demonstrate that the relative increase in lipolysis that normally occurs during early starvation (between 14 and 22 h of fasting) was blunted in women compared with men. However, basal (14-h fast) whole body lipolytic rates were much greater in women than in men, and the absolute increase in the lipolytic rate was the same in both groups. Therefore, the attenuated increase in lipolysis during fasting in women did not compromise fatty acid availability as a fuel in these subjects. In fact, the relatively blunted lipolytic response in women may be beneficial by preventing excessive, and potentially harmful, increases in plasma fatty acid concentrations (44). In contrast, the decrease in glucose production during fasting was similar in both groups. It is not known whether the observations in substrate kinetics made during this brief period of fasting would persist with more prolonged starvation.

The mechanism(s) responsible for the gender differences in lipid metabolism in response to brief fasting is not known but could be related to differences in hormonal responses and adipose tissue sensitivity to hormone action. Catecholamines and insulin are the two major hormones that regulate lipid and glucose kinetics during fasting. Data from previous studies suggest that the increase in lipolysis during short-term fasting is related to an increase in plasma epinephrine concentration and increased adipose tissue sensitivity to epinephrine-mediated lipolysis (31, 48), in conjunction with a decrease in plasma insulin concentration and decreased adipose tissue sensitivity to the antilipolytic effect of insulin (27). In the present study, plasma epinephrine concentrations increased during continued fasting in men but not in women, which would enhance lipolytic activity in men. In contrast, the relative (but not absolute) decline in circulating insulin concentration was greater in women than in men, which would enhance lipolytic activity in women. We did not assess adipose tissue sensitivity to epinephrine and insulin, which would further elucidate the impact of changes in plasma hormone concentrations on lipolysis of adipose tissue triglycerides. The greater relative increase in lipolysis observed in men than in women suggests that an increase in circulating epinephrine and adipose tissue lipolytic sensitivity to catecholamines is very important for stimulating lipolysis during early fasting. Indeed, acute β-adrenergic blockade at 84 h of fasting in men decreases whole body lipolytic rates to basal (12-h fasting) values (31).

Our female subjects were studied during the follicular phase of their menstrual cycle. It is unlikely that studying women during the luteal phase of their cycle would have affected the results of our study. Although previous studies have demonstrated that menstrual cycle phase can affect substrate kinetics during extreme physiological conditions, such as high-intensity exercise (50), glucose and fatty acid kinetics are the same during the follicular and luteal phases of the menstrual cycle during basal conditions, low-intensity exercise, or short-term fasting (6, 18, 50).

To match men and women on percent body fat, we recruited female subjects who were lean [body mass index (BMI) = 21 kg/m² and 24% body wt as fat] and
male subjects who were slightly overweight (BMI = 26 kg/m² and 23% body wt as fat), because women are generally fatter than men. However, these within-gender differences do not affect the interpretation of the results from our study, which was designed to evaluate the effect of gender, independent of adiposity, on substrate metabolism.

In summary, the results of the present study demonstrate the presence of gender differences in lipid but not glucose kinetics in men and women who were matched on percent body fat. Basal lipolytic rates (assessed by glycerol Ra) were greater in women than in men, whereas whole body glucose production and utilization were similar in both groups. During early fasting, the relative increase in whole body lipolytic rate was blunted in women compared with men, whereas the decline in the rate of glucose production was similar in both genders. These results demonstrate the need to study men and women separately when evaluating in vivo substrate kinetics in human subjects. Further studies are needed to determine the physiological and clinical importance of these gender differences in lipid kinetics.

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REFERENCES