Greater systemic lipolysis in women compared to men during moderate dose infusion of epinephrine and/or norepinephrine.

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Running Heading: Sex effects on catecholamine stimulated lipolysis

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ABSTRACT

Women have lower circulating catecholamine levels during metabolic perturbations such as exercise or hypoglycemia, but similar rates of systemic lipolysis. This suggests women may be more sensitive to the lipolytic action of catcholamines, while maintaining similar glucoregulatory effects. The aim of the current study, therefore, was to determine if women have higher rates of systemic lipolysis compared to men in response to matched peripheral infusion of catecholamines, but similar rates of glucose turnover. Healthy, non-obese women (n=11) and men (n=10) were recruited and studied on 3 separate days with the following infusions: epinephrine (Epi), norepinephrine (Norepi) or the two combined. Tracer infusions of glycerol and glucose were used to determine systemic lipolysis and glucose turnover, respectively. Following basal measurements of substrate kinetics, the catecholamine infusion commenced and measures of substrate kinetics continued for 60 mins. Catecholamine concentrations were similarly elevated in women and men during each infusion; epinephrine, 182-197 pg/ml and norepinephrine, 417-507 pg/ml. There was a significant sex difference in glycerol Ra and Rd with the catecholamine infusions (p<0.0001), mainly due to a significantly greater glycerol turnover during the first 30 minutes of each infusion: glycerol Ra during Epi only was 268 ±18 vs 206 ±21 μmol/min in women and men, respectively; during Norepi only 173 ±13 vs 153 ±17 μmol/min, respectively, and during Epi+Norepi 303 ±24 vs 257 ±21 μmol/min, respectively. No sex differences were observed in glucose kinetics under any condition. In conclusion, these data suggest that women are more sensitive to the lipolytic action of catecholamines, but have no difference in their glucoregulatory response. Thus, the lower catecholamine levels observed in women vs men during exercise and other metabolic perturbations, may allow women to maintain a similar or greater level of lipid mobilization while minimizing changes in glucose turnover.

Key Words: epinephrine, norepinephrine, lipolysis, sex differences, glucose kinetics
INTRODUCTION

Consistent and significant differences have been observed between women and men in their catecholamine response to metabolic stress. During exercise, or insulin-induced hypoglycemia, circulating levels of epinephrine, and in some instances norepinephrine, do not rise to the same extent in women as compared to men (4; 12; 13; 15; 23; 25; 26; 37; 56-58). A lesser increase in muscle sympathetic nerve activity (MSNA) has also been observed in women during hypoglycemia (12-14). Such observations are unlikely due to a sex difference in the “threshold” at which a catecholamine response is elicited. For example, during insulin-induced hypoglycemia, it has been demonstrated that the level of blood glucose required to elicit a significant counter-regulatory response is the same in women and men, rather, the magnitude of the response is reduced in women (14). In addition, maintenance of the same euglycemia in women and men during exercise, via exogenous glucose infusion, still results in the sex-based differences in catecholamine levels (15). Data suggest that the lower sympathetic response to hypoglycemia in women may be due to estrogen decreasing central sympathetic drive (51; 52). Despite a lower sympathetic response during metabolic stress women have similar, or greater, increases in circulating concentrations of glycerol and non-esterified fatty acids (NEFA; venous or arterialized measurements) (4; 13; 15; 25; 37; 56). Greater systemic rates of glycerol and NEFA appearance have also been reported {1316, 1235, 62, 2109, 3906}. Interestingly, these sex differences in the catecholamine response to exercise, and differences in lipid kinetics, are not observed in more elite athletes (46; 48). Nevertheless, these data suggest that for the majority of women, the lipolytic sensitivity to the action of catecholamines may be increased relative to men.

From the observations presented above, it might be expected that during a matched infusion of catecholamines women would show a greater lipolytic response compared to men. To date, however, there is very limited and inadequate data in this area. Jensen et. al.(29) determined NEFA kinetics in women vs men during a 2hr infusion of epinephrine and observed no sex difference in NEFA Ra with
measurements made over the final 60 mins of the infusion. With this study design, however, significant tachyphlaxia of the β−adrenergic receptors likely occurred by the time lipid kinetics were measured (60 mins after the onset of the infusion) (1), thus potentially obscuring any sex difference. Weber et. al. (61) measured substrate concentrations rather than substrate kinetics, in response to incremental doses of epinephrine (10 min duration each dose) and observed no sex difference on glycerol or NEFA concentrations. An incremental study design for hormone infusion is problematic, however, due to potential effects of the preceding dose on the response to the subsequent dose. It is noteworthy that neither of these studies controlled for hormonal status in women, nor gave any indication of habitual activity patterns or pre-study exercise of subjects, all factors that can potentially, independently, impact catecholamine action and/or sensitivity.

In addition to affects on lipid mobilization, catecholamines also stimulate glucose production by the liver and cause a decrease in glucose utilization at the periphery, predominantly in muscle (43; 44; 50). Effects on glucose production are due to direct and indirect actions (8; 9), as catecholamines can directly stimulate glycogenolysis (10) but also indirectly increase gluconeogenesis via delivery of lipolytic by-products, glycerol (increased precursor delivery) and NEFA’s (8; 10) as well as lactate. Although some increase in insulin can occur as a result of the increase in glucose production and concentration that results from an elevation in catecholamines this is limited due to the inhibition of insulin secretion by epinephrine and norepinephrine (11; 45). Furthermore, the small increase in insulin that can occur is for the most part insufficient to inhibit the effect of epinephrine on lipolysis (3). Of relevance, when tested in the overnight fasted state, women have been reported to have a lower glucose production in response to moderately intense exercise of greater than 30 minutes (26; 46) coincident with a lower catecholamine response.

The aim of the current study, therefore, was to determine if there are sex differences in lipolysis, but not glucose production, in response to sub-maximal doses of epinephrine (Epi),
norepinephrine (Norepi) or the two combined. It was hypothesized that for the same sub-maximal
dose of Epi and/or Norepi, women would have a greater rate of systemic lipolysis, but a similar rate
of glucose production, compared to men. It was further hypothesized that there would be a synergistic
effect of the Epi + Norepi combination in women only, such that lipolysis would be greater than the
sum of the two hormones infused individually.

MATERIALS AND METHODS

Subjects

Normal weight, healthy women and men (20-45 years) were recruited for the study (Table 1).
Female subjects were pre-menopausal, eumenorrheic and not using oral contraceptives. Medical
exclusions included past or present history of cardiovascular disease, high blood pressure, diabetes, any
hormonal imbalance or metabolic abnormality and use of oral contraceptives or other hormones.
Participants could be habitually active but were not highly trained (≤30 minutes of mild to moderate
intensity exercise/day). A total of 21 subjects (11 women, 10 men) took part in the study. Subject
characteristics are shown in Table 1. The study protocol was approved by the University of Colorado
Committee Institutional Review Board for the Protection of Human Subjects. All subjects read and
signed an informed consent form prior to admission into the study.

Preliminary assessments: A health and physical examination was completed on all subjects, including
blood and urine analysis, to confirm there was no medical reason for exclusion. Resting metabolic rate
(RMR) was measured using indirect calorimetry via a metabolic cart system (Sensormedics 2900,
Sensormedics, Yorba Linda, CA). Oxygen (O2) consumption and carbon dioxide (CO2) production
were used to calculate metabolic rate (30; 54). This RMR value was used to determine energy intake
of subjects during the period of pre-study diet control. Body composition was determined via dual
energy x-ray absorptiometry (Lunar Corp., Madison, WI)(41).

Pre-study diet and exercise control. Subjects were fed a controlled diet for 2 days prior to the study
day. All food was prepared by the General Clinical Research Center (GCRC) diet kitchen at the University of Colorado and subjects were required to consume breakfast in the GCRC with other food prepared to take away. No other food was permitted and subjects were required to consume all the food given. The only optional part of the diet were two food modules (200 kcal each, same composition as the overall diet) one or both of which the subjects could eat if they were hungry. The diet composition was 25% fat, 15% protein and 60% carbohydrate and initial energy intake was calculated at 1.6 – 1.75 x RMR based on subjects self-reported habitual activity level. Subjects were allowed to follow their usual activity routine for the first day of the diet and on the second day they refrained from any planned exercise. The aim of the diet control was to minimize within and between subject variation in the degree of energy and carbohydrate (glycogen) balance. In women and men the average energy intake was 50 and 51 kcals/kg FFM, respectively.

Study days

Subjects stayed overnight on the GCRC the evening before the study. Between 19:00 and 20:00 subjects consumed their evening meal. After this, subjects remained fasted until the end of the study the following day. Women were studied in the follicular phase of the menstrual cycle which was confirmed by measurement of serum estrogen and progesterone levels measured on the day of the study (progesterone had to be < 2.5 ng/ml).

Determination of glycerol and glucose kinetics. On the study day, intravenous (IV) catheter placement occurred between 6:45 and 7:30 am. An infusion IV was placed in an antecubital vein for delivery of stable isotopes. In the contralateral arm a sampling catheter was placed retrograde fashion into a dorsal hand vein, or if necessary, in to a wrist vein. The heated hand technique(35) was used to obtain arterialized blood samples. Initial blood samples were drawn for determination of background enrichment followed by a primed (2 μmol/kg), constant (0.09 μmol/kg/min) infusion of [1,1,2,2,3,-$^2$H$_5$]glycerol (Cambridge Isotopes, Andover, MA) and a primed (17.6 μmol/kg), constant (0.2
μmol/kg/min) infusion of [6,6-²H₂]glucose. All infusates were prepared by the Research Pharmacist at University Hospital, UCHSC, and were tested for sterility and pyrogenicity prior to use. Resting substrate kinetics were determined on blood samples taken over the last 30 minutes of a 120 minute rest phase (t 90, 100, 110 and 120 mins). The catecholamine infusion was then started and continued for 60 minutes up to 180 mins. Catecholamines were diluted in 0.9% saline containing 1 mg/ml ascorbic acid, to prevent oxidation. The infusion rate of epinephrine alone was 8 ng.kg⁻¹.min⁻¹, the infusion of norepinephrine alone was 8 ng.kg⁻¹.min⁻¹ in the first 4 subjects (2 men and 2 women) and 14 ng.kg⁻¹.min⁻¹ in the remaining subjects. This increase in norepinephrine infusion rate occurred as the circulating norepinephrine levels observed in the first 4 subjects were found to below the target level (similar to levels observed with moderate exercise, ~500 pg/ml). The infusion rate of the epinephrine and norepinephrine combined was the same as when administered individually. These infusion rates were selected to achieve circulating catecholamine levels similar to those observed during moderate exercise.

At the onset of the catecholamine infusion, the infusion rate of the glycerol was increased to 1.3 x rest in an attempt to avoid large fluctuations in tracer enrichment due to increased substrate turnover. Blood samples were drawn at t = 130, 140, 150, 160, 170, and 180 mins during the hormone infusion for the measurement of isotope enrichments and glycerol and glucose concentrations.

**Blood Pressure and Heart Rate Measurement:** Heart rate and blood pressure were monitored by an automatic blood pressure cuff placed on the arm used for sampling. Measurements were made immediately after the blood draws at t=0 and t=120 and immediately after each blood draw during the catecholamine infusions (t =130, 140, 150, 160, 170 and 180 mins).

**Respiratory gas exchange.** In the 30 mins prior to blood sampling at rest, a 15-20 min measurement of respiratory gas exchange was made via indirect calorimetry (Sensormedics 2900, Sensormedics, Yorba Linda, CA). During each hormone infusion a 20 min measurement of respiratory gas exchange was performed 25- 30 min after the start of the hormone infusion. Carbohydrate and fat oxidation
were calculated from the volume of O₂ consumed and volume of CO₂ expired after correcting for protein oxidation (30; 54). Protein oxidation was estimated from urinary nitrogen excretion with urine collected over the study period.

Determination of circulating hormone and substrate levels: Measurements of glycerol and glucose were made on all blood samples. Catecholamines (epinephrine and norepinephrine), insulin and glucagon were measured on samples drawn at 0, 100 and 120 mins of rest and at 140, 160 and 180 mins of the catecholamine infusion. Two to 3 ml of blood was added to EDTA tubes for the measurement of tracer enrichment and plasma substrate concentrations. Two and a half ml of whole blood was added to 40 μl of preservative (EGTA 3.6 mg plus glutathione 2.4 mg in distilled water), for plasma catecholamine determinations. Blood for glucagon measurement (2ml) was added to tubes containing EDTA plus 500 KIU Aprotinin. Samples were immediately placed on ice, spun and plasma separated. Approximately 2.5-3.5 ml whole blood was allowed to clot and the serum separated off after spinning. This was used for determination of the remaining hormone and substrate concentrations. All plasma, serum and supernatent samples were stored at -70°C until analysis. Plasma samples were analyzed for glycerol and glucose enrichment and concentration. Catecholamines were determined in duplicate by high-performance liquid chromatography with electrochemical detection (intra-assay CV’s 5.4% epinephrine, 4.5% norepinephrine) (6). Plasma samples were used for enzymatic assays of NEFA (Wako Chemical USA Inc., Richmond, VA; intra-assay CV 1.2%). Radioimmunoassays were used to determine serum insulin (Kabi Pharmacia, Piscataway, NJ), progesterone, estradiol, (Diagnostic Products Corporation, Los Angeles, CA), and glucagon (Linco Research Inc. St. Louis, MO). Samples were run in duplicate with intra-assay CVs of 5.2%, 7.5%, 6.0%, and 8.0%, respectively. Within subjects, samples from each study day were run in the same batch.

Determination of glycerol and glucose isotope enrichment and concentration: These were measured via gas-chromatography-mass spectrometry (GC-MS; GC Model 6890 and 5973N, Agilent, Palo
Alto, CA). The pentacetate derivative of glycerol and glucose were generated as follows. Samples were deproteinized with iced ethanol and the supernatent was dried in a speedvac at 50°C for 2.5 hours. Samples were then derivatized using 100 μl of acetic anhydride-pyridine solution (1:1) and heated for 30 min at 100°C. 100 μl of ethyl acetate was then added and the samples were vortexed, and transferred to GC-MS vials for analysis. Injector temperature of the GC-MS was set at 250°C and initial oven temperature was set at 150°C. The column used was an Agilent HP-5MS 0.25mm*30m with a 0.25mm film thickness. Oven temperature was increased 30°C/min until a final temperature of 250°C was achieved. Helium was used as the carrier gas with a 20:1 ml/min pulsed split injection ratio; transfer line temperature was set at 280°C, source temperature at 250°C, and quadruple temperature at 150°C, and methane chemical ionization (63) was used to monitor selective ions with mass-to-charge ratios of 159 (M+0 from natural glycerol), 164 (M+5 from [1,1,2,2,3,-2H5]glycerol) and 162 (M+3 from the [1,2,3,-13C3] glycerol internal standard) for glycerol and 331 (M+0 from natural glucose), 333 (M+2 from [6,6- 2H2]glucose) and 337 (M+6 from the U-13C glucose, internal standard) for glucose.

Natural glycerol standards were prepared from 5.4-1087 μmol/L and spiked with 105 μmol/L of the internal standard [1,2,3,-13C3]glycerol to generate the glycerol standard curve for determining glycerol concentration. The calibration curve was constructed by comparing the known ratio of glycerol: [1,2,3,-13C3]glycerol to the measured area ratio of 159:162. Natural glycerol in the samples was then determined by measuring the sample 159:162 area ratio, and using the linear equation obtained from the calibration curve to calculate the natural glycerol concentration in μmol/L. For the glucose calibration curve, natural glucose standards from 10-200mg/dL were prepared and spiked with 80 μg U-13C-glucose. The calibration curve was constructed by comparing the known ratio of glucose:U-13C-glucose to the measured area ratio of 331:337. Natural glucose concentration in the samples was then determined by measuring the sample 331:337 area ratio, and using the linear
equation obtained from the calibration curve to calculate the natural glucose concentration in mg/dL.

**Calculations.**

\[
Ra = F - pV \frac{[(C_2+C_1)/2][((E_2-E_1)/(t_2-t_1))]}{(E_2+E_1)/2} - F
\]

\[
Rd = Ra - pV(C_2-C_1)/(t_2-t_1)
\]

where, \(Ra\) = rate of appearance of tracee (\(\mu\)mol/min), \(F\) = infusion rate of tracer (\(\mu\)mol/min), \(pV\) = effective volume of tracee distribution (230 ml/kg bodyweight for glycerol and 100 ml/kg bodyweight for glucose) (47; 55), \(t_1\) = time 1 of sampling, \(t_2\) = time 2 of sampling, \(C_1\) = [tracee] at \(t_1\), \(C_2\) = [tracee] at \(t_2\), \(E_1\) = tracer enrichment (tracer:tracee ratio) at \(t_1\), \(E_2\) = enrichment at \(t_2\), \(Rd\) = rate of disappearance.

**Data analysis**

Subject characteristics were compared using an unpaired t-test. For substrate concentrations and kinetics, data was analyzed using a general linear multivariate model with repeated measurements. This was used to evaluate differences in the pattern of the time course of response between the sexes on each study day. In this model, time was included as the repeated within subject factor and between subject factors included sex (male or female) and infusion (Epi, Norepi or Epi + Norepi). The model evaluated 2 way interactions (time x sex and time x infusion), as well as any 3 way interaction (time x sex x infusion). Post-hoc analyses were performed using Bonferroni’s test. Data from one female subject was excluded on the Epi infusion day as the infusate did not include the epinephrine (as evidenced by no change in circulating Epi levels). For the glucose kinetic data, one male and one female had to be excluded due to analytical problems during sample analysis. Another female was excluded from this data set as her glucose turnover rates were 3 standard deviations above the group mean for no apparent reason.

For glycerol concentration and kinetics, there was a clear change in the pattern of response from rest, to the first 30 mins of the hormone infusion (T130-150) and then the last 30 mins of
infusion (T160-180). Physiologically, this may be explained by the onset of tachyphylaxia of the adrenergic β-receptors about 30 mins into the catecolamine infusion. Therefore, data over these time periods was averaged and further compared between the sexes and study days. For catecholamines, insulin and glucagon, values for the entire 60 min infusion were averaged and compared to average rest values.

Data for glycerol kinetics were expressed in absolute rates as well as relative to body weight, the more traditional method of data presentation. As adipose tissue is the major site of lipolysis, it could be considered that expressing data relative to fat mass, or statistically co-varying for fat mass, may be the best approach for comparing glycerol Ra. This was not necessary, however, as the men and women in this study had identical fat masses, and the difference in body weight was purely due to differences in FFM. For glucose kinetics, we expressed data in terms of body weight and FFM as organs (liver and kidney) are the source of glucose production in the body and lean tissue mass the main sight of glucose disposal. Results are presented as mean ± standard error of the mean (SEM) with statistical significance set at p < 0.05.

RESULTS

Hormone concentrations: At rest, women and men had similar epinephrine levels but women had significantly lower norepinephrine concentrations as compared to men (p< 0.0001 Table 2). Circulating catecholamine levels increased significantly above resting values as expected for the corresponding catecholamine infusion (p<0.0001). For the Epi or Epi + Norepi infusion, circulating epinephrine increased to similar levels in women and men; approximately 5.9-6.8 fold above resting levels. Circulating norepinephrine during the Norepi or combined Epi + Norepi infusions was still lower in women vs men, but the change from rest was not significantly different; an increase of 356 ± 35 pg/ml vs 359 ± 28 pg/ml respectively, during Norepi infusion, and an increase of 307 ± 35 pg/ml vs 346 ± 25 pg/ml, respectively, during the combined Epi + Norepi infusion. By 10 minutes of each
catecholamine infusion, the concentration of epinephrine was between 2 to 15 pg/ml of the final 60 min value and the corresponding values for norepinephrine were -3 to 44 pg/ml. These differences are within the analytical error range for HPLC analysis and demonstrate a rapidly achieved steady state for the infused hormone levels.

Insulin concentrations did not change significantly from rest with any of the catecholamine infusions. Resting values in women averaged 3.7 – 4.1 uU/ml on the 3 study days and 3.3 -4.7 uU/ml during the catecholamine infusions. In men, the corresponding values were 4.0 – 4.3 uU/ml at rest and 4.0 – 5.1 uU/ml with the infusions. Glucagon levels were also stable during the study. Average values for each study day at rest were 54-55 pg/ml in women, 60-63 pg/ml in men, and during infusions 53-56 pg/ml and 61-62 pg/ml, respectively.

**Blood pressure and heart rate:** Pre-infusion heart rate over the 3 days averaged 59 ±3 in women and 63 ±2 in men with the corresponding values for the Epi infusion being 68 ±3 and 67 ±2, respectively; for Norepi, 56 ±3 and 60 ±2, respectively and for Epi+Norepi, 65 ±3 68 ±2, respectively There were no significant changes in response to any infusion in either women or men. Blood pressure also did not change significantly with any infusion: pre-infusion 106/51 and 119/56 mmHg in women and men, respectively, with the corresponding values for the infusions being 104/45 and 114/49 mmHg for Epi, 110/56 and 121/56 for Norepi and 107/50 and 120/52 for Epi+Norepi.

**Glycerol kinetics:** Figure 1 shows the glycerol enrichment, expressed as moles percent excess (MPE%), at rest and during the 60 mins of each hormone infusion. Despite the increase in isotope infusion rate, glycerol MPE% fell during the first 30 mins of each catecholamine infusion and then remained more stable during the final 30 mins. Figures 2 and 3 show the glycerol concentration and glycerol Ra (absolute rates), respectively, throughout each experimental day. There was a significant effect of time (p <0.0001) due to an initial increase in glycerol concentration and glycerol Ra after the start of the hormone infusions followed by a decrease. For glycerol concentration and absolute Ra,
there was a significant time x sex (p < 0.0001) and time x infusion (p< 0.0001) interaction. Results were similar for glycerol Rd (time x sex interaction p<0.001, and time x infusion interaction p<0.0001) Post-hoc analysis revealed a significant difference between glycerol concentrations during the combined Epi + Norepi and Norepi infusions (p<0.0001) whereas for glycerol Ra and Rd, there was a significantly greater increase for Epi +Norepi vs Norepi (p <0.0001) and also Epi vs Norepi (Ra p=0.02 and Rd p=0.03).

Data were also compared between groups and treatments for glycerol Ra and Rd at rest, over the first 30 mins of hormone infusion and over the final 30 mins of hormone infusion (Table 3). Data are expressed in absolute terms and relative to body weight for comparison with other data in the literature. Women had a significantly greater glycerol Ra and Rd compared to men at rest when data were expressed relative to body weight (p=0.003) but not for absolute rates of glycerol Ra. Between 130-150 mins of hormone infusion, there was a significant effect of sex with respect to absolute glycerol Ra and Rd (p < 0.01) with greater differences observed when data were expressed per body wgt (p < 0.0001). Comparing kinetic data from 160 – 180 mins, however, resulted in no sex difference for absolute glycerol Ra and Rd whereas data were still significantly different between men and women when expressed per body weight (p<0.0001). The Norepi+Epi infusion, or Epi infusion alone, consistently resulted in a greater glycerol Ra and Rd vs the Norepi only infusion, the magnitude of difference tending to be greater for the 130-150 min time period compared to the 160-180 min time period.

Glucose kinetics: Glucose enrichment at rest and during the 60 mins of each hormone infusion were stable in women (1.33 ±0.19 and 1.30 ±0.19 MPE%, respectively) and men (1.57 ±0.10 and 1.57 ±0.09 MPE%, respectively). Table 4 shows glucose concentration and Ra compared between groups and treatments at rest, over the first 30 mins of hormone infusion and over the final 30 mins of hormone infusion. As glucose kinetics were stable over the duration of each measurement period,
glucose Ra and glucose Rd (data not shown) were essentially the same. Resting glucose concentration was significantly lower in women vs men (p<0.0001) and this sex difference persisted throughout the duration of each hormone infusion (p<0.01). An overall effect of infusion was observed for glucose concentration (t130-150 mins, p=0.014 and t160-180 mins p<0.001). For the first 30 mins, this was due to a higher glucose concentration during the Epi+Norepi vs Norepi infusion (p=0.013) whereas both the Epi+Norepi and Epi infusions resulted in higher glucose levels vs the Norepi infusion during the final 30 mins (p<0.001 and p=0.017, respectively). For data averaged over each time period, comparisons within each time frame showed no difference for glucose Ra by sex, or infusion. This was also true for glucose Rd. Results were the same for glucose kinetics expressed relative to fat-free mass (data not shown). It appeared that the significant changes in glucose concentration were not necessarily reflected in significant differences in glucose kinetics with the different hormone infusions. This was likely due to the relatively small changes in concentration along with almost all of the change occurring by the time the first blood sample was taken after the start of the hormone infusions (within 10 minutes). Glucose concentrations at rest vs 130 mins of Epi infusion were 80 ± 1 vs 86 ± 2 mg/dl in women and 85 ± 1 vs 87 ± 2 mg/dl in men, for the Norepi infusion, 80 ± 1 vs 83 ± 1 mg/dl and 85 ± 2 vs 88 ± 2 mg/dl, respectively; and for the Epi+Norepi infusion, 81 ± 1 vs 87 ± 2 mg/dl and 85 ± 1 vs 89 ± 2 mg/dl, respectively. Concentrations were then stable through the end of the study. Although there was a significant overall effect of time (p< 0.0001) for glucose Ra and Rd, changes were small and the most dynamic changes likely occurred in the first ten minutes of the infusions (before blood sampling commenced) with a rapid re-establishment of a relatively unchanged glucose turnover.

Non-esterified fatty acid concentrations: NEFA concentrations mirrored those of glycerol for all treatments and between women and men (Figure 4). There was a significant time by sex interaction (p<0.002) due to concentrations increasing more in women than men during the catecholamine
infusions. In both women and men, Epi and Epi+Norepi resulted in significantly greater NEFA levels over time compared to the Norepi infusion ($p = 0.045$ and $p = 0.003$, respectively).

*Energy Expenditure and Whole-body Substrate Oxidation:* The average metabolic rate before and during the hormone infusions is shown in Table 5. In both women and men, metabolic rate increased from rest to each hormone infusion, significantly so for Epi and Epi+Norepi ($p < 0.0001$). Absolute protein oxidation, estimated from urinary nitrogen excretion, was significantly higher in men vs women ($p < 0.001$) on all days (Epi: 0.056 ± 0.003 vs 0.042 ± 0.003 g/min, Norepi; 0.062 ± 0.005 vs 0.052 ± 0.006 g/min, and Epi+Norepi; 0.068 ± 0.008 vs 0.043 ± 0.003 g/min, respectively). Respiratory gas exchange was therefore adjusted for the sex specific protein oxidation rates, giving the non-protein RER (NPRER; Table 7). The NPRER significantly decreased during hormone infusions ($p < 0.0001$) and this decrease was significantly greater in women compared to men ($p < 0.001$). Using the NPRER to calculate whole body fat and carbohydrate (CHO) oxidation rates (g/min), revealed that fat oxidation increased significantly ($p < 0.0001$) whereas CHO oxidation decreased significantly ($p < 0.0001$) with hormone infusions. Nutrient oxidation relative to total oxidation (% of total) was calculated to allow for the differences in whole body metabolic rate between men and women. Pre-infusion, the percentage contribution of each nutrient to total oxidation was not different between women and men; fat 59-62%, CHO 17-21%, and protein 19-24%. There was a significant time x sex interaction for relative fat ($p < 0.0001$) and CHO ($p < 0.0001$) oxidation as women had a significantly greater contribution from fat to total oxidation compared to men during hormone infusions (Epi; 85 ±3% vs 74 ±3%, Norepi, 73 ±3% vs 68 ±4%, Epi+Norepi; 84 ±3% vs 71 ±6%, respectively) and a significantly lower CHO contribution (Epi; -3 ±3% and 8 ±3%, Norepi, 4 ±3% and 10 ±3%, Epi+Norepi; -2 ±3% and 7 ±5%, respectively). Relative protein oxidation decreased with time ($p < 0.0001$) this was not different between the sexes.

**DISCUSSION**

This study evaluated the effect of matched, moderate elevations in circulating catecholamines, similar to those observed during moderate exercise, on systemic glycerol and glucose kinetics in
women compared to men. In response to Epi alone or Epi plus Norepi, women had a greater increase in systemic glycerol release compared to men, implying a greater lipolytic response to the same circulating level of catecholamines. Systemic NEFA levels were also increased significantly more in women than men with each catecholamine infusion and this was accompanied by a greater increase in whole body lipid oxidation. With this moderate dose of Epi and/or Norepi, both women and men had a similar, but small increase in glucose production and glucose disposal. These data suggest that in women, their greater lipolytic sensitivity to catecholamines contributes to an increased capacity for lipid mobilization and utilization which likely enables them to accommodate changes in fuel requirements associated with metabolic perturbations with minimal changes in glucose turnover. This could be viewed as facilitating glucose conservation in women relative to men.

The only other study that has evaluated lipid kinetics in men and women during Epi infusion used isotopically labeled palmitate to measure NEFA release (29). Systemic NEFA concentrations and Ra were increased similarly in men and women over the final 60 minutes of a 2 hr infusion (Epi ~ 8 fold above resting levels) showing no sex difference in systemic NEFA release. With this study design, however, NEFA Ra may not appropriately reflect lipolysis, especially when trying to relate this to what occurs during exercise. For example, with catecholamine infusion under resting conditions, NEFA oxidation as well as blood flow, will not change to the same extent as during exercise. Consequently, there will likely be greater local accumulation of NEFA within the tissue of release, as well as potentially greater re-esterification of NEFA (5; 20; 21), meaning that NEFA Ra at rest may underestimate the lipolytic potential. Furthermore, it has been shown that at rest with greater than 60 minutes of hormone infusion, significant tachyphylaxia of the β-adrenergic receptors occurs (1). Indeed, in our current study, it appeared that tachyphylaxia occurred after 20-30 mins of catecholamine infusion as glycerol turnover markedly decreased in the final 30 minutes of each study. As the sex difference observed in glycerol kinetics in the current study occurred in the first 30 minutes of infusion,
and was no longer present in the final 30mins, the current data imply that tachyphylaxia may have been greater in women than men. This could also have been a factor contributing to the lack of a sex difference in NEFA kinetics in response to epinephrine reported by Jensen et. al. (29).

The data on circulating NEFA changes corroborate the observation of increased lipolysis in women vs men. Systemic NEFA levels were increased more in women than men with catecholamine infusions, suggesting increased mobilization of lipid fuel from tissue triglyceride stores, although this could only be definitively concluded with measures of NEFA Ra. Nevertheless, the elevation in systemic NEFA levels was accompanied by a greater increase in lipid oxidation in women vs men during hormone infusions. It should be noted, however, that in a few of the subjects, NPRER was below 0.70, leading to a slightly negative CHO oxidation using the stochiometric calculations of gas exchange. An NPRER under 0.70 implies non-oxidative contribution to gas exchange which under these experimental conditions is difficult to explain. Rather, the result could be related to slight errors in both the indirect calorimetry measures of gas exchange and the estimation of protein oxidation from urinary nitrogen excretion, resulting in an artifact that lowered NPRER below 0.70. In such instances, it is probably more appropriate to assume CHO oxidation equal zero rather than a negative value. In doing so, this does not change the observation of a sex difference in fat and CHO oxidation. Hence, these data support the conclusion that the increased lipolysis observed in women in response to catecholamine infusions is associated with an increase in circulating levels of lipid fuel (NEFAs) and also increased lipid utilization.

The impetus for the current investigation came from observations of sex-based differences in substrate metabolism in studies during metabolic stress including exercise and hypoglycemia (4; 12; 13; 15; 23; 25; 26; 37; 56-58). The endocrine regulation of lipid (and glucose) metabolism during metabolic stress is orchestrated by a number of complimentary hormonal changes implying that more than one factor may be involved in the sex-based differences in lipid mobilization and utilization.
Catecholamines are considered important regulators of lipolysis thus the observations of a significantly lower increase in these hormones, in particular epinephrine, in women relative to men during metabolic stress raises the issue of their role in sex-based differences in the regulation of lipolysis under such conditions. Nevertheless, other factors that could contribute to sex-based differences in lipid metabolism include insulin, growth hormone (GH), interleukin-6 (IL-6) and cortisol. With respect to insulin, sex differences in the fall in insulin with exercise could facilitate sex-differences in lipolysis but in general, data show no sex difference in this decline (23; 26; 46; 49). Despite the fact that women have higher GH levels than men at rest, importantly, the magnitude of the increase in GH from rest to exercise has been reported to be similar (62) or greater (23) in men vs women. Likewise, although there are sex differences in resting cortisol levels, cortisol increases with exercise are similar between men and women (15; 23; 26). Interleukin-6 has emerged as a potential lipolytic factor (59) and this cytokine is released from muscle during exercise (22; 39). It appears, however, that at the circulating levels of IL-6 commonly achieved with moderate exercise, concentrations are insufficient to elevate lipolysis, at least in men (24). Whether there are sex-differences in the lipolytic response to IL-6, and/or IL-6 changes with exercise are currently unknown. More recently, atrial natriuretic peptide (ANP) has been reported to mediate exercise induced lipolysis (37; 38) more so than catecholamines but women and men and women do not differ in their ANP response to exercise (37). A lack of a sex-based difference in the increase (or decrease) in these lipolytic factors during exercise or hypoglycemia does not exclude the possibility that they may interact with each other, to enhance lipolysis and lipid oxidation more in women than in men. This study did show, however, that sex-based differences in the lipolytic response to catecholamines, is likely one important factor determining the sex-based differences in lipid metabolism during metabolic stress.

The question arises as to what might be the tissues contributing to the sex difference in
catecholamine stimulated lipolysis. Systemic measures of glycerol Ra predominantly reflect lipolysis from peripheral locations, that is, SAT and skeletal muscle, as in both sexes at least 90% of the glycerol released from VAT lipolysis is cleared on first pass by the liver (27). Data suggests that lipoprotein lipase (LPL) mediated generation of glycerol from very-low-density lipoprotein triglyceride (VLDL-TG) hydrolysis is also unlikely to contribute measurably to systemic glycerol Ra after an overnight fast (17; 28). Although catecholamines can increase LPL activity in muscle (16; 40), and may decrease LPL activity in adipose tissue (42) our previous data, on exercise effects on muscle and adipose tissue LPL activity in men and women, suggest that sex-differences in LPL mediated VLDL-TG lipolysis is unlikely to contribute to the greater systemic glycerol Ra observed in women in the current study. Rather, data suggest that in women, peripheral tissue lipolysis is the source of the increased glycerol release.

As was hypothesized, no sex difference in glucose kinetics occurred with the catecholamine infusions used in the current study. Both women and men had only a very small change in glucose production and utilization with this moderate elevation in circulating catecholamines levels. This is not necessarily unexpected for norepinephrine alone as this is not a potent stimulator of glucose production acutely (9) whereas it might have been expected that the epinephrine would have increase increased glucose Ra to a larger extent (7; 44). Our observation of a minimal change in glucose kinetics with this moderate elevation in circulating catecholamine levels suggests that during moderate exercise, other factors determine the increase in glucose production that occurs. In a previous study (26) we observed that men had a significantly greater increase in glucose Ra and Rd compared to women during 90 mins of exercise at 60% of maximal oxygen uptake (85% of lactate threshold). Notably, measurements were made after an overnight fast, when liver glycogen levels would have been low, similar conditions to the current study. Although the men had a significantly greater exercise increase in epinephrine compared to women, they also had a greater increase in
circulating glucagon levels. We hypothesized that the increment in glucagon, in the face of similar insulin levels, was playing the dominant role in the exercise sex difference in glucose Ra confirming previous observations (60). Results from the current study support such a conclusion as during the catecholamine infusions, glucagon and insulin levels did not change, nor were they different between men and women, and we observed no sex difference in glucose kinetics with only a very small increase in glucose production.

Although women and men had similar rates of glucose disposal, total CHO, that is, glucose oxidation was lower in women than men suggesting a greater non-oxidative glucose disposal in women. We made a crude estimate of non-oxidative glucose disposal based on the data from the 9 women and 9 men for whom glucose Rd data was available, along with glucose oxidation. Two comparisons were made; one using the negative glucose oxidation estimated from the indirect calorimetry and urinary nitrogen calculations and the other assuming the negative glucose oxidation was equal to 0. Repeated measures (rest vs infusion) ANOVA, revealed a significant (p=0.012) and marginally significant (p=0.07), time*sex interaction (data expressed relative to FFM), respectively, for the two methods of calculating non-oxidative disposal. The data do, therefore, tend to suggest a higher non-oxidative glucose disposal in women vs men. Whether this was due to greater glycogen synthesis and/or a higher conversion of glucose to lactate in women relative to men could not be determined in the current study.

We chose to infuse norepinephrine, as well as epinephrine to determine the effect of the catecholamine combination on glycerol kinetics as both these hormones change significantly with exercise and more so in men than women. It was hypothesized that the combined infusion would act synergistically, ie resulting in a greater glycerol Ra than the combined, individual, effects of epinephrine or norepinephrine alone, and that this synergistic effect would be observed in women but not necessarily men. Our observations, however, did not support this hypothesis. Nevertheless, it is
acknowledged that the systemic delivery of norepinephrine does not mimic the dominant way in which norepinephrine will affect tissue lipolysis in response to an increase in sympathetic nervous system (SNS) activity (2), as occurs during exercise. Norepinephrine release at the nerve terminals within a tissue acts locally to induce physiologic changes including stimulation of lipolysis. The “spillover” of norepinephrine into the systemic circulation underestimates the quantity of norepinephrine released within a tissue. Nevertheless, the systemic delivery of norepinephrine can also stimulate metabolic effects (31; 53). In the current study, the increase in glycerol Ra with the level of norepinephrine infused was small in both men and women. Although this level was similar to circulating norepinephrine levels that may be observed with moderate exercise, it almost certainly under-represents what would have been released at the nerve terminals which would likely result in greater lipolysis. Had we used a high dose of norepinephrine alone, and in combination with the epinephrine, we may have seen a higher rate of lipolysis under both conditions (31). Whether a greater norepinephrine level is required to elicit a synergistic interaction with epinephrine is a possibility, therefore such an interaction cannot be ruled out in women and/or men.

It is possible that there are sex differences in the norepinephrine release and/or “spillover” following SNS activation and this is responsible for the sex differences in circulating norepinephrine levels with metabolic perturbations. Data from measures of MSNA in women and men in response to hypoglycemia, however, report that women have a lower sympathetic response to the same metabolic perturbation (12-14). Although this information is indirect, it does suggest that tissue norepinephrine release is indeed lower in women than men but does not address whether or not norepinephrine re-uptake is different between the sexes.

Although we attempted to minimize the change in glycerol enrichment during each hormone infusion study by increasing the glycerol infusion rate enrichments still fell during the initial infusion period. The non-steady state nature of the changes in glycerol kinetics with this study design are
unavoidable due to the inherent dynamic nature of the response to catecholamine stimulated lipolysis. Although not ideal for the application of tracer methodology, the non-steady state calculations allow for this for the most part (55) and have been shown to be robust for the measurement of glycerol turnover under conditions more dynamic than those observed in the current study (19). Unfortunately, the time that a steady state in lipolysis occurs in response to catecholamines stimulation is after tachyphylaxia has been established, at least under resting conditions (1). It is interesting that during exercise, lipolysis continues and actually increases over time with no evidence of tachyphylaxia despite continued elevations in epinephrine and norepinephrine. The reasons for this difference between tachyphylaxia in the resting and exercise conditions could be related to a number of factors. Firstly, the mediators of the tachyphalaxic response of β-adrenergic receptors are changed. It is not completely understood what may induce tacyphalaxia but local elevation in NEFA, and/or adenosine, as well as system increases in ketones have all been proposed as factors leading to the down regulation of the receptors. (32; 33). Down-regulation of the β–adrenergic receptors acutely by such factors may be mitigated if these metabolites can be removed from the cellular environment, for example during exercise, changes in blood flow, metabolic demand and substrate utilization may facilitate such a reduction in receptor exposure to antilipolytic factors. Alternatively, other hormones and factors previously discussed may be permitting continued lipolysis during exercise.

For studies that compare women and men directly, there is always an issue of how best to match the subjects and then how to express data. We matched women for age and physical activity level, as both factors can impact the metabolic response to sympathetic stimulation. Our subjects were not highly trained but were habitually active (25-30 minutes of mild to moderately intense aerobic activity/day) and we also pair matched men and women based on their self-reported activity level. Interestingly, the women and men matched in this way had similar absolute fat masses, with all the sex difference in body weight being due to differences in fat-free mass. We have observed this
previously in normal weight but more active women and men matched for habitual activity and fitness level (26). As adipose tissue is the major site of lipolysis, it might be considered that expressing data relative to fat mass, or statistically co-varying for fat mass, may be the best approach for evaluating sex differences glycerol Ra. This was not necessary, however, as women and men in this study had identical fat masses, so we expressed data in absolute terms (ie μmol/min). It was not felt appropriate to co-vary for fat mass, as with this small number of subjects we were unable to observe a significant correlation between fat mass and average glycerol Ra for each infusion (either the 60 mins average or the average for each 30 min period). Whether the data were expressed in absolute terms or relative to body weight, the more traditional way of expressing glycerol kinetic data, we observed a higher rate of glycerol release in response to Epi or Epi + Norepi in women vs men.

In conclusion, this study demonstrated a significantly greater increase in systemic glycerol Ra in response to the same moderate elevation in circulating epinephrine and/or norepinephrine in women vs men. This suggests that women have a greater increase in peripheral lipolysis in response to catecholamines compared to men, and that women are more sensitive to the lipolytic action of catecholamines. While sex differences in glycerol kinetics were observed no sex differences occurred in glucose production or utilization. Thus, the lower catecholamine levels observed in women vs men during exercise and other metabolic perturbations, may allow women to achieve a similar or greater level of lipid mobilization and utilization compared to men, while facilitating relative glucose conservation.
ACKNOWLEDGEMENTS

The authors would like to thank all the subjects who volunteered for the study for their time and cooperation. We would also like to thank the GCRC nursing, dietary and laboratory staff for their valuable assistance as well.

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Figure Legends.

Figure 1. Glycerol tracer enrichment, expressed as mole percent excess (MPE%). A. At rest (90 to 120 mins) and during Epi infusion (130-180 mins). B. At rest (90 to 120 mins) and during Norepi infusion (130-180 mins). C. At rest (90 to 120 mins) and during Epi+Norepi infusion (130-180 mins).

Figure 2. A. Glycerol concentration at rest (90 to 120 mins) and during Epi infusion (130-180 mins). B. Glycerol concentration at rest (90 to 120 mins) and during Norepi infusion (130-180 mins). C. Glycerol concentration at rest (90 to 120 mins) and during Epi+Norepi infusion (130-180 mins). Significant time x infusion interaction (p<0.0001) and time x sex interaction (p<0.0001). Post-hoc comparisons revealed a significant difference between Epi+Norepi vs Norepi (p<0.0001).

Figure 3. A. Glycerol rate of appearance (Ra) at rest (100 to 120 mins) and during Epi infusion (130-180 mins). B. Glycerol Ra at rest (100 to 120 mins) and during Norepi infusion (130-180 mins). C. Glycerol Ra (100 to 120 mins) and during Epi+Norepi infusion (130-180 mins). Significant time x infusion interaction (p<0.0001) and time x sex interaction (p<0.0001). Post-hoc comparisons revealed a significant difference between Epi vs Norepi (p=0.02) and Epi+Norepi vs Norepi (p<0.0001).

Figure 4. A. Non-esterified fatty acid concentration at rest (90 to 120 mins) and during Epi infusion (130-180 mins). B. Non-esterified fatty acid concentration at rest (90 to 120 mins) and during Norepi infusion (130-180 mins). C. Non-esterified fatty acid concentration (90 to 120 mins) and during Epi+Norepi infusion (130-180 mins). Significant time x infusion interaction (p<0.001) and time x sex interaction (p<0.002). Post-hoc comparisons revealed a significant difference between Epi vs Norepi (p=0.045) and Epi+Norepi vs Norepi (p=0.003).
Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>WOMEN (n = 11)</th>
<th>MEN (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>30 ± 8</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.7 ± 1.7</td>
<td>23.5 ± 1.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>59.4 ± 5.0 a</td>
<td>74.0 ± 7.6</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>24.0 ± 1.7 b</td>
<td>19.1 ± 1.3</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>14.3 ± 2.3</td>
<td>14.3 ± 3.9</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>45.1 ± 3.9 a</td>
<td>59.7 ± 5.4</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.74 ± 0.47</td>
<td>0.84 ± 0.37</td>
</tr>
</tbody>
</table>

Mean values ± SD. BMI = body mass index.

HOMA-IR = homeostasis model of insulin resistance: [fasting glucose (mg/dl)/fasting insulin (μU/ml)]/405

(34)

Women lower than men; a p < 0.0001, women higher than men b p < 0.0001.
Table 2. Circulating catecholamine levels at rest and during hormone infusion for each study day.

Mean values ± SEM.

<table>
<thead>
<tr>
<th>INFUSION</th>
<th>EPI</th>
<th>NOREPI</th>
<th>EPI + NOREPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[E] [NE]</td>
<td>[E] [NE]</td>
<td>[E] [NE]</td>
</tr>
<tr>
<td>Women before infusion</td>
<td>29 ± 6 92 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28 ± 6 105 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31 ± 4 110 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Men</td>
<td>30 ± 4 145 ± 14</td>
<td>32 ± 4 151 ± 18</td>
<td>30 ± 4 139 ± 11</td>
</tr>
<tr>
<td>Women during infusion</td>
<td>193 ± 18&lt;sup&gt;d&lt;/sup&gt; 99 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 ± 5 461 ± 29&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>183 ± 14&lt;sup&gt;d&lt;/sup&gt; 417 ± 35&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Men</td>
<td>188 ± 11&lt;sup&gt;d&lt;/sup&gt; 154 ± 16</td>
<td>31 ± 4 507 ± 31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>197 ± 16&lt;sup&gt;d&lt;/sup&gt; 485 ± 29&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

EPI = epinephrine infusion only, NOREPI = norepinephrine infusion, EPI + NOREPI = combined epinephrine + norepinephrine infusion.

[E] = circulating epinephrine concentration  [NE] = circulating norepinephrine concentration

Significant main effect of sex;  <sup>a</sup> p < 0.0001,  <sup>b</sup> p < 0.01

Significant main effect of infusion on both circulating epinephrine and norepinephrine concentrations,  <sup>c</sup> p < 0.0001

Post hoc analysis: epinephrine concentration greater during Epi and Epi +Norepi infusions vs Norepi alone,  <sup>d</sup> p < 0.0001; norepinephrine concentration greater during Norepi and Epi +Norepi infusions vs Epi alone,  <sup>e</sup> p < 0.0001.
Table 3. Average glycerol rate of appearance and disappearance at rest and during the first and second 30 mins of each hormone infusion

<table>
<thead>
<tr>
<th></th>
<th>Average Rest</th>
<th>Average 130-150 mins infusion</th>
<th>Average 160-180 mins infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Norepi</td>
<td>Epi</td>
</tr>
<tr>
<td><strong>Ra (μmol⁻¹min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>110 ± 8</td>
<td>117 ± 13</td>
<td>110 ± 16</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>108 ± 12</td>
<td>94 ± 10</td>
<td>110 ± 11</td>
</tr>
<tr>
<td><strong>Ra (μmol⁻¹kg body weight⁻¹min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>1.87 ±0.15 b</td>
<td>1.97 ±0.22 b</td>
<td>1.82 ±0.25 b</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>1.46 ± 0.16</td>
<td>1.27 ± 0.15</td>
<td>1.50 ± 0.16</td>
</tr>
<tr>
<td><strong>Rd (μmol⁻¹min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>103 ± 7</td>
<td>112 ± 12</td>
<td>105 ± 16</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>103 ± 10</td>
<td>91 ± 10</td>
<td>106 ± 10</td>
</tr>
<tr>
<td><strong>Rd (μmol⁻¹kg body weight⁻¹min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>1.75 ±0.15 c</td>
<td>1.90 ±0.21 b</td>
<td>1.76 ±0.24 c</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>1.38 ± 0.14</td>
<td>1.23 ± 0.14</td>
<td>1.44 ± 0.15</td>
</tr>
</tbody>
</table>
Mean values ± SEM.

EPI = epinephrine infusion only, NOREPI = norepinephrine infusion, EPI + NOREPI = combined epinephrine + norepinephrine infusion.

Significant main effect of sex: a p <0.0001, b =0.003, c =0.004, d p <0.01.

Significant difference vs Norepi infusion over the same time period: e p <0.0001, f <0.001, g =0.002, h =0.005, i = 0.02, j =0.03, k = p <0.05, l = 0.055
Table 4. Average glucose concentrations and rate of appearance and disappearance at rest and during the first and second 30 mins of each hormone infusion

<table>
<thead>
<tr>
<th></th>
<th>Average Rest</th>
<th>Average 130-150 mins infusion</th>
<th>Average 160-180 mins infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Norepi</td>
<td>Epi</td>
</tr>
<tr>
<td></td>
<td>Norepi</td>
<td>Epi</td>
<td>Norepi</td>
</tr>
<tr>
<td><strong>Concentration (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>81 ± 1</td>
<td>80 ± 1</td>
<td>81 ± 1</td>
</tr>
<tr>
<td></td>
<td>82 ± 1</td>
<td>87 ± 1</td>
<td>87 ± 1</td>
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<tr>
<td></td>
<td>88 ± 2</td>
<td>82 ± 1</td>
<td>82 ± 1</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>85 ± 1</td>
<td>85 ± 2</td>
<td>85 ± 1</td>
</tr>
<tr>
<td></td>
<td>87 ± 1</td>
<td>87 ± 1</td>
<td>91 ± 2</td>
</tr>
<tr>
<td></td>
<td>87 ± 1</td>
<td>86 ± 2</td>
<td>86 ± 2</td>
</tr>
<tr>
<td><strong>Ra (μmol⁻¹kg body weight⁻¹min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>11.2 ± 0.9</td>
<td>10.5 ± 0.7</td>
<td>11.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>11.6 ± 1.1</td>
<td>10.4 ± 0.8</td>
<td>11.3 ± 0.8</td>
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<tr>
<td></td>
<td>11.6 ± 0.8</td>
<td>10.7 ± 0.6</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>11.4 ± 0.6</td>
<td>11.0 ± 0.7</td>
<td>11.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>11.0 ± 0.7</td>
<td>11.0 ± 0.7</td>
<td>11.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>11.9 ± 0.8</td>
<td>11.3 ± 0.6</td>
<td>11.7 ± 0.7</td>
</tr>
</tbody>
</table>

Mean values ± SEM.  EPI = epinephrine infusion only, NOREPI = norepinephrine infusion, EPI + NOREPI = combined epinephrine + norepinephrine infusion.

Significant main effect of sex;  a p < 0.0001,  b p < 0.01. Significant main effect of infusion,  c p < 0.001,  d p = 0.014

Post hoc analysis: significant difference between Epi + Norepi vs Norepi,  e p = 0.013, Epi vs Norepi  f p = 0.017 and Epi + Norepi vs Norepi  g p < 0.001
Table 5. Metabolic rate and non-protein respiratory exchange ratio before and during the final 30 minutes of hormone infusion

<table>
<thead>
<tr>
<th>INFUSION</th>
<th>EPI</th>
<th>NOREPI</th>
<th>EPI + NOREPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MR (kcal/min)</td>
<td>NPRER</td>
<td>MR (kcal/min)</td>
</tr>
<tr>
<td>Women</td>
<td>before infusion</td>
<td>0.94 ± 0.02</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>Men</td>
<td>1.21 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 ± 0.01</td>
<td>1.22 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Women</td>
<td>during infusion</td>
<td>1.03 ± 0.03</td>
<td>0.70 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Men</td>
<td>1.33 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74 ± 0.01</td>
<td>1.24 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SEM.

EPI = epinephrine infusion only, NOREPI = norepinephrine infusion, EPI + NOREPI = combined epinephrine + norepinephrine infusion.

MR = metabolic rate, NPRER = non-protein respiratory exchange ratio.

Significant main effect of sex: <sup>a</sup> p < 0.0001. Significant time by sex interaction <sup>b</sup> p = 0.001
Figure 1. 

A

B

C
Figure 2.

A

B

C
Figure 3.

A

B

C
Figure 4.  

A

B

C