Effects of fasting on insulin action and glucose kinetics in lean and obese men and women

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Submitted 15 November 2006; accepted in final form 3 August 2007

Bergman BC, Cornier M-A, Horton TJ, Bessesen DH. Effects of fasting on insulin action and glucose kinetics in lean and obese men and women. Am J Physiol Endocrinol Metab 293: E1103–E1111, 2007. First published August 7, 2007; doi:10.1152/ajpendo.00613.2006.—The development of insulin resistance in the obese individual could impair the ability to appropriately adjust metabolism to perturbations in energy balance. We investigated a 12- vs. 48-h fast on hepatic glucose production (Ra), peripheral glucose uptake (Rd), and skeletal muscle insulin signaling in lean and obese subjects. Healthy lean [n = 14; age = 28.0 ± 1.4 yr; body mass index (BMI) = 22.8 ± 0.42] and nondiabetic obese [n = 11; age = 34.6 ± 2.3 yr; BMI = 36.1 ± 1.5] subjects were studied following a 12- and 48-h fast during 2 h of rest and a 3-h 40 mU·m−2·min−1 hyperinsulinemic-euglycemic clamp (HEC). Basal glucose Ra decreased significantly from the 12- to 48-h fast (lean 1.96 ± 0.23 to 1.63 ± 0.15; obese 1.23 ± 0.07 to 1.07 ± 0.07 mg·kg−1·min−1; P = 0.004) and was equally suppressed during the HEC after both fasts. The increase in glucose Rb during the HEC after the 12-h fast was significantly decreased in lean and obese subjects after the 48-h fast (lean 9.03 ± 1.17 to 4.16 ± 0.34, obese 6.10 ± 0.77 to 3.56 ± 0.30 mg·kg·FFM−1·min−1; P < 0.001). After the 12- but not the 48-h fast, insulin-stimulated AKT Ser473 phosphorylation was greater in lean than obese subjects. We conclude that 1) 48 h of fasting produces a marked decline in peripheral insulin action, while suppression of hepatic glucose production is maintained in lean and obese men and women; and 2) the magnitude of this decline is greater in lean vs. obese subjects.

METHODS

Subjects

Healthy lean men and women and nondiabetic obese men and women were recruited for this study. Subjects gave informed consent and were excluded if they smoked or had diabetes, hyperlipidemia, liver, or kidney disease or were taking medications that affect glucose or lipid metabolism and/or regularly engaged in moderate to vigorous exercise (>5 h/wk). Control subjects were excluded if they had a body mass index (BMI) <20 kg/m² or >25 kg/m² and had a waist circumference >40 in. for men and >35 in. for women. Obese subjects were excluded if they had a BMI <30 kg/m² or a waist circumference >40 in. for men and <35 in. for women. Subjects were weight stable in the 6 mo before participation in this research study. Women were taking oral contraceptives and were studied in the week they were not taking the active pill to minimize effects of different oral contraceptives on substrate metabolism. This study was approved by the Colorado Multiple Institution Review Board at the University of Colorado at Denver and Health Sciences Center.

Experimental Design

After preliminary screening, subjects participated in two metabolic trials separated by 1–2 mo. Subjects completed the trials in a randomized order following either a 12-h overnight fast or a 48-h fast; trials were performed at the General Clinical Research Center (GCRC). During the metabolic trials, glucose kinetics were measured under basal conditions and during an HEC.

Preliminary Testing

Subjects reported to the GCRC for the screening procedures following a 12-h overnight fast. Subjects were given a health and physical exam, followed by fasting blood measures. Body composition was determined using dual-energy X-ray absorptiometry analysis (Lunar DPX-IQ, Lunar, Madison, WI). Resting metabolic rate (RMR) was measured using indirect calorimetry (Sensormedics 2900, Sensormedics, Yorba Linda, CA). Subjects rested supine for 30 min, and then a ventilated canopy was placed over their head and measurements continued for 15–20 min (48). Diabetes was excluded with a

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75-g oral glucose tolerance test (glucose concentration >200 mg/dl after 2 h).

Diet Control

Three-day diet records were analyzed for each subject to determine macronutrient and energy content of their habitual diet. Dieticians used the RMR with an activity factor of 1.4, along with each subject’s typical dietary intake, to construct a 3-day diet that was provided by the GCRC kitchen to each subject before both metabolic studies. The macronutrient composition of the diet was 30% fat, 15% protein, and 55% carbohydrate. Subjects during the run-in period only consumed food provided by the GCRC. Subjects were asked to maintain normal daily physical activity during the period of dietary control and refrain from planned exercise during their stay at the GCRC. Preliminary nutritional control ensured subjects were close to energy balance, and, therefore, differences in energy status and glycogen stores before testing were minimized between and within subjects. Subjects spent the night before the 12-h fasting study and spent the entire 48-h fast at the GCRC.

Fasting Details

For the 48-h fasting study, subjects remained resident at the GCRC to ensure compliance. Subjects were only allowed to consume water, ice, and flavored carbonated water. No diet sodas or “calorie-free” foods or beverages were allowed.

Metabolic Studies

Subjects had an intravenous catheter placed in an antecubital vein for infusion of isotopes, dextrose, and insulin. A sampling catheter was placed retrograde in a dorsal hand vein of the contralateral arm. For all blood samples, the heated hand technique (10) was used to arterio-ralize the blood. Background sampling began 30 min after sampling catheters were placed.

HEC

On the morning after completion of the overnight or 48-h fast, a blood sample was drawn for determination of background glucose enrichment as well as basal hormone and metabolite concentrations. For the measurement of glucose turnover, a primed (4.5 mg/kg) constant (0.03 mg·kg⁻¹·min⁻¹) infusion of [6,6-²H₂]glucose was initiated and continued through the end of the clamp. Resting measurements were made after 90 min of the constant infusion to allow for equilibration of the tracer in the glucose pool. Four blood samples for tracer determinations were taken during minutes 90–120 in the basal state. Samples were also taken at the end of the basal period for basal hormone and substrate levels. Indirect calorimetry was performed before starting basal blood sampling, using a respiratory canopy (Sensormedics 2900, Sensormedics). Following blood sampling and indirect calorimetry, a vastus lateralis muscle biopsy was taken immediately prior to the Bergstrom needle biopsy technique (4). Samples were then agitated at 4°C for 1 h and then spun at a relative centrifugal force of 16 for 15 min to pellet insoluble protein. The supernatant was saved and used to determine protein concentration (Pierce BCA kit, Pierce, Rockford, IL).

Western Blotting

A molecular weight standard, 30 µg of sample protein, and a skeletal muscle protein internal standard were run on a 7% Tris-HCl gel (Bio-Rad, Hercules, CA) and transferred to a polyvinylidene difluoride membrane. Primary antibody incubations were performed in either 5% nonfat milk or 5% bovine serum albumin overnight at 4°C, and a horseradish peroxidase-conjugated secondary antibody was incubated for 1 h at room temperature. Enhanced chemiluminescent substrate (Bio-Rad) was used to visualize the protein bands of interest. The intensity of protein bands was captured using an Alphalager 3300 (Alpha Innotech, San Leandro, CA), quantified using AlphaImager 3300 (Alpha Innotech, San Leandro, CA), and expressed relative to the skeletal muscle protein internal standard run on every gel. Phosphotyrosine, AKT Ser473, total AKT, and insulin receptor substrate-1 (IRS-1) antibodies were purchased from Cell Signaling Technology (Danvers, MA); IRS-1 Ser307 and Pan85 antibodies were from Upstate (Charlottesville, VA); insulin receptor antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA); and secondary antibodies were from Chemicon (Chemicon International, Temecula, CA).

Gas Chromatography-Mass Spectroscopy Methods

Glucose isotopic enrichment was measured using gas chromatography-mass spectrometry (GC-MS; GC model no. 5890, series II, and MS model no. 5989A, Hewlett-Packard). Briefly, 100 µl of [U-¹³C]glucose were added as an internal standard. Then, 100-µl plasma samples were precipitated of proteins using 1 ml of iced ethanol, vortexed, and kept at 4°C for 1 h. Samples were spun in a microcentrifuge for 1 min to pellet the proteins and then dried on a savant centrifuge evaporator. Dry samples were then prepared using the penta-acetate derivative by adding 200 µl of 1:1 acetic anhydride-pyridine. Samples were capped and heated for 1 h at 60°C before analysis. Ions with an m/z of 242, 243, 244, and 247 were monitored using selective ion monitoring for the calculation of concentration and enrichment.

Calculations

Calculations. RMR and respiratory exchange ratio (RER) values for the 48-h fast were corrected for oxygen consumption resulting
from ketone production data measured via blood accumulation (43). Rates of glucose appearance (Ra) and disappearance (Rd) and metabolic clearance rate were calculated using the Steele equation modified for stable isotopes during basal conditions (49) and as described by Finegood et al. (16) during the insulin clamp. Nonoxidative glucose disposal (NOGD) during the clamp was calculated from the difference in isotopically measured glucose Rd and carbohydrate oxidation measured by indirect calorimetry.

Statistics. Data are presented as means ± SE. Differences among fasting duration, HEC, and body habitus were analyzed using a repeated-measures ANOVA, with two 2-level within-subject factors (duration of fast and HEC) and one 2-level between-subjects factor (body habitus). When the interaction of fasting and HEC was significant, we performed a subset analysis on fasting and HEC conditions (SPSS, Chicago, IL). An α-level of 0.05 was used throughout.

RESULTS

Subject Characteristics

Anthropometric data for subjects are reported in Table 1. Outcome variables were similar between genders, so men and women were combined to increase statistical power, and data are reported as lean and obese only. Per design, lean subjects weighed significantly less (P < 0.001) and had a lower BMI than obese subjects (P < 0.001).

RER and Metabolic Rate

There was a significant effect of 48 vs. 12 h of fasting to decrease RER values during basal and HEC (Fig. 1, P < 0.001) and a significant effect of the HEC to increase RER values after 12 and 48 h of fasting (P < 0.001). Fasting for 48 h significantly blunted the increase in RER values during the HEC (P < 0.001). Body habitus did not change the effect of the fast and HEC on RER values.

RMR under basal conditions expressed as either oxygen consumption per fat-free mass (FFM) or energy expenditure did not change following the 48-h fast compared with the 12-h fast in lean or obese subjects (Table 2). The HEC also did not change metabolic rate in either lean or obese subjects during the 12- or 48-h fast.

Substrate Concentrations

Fasting for 48 h significantly decreased basal glucose concentration compared with the 12-h fast in lean and obese subjects (P < 0.001, Table 3). Glucose concentration was not significantly different during the HEC following the 12- or 48-h fast.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Age, yr</td>
<td>28.0±1.4</td>
<td>34.6±2.3†</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.2±3.0</td>
<td>102.7±3.5†</td>
</tr>
<tr>
<td>Height, in.</td>
<td>69±1.3</td>
<td>68.3±1.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.8±0.4</td>
<td>35.4±1.3†</td>
</tr>
<tr>
<td>%Body fat</td>
<td>24.5±2.3</td>
<td>42.3±2.5†</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>52±3.4</td>
<td>59.2±3.1</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>16.2±1.4</td>
<td>43.9±3.3†</td>
</tr>
<tr>
<td>Waist circumference, in.</td>
<td>33.2±1.1</td>
<td>44.1±1.4†</td>
</tr>
<tr>
<td>2-h OGTT glucose, mg/dl</td>
<td>95.6±9.1</td>
<td>120.5±8.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; OGTT, oral glucose tolerance test. †Significantly different than lean (P < 0.05).

48-h fast (P = 0.868) and was stable during the last 30 min of the clamp, with a coefficient of variation of 3.7 ± 0.04% for lean and 3.0 ± 1.2% for obese during the 12-h-fast HEC and 3.4 ± 0.07% for lean and 2.3 ± 0.06% for obese during the 48-h-fast HEC. Body habitus did not change the effect of fasting and HEC on glucose concentration.

Insulin concentration decreased significantly after the 48-compared with the 12-h fast (P < 0.001, Table 3). Basal insulin concentration was nonsignificantly lower in lean compared with obese subjects following both 12- and 48-h fasts (P = 0.13). During the HEC, insulin concentration increased from the basal state in all groups (P < 0.001), with no differences achieved during the HEC in lean and obese subjects.

FFA concentration was significantly increased after the 48-vs. the 12-h fast in the basal state (P < 0.001, Fig. 2A). The change in basal FFA concentration from 12 to 48 h of fasting was nonsignificantly increased in lean compared with obese subjects (P = 0.07). The HEC decreased FFA concentration after 12 and 48 h of fasting, but FFA concentration remained greater during the HEC after the 48-h fast (P < 0.001). Body habitus significantly changed the effect of fasting and the HEC on FFA concentration (P = 0.024), indicating that the ability of insulin to suppress FFA concentration was significantly greater in lean compared with obese subjects after the 48-h fast.

Basal glycerol concentration increased following the 48-compared with the 12-h fast in lean and obese subjects (P = 0.001, Table 3). Glycerol concentration decreased significantly during the HEC in both lean and obese subjects following the 12- and 48-h fasts (P < 0.001). Body habitus did not significantly change the effect of fasting and HEC on glycerol concentration. The decrease in glycerol concentration during the HEC was significantly greater in lean compared with obese subjects (P = 0.002).

β-Hydroxybutyrate (β-OH) was not different between lean and obese subjects after the 12-h fast and increased following the 48-h fast (Fig. 2B, P < 0.001). The increase in β-OH during the 48-h fast was significantly greater in lean compared
with obese subjects \( (P = 0.018) \). β-OH concentration decreased during the HEC compared with basal \( (P < 0.001) \) but remained greater after the 48-h fast in lean and obese subjects \( (P = 0.023) \). Body habitus significantly changed the interaction of fasting and HEC \( (P = 0.007) \).

Adiponectin concentration was significantly greater in lean compared with obese subjects under basal and HEC conditions after both 12- and 48-h fasts \( (P = 0.007) \). Adiponectin concentration decreased significantly in both lean and obese subjects \( (P = 0.009) \). Leptin concentration was significantly lower in lean compared with obese subjects under basal and HEC conditions after both 12- and 48-h fasts \( (P = 0.008) \). The HEC significantly increased leptin concentration in all subjects \( (P = 0.008) \), but the small absolute difference is not likely to be clinically meaningful. Body habitus did not change the effect of fasting and HEC on adiponectin and leptin concentration.

**Glucose Kinetics**

Basal and insulin-stimulated glucose enrichment were stable during the measurement period in each study \( (P = 0.004) \). Basal glucose \( R_{G} \) decreased from the 12- to the 48-h fast in lean and obese subjects \( (P = 0.003) \). Leptin concentration was significantly lower in obese compared with lean subjects in the basal state \( (P < 0.003) \). The HEC significantly decreased basal glucose \( R_{G} \) \( (P < 0.001) \) to values not significantly different from zero following both the 12- and 48-h fast in both lean and obese subjects. Body habitus significantly changed the effect of fasting and HEC on glucose \( R_{G} \) \( (P = 0.012) \), indicating a more pronounced decrease in glucose \( R_{G} \) after the 48-h fast with similar suppression by the HEC.

Basal glucose \( R_{G} \) also decreased from the 12- to the 48-h fast in both lean and obese subjects \( (P = 0.008) \). Basal glucose \( R_{G} \) data are reported normalized to FFM, the tissue principally involved in glucose uptake. Glucose \( R_{G} \) was not significantly different in obese compared with lean subjects under basal conditions. Glucose \( R_{G} \) increased during the HEC \( (P = 0.001) \) when compared with basal conditions after both 12- and 48-h fasts \( (P < 0.001) \). The increase in glucose \( R_{G} \) during the HEC was significantly lower than during the 12-h fast in lean and obese subjects \( (P < 0.001) \). Therefore, the 48-h fast equalized glucose \( R_{G} \) in lean and obese subjects. Body habitus did not significantly change the effect of fasting and HEC on glucose \( R_{G} \). Insulin-stimulated NOGD was significantly lower in obese vs. lean subjects after the 12-h fast \( (P = 0.03) \), but NOGD was lost in the obese. The ratio of serine-phosphorylated insulin receptor to total insulin receptor content was decreased significantly \( (P = 0.03) \) by insulin stimulation following the 12-h fast in obese subjects after the 48-h fast \( (P = 0.02) \). The only possible difference to explain alterations in insulin action between lean and obese subjects in the parameters measured was a nonsignificant decrease \( (P = 0.09) \) in the ratio of serine-phosphorylated AKT to total AKT content after the 12-h fast. No other significant differences were found between groups.

**DISCUSSION**

This study demonstrated that 48 h of fasting promotes substantial whole body insulin resistance in both lean and obese subjects. Insulin receptor content was nonsignificantly increased following insulin stimulation after the 12-h fast in both lean \( (P = 0.10) \) and obese \( (P = 0.06) \) subjects. This was maintained in lean subjects after the 48-h fast \( (P = 0.08) \) but was lost in the obese. The ratio of tyrosine-phosphorylated insulin receptor to total insulin receptor content was decreased significantly \( (P = 0.03) \) by insulin stimulation after the 12-h fast in obese subjects and after the 48-h fast in lean subjects \( (P = 0.02) \). The only possible difference to explain alterations in insulin action between lean and obese subjects in the parameters measured was a nonsignificant decrease \( (P = 0.09) \) in the ratio of serine-phosphorylated AKT to total AKT content after the 12-h fast. No other significant differences were found between groups.

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**Table 2. RMR in lean and obese men and women following 12 and 48 h of fasting under basal and insulin-stimulated conditions**

<table>
<thead>
<tr>
<th></th>
<th>Lean Basal 12 h</th>
<th>Obese Basal 12 h</th>
<th>Lean Ins. Stim 12 h</th>
<th>Obese Ins. Stim 12 h</th>
<th>Lean Basal 48 h</th>
<th>Obese Basal 48 h</th>
<th>Lean Ins. Stim 48 h</th>
<th>Obese Ins. Stim 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMR, ml O₂·kg⁻¹·min⁻¹</td>
<td>3.60±0.07</td>
<td>2.89±0.08†</td>
<td>3.55±0.01†</td>
<td>2.80±0.10†</td>
<td>3.85±0.09*</td>
<td>2.97±0.17†</td>
<td>3.58±0.07‡</td>
<td>2.88±0.18†</td>
</tr>
<tr>
<td>FFM·O₂·kg⁻¹·min⁻¹</td>
<td>4.84±0.18</td>
<td>4.99±0.16‡</td>
<td>4.82±0.15</td>
<td>4.84±0.16</td>
<td>5.07±0.19</td>
<td>5.08±0.15</td>
<td>4.79±0.16</td>
<td>4.89±0.15</td>
</tr>
<tr>
<td>Kcal/day</td>
<td>1,653±72</td>
<td>2,009±78§</td>
<td>1,629±91</td>
<td>1,985±86§</td>
<td>1,853±75*</td>
<td>2,054±125</td>
<td>1,736±69</td>
<td>1,986±127</td>
</tr>
</tbody>
</table>

Values are means ± SE. RMR, resting metabolic rate; Ins. Stim, insulin-stimulated condition. *Significantly different than 12 h \( (P < 0.05) \). †Significantly different than lean \( (P < 0.05) \). §Significantly different than basal \( (P < 0.05) \).

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**Table 3. Metabolite concentrations under basal and insulin-stimulated conditions in lean and obese subjects following 12 and 48 h of fasting**

<table>
<thead>
<tr>
<th></th>
<th>Lean Basal 12 h</th>
<th>Obese Basal 12 h</th>
<th>Lean Ins. Stim 12 h</th>
<th>Obese Ins. Stim 12 h</th>
<th>Lean Basal 48 h</th>
<th>Obese Basal 48 h</th>
<th>Lean Ins. Stim 48 h</th>
<th>Obese Ins. Stim 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>84.2±1.5</td>
<td>90.1±2.2</td>
<td>87.8±0.6</td>
<td>90.3±0.6</td>
<td>62.0±1.5*</td>
<td>74.5±3.1*</td>
<td>88.3±0.8</td>
<td>88.6±0.3</td>
</tr>
<tr>
<td>Insulin, pg/ml</td>
<td>5.5±0.6</td>
<td>8.3±1.4</td>
<td>50.9±3.0†</td>
<td>55.1±3.7†</td>
<td>3.4±0.2*</td>
<td>5.1±0.8*</td>
<td>53.0±4.1‡</td>
<td>49.8±2.8§</td>
</tr>
<tr>
<td>Glycerol, μM</td>
<td>62.6±2.1</td>
<td>70.2±5.5</td>
<td>22.5±2.4‡</td>
<td>34.3±3.7‡</td>
<td>110.6±10.6*</td>
<td>90.1±10.0*</td>
<td>37.8±2.6‡</td>
<td>53.8±4.8†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Main effects: glucose fasting \( P < 0.001 \), hyperinsulinemic-euglycemic clamp (HEC) \( P = 0.11 \), fast × clamp \( P = 0.002 \), insulin fasting \( P = 0.46 \), HEC \( P < 0.001 \), fast × clamp \( P = 0.82 \), glycerol fasting \( P = 0.001 \), HEC \( P < 0.001 \), fast × clamp \( P = 0.173 \). *Significantly different than 12 h \( (P < 0.05) \). †Significantly different than lean \( (P < 0.05) \). ‡Significantly different than basal \( (P < 0.05) \).
obese subjects, with the decrease in insulin action more pronounced in lean compared with obese subjects. At this dose of insulin (40 mU·m⁻²·min⁻¹), hepatic glucose output was completely suppressed after both fasts, whereas peripheral insulin action significantly declined in both lean and obese men and women.

In the basal state following the 12-h fast, whole body glucose and fat oxidation were not different between lean and obese subjects as previously reported (27). During the HEC, both groups showed a similar increase in carbohydrate oxidation (Fig. 1). This result was unexpected, considering the data of Kelley and Mandarino (28) suggesting that obese individuals are metabolically less flexible than lean subjects. Kelley and Mandarino suggested that metabolic inflexibility manifests in obesity from an inability to switch from predominantly fat uptake and oxidation during a fast to predominantly glucose uptake and oxidation during insulin-stimulated conditions. If obese subjects in this study were less metabolically flexible, we would expect to observe a less dramatic increase in carbohydrate oxidation during the HEC. From the perspective of whole body substrate utilization, our data suggest that obese individuals are metabolically flexible following a 12-h fast. However, following the 48-h fast, the increase in RER during the HEC was dramatically reduced compared with the 12-h fast in each group. Thus 48 h of fasting induces metabolic inflexibility in whole body substrate utilization in both lean and obese subjects.

Following prolonged fasting in lean subjects, others have reported RMR increased (33, 44), decreased (35, 46), or unchanged (8, 14, 15). In the present study, RMR expressed per kilogram of FFM did not change in lean or obese subjects following the 48- compared with the 12-h fast (Table 2). Thus there is poor agreement in the literature on how fasting affects RMR in lean individuals. Our data add to the puzzle and suggest that obese subjects do not change RMR during a closely monitored 48-h fast.

As previously reported (34, 40), glucose Ra was lower in obese subjects compared with lean controls following the 12-h

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**Fig. 2.** Free fatty acid (FFA; A), β-hydroxybutyrate (B-OH; B), adiponectin (C), and leptin (D) concentrations under basal and insulin-stimulated conditions in lean and obese subjects following 12 and 48 h of fasting. Values are means ± SE. Main effects: FFA fasting P < 0.001, HEC P < 0.001, fast × clamp P < 0.001, B-OH fasting P < 0.001, HEC P < 0.001, fast × clamp P < 0.001, adiponectin fasting P = 0.24, HEC P = 0.009, fast × clamp P = 0.85, leptin fasting P = 0.001, HEC P = 0.008, fast × clamp P = 0.003. ¥Significantly different than basal (P < 0.05). *Significantly different than 12 h (P < 0.05). #Significantly different than lean (P < 0.05).
Similar to data from Horowitz et al. (22), the reduction in glucose Ra during the 48- vs. 12-h fast was 50% less in obese (16.7% decrease) compared with lean subjects (30.6% decrease) in the basal state. During prolonged fasting, hepatic glucose production decreases largely because of decreased glycogenolysis with little change in gluconeogenesis (GNG) (42). Previous reports suggested that GNG is greater in obese vs. lean subjects (17, 34), potentially via increased GNG substrate delivery or stimulation by greater FFA concentration (23, 32). The less dramatic decrease in glucose Ra in obese subjects in the basal state after the 48- vs. 12-h fast may be caused by decreased suppression of GNG and/or a less dramatic decrease in glycogen content promoting hepatic glycogenolysis during the 48-h fast.

The insulin dose of 40 mU·m⁻²·min⁻¹ used during the HEC suppressed hepatic glucose production following the 12- and 48-h fasts in both lean and obese subjects. Suppression of hepatic glucose production was determined by similarly decreased glucose Ra in obese compared with lean subjects. These data do not agree with other reports that suggested decreased hepatic insulin action in obese compared with lean subjects following an overnight fast (40). Our data suggest that hepatic glucose production is similarly suppressible in lean and obese subjects before and after energetic perturbation.

As expected, glucose Rd was 34% less during the HEC in obese compared with lean subjects following the 12-h fast (Fig. 3C). Our results confirm those of previous studies that reported a significant decrease in insulin action with extended fasting (3, 19, 24, 36, 37). Interestingly, glucose Rd during the HEC was not different between lean and obese groups following the 48-h fast. Thus insulin action decreased to a greater extent in lean (58%) compared with obese subjects (44%). These glucose uptake data are consistent with decreased metabolic flexibility in obese compared with lean individuals (28).

One of the goals of this investigation was to try to identify steps in the insulin signaling cascade that might help explain changes in insulin action with extended fasting. Previous studies in humans reported decreased skeletal muscle insulin...
A possible mechanism for the more pronounced decrease in insulin action in lean vs. obese subjects during an HEC. We found decreased insulin-stimulated insulin receptor tyrosine phosphorylation in obese subjects following the HEC compared with the basal state (Table 4). A greater proportion of serine-phosphorylated AKT in lean compared with obese subjects after the 12-h fast was the only measurement that could explain increased insulin action in lean vs. obese subjects. Our data are similar to those of others who have found no differences in insulin-stimulated p85 protein content (29) and AKT content and activity (29) between lean and obese subjects. Our data suggest that changes in serine phosphorylation of AKT may have promoted insulin resistance and decreased glucose uptake in the obese subjects, which resulted in decreased glucose uptake and glycogen storage compared with lean subjects.

A possible mechanism for the more pronounced decrease in insulin action in lean vs. obese subjects could be a more dramatic increase in FFA concentration in lean subjects following the 48-h fast. FFA concentration (Fig. 2) was increased in both lean and obese subjects after a 48-h fast compared with lean subjects following the HEC compared with the basal state. FFA concentration did not change with 48 h of fasting in lean men and women (Fig. 2D). Thus there is general agreement that increased FFA concentration decreases insulin action in both lean and obese subjects. FFA infusion has been reported to decrease glycogen synthesis in rats (20) and humans (5). Similarly, we found that insulin-stimulated NOGD decreased 62% for lean subjects between the 12- and 48-h fast, suggesting that reduced glycogen storage may be involved in the decrease in glucose uptake (Fig. 3D). Additionally, acute FFA infusion abolishes the normal increase in IRS-1-associated phosphatidylinositol 3-kinase activity during insulin stimulation (13, 20). Therefore, it is possible that the more dramatic increase in FFA concentration during the fast may explain the greater fall in glucose R\textsubscript{g} in lean vs. obese subjects.

Another potential mechanism that may have played a role in decreased insulin action following the 48- vs. 12-h fast is the increased \(\beta\)-hydroxybutyrate concentration (Fig. 2B). Webber et al. (45) reported that acute elevation of \(\beta\)-hydroxybutyrate concentration to levels seen during a 72-h fast decreased forearm glucose uptake during an insulin clamp by 43%. The more dramatic increase in \(\beta\)-hydroxybutyrate concentration during the 48-h fast in lean vs. obese subjects may be implicated in the more dramatic decrease in insulin action in lean subjects.

Adiponectin is an insulin-sensitizing cytokine released from adipocytes (50). We were interested in determining whether adiponectin decreased during fasting and whether a change in adiponectin promoted alterations in insulin action. Others have reported that 4 days of caloric restriction (25) or 72 h of fasting (6) did not change basal adiponectin concentration in lean men. Similarly, we found that basal adiponectin concentration did not change with 48 h of fasting in lean men and women (Fig. 2C). We extend previous findings to suggest that 48 h of fasting also does not change basal adiponectin concentration in obese men and women. Similar to data reported by Yu et al. (51), we found a significant, but small, decrease in adiponectin during the HEC that was not affected by body habitus or fasting duration. The decrease was consistent among subjects but is not likely to be clinically relevant considering the negligible absolute difference. Therefore, adiponectin does not change during 48 h of fasting, and the small decrease during the HEC is unlikely to explain the observed decrease in insulin action.

Leptin concentration is related to body fat content; however, 24–36 h of fasting has been shown to acutely decrease leptin concentration without changes in fat mass (6, 21, 31). Our study corroborates these findings, as leptin concentration decreased in both lean and obese subjects after a 48-h fast compared with a 12-h fast (Fig. 2D). Thus there are other factors that regulate leptin concentration aside from body fat mass. Decreased whole body adipose tissue leptin production may explain our findings, as Klein et al. (30) reported that leptin concentration decreased during fasting in lean and obese women because of decreased net subcutaneous adipose tissue leptin production. It is unlikely that changes in leptin concentration are involved in alterations in insulin action in this study, because leptin decreased similarly between lean and obese subjects.

One important limitation to address with this study is that muscle biopsies were obtained 15–20 min after the HEC was stopped. Therefore, we may have missed subtle changes in insulin-signaling measures during the HEC because of this delay. Additionally, we only used one dose of insulin in this study, which completely inhibited glucose R\textsubscript{g}. However, it is possible that a lower insulin dose may have revealed subtle differences in hepatic insulin action between groups.
Conclusions

Forty-eight hours of fasting promoted substantial whole body insulin resistance in both lean and obese subjects. However, obese subjects had a less pronounced decrease in insulin action compared with lean subjects following the 48-h fast. A greater decrease in insulin action in lean subjects after the 48-h fast may be due to greater increases in FFA and β-hydroxybutyrate concentration compared with obese subjects. Furthermore, at this dose of insulin, the liver appears to remain insulin sensitive despite whole body insulin resistance in both lean and obese men and women.

GRANTS

This work was partially supported by the National Institutes of Health General Clinical Research Center Grant RR-00036 and National Institute of Diabetes and Digestive and Kidney Diseases grants to D. H. Bessenese (DK-02935), M. Cornier (K23-RR-016185), and B. C. Bergman (DK-059739).

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