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The effects of short-term overfeeding on insulin action in lean and reduced-obese individuals

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Abstract

Insulin resistance is clearly associated with obesity. However, the role of excess energy intake per se as opposed to increased fat mass in the development of insulin resistance has not been clearly defined. It may be that the nutrient load provided by short-term overfeeding is sufficient to induce measurable changes in insulin action in skeletal muscle and the liver. We examined the effects of 3 days of overfeeding on insulin action and glucose kinetics in 13 lean (body mass index, 20.9 ± 2.4 kg/m^2; 6 men, 7 women) and 9 reduced-obese (RO) (body mass index, 29.1 ± 2.2 kg/m^2; 4 men, 5 women) individuals. A two-step euglycemic hyperinsulinemic clamp study (5 and 40 mU m^{-2} min^{-1}) with a primed, constant infusion of [6,6-$^2H_2$]glucose was performed after 3 days of a weight-maintenance diet and again after 3 days of overfeeding by 50% (50% carbohydrate, 30% fat, 20% protein). At baseline, lean individuals were more insulin sensitive, as measured by glucose infusion rate, than RO individuals (12.08 ± 0.8 vs 7.62 ± 1.0 mg · kg^{-1} · min^{-1}, $P < .01$) with lean women being more insulin sensitive than lean men ($P < .01$). Overfeeding resulted in a reduction in glucose infusion rate in lean women (13.37 ± 1.3 to 11.42 ± 1.0 mg · kg^{-1} · min^{-1}, $P < .05$), but no change was noted in lean men or RO individuals. Basal and insulin-stimulated glucose disposal remained unchanged with overfeeding in all groups. Low-dose insulin suppression of endogenous glucose production was impaired after overfeeding in lean women (euenergetic, 1.92 ± 0.36 to 0.36 ± 0.16 mg · kg^{-1} · min^{-1}; overfeeding: 2.13 ± 0.17 to 0.86 ± 0.12 mg · kg^{-1} · min^{-1}; $P = .04$) but remained unchanged in the other groups. These findings demonstrate that insulin action is reduced in lean, obese-resistant women after short-term overfeeding primarily because of an inhibition of insulin-mediated suppression of endogenous glucose production, whereas short-term overfeeding does not appear to effect insulin action in lean men and RO individuals. This response may be indirectly involved in the ability of lean women to maintain weight in the face of an obesigenic environment.

1. Introduction

The prevalence of obesity has risen dramatically in the United States during the past 20 years. Although genes undoubtedly play an important role in the development of obesity, genetic influences would not be expected to change over such a short period. This suggests that environmental influences are likely playing a significant role in causing this epidemic and that it is likely the interaction of relevant genes with environmental factors that produces the obese state [1,2]. One of the most dramatic changes in the environment during the past 40 years has been the broad availability of relatively inexpensive, highly palatable food [3,4]. It is highly likely that most individuals intermittently experience brief, 1- to 3-day periods of positive energy balance when exposed to the modern Western diet. Why do not all people when exposed to highly palatable food eat in excess and become progressively more obese? The ability of an individual to sense and respond appropriately to these periods of positive energy balance may determine whether a particular individual becomes obese or remains lean. Clearly, endogenous regulatory mechanisms are recruited in response to periods of positive energy balance that help to attenuate weight gain. These could involve reductions in energy intake, increases in energy expenditure, or changes in nutrient metabolism. It may be that obesity resistance is characterized by greater partitioning of dietary nutrients toward skeletal muscle and liver where they may generate a greater "nutrient signal" that allows the individual to respond more accurately to the nutrient load. This nutrient signal may involve changes in insulin sensitivity.

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Although the effects of overfeeding on energy expenditure and substrate oxidation have been examined by a number of groups [5-15], the effects of overfeeding on insulin sensitivity and glucose metabolism have received less attention. Studies of overfeeding have generally used long periods of overfeeding that, in addition to producing changes in energy balance, produce changes in body composition that may have independent effects. In addition, long-term overfeeding may not provide insight into the adaptive processes that occur with more common brief periods of overeating. In addition, few studies published in this area have used the gold standard euglycemic hyperinsulinemic clamp to measure insulin action, and of these studies, none have examined the effects of short-term overfeeding.

We hypothesized that lean individuals who appear to be resistant to weight gain in an obesigenic environment, that is, obese resistant, would respond to 3 days of overfeeding with a reduction in whole-body insulin sensitivity. Specifically, we hypothesized that because of preferential delivery of dietary nutrients to skeletal muscle and liver in obesity-resistant individuals, overfeeding would result in a reduction in insulin-stimulated glucose disposal. In contrast, we reasoned that overfeeding reduced-obese (RO) individuals who are at high risk for weight gain, that is, obese prone, would have a greater tendency to deliver dietary nutrients to adipose tissue and as a result would not change insulin sensitivity in response to short-term overfeeding. Although we did not measure the partitioning of dietary nutrients directly, the effects of short term overfeeding on insulin sensitivity were determined in the present study.

2. Methods

2.1. Subjects

Lean (body mass index [BMI], 19-23 kg/m^2) and obese (BMI, 30-35 kg/m^2) healthy individuals aged 25 to 45 years were recruited and screened. The study was approved by the Colorado Multiple Institutional Review Board, and all subjects gave informed consent. Thirteen lean individuals (7 women, 6 men) and 9 obese individuals (5 women, 4 men) were enrolled into the study (Table 1).

2.2. Weight reduction

Obese individuals entered a weight loss program with a goal weight loss of 10% of their initial body weight. This was accomplished by ongoing supervision and intervention by General Clinical Research Center (GCRC) research dieticians. Actual weight loss was 10.1 ± 3.3 kg or 10.3% ± 0.9% (mean ± SD) of their initial body weight. Once the weight loss was achieved, the RO subjects were maintained at this new reduced weight (within ±2%) with close supervision by the GCRC research dieticians for at least 4 weeks before studies being performed.

2.3. Study design and measurements

Subjects first underwent baseline assessments, including measurements of resting metabolic rate (RMR) and respiratory quotient by hood indirect calorimetry (2900 metabolic cart, Sensormedics, Yorba Linda, CA) and body composition measurement by dual-energy x-ray absorptiometry (DPX whole-body scanner, Lunar Radiation, Madison, WI). Subjects were then started on a euenergetic, weight-maintenance diet for 7 days (days 1-7). Daily energy requirements were estimated as measured RMR times an activity factor of 1.3. Modest alterations in this estimate were made if suggested by 3-day food diaries or values calculated by the Harris-Benedict equation. Subjects were weighed daily and adjustments in the energy content of the diet were made if body weight changed by more than ±1%. Minor adjustments in energy intake were necessary in 2 lean and 2 RO individuals after 1 day of diet, but all subjects were weight stable at the time of the initial metabolic testing. As shown in Table 1, actual energy intake was not different than would have been predicted by the dietary reference intakes prediction equation [16]. On day 8, energy intake was increased by 50% (overfeeding diet) and was continued at this level for 3 days (days 8-10). The macronutrient composition of the diets was kept stable at 50% carbohydrate, 30% fat, and 20% protein. The polyunsaturated to saturated fat ratio (P/S ratio) fiber content, and cholesterol content of the diets were identical. All food was prepared and provided by the GCRC kitchen. Subjects presented to the GCRC every morning. They were weighed, ate breakfast, and picked up the remainder of their daily meals in coolers. Subjects were asked to maintain their usual pattern of physical activity and were regularly questioned regarding activity and compliance. Subjects were asked to not consume any alcoholic or additional energy-containing beverages during the study period.

On days 4 (euenergetic state) and 11 (overfed state), studies of insulin and glucose metabolism were performed after a 12-hour fast. Subjects were admitted to the GCRC the evening before the metabolic studies were performed. The following morning, a standard antecubital venous catheter was placed in 1 arm for infusions. A second catheter was

Table 1
Daily predicted and actual energy intake during euenergetic and overfeeding diet periods

EI (kJ/day)	Lean				Reduced-Obese			
	Women		Men		Women		Men	
	EU	OF	EU	OF	EU	OF	EU	OF
Predicted	7.74 ± 0.17	11.61 ± 0.26	10.43 ± 0.28	15.71 ± 0.43	8.84 ± 0.30	13.28 ± 0.45	11.65 ± 0.33	17.48 ± 0.49
Actual	7.69 ± 0.20	11.43 ± 0.26	10.19 ± 0.38	15.38 ± 0.51	8.40 ± 0.36	12.54 ± 0.55	11.24 ± 0.40	17.07 ± 0.62

Values are mean ± SEM. EI indicates energy intake; EU, euenergetic; and OF, overfeeding.

placed retrograde in a dorsal hand vein of the contralateral arm for sampling, using the heated hand technique to obtain arterialized venous blood [17]. Blood was then sampled for baseline assessments. A primed (19 μmol/kg), constant (0.22 μmol kg^{-1} min^{-1}) infusion of [6,6-$^2H_2$]glucose was used to measure glucose turnover. To examine insulin suppression of endogenous glucose production (EGP), a low-dose infusion of insulin was used. A primed, continuous infusion of insulin at 5 mU m^{-2} min^{-1} was initiated from time 120 to 220 minutes. The insulin infusion was then increased for maximal glucose utilization to 40 mU m^{-2} min^{-1} for an additional 100 minutes to assess peripheral glucose disposal. Blood samples were taken at 90, 100, 110, 200, 210, 220, 300, 310, and 320 minutes for steady-state measurements of metabolites and isotope enrichments. A 20% dextrose solution enriched with [6,6-$^2H_2$] glucose was infused and adjusted to maintain euglycemia at a blood glucose level of approximately 90 mg/dL.

2.4. Laboratory analyses

Plasma glucose level was measured immediately upon sampling during the euglycemic hyperinsulinemic clamp studies via a YSI glucose analyzer (YSI Inc, Yellow Springs, OH) [18]. Baseline and final assessments included blood for insulin, glucose, free fatty acids (FFAs), glycerol, and leptin. Insulin [19] (Clinical Assays Gamma Coat RIA, Kabi Pharmacia, Piscataway, NJ) was determined by radioimmunoassay. Standard enzymatic assays were used to measure FFAs (NEFA Kit, Wako Chemicals, Richmond, VA) and glycerol (Boehringer Mannheim Diagnostics, Amsterdam, The Netherlands).

2.5. Gas chromatography/mass spectroscopy

Glucose isotopic enrichment was measured by using gas chromatography/mass spectrometry (gas chromatograph model 5890 series II and MS model 5989A, Hewlett-Packard, Ramsey, MN). Briefly, 100 μg of [U-^{13}C] glucose was added to 100 μL plasma as an internal standard. Proteins were precipitated by vortexing with 1 mL iced ethanol and then kept at 4°C for 1 hour. Samples were spun in a microcentrifuge for 1 minute 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 hour at 60°C before analysis. Ions with an m/z of 242, 243, 244, and 247 were measured by using selective ion monitoring for the calculation of concentration and enrichment.

2.6. Calculations

Rates of glucose appearance (Ra) and disappearance (Rd) were calculated by using a modified Steele equation [20] as described by Finegood et al [21]. Basal Ra and Rd were calculated during the last 20 minutes of the baseline infusions (between 90 and 110 minutes) by using the following equations:

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

Insulin-suppressed Ra and insulin-stimulated Rd were calculated during the last 20 minutes of each insulin clamp phase. To account for the tracer in the "spiked" dextrose solution, the following equations were used for measurements made during the clamp:

$$Rd = \left[\frac{F}{(E_2+E_1)/2} - pVC_1\left[\frac{(E_2-E_1)/(t_2-t_1)}{(E_2-E_1)/2}\right] + \left\{\frac{Eg}{(E_2+E_1)/2} \times Ginf(t_1)\right\}\right] - \frac{V(C_2-C_1)}{(t_2-t_1)}$$
$$Ra = \left[\frac{F}{(E_2+E_1)/2} - pVC_1\left[\frac{(E_2-E_1)/(t_2-t_1)}{(E_2-E_1)/2}\right] + \left\{\frac{Eg}{(E_2+E_1)/2} \times Ginf(t_1)\right\}\right] - Ginf(t_1)$$

F is the infusion rate of tracer (milligrams per minute), p the percentage of the volume distribution of glucose that is

Table 2
Baseline subject characteristics

	Lean		Reduced obese	
	Women	Men	Women	Men
n	7	6	5	4
Age (y)*	30.6 ± 8.0	29.3 ± 7.6	38.2 ± 8.3	36.5 ± 7.0
Body weight (kg)*,†	54.9 ± 6.1	70.8 ± 7.2	85.3 ± 9.9	92.4 ± 7.0
BMI (kg/m^2)*	20.6 ± 1.8	21.3 ± 3.0	30.4 ± 2.6	27.5 ± 1.8
Fat mass (kg)*,†	14.1 ± 3.6	8.9 ± 2.8	36.1 ± 7.0	22.5 ± 7.6
Lean body mass (kg)*,†	40.9 ± 4.1	61.9 ± 4.4	49.2 ± 6.0	69.9 ± 14.2
RMR (kJ/d)†	5.15 ± 0.46	6.42 ± 0.56	5.78 ± 0.46	6.24 ± 0.63
RQ	0.9 ± 0.09	0.86 ± 0.07	0.87 ± 0.10	0.84 ± 0.08
Glucose (mg/dL)*,†	77.7 ± 4.6	85.7 ± 3.3	86.1 ± 4.0	90.5 ± 6.7
Insulin (μU/mL)*	4.3 ± 1.6	4.2 ± 1.1	6.1 ± 0.8	4.8 ± 1.1
FFAs (μEq/L)	862 ± 84	789 ± 90	747 ± 99	632 ± 111
Glycerol (μmol L^{-1} L^{-1})	63.5 ± 6.5	57.4 ± 7.0	61.8 ± 7.6	61.6 ± 8.6
Leptin (ng/mL)*,†	5.0 ± 1.5	1.2 ± 1.6	18.2 ± 1.8	3.2 ± 2.0

Values are number or mean ± SD. RQ indicates respiratory quotient.

* $P < .05$ for lean compared with reduced obese.

† $P < .05$ for women compared with men.

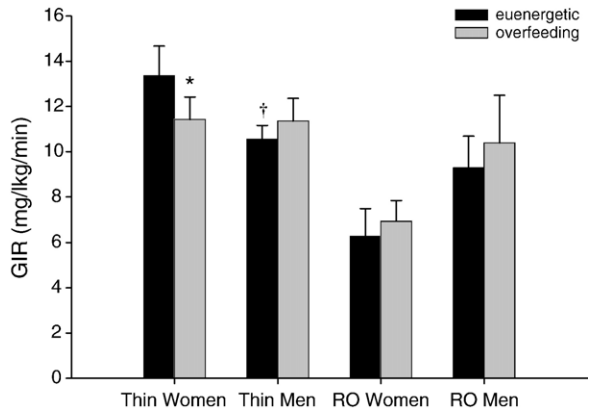

Fig. 1. The effects of overfeeding on insulin-stimulated GIR during a 40 mU m^{-2} min^{-1} insulin infusion. Overfeeding resulted in a reduction in GIR in lean women (*P = .01), whereas no effect was noted in lean men or RO individuals. Lean individuals had significantly greater baseline GIR than RO individuals (P = .002), which was especially apparent within the women (P < .001). Lean women had greater baseline GIR than lean men ($^{\dagger}P$ = .078).

rapidly turning over (50%), V the estimated volume of distribution of glucose (180 mL/kg), Eg the enrichment of the glucose infusate, Ginf(t_1) the rate of infusion of exogenous glucose at time t_1, t_1 the time 1 of sampling, t_2 the time 2 of sampling, C_1 is [glucose] at t_1, C_2 [glucose] at t_2, E_1 the plasma enrichment at t_1, and E_2 the enrichment at t_2. Ra is expressed as milligrams per kilogram per minute, whereas Rd is expressed as milligrams per $kilogram_{lbm}$ per minute.

2.7. Statistical analyses

Unless indicated, data are presented as mean ± SEM. Statistical analyses were done using SAS software (SAS Institute, Cary, NC). Significance tests were 2 sided with significance set at level .05. Linear mixed statistical models with a random subject effect were used to account for repeated measurement of subjects [22]. After considering several mixed models to allow for different variances between groups or sexes, and to allow for more complex correlation structures, the model including simply a random effect for subject to account for repeated measures was found to be sufficient, and results were robust to the choice of model. Examination of residuals indicated normality assumptions were satisfied. Using this model, we estimated least squares means as well as contrasts comparing means for each man/woman by lean/RO group on euenergetic and overfeeding diets. These analyses are similar to calculating the mean change from euenergetic to overfeeding and carrying out simple analyses such as t tests or analysis of variance but adjust for repeated measurement of subjects. Similar analyses were used to examine the overall effects of lean/RO combining sex and of sex combining weight groups. Interactions were used to assess differences among the groups in the change from euenergetic to overfeeding.

3. Results

Subject characteristics are summarized in Table 2. The RO individuals were older and had greater body weight, BMI, and percent body fat compared with the lean individuals. There was also a significant sex difference in body weight, fat mass, and lean body mass. The RO individuals had greater fasting glucose, insulin, and leptin concentrations. There was also a sex difference in glucose and leptin concentrations. Three days of overfeeding did not lead to significant changes in body weight.

The effects of overfeeding on insulin-mediated whole-body glucose uptake as measured by glucose infusion rate

Table 3
The effects of overfeeding on basal and insulin-mediated glucose Ra and Rd

	Glucose Ra		Glucose Rd	
	Basal ($mg \cdot kg^{-1} \cdot min^{-1}$)	Insulin suppressed ($mg \cdot kg^{-1} \cdot min^{-1}$)	Basal ($mg \cdot kg_{lbm}^{-1} \cdot min^{-1}$)	Insulin stimulated ($mg \cdot kg_{lbm}^{-1} \cdot min^{-1}$)
Lean				
Women				
Eucaloric	1.92 ± 0.25	0.36 ± 0.16	2.32 ± 0.55	18.08 ± 2.04
Overfeeding	2.13 ± 0.17	0.86 ± 0.12*	2.99 ± 0.66	16.39 ± 1.44
Men				
Eucaloric	2.25 ± 0.31	0.98 ± 0.15†	2.46 ± 0.51	12.16 ± 0.57†
Overfeeding	2.38 ± 0.47	1.15 ± 0.16	2.98 ± 0.60	12.86 ± 1.06
Reduced Obese				
Women				
Eucaloric	1.40 ± 0.25	0.66 ± 0.15	2.66 ± 0.55	10.19 ± 1.43
Overfeeding	1.64 ± 0.45	0.49 ± 0.16	2.83 ± 0.66	10.81 ± 0.96
Men				
Eucaloric	2.08 ± 0.44	0.78 ± 0.31	2.49 ± 0.62	12.40 ± 1.00
Overfeeding	1.58 ± 0.43	0.64 ± 0.39	1.64 ± 0.74	10.98 ± 1.72

Values are mean ± SEM. Insulin-suppressed glucose production (Ra) was measured during a low-dose (5 mU m^{-2} min^{-1}) insulin infusion. Insulin-stimlulated glucose disposal (Rd) was measured during a high-dose (40 mU m^{-2} min^{-1}) insulin infusion.

* P < .05 for overfeeding compared with euenergetic feeding.

† P < .05 for lean men compared with lean women.

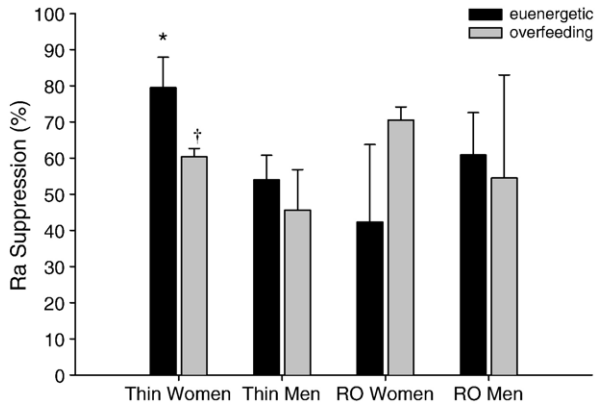

Fig. 2. The effects of overfeeding on insulin-suppressed EGP (Ra) during a low-dose (5 mU m^{-2} min^{-1}) insulin infusion. Lean women had significantly lower baseline insulin-suppressed glucose Ra than lean men (*P = .018). Overfeeding resulted in a dramatic reduction in insulin-mediated suppression of glucose Ra in lean women ($^{\dagger}P$ = .04), whereas no effect was noted in lean men or RO individuals.

(GIR) during the 40 mU m^{-2} min^{-1} insulin infusion are shown in Fig. 1. Steady-state plasma glucose and insulin levels during the hyperinsulinemic clamp studies did not differ between groups or between diet phases (87 mg/dL and 55 μU/mL, respectively). In the euenergetic state, lean individuals were more insulin sensitive than RO individuals (12.08 ± 0.8 vs 7.62 ± 1.0 mg·kg^{-1}·min^{-1}, P = .002), which was especially apparent within women (P < .001). Within lean individuals, women were more insulin sensitive than men (13.37 ± 1.3 vs 10.56 ± 0.6 mg·kg^{-1}·min^{-1}, P < .01). Overall, overfeeding did not alter insulin sensitivity in either lean (12.08 ± 0.8 to 11.40 ± 0.7 mg·kg^{-1}·min^{-1}, P = .27) or RO individuals (7.62 ± 1.0 to 8.47 ± 1.2 mg·kg^{-1}·min^{-1}, P = .19). When examined separately, however, lean women had a significant 15% reduction in GIR with overfeeding (13.37 ± 1.3 to 11.42 ± 1.0 mg·kg^{-1}·min^{-1}, P = .01), whereas no overfeeding effect was noted in lean men (10.56 ± 0.6 to 11.36 ± 1.0 mg·kg^{-1}·min^{-1}), RO women (6.28 ± 1.2 to 6.94 ± 0.9 mg·kg^{-1}·min^{-1}), or RO men (9.29 ± 1.4 to 10.39 ± 2.1 mg·kg^{-1}·min^{-1}).

The effects of overfeeding on endogenous and insulin-suppressed glucose production rates (Table 3) were estimated using the glucose rate of appearance (Ra) in the basal state and during a low-dose (5 mU m^{-2} min^{-1}) insulin infusion. In the euenergetic state, basal Ra was similar across groups and sex. Lean women, however, had lower insulin-suppressed Ra than lean men (0.36 ± 0.16 vs 0.98 ± 0.15 mg·kg^{-1}·min^{-1}, P = .009), which was associated with a greater percent suppression of Ra (79.5% ± 9.6% vs 54.0% ± 7.9%, P = .04). There was no difference, however, between lean and RO groups. Overall, overfeeding did not significantly affect basal Ra; however, insulin suppression of Ra was reduced with overfeeding from 80% to 60% in lean women (euenergetic, 1.92 ± 0.36 to 0.36 ± 0.16 mg·kg^{-1}·min^{-1}; overfeeding, 2.13 ± 0.17 to 0.86 ± 0.12 mg·kg^{-1}·min^{-1}, P = .04), whereas remaining unchanged in the other groups (Fig. 2).

The effects of overfeeding on basal and high-dose (40 mU m^{-2} min^{-1}) insulin-stimulated glucose disposal (Rd) are also shown in Table 3 and are reported in relationship to lean body mass (mg kg_{lbm}^{-1} min^{-1}). In the euenergetic state, lean women had greater insulin-stimulated Rd than lean men (18.08 ± 2.04 vs 12.16 ± 0.57 mg kg_{lbm}^{-1} min^{-1}, P = .005). Lean individuals had significantly greater insulin-stimulated glucose uptake rates compared with RO individuals (15.12 ± 0.91 vs 11.29 ± 1.01 mg kg_{lbm}^{-1} min^{-1}, P = .013), especially when comparing lean with RO women (P < .001). Basal and insulin-stimulated Rd, however, remained unchanged with overfeeding in all groups.

There were no significant differences in basal or insulin-suppressed concentrations of FFAs or glycerol among the groups. As would be expected, overfeeding resulted in an overall reduction in basal FFA and glycerol concentrations (FFA, 774 ± 47 to 516 ± 46 μEq/L; glycerol, 61.1 ± 3.4 to 44.5 ± 3.0 μmol L^{-1} L^{-1}; P < .001) with significantly greater reductions in the men as compared with the women (P < .001). This was associated with a significant reduction in insulin suppression of FFA and glycerol after overfeeding

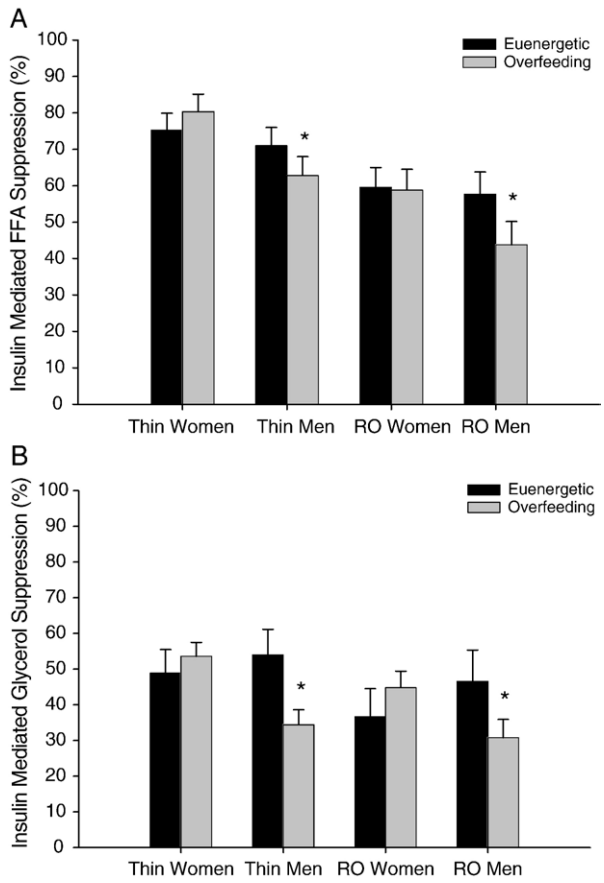

Fig. 3. The effects of overfeeding on insulin suppression of FFA and glycerol concentrations during a low-dose (5 mU m^{-2} min^{-1}) insulin infusion. Both lean and RO men had a significant reduction in insulin suppression of FFA and glycerol after overfeeding (*P < .05).

in the men (FFA, 64.3% ± 3.9% to 53.3% ± 4.1%; glycerol, 51.0% ± 3.0% to 33.0% ± 3.0%), whereas no changes were noted in the women (FFA, 67.5% ± 3.6% to 69.5% ± 3.7%; glycerol, 43.8% ± 2.8% to 50.0% ± 2.8%) as shown in Fig. 3.

4. Discussion

The present study was performed to examine the effects of short-term overfeeding on whole-body insulin action and glucose metabolism in lean and RO individuals. The data demonstrate that lean women respond to overfeeding with a reduction in whole-body insulin sensitivity, which can be primarily explained by relative hepatic insulin resistance. Short-term overfeeding in lean men and RO individuals, however, did not alter whole-body insulin sensitivity. Basal lipolysis appears to be suppressed with overfeeding, being especially dramatic in men. These findings are consistent with the notion that lean women have specific metabolic responses to short-term overfeeding that may promote a return to energy balance.

The present data demonstrate that lean women, who were screened to be resistant to weight gain and obesity, respond to short-term overfeeding with relative whole-body insulin resistance as noted by the reduction in GIR during the euglycemic hyperinsulinemic clamp. This resistance is not accounted for by a reduction in glucose uptake as noted by the Rd data, but by a relative impairment in insulin-mediated suppression of EGP as seen with the Ra data. Overfeeding, however, did not alter peripheral or hepatic insulin action in lean men or RO individuals. Prior studies have found quite variable effects of overfeeding on insulin sensitivity. Although it has been shown in a number of studies that overfeeding results in elevated fasting insulin concentrations in the setting of normal glucose concentrations, a few studies have found no such effects [10,23-28]. It has also been shown that the insulin response to a meal or a glucose load is impaired with overfeeding [23,27,29,30]. These same studies, however, have found that the glucose response to a meal or to a glucose load is unchanged or even reduced. Carbohydrate overfeeding has been shown to increase hepatic glucose output yet inhibit hepatic gluconeogenesis in the setting of increased insulin levels [24], but no study has specifically examined the effects of mixed overfeeding on EGP. Differences in insulin sensitivity in response to long-term overfeeding have not been seen between lean and obese individuals. No other studies have been done using the steady-state conditions of an insulin clamp to measure insulin sensitivity in response to short-term overfeeding and none have examined sex differences.

As would be expected and has been shown in previous studies, basal FFA concentrations were significantly reduced during overfeeding [24,26,28,31]. Although the effects of overfeeding on glycerol metabolism have been found to be variable [23,32], we found that the effects of overfeeding on glycerol levels paralleled the effects on FFA. Although we did not use tracer techniques to determine rates of lipolysis, the finding that both FFA and glycerol concentrations paralleled each other suggest that these effects were due to changes in lipolysis. Interestingly, overfeeding resulted in greater suppression of basal FFA and glycerol in men as compared with women. In addition, insulin suppression of FFA and glycerol was impaired following overfeeding in men only, suggesting development of insulin resistance at the level of adipose tissue in the men.

The mechanisms that underlie these effects of overfeeding on insulin and glucose metabolism are not clear. Endogenous glucose production and insulin-mediated suppression of EGP is thought to be in part modulated by circulating FFA levels [28,33]. Changes in circulating FFA, however, cannot explain the changes in insulin action seen in this study, as FFA levels were overall reduced with overfeeding. It may be that those individuals that deliver more dietary nutrients, especially fat, to liver, skeletal muscle, or even brain may "sense" this nutrient load more effectively, resulting in the induction of insulin resistance and preferential fat oxidation. Activation of nutrient sensors in the liver, for example, may be responsible for the overfeeding effects on EGP seen in thin women.

The present study examined the effects of 3 days of overfeeding, whereas studies of overfeeding have generally used prolonged periods of overfeeding (weeks to months) that produce changes in body composition that may have independent effects. Whereas small positive increments in energy balance for a long period may certainly underlie the development of obesity, short-term overfeeding may also lead to weight gain and obesity [34]. Clinical experience and a limited body of literature suggest that short periods of large positive energy balance that are inadequately compensated for can produce the gradual weight gain seen in many Americans. In fact, we all experience brief periods where energy intake far exceeds energy expenditure for short periods [35]. These brief periods lasting from one meal to several days regularly occur on holidays, periods of celebration, or vacations. Some authors have emphasized the capacity of humans to consume large quantities of food far in excess of daily energy requirements [36]. We think that studies of short-term overfeeding may provide relevant insights into how energy balance is maintained.

A large number of previous studies have examined the mechanisms that underlie the weight gain that is seen in obese and obesity-prone individuals [12,32,37-39]. However, far less attention has been paid to the mechanisms that promote thinness or "obese resistance." Epidemiological evidence suggests that the genetic contribution to the obese-resistant phenotype may be as strong or perhaps even stronger than the genetic contribution to the obese phenotype [40,41]. Bjorntorp [42] and Baghaei et al [43] have begun examining potential mechanisms underlying the maintenance of thinness in an obesigenic environment. It may be that important insights into the most biologically

relevant weight regulatory systems can be gained from studies in lean, obese-resistant individuals.

There are a few limitations to this study that need to be discussed. First, simply finding a difference in insulin sensitivity in response to short-term overfeeding in lean women does not necessarily mean that these differences are responsible for the maintenance of leanness in these individuals. Many other factors that were not studied including differences in physical activity, differences in energy intake, or subtle differences in basal energy expenditure could also play important roles. Second, the choice of "reduced obese" subjects as a model of "obesity proneness" could be criticized. It may be that the reduced state has unique characteristics that are different from preobesity. In addition, these individuals were heavier and older than the lean individuals, which may each have independent effects. It would be extremely difficult if not impossible, however, to "weight reduce" overweight or obese individuals to a comparable BMI to that of very lean individuals. Finally, although the study was powered adequately to examine the effects of overfeeding on changes in whole-body insulin sensitivity between groups, it was not powered to examine sex differences; however, despite this shortcoming, significant sex-overfeeding interactions were found.

In conclusion, these findings demonstrate that 3 days of 50% overfeeding results in relative whole-body insulin resistance in lean, obese-resistant women. This insulin resistance can be explained by resistance of insulin to adequately suppress EGP. On the other hand, short-term overfeeding in lean men and RO individuals, who are at high risk for weight gain and obesity, does not result in changes in whole-body insulin action. Finally, men appear to develop relative adipose tissue resistance to insulin in response to overfeeding, that is, impaired insulin suppression of FFA and glycerol concentrations following overfeeding.

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