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Eight Weeks of Overfeeding Alters Substrate Partitioning Without Affecting Metabolic Flexibility in Men

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Abstract

Background/Objective—Impairments in metabolic flexibility and substrate handling are associated with metabolic syndrome. However, it is unknown whether metabolic inflexibility causes insulin resistance. We therefore measured metabolic flexibility and substrate handling before and after 8 weeks of overfeeding in initially healthy adults, as a model of the early stages of insulin resistance.

Subjects/Methods—Twenty-nine healthy men (27 ± 5 years old; BMI 25.5 ± 2.3 kg/m²) were overfed by 40% above baseline energy requirements for 8 weeks and gained 7.6 ± 2.1 kg of weight. Before and after overfeeding, energy expenditure, substrate oxidation, and metabolic flexibility were measured in 2 ways: a) during 1 day of eucaloric feeding in a whole-room indirect calorimeter, and b) during a two-step hyperinsulinemic-euglycemic clamp.

Results—Eight weeks of overfeeding decreased insulin sensitivity at low and high doses of insulin ($p=0.001$ and $p=0.06$, respectively). This was accompanied by decreases in the respiratory quotient (RQ) while sleeping (0.877 ± 0.020 to 0.864 ± 0.026 ; $p=0.05$) and at low insulin levels during the clamp (0.927 ± 0.047 to 0.907 ± 0.032 ; $p=0.01$). Overfeeding did not affect metabolic flexibility as measured during a clamp ($p=0.17$), but it tended to increase 24-hour metabolic flexibility (awake – sleep RQ) as measured by chamber by 0.010 ± 0.028 ($p=0.08$). In terms of substrate oxidation, overfeeding increased protein oxidation (13 ± 23 g/day; $p=0.003$) and tended to increase fat oxidation (6 ± 16 g/day; $p=0.07$), but did not affect carbohydrate oxidation ($p=0.64$). Individuals with greater metabolic adaptation to overfeeding had higher carbohydrate oxidation rates ($r=0.66$, $p=8 \times 10^{-5}$) but not fat oxidation rates ($p=0.09$).

Conclusions—The early stages of insulin resistance are accompanied by modest declines in the RQs during sleep and during a clamp, with no changes in fasting RQ or signs of metabolic inflexibility. Our data therefore suggest that metabolic inflexibility does not cause insulin resistance.

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Conflicts of Interest Statement:

CMP, BZ, and DLJ have nothing to disclose. ER reports personal fees from Energesis as a consultant, outside the submitted work.

Keywords

overfeeding; overeating; metabolic flexibility; substrate oxidation; carbohydrate; fat; metabolic adaptation

INTRODUCTION

Metabolic flexibility (MF) is the capacity to adjust substrate oxidation rates in response to changes in fuel availability (1). It represents the plasticity in switching between oxidizing fatty acids and carbohydrates. *In vivo*, MF is operationally defined as the increase in respiratory quotient (RQ) between fasting and postprandial states, as measured either in response to a mixed meal or during a hyperinsulinemic-euglycemic clamp (1). Impairments in MF have been reported to occur in obesity (2, 3), insulin resistance and prediabetes (4, 5), and diabetes (6–8). This “metabolic inflexibility” typically manifests itself as both lower fat oxidation in the fasting state (higher fasting RQ) and impaired stimulation of carbohydrate oxidation during feeding (lower postprandial RQ), resulting in a smaller difference in RQ between fasting and feeding (1, 3, 4, 6–10). However, some studies have reported normal RQs during fasting in individuals with obesity or diabetes, with the implication that mostly postprandial substrate oxidation is altered (3, 9, 11).

A related oxidative factor that has been invoked to explain the development of metabolic syndrome is a lower capacity to upregulate fat oxidation in response to fat overload. Such impaired fat oxidation promotes the accumulation of acyl-CoA and its derivatives, leading to lipotoxicity, ectopic fat deposition, and insulin resistance (1, 2, 12–14). For instance, women with obesity who were placed on a high-fat diet were unable to increase fat oxidation and therefore stored more of the excess lipid, relative to lean controls (14). This is supported by other studies reporting impaired fatty acid oxidation in individuals with obesity (3, 15, 16). Moreover, long-term longitudinal studies find that individuals with higher 24-hour RQs (indicative of lower fat oxidation) gain more weight over time, even after controlling for acute energy balance or energy expenditure (17, 18). Thus, the ability to maintain high rates of fat oxidation in the face of positive energy balance (or a high-fat diet) may enable greater metabolic adaptation and help resist body weight gain.

The physiologic mechanisms responsible for these impairments in substrate oxidation have been linked to defects in glucose transport (8, 9, 19, 20), failure of insulin to suppress fatty acid release from adipose tissue (12, 19, 21, 22), lower glucose oxidation rates within skeletal muscle (21), failure to suppress hepatic glucose production (19, 21), and mitochondrial dysfunction (23, 24). However, whether metabolic inflexibility merely reflects the net effect of physiologic changes on fuel availability—or whether it reflects an intrinsic oxidative defect—is unknown. Some studies have shown that adjusting MF for the glucose disposal rate (GDR) (9, 11, 25) or measuring MF at matched GDR (8) causes the differences to vanish between lean individuals and individuals with obesity or diabetes, which suggests that metabolic inflexibility stems mostly from lower rates of glucose transport. Conversely, Færch et al. found that MF is still lower in individuals with prediabetes even after adjusting for both insulin sensitivity and BMI, suggesting instead that metabolic inflexibility may

precede the onset of insulin resistance (5). In addition, Thorburn et al. (26) reported that individuals with diabetes still have lower glucose oxidation rates and consequently higher nonoxidative glucose disposal rates even at matched GDR. Since fat oxidation rates were identical at matched GDR in this study, this suggests that at least part of metabolic inflexibility is driven by impairments in carbohydrate partitioning.

Taken together, it is thus unclear when and how impairments in MF arise, and the degree to which they are intrinsic defects versus reflections of fuel availability. We therefore investigated metabolic flexibility and substrate oxidation in lean, healthy men who were overfed by 40% for 8 weeks, as a model of the early stages of insulin resistance. We hypothesized that after overfeeding, metabolic flexibility and substrate oxidation would be impaired to a larger extent than insulin sensitivity, suggesting that metabolic inflexibility develops prior to the onset of insulin resistance.

SUBJECTS AND METHODS

Participants and Dietary Intervention

This study was approved by Pennington Biomedical Research Center (PBRC)'s institutional review board, registered on clinicaltrials.gov (NCT01672632), and conducted in accord with the Helsinki Declaration of 1975. All participants provided written informed consent prior to enrolling in the study. Primary outcome results have been described in detail (27). Briefly, males and females aged 20–40 years with a BMI between 22.5 and 32.5 kg/m² were eligible to participate. Exclusion criteria included significant medical problems, evidence of chronic disease, and use of certain medications or substances, as previously described (27). Only males (N=29 out of 35 completers) are included in this analysis. Participants were overfed by 40% for 8 weeks (56 days), with metabolic testing performed for 3 days before and after the overfeeding intervention under eucaloric conditions. Prior to baseline testing, participants' energy expenditure was measured over a 2-week period using doubly labeled water (DLW) (28). During the second week, they were fed a eucaloric diet to establish their weight maintenance energy intake (29). The average of the weight maintenance energy intake during the second week and energy expenditure over 2 weeks was multiplied by 1.4 to account for physical activity and then rounded to the closest 419 kJ/day (100 kcal/day) to determine each individual's overfeeding prescription. Meals were prepared by the PBRC metabolic kitchen and were composed of 41% carbohydrate, 15% protein, and 44% fat (40% saturated, 37% monounsaturated, and 23% polyunsaturated fatty acids), yielding a food quotient (FQ=respiratory quotient of the diet) of 0.832. Participants ate all meals (3 per day, 7 days a week) at PBRC under supervision and were not allowed to consume any other food, but were otherwise free living. During the 3 days of post-intervention testing, participants were fed a new eucaloric diet according to their new body weight, using the energy expenditure equation derived in (29). Body composition was measured by DXA (QDR 4500A; Hologic, Bedford, MA).

Respiratory Chamber

Before and after overfeeding, 24-hour energy expenditure and substrate oxidation were measured at thermoneutrality in a whole-room indirect calorimeter (30). Participants entered

the chamber at 8 am, were fed 3 meals and 1 snack, and were not allowed to exercise. Sleep energy expenditure (SEE) and sleep RQ were assessed between 2:00 and 5:00 a.m. and included all minutes during which activity as measured by radar was <1%. Oxygen and carbon dioxide production and 24-hour urinary nitrogen excretion were used to calculate energy expenditure, as well as oxidation of carbohydrate, fat, and protein (31). Since RQ is influenced by energy balance, values were adjusted for energy balance by regressing RQs (24-hour, awake, or sleep) versus energy balance (24-hour energy intake minus energy expenditure). Chamber metabolic flexibility was defined as the difference between the awake RQ and sleep RQ. The thermic effect of food (TEF) was determined by subtracting SEE from the y-intercept of the regression of EE versus % activity, using the data averaged in 15-minute intervals, and was expressed as a percentage of energy intake (32).

Hyperinsulinemic-Euglycemic Clamp

Insulin sensitivity was measured using a two-step hyperinsulinemic-euglycemic clamp. Insulin was infused for 180 min at 10 mU/min m² (low-dose insulin), followed by 150 min at 50 mU/min m² (high-dose insulin). Infusion of a 20% glucose solution was adjusted to maintain plasma glucose at 90 mg/dL. Insulin sensitivity was assessed as the glucose infusion rate (GIR) during the final 30 min of each step of the clamp (steady-state) (33) and was expressed per kg of estimated metabolic weight (fat-free mass + 17.7 kg) (34). Resting metabolic rate and RQ were measured using a DeltaTrac hood indirect calorimeter (Sensor Medics, Yorba, CA) for 30 min fasting and for 30 min during each steady-state period of the clamp. The oxidative component of GIR was calculated as described by Jequier et al (31) while the nonoxidative component was calculated as the difference between total GIR and its oxidative component. Clamp whole-body metabolic flexibility was defined as the difference between the high-dose or low-dose steady-state RQ and the fasting RQ.

Statistical Analyses

Statistical analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC), Excel, and Mathematica 10.0 (Wolfram Research, Champaign, IL). All data are presented as mean ± standard deviation. Changes between baseline and post-overfeeding values were analyzed by paired, two-tailed t-tests or Wilcoxon signed rank tests, depending on the normality of the data as determined using the Shapiro-Wilk test. Associations between RQs or MFs and other metabolic parameters were analyzed using linear regression. For all statistical tests, the false positive rate was set at $\alpha=0.05$. Lastly, correlation analyses were performed among multiple variables using the Bonferroni correction applied to 63 exploratory variables; all other data are non-exploratory and were analyzed for significance without the Bonferroni correction. All p-values in this manuscript are reported as raw (unadjusted) values.

RESULTS

Participant Characteristics

Twenty-nine men aged 27 ± 5 years with a mean BMI of 25.5 ± 2.3 kg/m² completed the 8-week overfeeding protocol, as reported in (27). During the overfeeding phase, participants consumed an average of 17.73 ± 1.97 MJ/day ($4,235 \pm 470$ kcal/day), versus 12.78 ± 1.66 MJ/day ($3,054 \pm 396$ kcal/day) at baseline. As shown in Table 1, the mean weight gain was

7.6 ± 2.1 kg, of which about 55% was fat mass (FM; 4.2 ± 1.4 kg; $p=1 \times 10^{-15}$) and the remaining was fat-free mass (FFM; 3.4 ± 1.5 kg; $p=2 \times 10^{-12}$). These changes were accompanied by increases in visceral adipose tissue ($p=9 \times 10^{-10}$) and total cholesterol ($p=4 \times 10^{-7}$), whereas the increase in intrahepatic lipid was not significant ($p=0.20$). As previously reported (27), insulin sensitivity at low-dose insulin decreased by 18% (2.87 ± 0.94 vs. 2.35 ± 0.07 mg/min/kg; $\Delta = -0.42 \pm 0.65$ mg/min/kg; $p=0.001$), whereas insulin sensitivity at high-dose insulin only trended towards a decrease after 8 weeks of overfeeding (11.51 ± 2.54 vs. 10.91 ± 2.46 mg/min/kg; $\Delta = -0.60 \pm 1.56$ mg/min/kg; $p=0.06$).

Respiratory Quotients and Metabolic Flexibility

RQs were measured both through a one-day stay in a respiratory chamber (chamber RQs; Figure 1A) and using an indirect hood calorimeter before and during a 2-step hyperinsulinemic-euglycemic clamp (clamp RQs; Figure 1B). As shown in Figure 1A, sleep RQ decreased from 0.877 ± 0.020 at baseline to 0.864 ± 0.026 after 8 weeks of overfeeding ($\Delta \text{ RQ} = -0.013 \pm 0.034$; $p=0.05$). By contrast, there were no statistically significant changes in the 24-hour RQ (0.896 ± 0.019 vs. 0.891 ± 0.025 ; $\Delta \text{ RQ} = -0.005 \pm 0.025$; $p=0.29$) or the awake RQ (0.900 ± 0.022 vs. 0.896 ± 0.027 ; $\Delta \text{ RQ} = -0.004 \pm 0.025$; $p=0.39$) in response to overfeeding.

Figure 1B shows the fasting RQ and the RQs measured during low-dose insulin (low ins) and high-dose insulin (high ins) infusions during a 2-step clamp. The fasting RQ was unchanged in response to overfeeding (0.874 ± 0.044 vs. 0.866 ± 0.032 ; $\Delta \text{ RQ} = -0.008 \pm 0.044$; $p=0.34$). During insulin and glucose infusion, the RQ during low-dose insulin infusion was lower by -0.020 ± 0.039 in response to overfeeding (0.927 ± 0.047 vs. 0.907 ± 0.032 ; $p=0.01$). Similarly, the RQ during high-dose insulin infusion tended to be lower by -0.017 ± 0.043 after 8 weeks of overfeeding (0.973 ± 0.046 vs. 0.957 ± 0.039 ; $p=0.08$). Notably, this trend in the high-dose insulin RQ data becomes significant if a single outlier (RQ=0.838; >3 SD from the mean) is excluded (0.979 ± 0.038 to 0.957 ± 0.037 ; $\Delta \text{ RQ} = -0.022 \pm 0.036$; $p=0.01$).

As shown in Figure 1C, overfeeding tended to increase the 24-hour metabolic flexibility (awake RQ – sleep RQ) as measured by respiratory chamber from 0.023 ± 0.022 to 0.033 ± 0.025 ($\Delta \text{ MF} = 0.010 \pm 0.028$; $p=0.08$). As shown in Figure 1A, the increase in 24-hour metabolic flexibility was driven more by decreases in the sleep RQ than by changes in the awake RQ. However, overfeeding did not impact metabolic flexibility as measured by the clamp method (Figure 1D); MF values were unchanged at low insulin (0.054 ± 0.036 vs. 0.040 ± 0.041 ; $\Delta \text{ MF} = -0.014 \pm 0.045$; $p=0.17$) and high insulin (0.098 ± 0.040 vs. 0.089 ± 0.048 ; $\Delta \text{ MF} = -0.009 \pm 0.045$; $p=0.41$) levels.

24-Hour Substrate Oxidation

Figure 2 shows that overfeeding enhanced 24-hour protein oxidation from 94 ± 18 g/day at baseline to 108 ± 20 g/day post-overfeeding ($\Delta = 13 \pm 23$ g/day; $p=0.003$); this increase was still significant after adjusting for increased energy intake based on weight gain ($p=0.05$). Fat oxidation also tended to increase, raising from 45 ± 18 g/day at baseline to 51 ± 19 g/day following overfeeding ($\Delta = 6 \pm 16$ g/day; $p=0.07$), in line with the changes in body weight.

However, 24-hour carbohydrate oxidation remained unchanged (329 ± 46 vs. 335 ± 53 g/day; $\Delta = 6 \pm 52$ g/day; $p=0.64$) in response to overfeeding.

Oxidative and Nonoxidative Glucose Disposal

Previously, we reported that 8 weeks of overfeeding decreased insulin sensitivity at low levels of insulin by 18% ($p=0.001$) and by a marginal 5% ($p=0.06$) at high levels of insulin (27). Here we investigated the differences between oxidative and nonoxidative components of glucose disposal to probe carbohydrate partitioning. As depicted in Figure 3A, the oxidative disposal rate at low-dose insulin declined slightly by -0.16 ± 0.69 mg/min/kg (from 2.30 ± 0.56 to 2.14 ± 0.47 mg/min/kg; $p=0.03$), whereas the nonoxidative disposal rate decreased more substantially by -0.48 ± 0.69 mg/min/kg (from 1.24 ± 0.74 to 0.76 ± 0.66 mg/min/kg; $p=0.01$). However, both the oxidative component (3.18 ± 0.75 vs. 3.05 ± 0.74 mg/min/kg; $\Delta = -0.13 \pm 0.72$ mg/min/kg; $p=0.20$) and nonoxidative component (8.97 ± 1.92 vs. 8.87 ± 1.77 mg/min/kg; $\Delta = -0.10 \pm 1.22$ mg/min/kg; $p=0.69$) of glucose disposal under high-dose insulin concentrations were unchanged (Figure 3B).

Thermic Effect of Food and Thermic Effect of Glucose

We also investigated postprandial thermic responses—namely, the thermic effects of a mixed meal and of glucose. As shown in Figure 4A, the thermic effect of one day of feeding (mixed meals) as measured by respiratory chamber method was unaffected by long-term overfeeding (8.1 ± 4.3 to $9.4 \pm 5.3\%$; $\Delta = 1.3 \pm 5.6\%$; $p=0.27$). Similarly, the thermic effect of glucose as measured during the high-dose insulin stage of the clamp did not change at the end of the 8-week intervention ($3.1 \pm 2.1\%$ vs. $2.6 \pm 3.2\%$; $\Delta = -0.4 \pm 3.2\%$; $p=0.49$) (Figure 4B).

Relationship To Metabolic Adaptation

Lastly, we tested whether alterations in substrate oxidation may be linked to metabolic adaptation, defined here as the increase in sleeping energy expenditure beyond that which can be explained by weight gain (change in $SEE_{\text{measured}} - SEE_{\text{predicted}}$) (35). Metabolic adaptation correlated most strongly with the change in sleep RQ in response to overfeeding ($r=0.66$, 95% CI: 0.38–0.83; $p=8 \times 10^{-5}$). Namely, participants whose sleep RQs remained higher after overfeeding had higher levels of metabolic adaptation. This translated into clear differences in substrate oxidation. Metabolic adaptation correlated with increases in carbohydrate oxidation rates ($r=0.64$, 95% CI: 0.35–0.82; $p=0.0001$; Figure 5A), but not with changes in fat oxidation rates after Bonferroni correction ($p=0.09$; Figure 5B). However, there was no correlation between the percent of body weight gained during overfeeding and either baseline metabolic flexibility as measured by the chamber ($p=0.95$) or the change in metabolic flexibility ($p=0.16$).

DISCUSSION

Mounting evidence links impairments in metabolic flexibility—the ability to switch between oxidizing lipid and carbohydrate—to obesity, insulin resistance, and diabetes. Moreover, nondiabetic adults with higher 24-hour RQs gain more weight than those with lower RQs, even after adjusting for energy balance or energy expenditure (17, 18). Together, this

suggests that alterations in substrate handling may drive weight gain and the development of metabolic syndrome. However, it is unclear whether metabolic inflexibility is a cause or consequence of insulin resistance. We therefore investigated the impact of 8 weeks of 40% overfeeding on insulin sensitivity, metabolic flexibility, and substrate oxidation in 29 healthy men.

Eight weeks of overfeeding with significant weight gain decreased insulin sensitivity and lowered or tended to lower the RQs during a two-stage clamp, but did not change the fasting RQ. The decline in RQ with glucose infusion but not in the fasting state suggests some impairments in postprandial carbohydrate handling. However, the changes in metabolic flexibility as measured by the clamp method did not reach significance, suggesting that insulin resistance takes root before metabolic flexibility starts to decline. This conflicts with the limited data available from other studies. One study reported a decline in metabolic flexibility in adults aged 35–65 after 4 weeks of high-fat overfeeding (36), while a 3-week study in middle-age overweight and obese individuals reported a decrease in metabolic flexibility on a eucaloric high-fat diet but no changes in insulin sensitivity (37). One reason for the discrepancy may be that we investigated the change in metabolic flexibility in significantly younger, initially non-insulin resistant individuals, rather than older and/or more overweight individuals. The compensatory metabolic responses may therefore differ by age and/or health status. A second reason may be that we measured metabolic flexibility under eucaloric conditions, rather than in response to a high-calorie or high-fat challenge.

It is important to realize that measuring metabolic flexibility via the clamp method induces a non-physiologic, steady state condition wherein plasma glucose is normal but insulin levels are held at high constant levels, thus suppressing circulating free fatty acids. It is worth considering whether metabolic flexibility should be instead measured under daily physiologic conditions—that is, measured by respiratory chamber—over the course of a normal day and including all feeding episodes. Such conditions avoid the confounding factor of different glucose disposal rates during a clamp and avoid non-physiologically high levels of insulin. Moreover, the clamp method of measuring metabolic flexibility misses out on the dynamic “real-world” nature of substrate oxidation, including non-steady state conditions and night-day differences in substrate oxidation, which may be affected by sleep-wake cycles and/or the circadian clock. For example, we should not assume that substrate oxidation at night while subjects are asleep is the same as when they are awake and fasting during the daytime. Indeed, in our study, whole-body metabolic flexibility measured by the chamber method tended to improve, rather than to decrease, with overfeeding. The increase in 24-hour metabolic flexibility was driven by a decrease in the sleep RQ, implying increased fat oxidation at night. Interestingly, we found that overfeeding affected only the sleep RQ but not the awake RQ. Individuals with a family history of diabetes are known to have a lower sleep RQ but not awake RQ than GDR-matched control subjects (23), so a change in substrate oxidation during sleep may be a hallmark of the first stages of insulin resistance.

In the present study, we also found that the early stages of insulin resistance appear to be driven more by declines in nonoxidative glucose metabolism, than by changes in oxidative metabolism. Consistent with this observation, Felber et al. reported that the declines in both

components of glucose disposal in obese adults were due to higher fat oxidation rates during insulin infusion (i.e., a lower RQ) (38). They hypothesized that hyperglycemia accompanying insulin resistance may serve to compensate for these defects in nonoxidative disposal (38). This explanation is plausible, as Thorburn et al. showed that when the GDR is raised to match that of nondiabetic controls, adults with diabetes have higher nonoxidative disposal and similar fat oxidation rates (26).

We also investigated how long-term overfeeding altered 24-hour substrate oxidation rates. Protein oxidation was modestly increased, as reported in other overfeeding studies in initially lean healthy adults (39–41), even after adjusting for increased energy requirements. Fat oxidation tended to be higher, but this difference did not reach statistical significance. However, carbohydrate oxidation in response to a eucaloric diet was not significantly different from baseline. Previous studies have reported that in response to a hypercaloric challenge, shifts in substrate oxidation are dominated by the need to maintain carbohydrate balance, due to its limited storage capacity (reviewed in (41)). Increases in carbohydrate intake are therefore buffered by almost equal increases in carbohydrate oxidation, even at carbohydrate excesses of up to roughly 30–50% of daily energy expenditure, before net *de novo* lipogenesis becomes physiologically important (14, 40–46). Whereas carbohydrate intake stimulates its own oxidation (44), several studies have shown that fat intake does not stimulate its own oxidation—at least not in the short term in lean healthy adults (14, 43, 47, 48). For instance, two studies found that 50% carbohydrate overfeeding increased carbohydrate oxidation about two-fold to match carbohydrate intake, and this change was accompanied by an increase in energy expenditure, whereas 50% fat overfeeding increased fat oxidation rates by only ~20% with no change in energy expenditure (43, 46).

Because carbohydrate balance is tightly regulated whereas fat balance is not, it has thus been proposed that obesity may be driven by a failure to achieve fat balance (38, 42). Indeed, studies in humans report that energy balance strongly correlates ($r=0.72-0.96$) with fat balance in lean adults, but not with carbohydrate or protein balance (47, 49). However, we found no evidence supporting the hypothesis that metabolic adaptation is linked to higher fat oxidation rates. Instead, our data suggest that metabolic adaptation is mediated through increases in carbohydrate oxidation, not fat oxidation. Other overfeeding studies have hinted at similar results, finding that the increase in CHO oxidation comes at the expense of a decrease in fat oxidation (40, 41, 43, 45, 46, 50).

In sum, we found that the early stages of insulin resistance in initially healthy individuals are accompanied by modest declines in RQ during sleep and during a clamp. However, metabolic flexibility is unchanged and may even be higher when measured during a 24-hour stay in a respiratory chamber. Importantly, this suggests that impairments in insulin sensitivity and alterations in substrate oxidation—particularly during sleep and feeding—appear before declines in metabolic flexibility become apparent.

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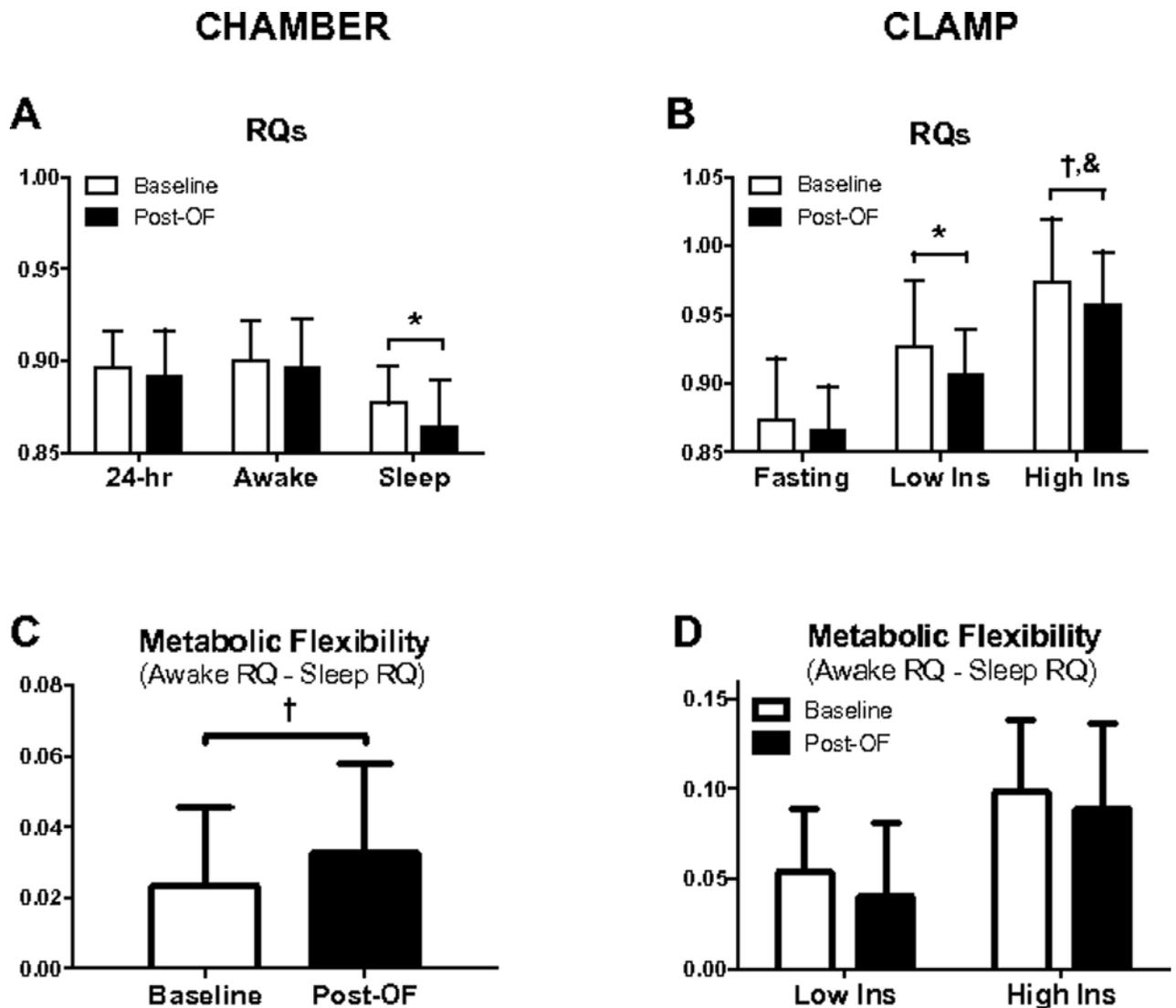


Figure 1.

(A) Overfeeding (Post-OF) decreased sleep RQ ($p=0.05$), whereas the 24-hour ($p=0.29$) and awake ($p=0.39$) RQs remained unchanged relative to baseline. (B) Overfeeding also lowered the RQ in response to low-dose insulin (low ins) during a clamp ($p=0.01$) and tended to lower the RQ during high-dose insulin (high ins) infusion ($p=0.07$), but the change in fasting RQ was not significant ($p=0.34$). (C) Metabolic flexibility as measured by respiratory chamber (awake RQ – sleep RQ) tended to be higher following overfeeding ($p=0.08$). (D) Metabolic flexibility as measured by clamp (RQ during insulin infusion – fasting RQ) was unchanged ($p=0.17$ and $p=0.41$, respectively). * $P < 0.05$. † $P < 0.10$. & Result becomes significant after the removal of a single > 3 SD outlier.

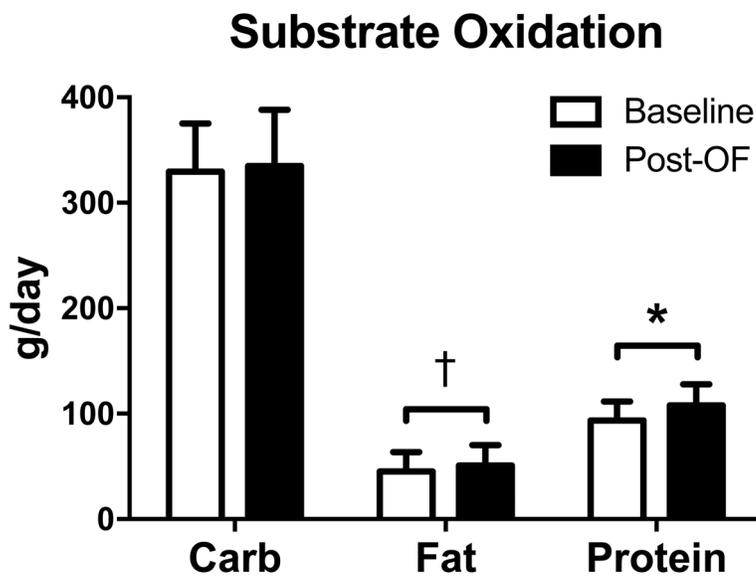


Figure 2. Long-term overfeeding increased 24-hour protein oxidation ($p=0.003$) and similarly tended to increase fat oxidation ($p=0.07$) in response to 1 day of eucaloric feeding, but carbohydrate oxidation was unaltered ($p=0.64$). * $P < 0.05$. † $P < 0.10$.

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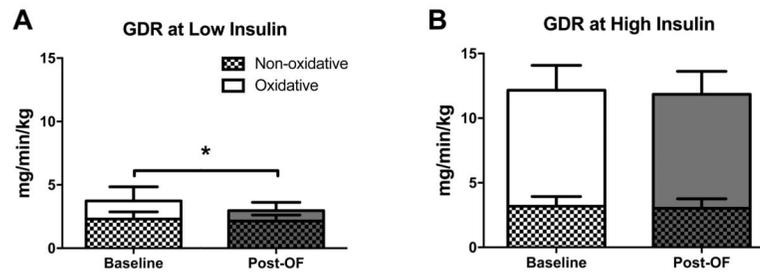


Figure 3.

(A) Overfeeding slightly decreased nonoxidative glucose disposal ($p=0.03$) and substantially decreased oxidative glucose disposal ($p=0.01$) at low-dose insulin infusion during a clamp.

(B) Both oxidative ($p=0.20$) and nonoxidative ($p=0.69$) glucose disposal were not significantly changed at high-dose insulin. * $P < 0.05$.

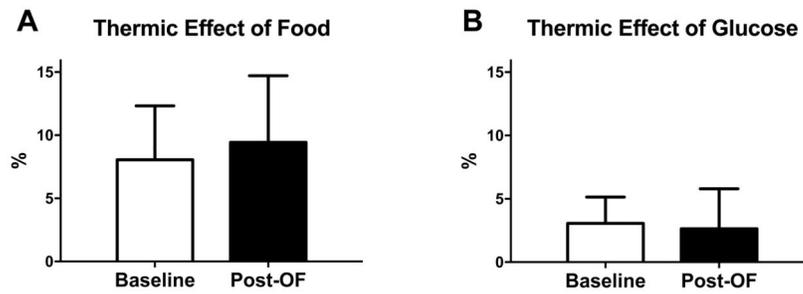


Figure 4.

Eight weeks of overfeeding did not increase (A) the thermic effect of food (1 day of eucaloric feeding of mixed meals), as measured in a respiratory chamber ($p=0.27$), or (B) the thermic effect of glucose in response to high-dose insulin and glucose infusion during a clamp ($p=0.49$).

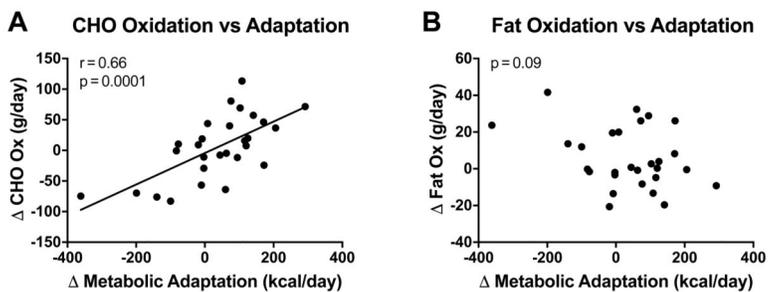


Figure 5. (A) Individuals with higher levels of metabolic adaptation (defined as the change in the difference between measured and predicted values of sleeping energy expenditure) had larger increases in their carbohydrate oxidation rates ($p=0.66$). (B) Changes in fat oxidation rates did not correlate with metabolic adaptation to overfeeding after Bonferroni correction ($p=0.09$).

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Table 1Participant Characteristics (N=29). Data are mean \pm SD.

	Baseline	Change	P Value
Demographics			
Age (yrs)	27 \pm 5	-	-
Weight (kg)	81.9 \pm 10.3	7.6 \pm 2.1	1 \times 10 ⁻¹⁶ *
BMI (kg/m ²)	25.5 \pm 2.3	2.3 \pm 0.7	2 \times 10 ⁻¹⁶ *
% Body Fat	19.4 \pm 4.9	2.9 \pm 1.2	5 \times 10 ⁻¹⁴ *
FFM (kg)	65.9 \pm 7.3	3.4 \pm 1.5	2 \times 10 ⁻¹² *
FM (kg)	16.0 \pm 4.8	4.2 \pm 1.4	1 \times 10 ⁻¹⁵ *
Fat Depots			
Visceral adipose tissue (kg)	0.58 \pm 0.49	0.36 \pm 0.19	9 \times 10 ⁻¹⁰ *
Intra-hepatic lipid (%)	1.5 \pm 3.2	0.7 \pm 2.8	0.20
Cardiovascular Disease Risk Factors			
Systolic Blood Pressure (mm Hg)	114 \pm 7	2 \pm 7	0.24
Diastolic Blood Pressure (mm Hg)	71 \pm 6	1 \pm 5	0.12
Heart Rate (beats/min)	66 \pm 9	2 \pm 7	0.13
Total Cholesterol (mg/dl)	171 \pm 25	25 \pm 20	4 \times 10 ⁻⁷ *
LDL (mg/dl)	99 \pm 23	21 \pm 17	6 \times 10 ⁻⁶ *
HDL (mg/dl)	55 \pm 12	2 \pm 7	0.20
Triglycerides (mg/dl)	87 \pm 42	9 \pm 55	0.47
Carbohydrate Metabolism			
Fasting Glucose (mg/dl)	91 \pm 7	2 \pm 6	0.12
Fasting Insulin (mU/l)	5.4 \pm 4.0	0.5 \pm 2.6	0.35
GIR at low insulin (mg/kg/min)	2.87 \pm 0.94	-0.42 \pm 0.65	0.001 *
GIR at high insulin (mg/kg/min)	11.51 \pm 2.54	-0.60 \pm 1.56	0.06
Energy Metabolism			
24-hour EE (kcal/24 hr)	2211 \pm 156	134 \pm 152	7 \times 10 ⁻⁵ *
SEE (kcal/24 hr)	1786 \pm 157	106 \pm 139	0.0004 *
Physical Activity (kcal/24 hr)	235 \pm 107	-9 \pm 97	0.62

*
P < 0.05