Published in final edited form as:

Appl Physiol Nutr Metab. 2013 May ; 38(5): 512–519. doi:10.1139/apnm-2012-0266.

Rapid development of systemic insulin resistance with overeating is not accompanied by robust changes in skeletal muscle glucose and lipid metabolism

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Abstract

Prolonged overeating and the resultant weight gain are clearly linked with the development of insulin resistance and other cardiometabolic abnormalities, but adaptations that occur after relatively short periods of overeating are not completely understood. The purpose of this study was to characterize metabolic adaptations that may accompany the development of insulin resistance after 2 weeks of overeating. Healthy, nonobese subjects ($n = 9$) were admitted to the hospital for 2 weeks, during which time they ate ~ 4000 kcal $\cdot$ day $^{-1}$ (70 kcal $\cdot$ kg $^{-1}$ fat free mass $\cdot$ day $^{-1}$). Insulin sensitivity was estimated during a meal tolerance test, and a muscle biopsy was obtained to assess muscle lipid accumulation and protein markers associated with insulin resistance, inflammation, and the regulation of lipid metabolism. Whole-body insulin sensitivity declined markedly after 2 weeks of overeating (Matsuda composite index: 8.3 ± 1.3 vs. 4.6 ± 0.7 , $p < 0.05$). However, muscle markers of insulin resistance and inflammation (i.e., phosphorylation of IRS-1-Ser³¹², Akt-Ser⁴⁷³, and c-Jun N-terminal kinase) were not altered by overeating. Intramyocellular lipids tended to increase after 2 weeks of overeating (triacylglyceride: 7.6 ± 1.6 vs. 10.0 ± 1.8 nmol $\cdot$ mg $^{-1}$ wet weight; diacylglyceride: 104 ± 10 vs. 142 ± 23 pmol $\cdot$ mg $^{-1}$ wet weight) but these changes did not reach statistical significance. Overeating induced a 2-fold increase in 24-h insulin response (area under the curve (AUC); $p < 0.05$), with a resultant $\sim 35\%$ reduction in 24-h plasma fatty acid AUC ($p < 0.05$). This chronic reduction in circulating fatty acids may help explain the lack of a robust increase in muscle lipid accumulation. In summary, our findings suggest alterations in skeletal muscle metabolism may not contribute meaningfully to the marked whole-body insulin resistance observed after 2 weeks of overeating.

Keywords

overfeeding; insulin sensitivity; triglycerides

Introduction

Abundant high-caloric food and a sedentary lifestyle have contributed to the increased prevalence of obesity and obesity-related diseases, such as cardiovascular disease and diabetes. Insulin resistance, hyperlipidemia, and high ectopic fatty acid accumulation in skeletal muscle and liver are just a few of the metabolic abnormalities common in obesity. Although the metabolic abnormalities in obesity are reasonably well defined, the metabolic responses during the early stages of weight gain are far less clear. Improved understanding of the metabolic changes that occur in response to overeating may shed light on the development of obesity-related metabolic complications, even before meaningful weight gain.

In the first few days of overeating (i.e., ~5 days) fasting plasma insulin concentration has been found to increase significantly (Adochio et al. 2009; Brøns et al. 2009), which provides a crude assessment of systemic insulin resistance. However, in these same studies, measures of peripheral insulin resistance did not change (Adochio et al. 2009; Brøns et al. 2009). Because nearly all insulin-mediated peripheral glucose uptake is directed to skeletal muscle, these findings suggest that the very early stages of overeating (~5 days) may not provide a great enough perturbation to impair skeletal muscle insulin action. In animal models of overfeeding, mice have been reported to retain skeletal muscle insulin sensitivity after 2 weeks of overeating, despite a profound reduction in hepatic insulin sensitivity and a considerable increase in fat mass (Kleemann et al. 2010). However, whether the same is true in the first 2 weeks of overeating and weight gain in human subjects is not clear.

Ectopic lipid accumulation in skeletal muscle and liver can contribute to insulin resistance through the accumulation of fatty acid intermediates such as diacylglycerides (DAG) (Yu et al. 2002; Kumashiro et al. 2011). Accumulation of fatty acid intermediates can result in the activation of inflammatory factors, such as c-Jun-N-terminal kinase (JNK), that reduce insulin signaling (Hirosumi et al. 2002). Lipid can accumulate in the liver in response to overeating even before a significant increase in body weight (Sobrecases et al. 2010), but it is not clear if skeletal muscle responds in a similar manner.

The primary aim of this study was to characterize systemic metabolic alterations that occur in response to 2 weeks of overeating in healthy nonobese adults. An additional objective of this study was to assess the effects of overeating on skeletal muscle lipid metabolism.

Materials and methods

Subjects

Nine healthy, nonobese subjects (7 men and 2 women; initial body mass index, 23.9 ± 0.3 kg·m⁻²; age, 24 ± 1 years) participated in a 2-week overfeeding study. All subjects were determined to be healthy by a comprehensive medical exam prior to participation in this study and were not taking any medications or participating in any regular physical activity or exercise program (<2 h of physical activity per week, based on self-report). All subjects provided written, informed consent prior to participation in this study. All procedures of this study were approved by the Institutional Review Board of the University of Michigan.

General study design

We conducted a battery of metabolic tests before (Baseline) and after a 2-week overeating period. During the Baseline trial, subjects were admitted to the Michigan Clinical Research Unit of the University of Michigan Hospital for an overnight stay. A meal tolerance test (MTT) was administered at 0900 h, with an overnight fasting blood sample obtained immediately before the meal, and additional samples collected every 20 min for 2 h after the meal for assessment of plasma glucose and insulin concentrations. Subjects were instructed to consume the entire meal as quickly as tolerable. The meal consisted of 10 kcal·kg⁻¹ body weight, 50% carbohydrate, 15% protein, and 35% fat. In addition to the blood samples collected during the MTT, we also collected blood samples every 2 h for 24 h to assess the 24 h profile for plasma concentrations of insulin, nonesterified fatty acid (NEFA), and triacylglyceride. In addition to the meal ingested for the MTT, subjects were also provided 2 additional meals (at 1200 and 1800 h) to maintain energy balance. The total caloric intake was 41 ± 1.5 kcal·kg⁻¹ fat-free mass (FFM) for the Baseline trial. Subjects remained in the hospital room overnight and the next morning, after an overnight fast, resting metabolic rate (RMR) was measured via indirect calorimetry (Vmax Encore, Sormedics, San Diego, Calif., USA) at 0600 h, and then a muscle biopsy sample was obtained from the vastus lateralis at 0800 h. Muscle samples were cleaned in sterile saline and immediately frozen in liquid nitrogen and stored at -80 °C until further analysis. Body composition was determined by Dual-Energy X-ray Absorptiometry (DEXA) scan and then the subjects were released from the hospital.

Approximately 1 week after the Baseline trial, subjects were re-admitted to the hospital for the entire duration of a 2-week overeating intervention (i.e., subjects did not leave the hospital during this 2-week period). During this 2-week overeating period, subjects consumed ~4000 kcal·day⁻¹ (70 kcal·kg⁻¹ FFM·day⁻¹; 50% carbohydrate, 35% fat, and 15% protein), and were limited to 1500 steps·day⁻¹ as monitored by a pedometer. The daily diet was provided as 3 meals (at 0800, 1200, 1900 h) and 4 snacks (at 1000, 1400, 1600, 2200 h).

On the morning of the 13th day of the overeating period, subjects performed a MTT after an overnight fast. The meal ingested during this MTT was identical, in both composition and quantity of calories, to the one administered during the MTT of the Baseline trial to make a more direct comparison of meal glucose tolerance in response to an identical carbohydrate load. Also starting in the morning of the 13th day of overeating, blood samples drawn every 2 h for 24 h to reassess plasma profiles of insulin, NEFA, and triacylglyceride concentrations. The next morning, RMR was measured again via indirect calorimetry and a muscle biopsy sample was obtained after an overnight fast as described above during the Baseline trial. Body composition was again determined by DEXA. After completion of all of the procedures on the 14th day of their hospital stay, the subjects were discharged.

Analytical procedures

Plasma substrate and insulin concentrations—Standard commercial kits were used to measure plasma concentrations of glucose (glucose oxidase method, Thermo Scientific, Middletown, Va., USA), NEFA (Wako Chemicals USA, Richmond, Va., USA), and triacylglyceride (Sigma, St. Louis, Mo., USA). Plasma insulin concentration was measured by radioimmunoassay (human insulin-specific RIA; Millipore, Billerica, Mass., USA). Cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by chemiluminescent assays (Siemens, Frimley, Camberley, UK). C-reactive protein measurement was performed using the Human C-Reactive Protein (CRP) Elisa Kit (Invitrogen, Fredrick, Md., USA).

Intramycellar triacylglyceride (IMTG) and DAG—Frozen muscle samples were rapidly homogenized in ice-cold saline and lipids then extracted (Bligh and Dyer 1959). Internal lipid markers for IMTG, DAG, monoacylglycerol, NEFA, phospholipids, and cholesterol esters with fatty acid moieties of odd carbon number were added at the start of extraction, for subsequent purity and recovery determinations (NuChek, Elysian, Minn., USA; Avanti Polar Lipids, Alabaster, Ala., USA). Processed samples were applied to a hexane-equilibrated, aminopropyl solid phase extraction cartridge (Supelco, St. Louis, Mo., USA), and individual lipid species were eluted (Bodenec et al. 2000). Fatty acid methyl esters (FAMES) were generated by alkaline methanolysis (Bodenec et al. 2000), and the resultant FAMES were measured by gas chromatography and electron-impact mass spectrometry (Agilent 6890A GC and 5973 N MSD, Palo Alto, Calif., USA).

Immunoblot analysis—Cytosolic and crude membrane fractions of muscle homogenates were used for electrophoresis analysis of protein contents in muscle using standard immunoblotting techniques (Burnette 1981). The blots were developed with enhanced chemiluminescence (Amersham Biosciences, Piscataway, N.J., USA) and the bands for the proteins of interest were quantified using AlphaEaseFC (AlphaInnotech, San Leandro, Calif., USA). Blots were probed with the following antibodies: α -DGAT1 (cat. no. NB110-41487; Novus Biologicals, Littleton, Colo., USA), α -GPAT1 (a gift from Dr. Rosalind Coleman), SCD1 (cat. no. SCD11-A; Alpha Diagnostic International), SREBP-1c (cat. no. sc366; Santa Cruz Biotechnology, Santa Cruz, Calif., USA), α -CD36/FAT (cat. no. 9154; Santa Cruz Biotechnology), α -ATGL (cat. no. 2138; Cell Signaling Technology, Danvers, Mass., USA), α -HSL (cat. no. 4107; Cell Signaling Technology), α -p-HSL Ser660 (cat. no. 4126; Cell Signaling Technology), α -p-HSL Ser563 (cat. no. 4139; Cell Signaling Technology), and α -p-HSL Ser565 (cat. no. 4137; Cell Signaling Technology).

Calculations

Homeostatic model assessment of insulin resistance (HOMA-IR)—HOMA-IR was calculated by the following equation: $\text{HOMA-IR} = (\text{fasting plasma insulin} \times \text{fasting plasma glucose})/k$; where k represents a constant 405 (Matthews et al. 1985).

Matsuda Composite Index of Insulin Sensitivity—Matsuda Composite Index of Insulin Sensitivity was calculated using the following equation: $10\,000/[(\text{fasting plasma insulin concentration} \times \text{fasting plasma glucose concentration}) \times (\text{mean plasma insulin concentration} \times \text{mean plasma glucose concentration})]^{1/2}$; where mean plasma glucose and insulin concentrations are the average plasma glucose and insulin during the 2-h meal tolerance test (Matsuda and DeFronzo 1999). Although the hyperinsulinemic-euglycemic clamp technique is the gold-standard for measuring insulin sensitivity, the Matsuda Composite Index has been found to compare very favorably with the clamp procedure (Matsuda and DeFronzo 1999). Furthermore, it should be noted that the Matsuda index was derived for use with an oral glucose tolerance test, but it has been found to be equally as effective with a meal tolerance test (Selimoglu et al. 2009).

Resting metabolic rate—Resting metabolic rate was calculated using the rates of carbon dioxide production and oxygen uptake (measured via indirect calorimetry after an overnight fast), and the equation of Weir (1949).

Area under the curve (AUC)—AUC was calculated using the trapezoidal rule with zero as the Baseline.

Statistical analysis—Paired Student's t tests was used to determine significant changes after overeating compared with Baseline. A 2-way ANOVA with Tukey's post hoc analysis

was used to assess differences in 24-h plasma concentrations of insulin, NEFA, and triacylglyceride, before and after overeating. α level was set to 0.05 to distinguish statistical significance. All statistical analysis was performed using SigmaPlot software for Windows version 11.0. All data are presented as means $\pm$ SE.

Results

Weight gain, body composition, and RMR with overeating

By the end of the 2-week overeating period, subjects gained an average of 2.4 ± 0.6 kg in body weight ($p < 0.05$). The majority of this weight gain was fat mass (1.6 ± 0.3 kg; $p < 0.05$), and there was no significant change in fat-free mass. Two weeks of overeating tended to increase RMR slightly ($p = 0.09$). Summary data for body weight, body composition, and RMR for our subjects before and after the overeating are provided in Table 1.

Markers of insulin sensitivity

Although fasting plasma glucose concentration was well maintained after 2 weeks of overeating (Table 2), fasting plasma insulin concentration doubled ($p < 0.05$; Table 2). As a result, HOMA-IR was also significantly elevated after overeating ($p < 0.05$; Table 2). Impaired insulin action after 2 weeks of overeating was also confirmed by calculating the Matsuda Composite Index during the meal tolerance test ($p < 0.05$; Fig. 1). Despite this rapid development in whole-body insulin resistance with overeating, our findings suggest that this 2-week exposure to overeating may not have impaired skeletal muscle insulin action. Elevated (noninsulin-stimulated) basal phosphorylation of insulin receptor substrate-1 (IRS-1) at serine residue 312 (p-IRS-1 Ser³¹²) (Aguirre et al. 2000) and markers of increased activation of intracellular inflammatory pathways, such as increased phosphorylation of JNK (p-JNK) (Hirosumi et al. 2002; Masharani et al. 2011) are known to be tightly associated with insulin resistance. Here we found basal p-IRS-1 Ser³¹² and p-JNK in skeletal muscle were both unaffected by overeating (Fig. 2). Similarly, elevated basal phosphorylation of Akt (p-Akt Ser⁴⁷³) in skeletal muscle has also been linked with insulin resistance (Liu et al. 2009), yet the phosphorylation of Akt at Ser⁴⁷³ in skeletal muscle remained unchanged after overeating (Fig. 2).

Intramyocellular lipids

Although we found a trend for an increase in IMTG concentration after 2 weeks of overeating (7.6 ± 1.6 vs. 10.0 ± 1.8 nmol·mg⁻¹ wet weight), this increase did not quite reach statistical significance ($p = 0.11$). Similarly, accumulation of DAG in skeletal muscle also did not increase significantly (104 ± 10 vs. 142 ± 23 pmol·mg⁻¹ wet weight; $p = 0.26$). In concert with the IMTG and DAG data, the protein abundance of the lipid synthesizing enzymes glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerides acyltransferase (DGAT) also did not change in skeletal muscle with overeating (Fig. 3). Protein abundance of lipolytic enzymes adipose triacylglyceride lipase (ATGL) and hormone sensitive lipase (HSL) in skeletal muscle did not change with overeating and the phosphorylation state of HSL associated with augmented lipolytic activity (p-HSL Ser⁵⁶³ Ser⁶⁶⁰) and suppression of lipolytic activity (p-HSL Ser⁵⁶⁵) were also not different after the overeating period (Fig. 4). Finally, we found that neither membrane-bound nor total protein abundance of the key fatty acid transporter, FAT/CD36, were altered by overeating (Fig. 3).

Plasma concentrations of insulin, NEFA, and blood lipid profiles

Overeating resulted in a chronic elevation in plasma insulin concentration throughout the day (Fig. 5A) and 24 h plasma insulin concentration AUC nearly doubled (311 ± 34 μ U·mL⁻¹·h⁻¹ vs. 601 ± 93 μ U·mL⁻¹·h⁻¹, at Baseline and 2 weeks, respectively, $p < 0.05$). In

conjunction with this chronic hyperinsulinemia, overeating suppressed plasma NEFA concentration throughout much of the day—this was especially evident in the overnight period (Fig. 5B)—and 24 h plasma NEFA AUC was suppressed by about 35% (487 ± 70 mmol·L⁻¹·min⁻¹ vs. 317 ± 47 mmol·L⁻¹·min⁻¹ at Baseline and 2 weeks, respectively, $p < 0.05$). Plasma NEFA concentration after an overnight fast was also reduced after overeating (Table 2). In contrast, overeating did not affect plasma triacylglyceride concentration after an overnight fast (Table 2), but plasma triacylglyceride concentrations were elevated for much of the day while overeating compared with Baseline (Fig. 5C). Additionally, 2 weeks of overeating increased plasma total cholesterol concentration (152 ± 10 mg·dL⁻¹ vs. 177 ± 11 mg·dL⁻¹, at Baseline and 2 weeks, respectively, $p < 0.05$). The increase in total cholesterol was due to both an increase in HDL-cholesterol (39 ± 2 mg·dL⁻¹ vs. 47 ± 4 mg·dL⁻¹, $p < 0.05$) and LDL-cholesterol (97 ± 10 mg·dL⁻¹ vs. 117 ± 11 mg·dL⁻¹), but this increase in LDL-cholesterol did not quite reach statistical significance ($p = 0.06$).

Hepatic and plasma inflammatory markers

Compared with Baseline levels, 2 weeks of overeating doubled the plasma concentrations of ALT (16.1 ± 2.2 IU·L⁻¹ vs. 35.1 ± 5.8 IU·L⁻¹, $p < 0.05$), and also significantly increased AST concentration (19.3 ± 1.3 IU·L⁻¹ vs. 24.5 ± 1.7 IU·L⁻¹, $p < 0.05$). Despite these elevations in markers of liver inflammation, both AST and ALT concentrations remained in the “normal” range after overfeeding for all subjects. Additionally, 2 weeks of overeating did not significantly affect plasma CRP concentration (0.5 ± 0.2 mg·L⁻¹ vs. 0.7 ± 0.2 mg·L⁻¹, Baseline vs. 2 weeks, respectively $p = 0.52$).

Discussion

One of the major findings of this study was that despite the increase in whole-body insulin resistance after 2 weeks of over-eating, we did not find evidence for changes in markers of insulin signaling in skeletal muscle. Therefore, our findings support the notion that in the early stages of caloric excess and weight gain, insulin responsive tissues other than skeletal muscle (e.g., liver) may develop insulin resistance in advance of metabolic alterations in muscle that can lead to impaired insulin action. Additionally, the hyperinsulinemia observed throughout the day while overeating, because of the large and frequent meals, likely had important metabolic consequences in our subjects.

Our finding that even relatively short-term exposure to overeating can induce systemic insulin resistance agrees with previous studies (Faeh et al. 2005; Brøns et al. 2009; Tam et al. 2010). Systemic insulin resistance can develop as a consequence of reduced insulin action in 1 or more of the key insulin-responsive tissues (e.g., skeletal muscle, liver, adipose tissue). In frank diabetes, it is common for all of these insulin-responsive tissues to be markedly resistant to the effects of insulin in regulating glucose uptake (skeletal muscle and adipose tissue) and glucose production (liver) (Korenblat et al. 2008). However, the progressive alterations in insulin resistance that occur in the early stages of weight gain are not clearly understood. Although we did not directly measure insulin sensitivity in skeletal muscle in our study, we did find that key markers associated with impaired muscle insulin action remained unaffected after 2 weeks of overeating. For example, in the basal state (noninsulin-stimulated condition) we found no changes in the phosphorylation of IRS-1 Ser³¹², Akt Ser⁴⁷³, and JNK, all of which have been previously found to be associated with impaired insulin action (Aguirre et al. 2000; Hirosumi et al. 2002; Liu et al. 2009; Masharani et al. 2011). We interpret these indirect findings to suggest that insulin signaling in skeletal muscle in the fasting state may not be affected in the early stages of weight gain. In contrast, the liver may be more susceptible to the development of insulin resistance in the earlier stages of weight gain (Brøns et al. 2009). Kleemann et al. (2010) assessed the

progressive sequence of the development of insulin resistance in liver, white adipose tissue, and skeletal muscle in mice on a high-fat diet, and found that liver was the first to develop insulin resistance, followed by adipose tissue. Results from human studies also support the hypothesis that insulin resistance may first emerge in the liver. Brøns et al. found increased hepatic insulin resistance after 5 days of overeating using a hyperinsulinemic–euglycemic clamp (Brøns et al. 2009). Additionally, other investigators have found that overeating significantly increased fasting insulin (Adochio et al. 2009) and HOMA-IR (Brøns et al. 2009; Tam et al. 2010), which are believed to largely reflect hepatic insulin sensitivity (Muniyappa et al. 2008).

If overeating does induce the development of insulin resistance in liver and adipose tissue in advance of skeletal muscle, it is possible that metabolic alterations in liver and adipose tissue may contribute the eventual development of impaired insulin action within skeletal muscle that occurs with more prolonged overeating and greater weight gain. In obesity, the relationship between altered adipose tissue metabolism and the development of skeletal muscle insulin resistance is well established (Ferrannini et al. 1983; Ivy et al. 1988). For example, the excessive fatty acid mobilization from adipose tissue found in obesity is known to be a key contributor to the development of impaired insulin sensitivity in muscle (Schenk et al. 2009). Additionally, skeletal muscle insulin resistance in obesity is also linked with an increased systemic inflammatory status resulting from alterations in the secretion of cytokines from adipose tissue and liver (Stienstra et al. 2011). Our findings that neither fatty acid concentration nor our marker of increased systemic inflammation (i.e., CRP) were elevated after our 2-week overeating intervention suggests that perhaps a more prolonged energy surplus, and (or) a greater gain in body fat are required to induce an appreciable change in systemic factors known to impair insulin action in muscle. Indeed, a slightly longer period of overeating has been found to augment systemic inflammation; Tam et al. (2010) found increased plasma concentration of CRP, as well as monocyte chemotactic protein (MCP-1) in response to 1 month of overeating, which was accompanied by peripheral (i.e., skeletal muscle) insulin resistance. Similarly, a more prolonged period of overeating that results in a greater accrual of fat mass and the suppression of the potent antilipolytic effects of insulin thereby augmenting systemic fatty acid availability (Faeh et al. 2005) can suppress insulin action in skeletal muscle.

Because our participants ate ~150 g of fat per day, we anticipated we would find a relatively large lipid accumulation within skeletal muscle, perhaps comparable to what we (Li et al. 2011) and others (Goodpaster et al. 1997) commonly report in obesity. However, in contrast to our expectations, we did not find a robust increase in the concentration of either muscle triacylglyceride or diacylglyceride. Previous work from our lab (Li et al. 2011) and others (Thrush et al. 2009) suggest that fatty acid supply for esterification within skeletal muscle, rather than increased lipogenic capacity, is largely responsible for the accumulation of muscle lipids. After meals, most of the dietary fat is delivered to tissues as triacylglycerides packaged within circulating lipoproteins (i.e., chylomicrons), and indeed we found pronounced hyperlipidemia throughout much of the day in our subjects. Lipoprotein lipase (LPL), which resides on the luminal surface of the capillaries within skeletal muscle and adipose tissue, is the enzyme responsible for catalyzing the hydrolysis of the triacylglycerides, liberating the meal-derived fatty acids that may be taken up by the local tissues. Muscle LPL activity has been reported to be suppressed by insulin (Lithell et al. 1978; Kiens and Richter 1998), which provides an attractive explanation for our finding that muscle lipid accumulation did not increase despite abundant postprandial plasma triacylglycerides (i.e., the chronic hyperinsulinemia in our subjects may have inhibited muscle LPL, thereby limiting the availability and uptake of meal-derived fatty acids within skeletal muscle). However, the regulation of muscle LPL activity and the distribution of meal-derived fat into skeletal muscle appear to be more complicated than this. Conflicting

evidence suggests that insulin may have no effect on skeletal muscle LPL activity (Yost et al. 1995) or may actually increase it (Ruge et al. 2009). Additionally, although the uptake of meal-derived fat into skeletal muscle has been reported to be negligible by some investigators (Jensen et al. 2003a), others have reported measurable extraction and storage of meal fat into skeletal muscle (Bickerton et al. 2007; Ruge et al. 2009; Labbé et al. 2011). In contrast to these equivocal reports in skeletal muscle, it is generally accepted that insulin augments adipose tissue LPL activity (Sadur and Eckel 1982; Sadur et al. 1984; Semenkovich et al. 1989). This increase in adipose tissue LPL combined with the insulin-mediated reduction in intra-cellular lipolysis (Burns et al. 1979) results in a large fatty acid gradient across the adipocyte membrane, favoring uptake of a substantial proportion of meal-derived fatty acids into adipose tissue (Evans et al. 2002; Jensen et al. 2003b; Labbé et al. 2011). We surmise that although some of the meal-derived lipids were likely being extracted by skeletal muscle in our study, the vast majority of the meal-derived fat was likely extracted and stored in adipose tissue. Albumin-bound NEFA are also a very important substrate for the synthesis of muscle lipids (Coppack et al. 1999), and plasma NEFA concentration was suppressed throughout the day and night with overeating in our study, likely a consequence of the potent antilipolytic effects from the persistent elevation in plasma insulin concentration with large and frequent meals. Together, this chronically low plasma NEFA availability in combination with the likelihood that the vast majority of meal-derived fatty acids were being directed into adipose tissue and perhaps liver (see below), rather than skeletal muscle, help explain why we did not find a robust increase in muscle lipid accumulation in our study. We cannot rule out the possibility that overeating did increase muscle lipid synthesis, but lipids did not accumulate in muscle because the increased synthesis rate was matched by a similar increase in the rate of lipolysis. Our measurements of proteins involved in the synthesis (i.e., DGAT and GPAT) and degradation (i.e., ATGL, HSL) of muscle lipids do not support the notion that overeating evoked cellular adaptations to alter muscle lipid turnover. However, our measurements were limited to assessments of protein abundance of the synthesizing enzymes and the abundance and phosphorylation–activation state of the lipolytic enzymes, so our findings are not conclusive.

A substantial amount of meal-derived fat is also taken up by the liver (Labbé et al. 2011). Once taken up by liver, the major fates of these fatty acids include being oxidized for energy, esterified into triacylglycerides for intrahepatic storage, or the esterified fatty acids may be released into the systemic circulation within very-low-density lipoproteins. Jensen et al. reported that nearly 50% of dietary fat is oxidized within 24 h after ingestion (Jensen et al. 2003a), and because the subjects in their study were on bed rest after the meals, skeletal muscle energy expenditure would have been quite low, so a high proportion of this oxidation was likely occurring in the liver. In general, hepatic fatty acids that are not oxidized are esterified into triacylglycerides, and over-feeding has been reported to increase hepatic triacylglyceride storage in humans (Sobrecases et al. 2010). However, this increase in hepatic lipid content is not solely attributed to the uptake and subsequent storage of dietary fat, *de novo* lipogenesis can also contribute, especially in response to excessive dietary carbohydrate intake (Minehira et al. 2004; Faeh et al. 2005). Hepatic steatosis has been linked with rather severe hepatic insulin resistance (Gastaldelli et al. 2007; Korenblat et al. 2008). We did not directly measure hepatic lipid content, but we did find that plasma AST and ALT increased significantly, which are commonly used clinically as crude markers of hepatic steatosis (Shah et al. 2009; Rodriguez-Hernandez et al. 2010). One week of overeating a diet similar to ours (i.e., high in both carbohydrate and fat) has been found to induce a 2- to 3-fold increase in hepatic lipid content, which was accompanied by a 70% increase in plasma ALT concentration (Sobrecases et al. 2010). Although the significant elevations we observed in AST and ALT with overeating in our study did not increase into the “abnormal” range, the notable increase in these markers of steatosis and liver damage,

suggest that our brief overeating intervention was likely compromising normal liver function.

Our observation that RMR (in the overnight fasted state) tended to increase after 2 weeks of overeating is consistent with findings from many other overeating studies (Forbes et al. 1986; Diaz et al. 1992; Tremblay 1992). In many cases, the higher RMR after overeating and weight gain has been accompanied by an increase in FFM, and this increase in metabolically active tissue could account for the elevated resting energy expenditure. However, FFM did not increase significantly with overeating in our relatively short overfeeding intervention. Therefore, our findings suggest that overeating augmented energy-requiring metabolic processes independently of any change in the accrual of more metabolically active tissue. The chronic hyperinsulinemia may have contributed to the increased in RMR in our participants. For example, insulin can increase Na^+/K^+ ATPase activity (Sweeney et al. 1998), and the energy required by ion pumps to maintain resting membrane potential is known to be a major contributor to resting energy expenditure (Gregg and Milligan 1980, 1982; Lyon et al. 1995). Consistent with the potential role of this ion pump to contribute to the increased resting energy expenditure found in our study, overfeeding has been found to increase the activity of Na^+/K^+ ATPase in the liver and skeletal muscle of mice (Flier et al. 1981). Other factors that may contribute to the increase in RMR that we found with overeating include the process of de novo lipogenesis (Griffin and Sul 2004), changes in sympathetic nervous system activity (O'Dea et al. 1982; Gentile et al. 2007) and other the neuroendocrine responses to overeating (Gautron and Elmquist 2011).

An important aspect of our study design was that in addition to the very high daily energy intake, our subjects were also tremendously inactive (~1500 steps per day). While this was not bed rest, it is certainly possible that the relative inactivity contributed to some of the metabolic adaptations we observed. It is important to note that our subjects were all very sedentary before their participation in our study. Our eligibility criteria required they not participate in structured physical activity programs and they accumulate <2 h of physical activity per week; most did not engage in any planned physical activity at all and they did not have active lifestyles or physically demanding occupations. Therefore, while 1500 steps·day⁻¹ was likely less than our subjects' habitual daily physical activity levels, the magnitude of the reduction in physical activity below their norm was far less than if we had recruited highly active participants. Along these lines, a recent study that parallels our study rather well enrolled habitually active adults (i.e., >10 000 steps·day⁻¹) to dramatically reduce their daily physical activity for 2 weeks (i.e., to ~1500 steps·day⁻¹) without overeating (Krogh-Madsen et al. 2010). Very interestingly, they found that the marked reduction in physical activity induced a reduction in peripheral (i.e., skeletal muscle) insulin action, and markers for impaired insulin signaling in skeletal muscle, without evoking hepatic insulin resistance (Krogh-Madsen et al. 2010), which was basically the opposite of the adaptations we report here. Therefore, it appears that even a relatively brief period of physical inactivity has particularly profound metabolic consequences within skeletal muscle of people who are habitually very active, but without overeating and without weight gain this relatively brief reduction in physical activity does not affect liver insulin action. Complementary findings from our study logically suggest that the effects of physical inactivity on skeletal muscle metabolism may not be as evident in people who live habitually sedentary lives, and it was likely the energy surplus (i.e., overeating) in our study that was largely responsible for the whole-body insulin resistance we observed. Nonetheless, we acknowledge that we cannot definitively distinguish between the effect of overeating from the effects of low physical activity, and our findings must be interpreted as such.

Overall, the present findings expand our understanding about metabolic adaptations that occur in the early stages of overeating. We found that just a few days of overeating suppressed insulin sensitivity at the whole body-level, but this was not accompanied by changes in markers of impaired insulin action in skeletal muscle, supporting findings from animal studies that indicate insulin resistance may develop first in other tissues, such as liver and adipose tissue. Even this relatively short exposure to overeating elevated markers of hepatic steatosis and abnormal liver function (i.e., liver enzymes AST and ALT). We were surprised to find that despite eating ~150 grams of dietary fat (~2-fold greater than most dietary recommendations), we did not find a robust increase in skeletal muscle lipid accumulation. From our indirect assessments, we surmised that a lipid may not have accumulated in muscle as a consequence of (i) the majority of meal-derived triacylglycerides (packaged within chylomicrons) may have been preferentially channeled toward storage in adipose tissue, and (ii) the chronically low NEFA concentration limited substrate availability for esterification and storage in muscle. Our findings suggest the chronic (i.e., 24-h) hyperinsulinemia that occurs with overeating may play an important role in regulating changes in substrate metabolism and resting energy expenditure, even in advance of substantial weight gain.

Acknowledgments

This work would not be possible without the expert technical assistance from Kathleen Symons and the efforts of our research dietitians and study coordinators, Lisa Locke Michael and Suzette Howton. This work was supported by National Institutes of Health (NIH) Grant R01 DK71955, with additional support from the Michigan Clinical Research Unit (NIH-UL1RR024986), and the Human Phenotyping Core of the Michigan Nutritional Obesity Research Center (P30-DK-089503).

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Fig. 1. The Matsuda composite index of insulin sensitivity measured during a meal tolerance test before and after 2 weeks of overeating. *, Significantly different from Baseline, $p < 0.05$.

Fig. 2.

(A) Protein abundance of phosphorylated proteins in the insulin signaling pathway: insulin receptor substrate-1 (IRS-1) phosphorylated at Serine 312, c-Jun N-terminal kinase (JNK) phosphorylated at Tyrosine 185, and Akt phosphorylated at Serine 472 from skeletal muscle biopsy samples obtained after an overnight fast. (B) Representative blots.

Fig. 3.

(A) Protein abundance of triacylglyceride synthesis enzymes glycerol-phosphate acyltransferase (GPAT), diacylglyceride acyltransferase (DGAT), and stearoyl-CoA desaturase-1 (SCD-1), transcription factor for lipid metabolism-related genes stearoyl response element binding protein-1a (SREBP-1a), and abundance of fatty acid transporter CD36 (FAT/CD36) on the crude membrane fraction (total abundance of FAT/CD36 did not change) measured in skeletal muscle biopsy samples obtained after an overnight fast before and after 2 weeks of overeating. (B) Representative blots before and after overeating.

Fig. 4.

(A) Protein abundance of lipolytic enzymes adipose triacylglyceride lipase (ATGL) and hormone sensitive lipase (HSL) measured in skeletal muscle biopsy samples obtained after an overnight fast before and after 2 weeks of overeating. Phosphorylation of HSL at Serine 565 (Ser⁵⁶⁵) is associated with an inhibition of lipolytic activity whereas phosphorylation at Serine residues 563 and 660 (Ser⁵⁶³ and Ser⁶⁶⁰) is associated with augmented lipolytic activity. (B) Representative blots.

Fig. 5. (A) Plasma insulin concentration, (B) plasma non-esterified fatty acid (NEFA) concentration, and (C) plasma triacylglyceride concentration over 24 h before and after 2 weeks of overeating. *, Significantly different from Baseline at that time, $p < 0.05$.

Table 1

Subject characteristics before and after 2 weeks of overeating.

	Baseline	2 wk
Body weight (kg)	75.0±2.6	77.1±2.7*
BMI (kg·m ²)	23.9±0.3	25.2±0.3*
Body fat percentage (%)	26.4±2.9	27.5±2.5*
Fat mass (kg)	19.8±2.4	21.2±2.0*
Fat free mass (kg)	55.2±2.7	55.9±2.8
Resting metabolic rate (kcal·kg ⁻¹ FFM·d ⁻¹)	28.2±0.5	29.4±0.9 [†]

Note: Values are means ± SE. BMI, body mass index; FFM, fat-free mass.* Significantly different from Baseline, $p < 0.05$.[†] $p = 0.09$ compared with Baseline.

Table 2

Plasma substrate and insulin concentrations before and after 2 weeks of overeating.

	Baseline	2 wk
Fasting plasma glucose (mg·dL ⁻¹)	86±3	83±2
Fasting plasma insulin (μU·mL ⁻¹)	6.6±0.9	12.1±3.1 *
HOMA-IR	1.4±0.2	2.6±0.7 *
Fasting plasma NEFA (mmol·L ⁻¹)	0.39±0.02	0.18±0.02 *
Fasting plasma triacylglyceride (mmol·L ⁻¹)	0.97±0.13	1.16±0.23

Note: Values are means ± SE. HOMA-IR, homeostatic model assessment of insulin resistance; NEFA: nonesterified fatty acid.* Significantly different from Baseline, $p < 0.05$.