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Weight Loss Reduces Liver Fat and Improves Hepatic and Skeletal Muscle Insulin Sensitivity in Obese Adolescents

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Obesity in adolescents is associated with metabolic risk factors for type 2 diabetes, particularly insulin resistance and excessive accumulation of intrahepatic triglyceride (IHTG). The purpose of this study was to evaluate the effect of moderate weight loss on IHTG content and insulin sensitivity in obese adolescents who had normal oral glucose tolerance. Insulin sensitivity, assessed by using the hyperinsulinemic–euglycemic clamp technique in conjunction with stable isotopically labeled tracer infusion, and IHTG content, assessed by using magnetic resonance spectroscopy, were evaluated in eight obese adolescents (BMI ≥95th percentile for age and sex; age 15.3 ± 0.6 years) before and after moderate diet-induced weight loss (8.2 ± 2.0% of initial body weight). Weight loss caused a 61.6 ± 8.5% decrease in IHTG content (P = 0.01), and improved both hepatic (56 ± 18% increase in hepatic insulin sensitivity index, P = 0.01) and skeletal muscle (97 ± 45% increase in insulin-mediated glucose disposal, P = 0.01) insulin sensitivity. Moderate diet-induced weight loss decreases IHTG content and improves insulin sensitivity in the liver and skeletal muscle in obese adolescents who have normal glucose tolerance. These results support the benefits of weight loss therapy in obese adolescents who do not have evidence of obesity-related metabolic complications during a standard medical evaluation.

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INTRODUCTION

The prevalence rate of childhood obesity has more than tripled in the last 30 years (1) and is likely responsible for the marked increase in obesity-related metabolic complications observed in the pediatric population, such as impaired oral glucose tolerance, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD) (2–5). Obese adolescents are particularly prone to metabolic derangements because of decreased insulin sensitivity that occurs during puberty (6–8). We have recently found that NAFLD is an important marker of insulin resistance in obese adolescents, and that obese children with NAFLD have impaired insulin action in both liver and skeletal muscle compared with obese children who are matched on age, sex, Tanner stage, BMI, and percent body fat (9).

In adults, moderate weight loss decreases intrahepatic triglyceride (IHTG) content and improves insulin action in liver and skeletal muscle (10–12). Weight loss also improves surrogate measures of whole-body insulin sensitivity, derived from fasting plasma glucose and insulin concentrations and oral glucose tolerance testing, in obese adolescents (13–18). These studies, however, did not provide information on the effects of weight loss on IHTG content and insulin action in specific organs.

The purpose of the present study was to evaluate the effect of moderate weight loss on IHTG content, basal glucose kinetics, and insulin sensitivity in skeletal muscle and liver in obese adolescents. Magnetic resonance spectroscopy was used to determine IHTG content, and stable isotopically labeled tracer infusion in conjunction with the hyperinsulinemic–euglycemic clamp technique were used to assess basal substrate kinetics and insulin action in liver and skeletal muscle before and after weight loss.

METHODS AND PROCEDURES

Study subjects

Subjects were recruited from outpatient clinics of St Louis Children's Hospital, local pediatric offices, and our current database of eligible volunteers. Eight obese adolescents (BMI ≥95th percentile for age and sex; 15.3 ± 0.6 years old; Tanner stage 4.4 ± 0.3; 7 boys, 1 girl; 6 whites and 2 African Americans) participated in this study. These subjects represent a subset of subjects whose baseline metabolic data were previously reported as part of another study (9).

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All subjects underwent a medical evaluation, which included a history and physical examination, 2-h oral glucose tolerance test, and blood tests. Subjects who had impaired fasting glucose concentration, diabetes, severe hypertriglyceridemia (>300 mg/dl), or history of liver disease other than NAFLD were excluded. None of the subjects consumed alcohol, smoked tobacco products, or took medications that are known to cause hepatic steatosis or to affect glucose or lipid metabolism.

The study was approved by the Human Research Protection Office and the General Clinical Research Center Advisory Committee of Washington University School of Medicine in St Louis, MO. All subjects agreed to participate in the study after a detailed explanation of the study was provided to them and their parents. Written informed consent was obtained from each subject's parent(s), and written informed assent was obtained from each subject before enrollment in the study.

Body composition assessments

Total body fat mass and fat-free mass (FFM) were determined by using dual-energy X-ray absorptiometry (Delphi W densitometer equipped with version 12.4 software; Hologic, Waltham, MA) (19). Total abdominal, subcutaneous abdominal, and intra-abdominal fat volumes were determined by using magnetic resonance imaging with a 1.5 T scanner (Siemens, Iselin, NJ). Eight 10-mm-thick axial images were obtained beginning at the L4-L5 interspace and analyzed for subcutaneous and intra-abdominal fat content by using Analyze 6.0 software (Mayo Foundation, Biomedical Imaging Resource, Rochester, MN); the volume of fat was calculated for each slice and the values were added. IHTG content was determined by using proton magnetic resonance spectroscopy with a 1.5 T scanner (MAGNETOM Sonata; Siemens, Erlangen, Germany) (20).

Hyperinsulinemic–euglycemic clamp

A one-stage hyperinsulinemic–euglycemic clamp procedure was performed 1–2 weeks after assessment of body composition. The female subject was studied during the follicular phase of her menstrual cycle. Subjects were instructed to continue their regular diet, to abstain from exercise for 3 days before the study, to avoid caffeine for 1 day before the study, and to fast (except for water) for 12 h before their admission to the General Clinical Research Center at 0600 hours on the morning of the study. At 0700 hours, a catheter was inserted into an antecubital vein to infuse stable isotopically labeled glucose tracer, insulin, and dextrose. Another catheter was inserted into a contralateral hand vein, which was heated to 55 °C by using a thermostatically controlled box, to obtain arterialized blood samples (21). At 0800 hours, after a baseline blood sample was obtained to determine the background plasma glucose tracer-to-tracee ratio (TTR), a primed, continuous infusion of [6,6-$^2H_2$]glucose (priming dose: 22.5 μmol/kg; infusion rate: 0.25 μmol/kg/min), dissolved in 0.9% NaCl solution, was initiated and maintained for 360 min. At 180 min, the hyperinsulinemic–euglycemic clamp procedure was started and continued for 180 min. Insulin was infused at a rate of 40 mU/m^2/min (initiated with a two-step priming dose of 160 mU/m^2/min for 5 min, followed by 80 mU/m^2/min for 5 min). Dextrose (20%) was infused at a variable rate to maintain plasma glucose concentration at 100 mg/dl. The dextrose solution was enriched with [6,6-$^2H_2$]glucose to ~2.5% to minimize changes in plasma glucose TTR during the clamp procedure (22). The infusion rate of [6,6-$^2H_2$]glucose was decreased by 50% (to 0.125 μmol/kg/min) during the clamp procedure (from 180 to 360 min) to account for the expected decline in hepatic glucose production (9,23). Blood samples were taken every 10 min during the last 30 min of the basal period and the clamp procedure to determine plasma glucose and insulin concentrations and glucose TTR. Blood samples were taken every 10 min between 190 and 360 min to monitor glucose concentration. After the clamp procedure was completed, subjects were given a regular meal. The dextrose infusion was tapered and stopped and subjects were discharged from the General Clinical Research Center when blood glucose concentrations were stable for at least 1 h after stopping the dextrose infusion.

Weight loss program

Subjects received individual behavioral therapy sessions with a psychologist who has considerable experience in obesity therapy for adolescents. The principles of the behavior modification program were adapted from the Healthy Habits weight loss program developed for this age group to set behavioral goals for reducing calorie intake and gradually increasing physical activity (24). Parents participated in the initial session, and their continuing involvement was flexible based on the adolescent's age, the family's preference, and at the psychologist's discretion. Adolescents were encouraged to self-monitor and maintain records of their food and beverage intake and physical activity; they were provided self-monitoring forms and a copy of *The Calorie King® Calorie Fat & Carbohydrate Counter* (25) to assist with estimating calorie and fat intake. At each meeting, adolescents were weighed, and the link between their weight change and energy-balance behaviors was addressed. Adolescents were encouraged to gradually reduce their caloric intake to ~1,200–1,500 kcal/day. Specific dietary recommendations were individualized, based on the adolescent's eating pattern and dietary preferences.

When subjects lost at least 5% of their body weight and were weight stable for at least 4 weeks, body composition analyses and the hyperinsulinemic–euglycemic clamp procedure performed before weight loss therapy were repeated.

Sample analyses

Plasma glucose concentration was determined by using an automated glucose analyzer (YSI 2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentration was measured by radioimmunoassay (Linco Research, St Louis, MO). Plasma free fatty acid (FFA) concentrations were quantified by using gas chromatography (HP 5890 Series II GC; Hewlett-Packard, Palo Alto, CA) after adding heptadecanoic acid to plasma as an internal standard (26).

Plasma glucose TTR was determined by using electron impact ionization gas chromatography–mass spectrometry (Agilent Technologies/HP 6890 Series GC System–5973 Mass Selective Detector; Hewlett-Packard) as previously described (27). After forming the heptafluorobutyryl derivative of glucose, plasma glucose TTR was determined by selectively monitoring ions at *m/z* 519 and 521.

Calculations

Metabolic and isotopic steady states were achieved during the last 30 min of the basal period (between 150 and 180 min) and the hyperinsulinemic–euglycemic clamp procedure (between 330 and 360 min). Therefore, total (endogenous and exogenous) glucose rate of appearance (Ra) in plasma during basal conditions and the hyperinsulinemic–euglycemic clamp procedure was calculated by dividing the glucose tracer infusion rate by the average plasma glucose TTR between 150 and 180 min (basal) and 330 and 360 min (clamp). Basal endogenous glucose Ra was calculated by using Steele's equation for steady-state conditions (28). It was assumed that glucose rate of disappearance from plasma was equal to total glucose Ra in the steady state.

Hepatic insulin sensitivity was assessed by using the hepatic insulin sensitivity index, which is the inverse of the product of the basal hepatic glucose production rate (in μmol/kg FFM/min) and fasting plasma insulin concentration (in mU/l) (29,30). Skeletal muscle insulin sensitivity was determined as the increase in glucose rate of disappearance during insulin infusion (9).

Statistical analysis

Data were tested for normality, and Student's *t*-test for paired samples (for normally distributed variables) or Wilcoxon's signed-rank test (for non-normally distributed variables) was used to evaluate differences before and after weight loss. Relationships between variables of interest were assessed by using Pearson's (for normally distributed variables) or Spearman's (for non-normally distributed variables) correlation coefficients. An analysis of variance with repeated measures was used to

Table 1 Body composition of study participants before and after weight loss

	Baseline	After weight loss	Percent change
Body weight (kg)	106.3 ± 7.4	96.8 ± 5.4*	−8.2 ± 2.0
BMI (kg/m^2)	35.7 ± 1.4	32.2 ± 1.0**	−9.6 ± 1.6
BMI (*z*-score)	2.4 ± 0.2	2.1 ± 0.3***	−12.8 ± 2.4
Fat-free mass (kg)	65.0 ± 6.2	62.5 ± 5.5	−3.1 ± 1.9
Fat mass (kg)	39.1 ± 1.9	32.9 ± 1.7**	−8.5 ± 1.8
Fat mass (%)	40.3 ± 2.4	35.0 ± 2.7**	−12.6 ± 4.9
Total abdominal fat (cm^3)[a]	4,997 ± 230	4,382 ± 443	−13.1 ± 5.4
Subcutaneous abdominal fat (cm^3)[a]	3,837 ± 190	3,487 ± 361	−10.0 ± 6.3
Intra-abdominal fat (cm^3)[a]	1,071 ± 171	744 ± 139*	−31.3 ± 8.5
Intrahepatic triglyceride content (%)	17.5 ± 6.0	5.3 ± 2.3**	−61.6 ± 8.5

Values are means ± s.e.m.
[a]Data represent values obtained from seven of the eight subjects because technical problems precluded obtaining images of abdominal fat in one subject.
Value significantly different from baseline value, *P < 0.05, **P = 0.01, ***P = 0.001.

evaluate the statistical significance of differences in glucose rate of disappearance during basal conditions and insulin infusion between the two time points. A two-tailed P value ≤0.05 was considered statistically significant. Statistical analyses were performed by using SPSS (version 13.0; SPSS, Chicago, IL). All data are presented as means ± s.e.m.

RESULTS

Characteristics and body composition of the study participants

Subjects lost 8.2 ± 2.0% body weight and 9.6 ± 1.6% BMI during the weight loss therapy program, which resulted in a concomitant decrease in fat mass and intra-abdominal fat volume, but no significant change in FFM (Table 1). In addition, IHTG content decreased by >60% with weight loss (P = 0.01). The decrease in IHTG content correlated directly with initial IHTG content (r = 0.93, P < 0.01) but was not related to the reductions in fat mass or intra-abdominal fat volume (P > 0.2). The decrease in IHTG content resulted in a resolution of steatosis (<5% IHTG content) in half (2/4) of the subjects with NAFLD.

Metabolic variables

Weight loss decreased fasting plasma insulin concentration but did not significantly change fasting plasma glucose or FFA concentrations (Table 2). Weight loss tended to improve plasma lipid and transaminase concentrations, but most of these differences were not statistically significant because of the small number of subjects and variability in the data (Table 2).

Glucose kinetics and insulin sensitivity

Weight loss decreased total basal glucose Ra from 937 ± 34 to 852 ± 49 μmol/min (P < 0.05), but glucose Ra expressed per kg FFM did not change (15.2 ± 1.4 and 14.1 ± 0.8 μmol/kg FFM/min before and after weight loss, respectively, P = 0.21).

Table 2 Effect of weight loss on metabolic variables

	Baseline	After weight loss	Percent change
Fasting glucose (mg/dl)	93.1 ± 2.8	91.2 ± 2.3	−1.9 ± 1.1
Fasting insulin (mU/l)	26.1 ± 4.5	16.5 ± 2.0*	−31.9 ± 7.9
$HOMA_{IR}$	6.0 ± 0.9	3.8 ± 0.5**	−33.0 ± 8.1
Free fatty acids (μmol/l)	435 ± 40	420 ± 61	−3.5 ± 10.8
Total cholesterol (mg/dl)	151.9 ± 8.7	132.8 ± 9.4**	−12.3 ± 4.9
LDL-cholesterol (mg/dl)	81.4 ± 7.4	74.8 ± 6.9	−7.3 ± 5.6
HDL-cholesterol (mg/dl)	44.3 ± 4.6	39.9 ± 3.0	−7.8 ± 4.1
Triglyceride (mg/dl)	131.1 ± 29.6	89.8 ± 13.7	−11.8 ± 18.7
Alanine aminotransferase (IU/l)	16.9 ± 5.2	7.5 ± 0.8	−56.0 ± 11.0
Aspartate aminotransferase (IU/l)	32.5 ± 11.5	17.8 ± 1.0	−42.6 ± 14.0

Values are means ± s.e.m.
HDL, high-density lipoprotein; $HOMA_{IR}$, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.
Value significantly different from baseline, *P < 0.01, **P = 0.05.

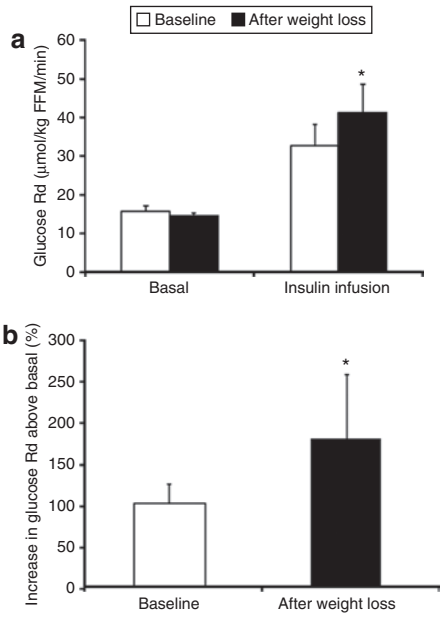

Figure 1 Effect of weight loss on glucose kinetics. (**a**) Glucose rate of disappearance (Rd) from plasma during basal conditions and during insulin infusion (hyperinsulinemic–euglycemic clamp procedure) in obese adolescents before and after weight loss. (**b**) Skeletal muscle insulin sensitivity, determined as the ability of insulin infusion to stimulate glucose Rd above basal levels before and after weight loss. *Value significantly different from baseline, P < 0.01. Values are means ± s.e.m. FFM, fat-free mass.

Weight loss resulted in significant increases in both skeletal muscle and hepatic insulin sensitivity (P < 0.05). Nonetheless, insulin infusion caused a greater increase in glucose rate of disappearance after than before weight loss (Figure 1), despite a trend toward a lower plasma insulin concentration during the clamp procedure after than before weight loss (86.1 ± 6.0 and 99.3 ± 4.8 mU/l, respectively, P = 0.09) and a lower plasma

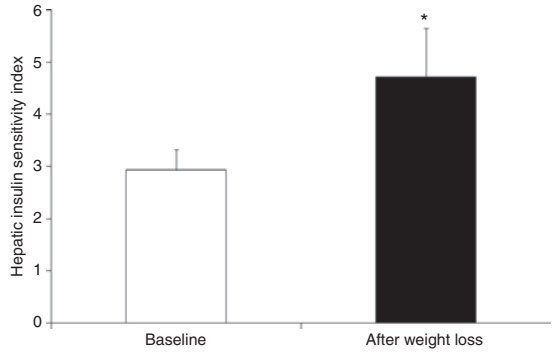

Figure 2 Hepatic insulin sensitivity index in obese adolescents before and after weight loss. Values are means ± s.e.m. *Value significantly different from baseline, $P = 0.01$.

glucose concentration after than before weight loss (94.1 ± 1.7 and 98.6 ± 1.2 mg/dl, respectively, $P < 0.05$). Hepatic insulin sensitivity, assessed by the hepatic insulin sensitivity index, was also greater after than before weight loss (4.7 ± 0.9 vs. 2.9 ± 0.4, $P = 0.01$) (**Figure 2**).

DISCUSSION

Insulin resistance is a common complication of obesity and an important risk factor for diabetes and other metabolic complications. In the present study, we found that moderate weight loss increases both liver and skeletal muscle insulin sensitivity in obese adolescents. Our subjects had normal oral glucose tolerance and fasting plasma glucose concentrations. Therefore, the improvement in insulin sensitivity was not detectable by routine blood tests and required a sophisticated assessment of insulin action by evaluating glucose kinetics during basal conditions and experimentally induced hyperinsulinemia. These results demonstrate that moderate (~8%) weight loss has important beneficial metabolic effects in obese adolescents who do not have obvious evidence of metabolic abnormalities after evaluation by a standard medical examination.

In the present study, we recruited obese adolescents who had normal glucose tolerance and used the hyperinsulinemic–euglycemic clamp technique to identify subtle changes in tissue-specific insulin action. The improvement in insulin sensitivity observed after weight loss is consistent with the results from previous studies conducted in obese adults who also had normal oral glucose tolerance (11,31–34). An earlier study conducted in obese prepubertal and peri-pubertal children demonstrated that ~7% reduction in body weight, induced by 14 days of severe calorie restriction, also increased insulin-mediated whole-body glucose disposal (35). The mechanisms responsible for the improvement in insulin sensitivity caused by weight loss are not entirely clear. The accumulation of IHTG is a major determinant of hepatic and skeletal muscle insulin resistance in both adolescents (9) and adults (23). Therefore, the reduction in IHTG content after weight loss could have contributed to the improvement in insulin action in our obese adolescents.

Weight loss caused >60% reduction in IHTG content, which was much greater than the relative decrease in total body fat, abdominal subcutaneous fat, and intra-abdominal fat masses. In addition, the relative decrease in IHTG was directly related to baseline content. These findings are consistent with observations made previously after modest weight loss in adult subjects (36). We have recently found that IHTG content responds rapidly to calorie restriction; 48 h of a low-calorie diet resulted in ~25% reduction in IHTG and only a small 1.5% reduction in body weight (10). The mechanism(s) responsible for IHTG depletion after weight loss is not known but must involve a change in the balance between IHTG formation (i.e., hepatic FFA uptake and *de novo* lipogenesis) and removal (fatty acid oxidation and very low-density lipoprotein–triglyceride secretion). Our findings imply that a decrease in basal FFA delivery to the liver is not critical for weight loss–induced decrease in IHTG content because plasma FFA concentrations did not change. However, data from a recent study that evaluated hepatic fatty acid uptake by using positron emission tomography found that moderate weight loss (~11% of initial body weight) in obese adults reduced both IHTG content and hepatic FFA uptake from the circulation, even though plasma FFA concentrations did not change (11). Therefore, it is possible that factors that regulate tissue FFA uptake from plasma influence ectopic triglyceride accumulation, independently of plasma FFA concentrations.

In conclusion, the results from this study demonstrate that moderate diet-induced weight loss reduces IHTG content and improves insulin sensitivity in both the liver and skeletal muscle in obese adolescents. Moreover, the improvement in insulin-mediated glucose metabolism occurred even though subjects had normal glucose tolerance at baseline.

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DISCLOSURE

The authors declared no conflict of interest.

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