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Background. An increase in intrahepatic triglyceride (IHTG) is the hallmark feature of nonalcoholic fatty liver disease (NAFLD) and is decreased by weight loss. Hepatic de novo lipogenesis (DNL) contributes to steatosis in people with NAFLD. The physiological factors that stimulate hepatic DNL and the effect of weight loss on hepatic DNL are not clear. **Methods.** Hepatic DNL, 24-h integrated plasma insulin and glucose concentrations, and both liver and whole-body insulin sensitivity were determined in people who were lean ($n = 14$), obese with normal IHTG content (Obese, $n = 26$) and obese with NAFLD (Obese-NAFLD, $n = 27$). Hepatic DNL was assessed by using the deuterated water method corrected for the potential confounding contribution of adipose tissue DNL. Liver and whole-body insulin sensitivity were assessed by using the hyperinsulinemic-euglycemic clamp procedure in conjunction with glucose tracer infusion. Six subjects in the Obese-NAFLD group were also evaluated before and after 10% diet-induced weight loss. **Results.** The contribution of hepatic DNL to IHTG-palmitate was 11%, 19% and 38% in the Lean, Obese and Obese-NAFLD groups, respectively. Hepatic DNL was inversely correlated with hepatic and whole-body insulin sensitivity, but directly correlated with 24-h plasma glucose [...]

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Insulin resistance drives hepatic *de novo* lipogenesis in nonalcoholic fatty liver disease

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ABSTRACT

Background. An increase in intrahepatic triglyceride (IHTG) is the hallmark feature of nonalcoholic fatty liver disease (NAFLD) and is decreased by weight loss. Hepatic *de novo* lipogenesis (DNL) contributes to steatosis in people with NAFLD. The physiological factors that stimulate hepatic DNL and the effect of weight loss on hepatic DNL are not clear.

Methods. Hepatic DNL, 24-h integrated plasma insulin and glucose concentrations, and both liver and whole-body insulin sensitivity were determined in people who were lean (n=14), obese with normal IHTG content (Obese, n=26) and obese with NAFLD (Obese-NAFLD, n=27). Hepatic DNL was assessed by using the deuterated water method corrected for the potential confounding contribution of adipose tissue DNL. Liver and whole-body insulin sensitivity were assessed by using the hyperinsulinemic-euglycemic clamp procedure in conjunction with glucose tracer infusion. Six subjects in the Obese-NAFLD group were also evaluated before and after 10% diet-induced weight loss.

Results. The contribution of hepatic DNL to IHTG-palmitate was 11%, 19% and 38% in the Lean, Obese and Obese-NAFLD groups, respectively. Hepatic DNL was inversely correlated with hepatic and whole-body insulin sensitivity, but directly correlated with 24-h plasma glucose and insulin concentrations. Weight loss decreased IHTG content, in conjunction with a decrease in hepatic DNL and 24-h plasma glucose and insulin concentrations.

Conclusions. These data suggest hepatic DNL is an important regulator of IHTG content, and that increases in circulating glucose and insulin stimulate hepatic DNL in people with NAFLD. Weight loss decreases IHTG content, at least in part, by decreasing hepatic DNL.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common complication of obesity, and is associated with multi-organ insulin-resistance (1, 2), dyslipidemia (high plasma triglyceride and low HDL-cholesterol concentrations) (3, 4), and an increased risk of diabetes and coronary heart disease (5, 6). The hallmark feature of NAFLD is an increase in IHTG content, which accumulates when the rate of hepatic triglyceride (TG) production is greater than the combined rates of TG export in very-low-density lipoprotein (VLDL) particles and intrahepatic oxidation of triglyceride-derived fatty acids. Data from a series of studies have shown that the rate of VLDL-TG secretion is increased (2, 7, 8) and hepatic fatty acid oxidation is likely normal or increased in people with NAFLD (9-11), demonstrating that an increase in IHTG production rather than a decrease in IHTG mobilization is the primary mechanism for developing and maintaining hepatic steatosis. Fatty acids used for IHTG production are derived from: i) fatty acids released into the systemic and portal circulations by lipolysis of TGs in subcutaneous and intra-abdominal adipose tissues, ii) fatty acids released into the systemic circulation by postprandial lipolysis of TGs in chylomicrons, iii) hepatic lipolysis of TGs in plasma lipoproteins delivered to the liver, and iv) fatty acids synthesized *de novo* from non-lipid precursors in the liver (12, 13).

The *de novo* synthesis of fatty acids in the liver involves a complex cytosolic polymerization process in which acetyl-CoA is converted to malonyl-CoA, which then undergoes several cycles of condensation, decarboxylation, and reduction reactions to form one palmitate molecule. *De novo* lipogenesis (DNL) in the liver can be measured *in vivo* by using isotopically labeled tracers to assess the synthesis rate of palmitate secreted in liver-derived TGs into plasma. Studies using this approach reported that DNL accounts for 15% to 26% of IHTG-palmitate production in people with NAFLD (12, 14-16) and 1% to 10% of IHTG-palmitate production in people who are either lean or obese with normal IHTG content (14, 17-20). It is possible, however, that these studies underestimated the contribution from DNL because: i) most studies were conducted when participants were in the fasted state when DNL is lowest

(19, 21); ii) the duration of isotope administration was not long enough to detect newly synthesized fatty acids that were incorporated into and released from slowly turning over IHTG pools (22, 23), a concept supported by the results of a recent clinical trial that administered deuterated water (D₂O) for a more prolonged period and found a greater contribution of DNL to IHTG production in people with nonalcoholic steatohepatitis than reported previously (24); and iii) the correction factor used to account for the contribution of fatty acids made by DNL in adipose tissue (i.e. fatty acids made *de novo* in adipose tissue that were released into the bloodstream and subsequently incorporated into VLDL-TG in the liver) was too high because it was assumed that all plasma free fatty acids (FFAs) made by DNL were derived from adipose tissue without any contribution of plasma FFAs from lipolysis of circulating TGs produced by the liver (14).

Although the mechanism(s) responsible for the increase in hepatic DNL in people with NAFLD is not known, it is possible that increases in circulating insulin and glucose associated with hepatic and whole-body insulin resistance are involved in stimulating hepatic DNL because insulin and glucose activate SREBP-1c and ChREBP, respectively, (25-30) which transcriptionally activate genes involved in DNL. This mechanism implies differential effects of insulin action on specific metabolic functions in the liver, manifested by insulin resistance with respect to the suppression of hepatic glucose production, but preserved insulin sensitivity with respect to the SREBP-1c pathway that stimulates fatty acid synthesis. Studies conducted in primary hepatocytes and in rodent models of obesity and diabetes support the notion of selective pathway-specific insulin resistance in the liver (31-34).

The purpose of the present study was to: 1) determine hepatic DNL, measured over a prolonged period (3 to 5 weeks) of daily D₂O ingestion and corrected for the contribution of fatty acids made *de novo* in adipose tissue, in three distinct cohorts who were lean with normal oral glucose tolerance and normal IHTG content (Lean), obese with normal oral glucose tolerance and normal IHTG content (Obese), or obese with abnormal oral glucose tolerance and NAFLD

(Obese-NAFLD); 2) determine the relationships among hepatic DNL and IHTG content and key factors that are likely involved in regulating DNL, namely liver and whole-body insulin sensitivity and integrated 24-h plasma insulin and glucose concentrations; and 3) determine the effect of moderate (10%) weight loss on hepatic DNL, IHTG content, liver and whole-body insulin sensitivity and integrated 24-h plasma insulin and glucose concentrations. Liver and whole-body insulin sensitivity were assessed by using the hyperinsulinemic-euglycemic clamp (HEC) procedure in conjunction with stable isotopically labeled glucose tracer infusion and a 24-h integration of plasma insulin and glucose concentrations was assessed by serial blood sampling. We hypothesized that: 1) hepatic DNL would negatively correlate with hepatic and whole-body insulin sensitivity and positively correlate with 24-h plasma glucose and insulin concentrations and IHTG content; and 2) moderate weight loss would decrease hepatic DNL in concert with improvements in insulin sensitivity and a decrease in the integrated 24-h plasma insulin and glucose concentrations.

RESULTS

Body composition and metabolic characteristics

The Obese and Obese-NAFLD groups were matched on percent body fat, but intra-abdominal adipose tissue (IAAT) volume and IHTG content were much greater in the Obese-NAFLD than in the Obese group (Table 1). In addition, fasting plasma TG, glucose and insulin concentrations, HbA1c, 2-h OGTT plasma glucose, and the integrated 24-h plasma glucose and insulin AUC values were greater, and hepatic and whole-body insulin sensitivity were lower, in the Obese-NAFLD than in the Obese group. Although many metabolic variables in the Obese group were not different than those in the Lean group, hepatic and whole-body insulin sensitivity were lower in the Obese group.

Hepatic DNL progressively increases with increases in insulin-resistant glucose metabolism

Total body water enrichment with deuterium after prolonged daily D₂O consumption was not different between groups, and was 1.80 ± 0.14 , 1.73 ± 0.07 and $1.80 \pm 0.07\%$ in the Lean, Obese and Obese-NAFLD groups, respectively; $P = 0.81$. DNL in adipose tissue TG was very small and not different between groups: $1.4 \pm 0.1\%$ of palmitate in adipose TG were derived from DNL, which represents the combined average of $1.7 \pm 0.5\%$, $1.2 \pm 0.1\%$, and $1.5 \pm 0.1\%$ in the Lean, Obese and Obese-NAFLD groups, respectively; $P = 0.26$. The proportion of plasma free palmitate produced by DNL ($8.8 \pm 0.8\%$) was more than 5-fold greater than the proportion of palmitate made by DNL in adipose tissue (Supplemental Figure 1). Accordingly, more than 80% of the labeled plasma free palmitate could not have come from adipose tissue fatty acids and must have come from the DNL pathway in liver and subsequently released into the bloodstream by lipolysis of TG in circulating liver-derived triglyceride-rich lipoproteins (TRL) (35). The relative contribution of hepatic DNL to total IHTG-palmitate synthesis (calculated from the palmitate made by DNL in circulating TRL-TG, after subtracting the contribution from palmitate made by DNL in adipose tissue) was lowest in the Lean group ($10.9 \pm 1.7\%$) and nearly 2-fold ($19.4 \pm 1.5\%$) and 3.5-fold ($38.5 \pm 2.0\%$) higher in the Obese and Obese-NAFLD groups, respectively (Figure 1A). The relative contribution of DNL to plasma TRL-TG palmitate was positively correlated with IHTG content (Figure 1B), negatively correlated with both whole-body and hepatic insulin sensitivity (Figure 1, C and D), and positively correlated with 24-h plasma insulin and glucose AUC values (Figure 1, E and F).

Moderate weight loss causes a marked decrease in hepatic DNL and IHTG content

Six subjects in the Obese-NAFLD group repeated the testing procedures after $10.3 \pm 0.8\%$ (range 7.3% - 12.1%) diet-induced weight loss. Body weight was stable throughout the periods of D₂O consumption before and after weight loss ($0.8 \pm 0.2\%$ change in weight during

both periods of D₂O administration) and total body water deuterium enrichment after weight loss was not different than values before weight loss (1.69 ± 0.23 and $1.78 \pm 0.14\%$ and, respectively; $P = 0.57$). Weight loss decreased the relative contribution of hepatic DNL to total IHTG-palmitate synthesis by $35 \pm 10\%$ and IHTG content by $50 \pm 8\%$ (Figure 2, A and B). Weight loss also improved hepatic and whole-body insulin sensitivity, decreased 24-h plasma glucose and insulin AUCs, and decreased HbA1c, and plasma triglyceride and LDL-cholesterol concentrations (Table 2).

DISCUSSION

Steatosis is the hallmark feature of NAFLD and precedes the progression to steatohepatitis and fibrosis (36). Accordingly, an understanding of the mechanisms responsible for the excessive accumulation of IHTG has important physiological and clinical implications. By using a prolonged oral D₂O administration approach to assess hepatic DNL and correcting for the contribution of fatty acids made by DNL in adipose tissue TG, we found a much larger contribution of DNL to IHTG formation in people with obesity and NAFLD than previously appreciated; on average, nearly 40% of plasma TRL-TG palmitate was derived from hepatic DNL and the relative contribution of DNL was directly correlated with IHTG content. We also found that people with obesity and normal IHTG content had a much greater contribution from hepatic DNL to IHTG formation than people who are lean. In addition, our data demonstrate a strong negative correlation between the rate of DNL and both whole-body and hepatic insulin sensitivity and positive correlations with integrated 24-h plasma glucose and insulin concentrations. An increase in hepatic and whole-body insulin sensitivity and a decrease in 24-h plasma glucose and insulin concentrations induced by moderate (10%) weight loss was also associated with a marked decrease in both hepatic DNL and IHTG content. Taken together, these data underscore the importance of DNL in the pathogenesis of hepatic steatosis and suggest increases in daily 24-h plasma glucose and insulin concentrations are major drivers of

increased DNL in people with obesity and NAFLD. Moreover, this clinical study supports the notion that selective hepatic insulin resistance demonstrated in cell systems and animal models (31, 33, 34) is involved in the pathogenesis of NAFLD in people.

The values we observed for the contribution of DNL to IHTG fatty acid production in our Lean, Obese and Obese-NAFLD groups were greater than those reported previously in similar study populations (12, 14-17, 22, 37, 38). The reason for the differences between our results and those of other studies is likely related to several factors. First, the long (3-5 week) duration of D₂O tracer administration in our study accounted for labelling of the liver TG pools that turn over slowly (12) and provided an integrated assessment of DNL during daily fasting and fed conditions over several weeks. In contrast, the assessment of hepatic DNL in most previous studies were conducted with a much shorter period of tracer administration and were conducted during fasting conditions when DNL is reduced. Second, we avoided overestimating the potential contribution of fatty acids synthesized *de novo* in adipose tissue that could have been released into the circulation and incorporated into VLDL-TG in the liver (14-17). We directly measured DNL-derived palmitate in adipose tissue TG, and estimated the incorporation of flux from this source of fatty acids into VLDL-TG in the liver. *De novo* lipogenesis accounted for less than 2% of palmitate in adipose triglycerides but 9% in plasma free palmitate, suggesting other sources of fatty acids, presumably spillover of fatty acids into the circulation during lipoprotein lipase-mediated lipolysis of TRL-TG derived from the liver, must contribute to the pool of circulating DNL-derived FFAs. Finally, we evaluated DNL in precisely defined cohorts who were segregated into distinct groups based on BMI, oral glucose tolerance and IHTG content. Therefore, it is possible that differences in study populations between our study and others contributed to differences in hepatic DNL.

The mechanism(s) responsible for the increase in hepatic DNL in people with NAFLD is not known. Our data suggest that daily 24-h increases in plasma insulin and glucose concentrations associated with insulin-resistant glucose metabolism contribute to the stimulation

of hepatic DNL. Data from studies conducted in cell systems and rodent models have shown insulin and glucose independently regulate hepatic DNL by activating SREBP-1c and ChREBP, respectively (25, 27-30, 39), which in turn activate nearly all the genes involved in hepatic DNL. Hepatic expression of SREBP-1c, ChREBP and other genes involved in lipogenesis are increased in people with hepatic steatosis compared with those who have normal IHTG content (40-44). We found positive relationships between hepatic DNL and the integrated 24-h plasma concentrations of both glucose and insulin and a negative relationship between hepatic DNL and the hepatic insulin sensitivity index. These findings support the notion of a dissociation between insulin action on hepatic glucose and lipid metabolism in people with NAFLD, manifested by insulin-resistant hepatic glucose metabolism but insulin sensitive hepatic lipogenesis. In addition, it is possible that the process of DNL itself produces toxic metabolites, such as diacylglycerol and ceramides, that can induce insulin resistance (45, 46) thereby establishing a positive feed-back loop in which insulin resistance stimulates hepatic DNL and hepatic DNL contributes to insulin resistance.

Our study has important implications in the development of drug therapies that modulate IHTG content. The therapeutic potential of modulating DNL to decrease IHTG content in people with NAFLD has been demonstrated in studies that involved novel pharmacologic agents. The data from one study showed liver-specific inhibition of acetyl-CoA carboxylase, a major regulator of hepatic DNL, caused a marked decrease in hepatic DNL and IHTG content in people with NAFLD (47). The influence of insulin action in the liver on IHTG content was demonstrated in a randomized controlled trial conducted in adults with insulin-treated type 2 diabetes, who were randomized to therapy with either peglispro (Eli Lilly and Co., Indianapolis, IN), a polyethylene glycolylated insulin analog, or insulin glargine (48). Treatment with peglispro, which has preferential action in the liver and decreased peripheral action than does insulin glargine (49) caused a 50% increase in IHTG content assessed at 26 and 52 weeks of therapy, whereas treatment with insulin glargine did not affect IHTG content. Collectively, these results

further underscore the important role of DNL in developing and maintaining steatosis in people with NAFLD.

Weight loss has profound effects on IHTG content. Even 48 h of calorie restriction causes a marked decline in IHTG content (50), and at any given percent weight loss, the relative decrease in IHTG content is much greater than the relative decrease in whole-body or intra-abdominal adipose tissues (51). In the present study, we found moderate (10%) weight loss caused a marked decrease in both hepatic DNL and IHTG content, suggesting a decrease in hepatic DNL is an important mechanism responsible for the weight loss induced decline in IHTG content.

In summary, we found that hepatic DNL, when assessed by a labeling protocol of sufficient duration to account for large, slowly turning over hepatic TG pools, is an important contributor to IHTG production and IHTG content in people with NAFLD. Moreover, by carefully selecting participants with a wide range in insulin sensitivity and IHTG content we were able to demonstrate the contribution of hepatic DNL to IHTG was negatively correlated with both hepatic and whole-body and insulin sensitivity and positively correlated with integrated 24-h plasma glucose and insulin concentrations. These data suggest increases in circulating glucose and insulin associated with insulin resistance promote hepatic DNL in people with NAFLD. In contrast, an improvement in insulin sensitivity and concomitant decrease in hepatic DNL are likely important contributors to the decline in IHTG content associated with moderate weight loss.

METHODS

Subjects

A total of 445 potential subjects were screened for this study. Sixty-seven men and women (mean age: 39 ± 1 years old; 14 men and 53 women) that were eligible and willing to participate and completed all baseline testing were included in the cross-sectional comparison of Lean, Obese and Obese-NAFLD subjects. The subject flow is shown in Supplemental Figure 2. Subjects were recruited by using the Volunteers for Health database at Washington University School of Medicine and by local postings between April 2016 and November 2018. All parts of this study were conducted in the Clinical Translational Research Unit (CTRU) and Center for Clinical Imaging Research (CCIR) at Washington University School of Medicine. Subjects completed a comprehensive screening evaluation, including a medical history and physical examination, standard blood tests, hemoglobin A1c (HbA1c), an oral glucose tolerance test (OGTT) and assessment of IHTG content by using magnetic resonance imaging (MRI) to determine eligibility. The following inclusion criteria were required for each cohort: 1) Lean group (n=14, 8 women): BMI 18.5-24.9 kg/m²; IHTG content $\leq 4\%$, serum TG concentration <150 mg/dl, fasting plasma glucose concentration <100 mg/dl, 2-hr OGTT plasma glucose concentration <140 mg/dl, and HbA1c $\leq 5.6\%$; 2) Obese group (n=26, 24 women): BMI 30-49.9 kg/m²; IHTG content $\leq 4\%$, serum TG concentration <150 mg/dl, fasting plasma glucose concentration <100 mg/dl, 2-hr OGTT plasma glucose concentration <140 mg/dl, and HbA1c $\leq 5.6\%$; and 3) Obese-NAFLD group (n=27, 21 women): BMI 30-49.9 kg/m²; IHTG content $\geq 6.0\%$ and HbA1c 5.7%-6.4% or fasting plasma glucose concentration ≥ 100 mg/dl or 2-hr OGTT plasma glucose concentration ≥ 140 mg/dl. Potential participants who had a history of diabetes or liver disease other than NAFLD, were taking medications that can affect metabolism or cause liver damage, or consumed excessive amounts of alcohol (>21 oz of alcohol per week for men and >14 oz of alcohol per week for women) were excluded.

Body composition analyses

Body fat mass and fat-free mass (FFM) were determined by using dual-energy X-ray absorptiometry (DXA, Lunar iDXA, GE Healthcare Lunar, Madison, WI). Abdominal subcutaneous adipose tissue and IAAT volumes and IHTG content was determined by using by magnetic resonance imaging (3-T superconducting magnet; Siemens, Iselin, NJ) as previously described (52, 53).

Integrated 24-h plasma glucose and insulin concentrations and insulin sensitivity

Subjects were admitted to the CTRU at 1700 h for ~48 hours and consumed a standard meal (50% carbohydrate, 35% fat, 15% protein) containing one-third of their estimated energy requirements (54) between 1800 h and 1900 h. At 0630 h the next morning on day 2, a catheter was inserted into an antecubital vein for 24-h serial blood sampling. Blood samples were obtained every hour from 0700 h to 2300 h on day 2 and from 0500 h to 0700 h on day 3, and additional blood samples were obtained every 30 min for 2 hours after each meal. Meals were provided at 0700 h, 1300 h, and 1900 h. Each meal contained one-third of the participant's energy requirements and were comprised of 50% carbohydrate, 35% fat, and 15% protein. A HEC procedure, in conjunction with stable isotopically labeled glucose tracer infusion, was conducted on day 3 to assess hepatic and whole-body insulin sensitivity. At 0700 h, a primed (8.0 $\mu\text{mol/kg}$) continuous (0.08 $\mu\text{mol/kg/min}$) infusion of [U- ^{13}C]glucose (Cambridge Isotope Laboratories Inc., Andover, MA) was started through the existing intravenous catheter. An additional catheter was inserted into a radial artery to obtain arterial blood samples. After the infusion of glucose tracer for 210 min (basal period), insulin was infused for 210 min at a rate of 50 mU/m² body surface area (BSA)/min (initiated with a two-step priming dose of 200 mU/m² BSA/min for 5 min followed by 100 mU/m² BSA/min for 5 min). The infusion of [U- ^{13}C]glucose was stopped during insulin infusion because of the expected decrease in hepatic glucose production (55). Euglycemia (~100 mg/dl) was maintained by variable infusion of 20% dextrose

enriched to ~1% with [U-¹³C]glucose. Blood samples were obtained before beginning the tracer infusion and every 6-7 minutes during the final 20 minutes (total of 4 blood samples) of the basal and insulin infusion periods.

De novo lipogenesis

Subjects consumed 50-mL aliquots of 70% D₂O (Sigma-Aldrich, St. Louis, MO), provided in sterile vials, every day for 3 to 5 weeks; aliquots of D₂O were consumed 3-4 times/day every day for the first 5 days (priming period) followed by two 50-mL doses daily. The final aliquot of D₂O was taken on the evening of day 2 of the inpatient CTRU admission. A blood sample obtained at 0700 h the following morning was used to determine body water D₂O enrichment and hepatic DNL. Compliance with D₂O consumption was monitored by interview at weekly visits with the study research coordinator, by counting the return of empty vials at each visit, and by evaluating D₂O enrichments in plasma (obtained on day 7 and weekly thereafter) and saliva (obtained on days 2, 4, and 11 and then weekly thereafter). To evaluate the potential confounding contribution of fatty acids made *de novo* in adipose tissue to our measurement of hepatic DNL, we measured DNL of palmitate in plasma FFA and in abdominal adipose tissue TGs. Abdominal subcutaneous adipose tissue was obtained by percutaneous biopsy during the basal stage of the HEC procedure as previously described (2).

Diet intervention and post-weight loss testing.

After baseline testing was completed, 7 subjects in the Obese-NAFLD group participated in a supervised weight loss program, supervised by our study dietitian and behavioral psychologist, involving weekly individual dietary and behavioral education sessions with all food provided as packed-out meals. The macronutrient content of the diet was comprised of ~50% of energy as carbohydrate, ~30% as fat, and ~15% as protein. The initial daily energy content of the diet provided 75% of estimated energy requirements (54); subsequent meals and energy

intake were adjusted weekly as needed to achieve a 0.5%-1% weight loss per week until ~10% weight loss was achieved, which took ~25 weeks. Once the targeted weight loss goal was achieved, dietary energy intake was modified to maintain a stable body weight for 3-4 weeks before the testing procedures performed at baseline were repeated. One subject withdrew from the study because they were unable to lose weight; data from 6 from subjects are reported.

Sample analyses

Plasma glucose concentration was determined by using an automated glucose analyzer (Yellow Spring Instruments Co, Yellow Springs, OH). Plasma insulin was measured by using electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics, Indianapolis, IN). Plasma TG and HDL-cholesterol concentrations were determined enzymatically by using colorimetric assays (Roche Diagnostics). Plasma LDL-cholesterol concentration was calculated according to the Friedewald formula (56). HbA1c was measured by using a turbidimetric inhibition immunoassay (Roche Diagnostics). Deuterium enrichment in total body water, deuterium enrichment and labeling pattern in plasma FFA, TRL-TG, and adipose tissue TG, and [U-¹³C]glucose enrichment in plasma glucose were determined by using gas-chromatography/mass-spectrometry (GC-MS) as described previously (57, 58).

Calculations

Plasma glucose and insulin area under the curve (AUC) over 24 h were calculated by using the trapezoidal method (59). The hepatic insulin sensitivity index (HISI) was calculated as the inverse of the product of plasma insulin concentration and endogenous glucose rate of appearance (Ra) into the systemic circulation, determined by dividing the glucose tracer infusion rate by the average plasma glucose tracer-to-tracee ratio (TTR) during the last 20 minutes of the basal period of the HEC procedure (1). Total glucose rate of disappearance (Rd) during insulin infusion was assumed to be equal to the sum of endogenous glucose Ra and the rate of

infused glucose during the last 20 min of the HEC procedure (1). The fractional contribution of DNL to palmitate in plasma FFA, plasma TRL-TG and adipose tissue-TG were calculated by mass isotopomer distribution analysis as described previously (17, 58). Circulating TRL-TG palmitate can be used to assess hepatic DNL because the fatty acid composition and source of fatty acids in IHTG and in circulating TRL-TG are the same (12). Accordingly, hepatic DNL was calculated as the measured total contribution of palmitate made by DNL in circulating TRL-TGs minus the estimated contribution of palmitate made by DNL in adipose tissue-TG that were released into the circulation and incorporated into TRL-TG in the liver. The contribution of palmitate made *de novo* in adipose tissue that were incorporated into circulating TRL-TG was estimated by: i) directly measuring the contribution of palmitate made by DNL in adipose tissue TGs; and ii) estimating the relative incorporation of the release of these fatty acids into the circulation that were then incorporated into VLDL-TG in the liver, based on the results from our previous studies that found that the contribution of systemic plasma FFA to VLDL-TG secreted by the liver was 75%, 65% and 40% in similar groups who were lean, obese with normal IHTG content and obese with NAFLD, respectively (2, 7, 60).

Statistical analyses

One-way analysis of variance (ANOVA) was performed to compare subject characteristics and outcome measures between Lean, Obese and Obese-NAFLD groups with Tukey's post-hoc procedure used to locate significant mean differences where appropriate. Student's t-test for paired samples was used to assess the statistical significance of differences in values before and after weight loss. Relationships among DNL, IHTG content and selected metabolic variables were evaluated by using linear and nonlinear regression analysis. In all instances a logarithmic regression curve provided the best-fit to the data. All statistical tests were 2-sided with a P-value <0.05 considered statistically significant. Data are reported as

means \pm SEM unless otherwise noted. Statistical analyses were performed by using SPSS (version 25, IBM, Armonk, NY).

Based on the inter-individual variability in hepatic DNL, assessed by using the deuterated water technique in people with obesity with normal IHTG content and in people with obesity and NAFLD, reported by others previously (14) and whole-body insulin sensitivity, assessed as the glucose rate of disappearance (Rd) during a hyperinsulinemic-euglycemic clamp procedure we reported previously reported (61), we estimated that 15-25 subjects per group would be needed to detect between-group differences in hepatic DNL of 7.5%-10% and between group differences in glucose Rd of 11-15 $\mu\text{mol/kg FFM/min}$ using a 2-sided test with a β -value of 0.9 and an α value of 0.05. Based on the values for hepatic DNL observed in the Obese-NAFLD group, we estimated that 6 subjects would be needed to detect a weight loss induced 14% decrease in hepatic DNL with a β -value of 0.90 and an α value of 0.05. These computations were performed using G*Power 3.1.9.2 (62).

Study approval

Subjects provided written, informed consent before participating in this study, which was approved by the Human Research Protection Office at Washington University School of Medicine in St. Louis, MO.

AUTHOR CONTRIBUTIONS

G.I.S., M.K.H. and S.K. designed the study. M.S., A.L.O., E.N., T.F., and B.W.P performed sample analyses, G.I.S., G.G.S., M.Y., M.C. and J.W.B. conducted the clinical studies, C.S. assessed IHTG content, and G.I.S. M.K.H. and S.K. interpreted the data and wrote the manuscript. All authors critically reviewed and edited the manuscript.

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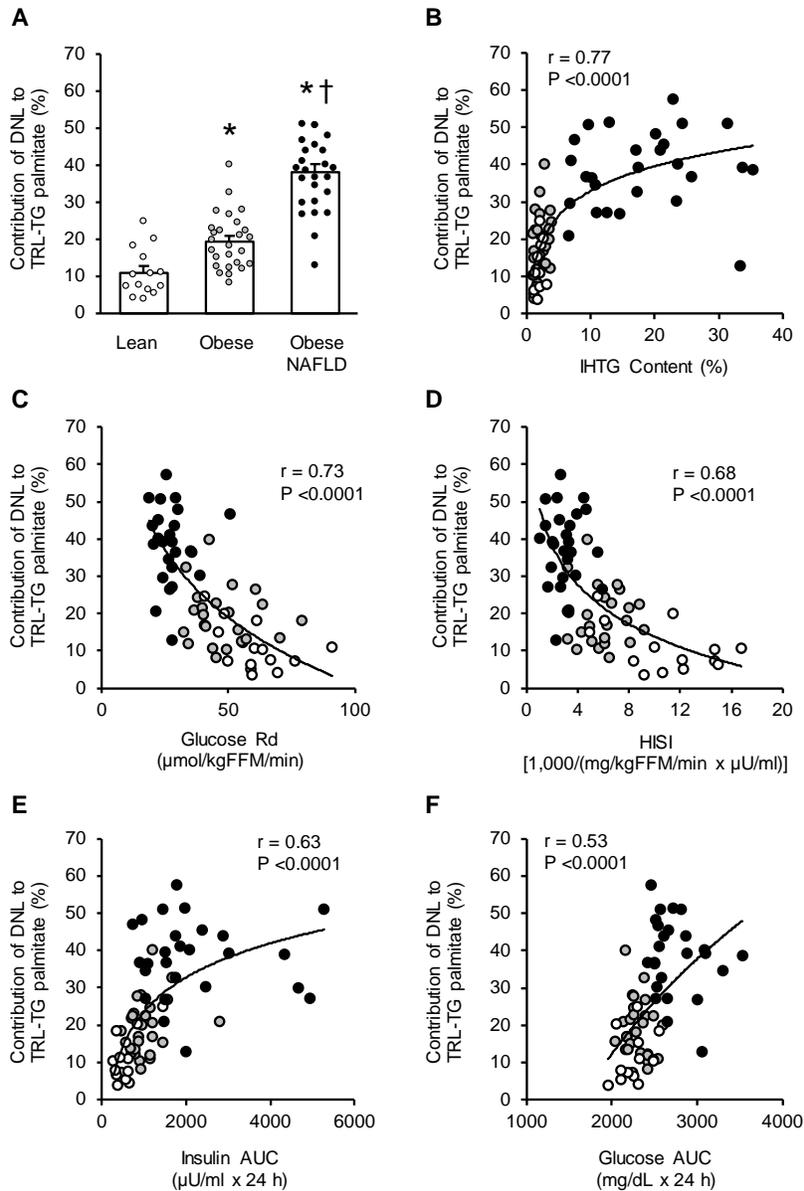


Figure 1. Relationships among hepatic *de novo* lipogenesis and metabolic characteristics.

Relative contribution of *de novo* lipogenesis (DNL) to IHTG content, assessed as palmitate produced by DNL measured in plasma triglyceride-rich lipoprotein triglycerides (TRL-TG) in three groups of subjects: i) lean with normal intrahepatic triglyceride (IHTG) content (Lean; $n=14$); ii) obese with normal IHTG content (Obese; $n=26$); and iii) obese with nonalcoholic fatty liver disease (Obese-NAFLD; $n=27$) (A). Values are means \pm SEM. One-way analysis of variance (ANOVA) was performed to compare subject characteristics and Tukey's post-hoc procedure was used to identify significant mean differences between groups where appropriate. *Value significantly different from the Lean group value, $P < 0.05$. †Value significantly different from the Obese group value, $P < 0.01$. Relationships between hepatic *de novo* lipogenesis (DNL), assessed as the percent contribution of DNL to plasma triglyceride-rich lipoprotein-triglyceride [TRL-TG] palmitate, and intrahepatic triglyceride (IHTG) content (B), whole-body insulin sensitivity, assessed as the glucose rate of disappearance (Rd) during a hyperinsulinemic-euglycemic clamp procedure (C), hepatic insulin sensitivity index (HISI) (D), and integrated 24-h areas under the curve (AUC) for plasma insulin (E) and glucose (F). Logarithmic regression analysis was used to determine lines of best-fit to the data. White, grey and black circles represent participants in the Lean, Obese and Obese-NAFLD groups, respectively.

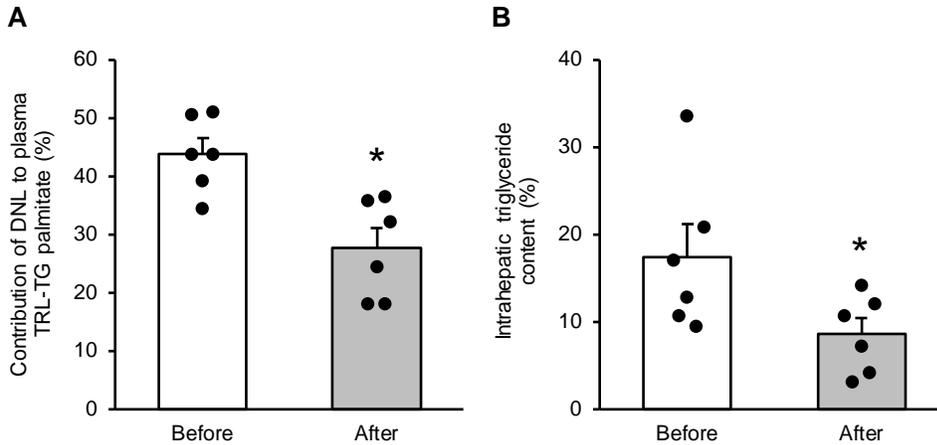


Figure 2. Moderate weight loss decreases hepatic *de novo* lipogenesis and intrahepatic triglyceride content in people with obesity and nonalcoholic fatty liver disease.

Relative contribution of *de novo* lipogenesis (DNL) to IHTG content, assessed as palmitate produced by DNL within plasma triglyceride-rich lipoprotein-triglyceride (TRL-TG) palmitate (**A**) and intrahepatic triglyceride content (**B**) before and after ~10% weight loss in 6 people with obesity and nonalcoholic fatty liver disease. Values are means \pm SEM. Circles represent individual values before and after weight loss. A Student's t-test for paired samples was used to assess the statistical significance of differences in values before and after weight loss. *Value significantly different from the Before value, $P < 0.05$.

Table 1. Body composition and metabolic characteristics of the study subjects

	Lean (n=14)	Obese (n=26)	Obese-NAFLD (n=27)
Body mass index (kg/m ²)	22.6 ± 0.4	37.0 ± 0.9 ^a	38.9 ± 0.9 ^a
Body fat (%)	29.4 ± 1.5	48.0 ± 1.2 ^a	47.7 ± 1.1 ^a
IHTG content (%)	1.8 ± 0.2	2.3 ± 0.2	18.0 ± 1.7 ^{a,b}
IAAT volume (cm ³)	400 ± 55	917 ± 71 ^a	1,864 ± 130 ^{a,b}
ASAT volume (cm ³)	937 ± 105	3,716 ± 215 ^a	3,644 ± 240 ^a
HbA1c (%)	5.0 ± 0.1	5.0 ± 0.1	5.7 ± 0.1 ^{a,b}
Triglyceride (mg/dl)	67 ± 8	67 ± 4	141 ± 13 ^{a,b}
HDL-cholesterol (mg/dl)	67 ± 4	55 ± 3 ^a	43 ± 2 ^{a,b}
LDL-cholesterol (mg/dl)	99 ± 6	99 ± 5	118 ± 6
Fasting glucose (mg/dl)	85 ± 1	88 ± 1	101 ± 2 ^{a,b}
Glucose: 2 h OGTT (mg/dl)	96 ± 5	106 ± 3	170 ± 6 ^{a,b}
Glucose: 24-h AUC (mg/dl x 24h)	2,260 ± 46	2,302 ± 27	2,732 ± 56 ^{a,b}
Fasting insulin (μU/ml)	5.2 ± 0.5	11.8 ± 1.4	27.1 ± 3.4 ^{a,b}
Insulin: 24-h AUC (μU/ml x 24h)	564 ± 81	1,059 ± 89	2,168 ± 252 ^{a,b}
Hepatic insulin sensitivity index	10.8 ± 1.0	5.8 ± 0.4 ^a	3.0 ± 0.2 ^{a,b}
Glucose Rd during the HECP (μmol/kg FFM/min)	60.8 ± 3.5	48.3 ± 2.4 ^a	27.6 ± 1.4 ^{a,b}

Data are expressed as mean ± SEM. Abbreviations: ASAT=abdominal subcutaneous adipose tissue; AUC=area under the curve; FFM=fat-free mass; HbA1c=hemoglobin A1c; HECP=hyperinsulinemic-euglycemic clamp procedure; IAAT=intra-abdominal adipose tissue; IHTG=intrahepatic triglyceride; OGTT=oral glucose tolerance test; Rd=rate of disappearance. One-way analysis of variance (ANOVA) was performed to compare subject characteristics; Tukey's post-hoc procedure used to identify significant mean differences between groups where appropriate.

^aValue significantly different from the corresponding value in Lean group, $P < 0.05$.

^bValue significantly different from the corresponding value in the Obese group, $P < 0.05$.

Table 2. Body composition and metabolic characteristics of the study subjects before and after 10% weight loss

	Before	After	P-value
Body mass index (kg/m ²)	37.7 ± 1.6	34.4 ± 1.6	-
Body fat (%)	47.2 ± 2.0	43.5 ± 2.0	0.02
HbA1c (%)	5.8 ± 0.2	5.4 ± 0.2	0.03
Triglyceride (mg/dl)	151 ± 11	118 ± 9	0.04
HDL-cholesterol (mg/dl)	44 ± 2	38 ± 1	<0.01
LDL-cholesterol (mg/dl)	122 ± 6	100 ± 9	0.02
Fasting glucose (mg/dl)	106 ± 6	99 ± 3	0.15
Glucose: 24-h AUC (mg/dl x 24h)	2,870 ± 117	2,559 ± 121	<0.001
Insulin: 24-h AUC (μU/ml x 24h)	2,135 ± 369	1,183 ± 137	0.02
Hepatic insulin sensitivity index	2.5 ± 0.4	4.9 ± 0.9	0.04
Glucose Rd during the HECP (μmol/kg FFM/min)	25.6 ± 1.7	40.7 ± 2.8	<0.01

Data are expressed as mean ± SEM. Abbreviations: AUC=area under the curve; FFM=fat-free mass; HbA1c=hemoglobin A1c; HECP=hyperinsulinemic-euglycemic clamp procedure; Rd=rate of disappearance. All P-values were calculated using Student's t-test for paired samples.