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Methods. A hyperinsulinemic-euglycemic clamp and a 3-hour oral glucose tolerance test were used to evaluate insulin sensitivity and insulin kinetics after glucose ingestion in three groups: i) lean with normal intrahepatic triglyceride (IHTG) and glucose tolerance (Lean-NL; n=14); ii) obese with normal IHTG and glucose tolerance (Obese-NL; n=24); and iii) obese with hepatic steatosis and prediabetes (Obese-NAFLD; n=22).

Results. Insulin sensitivity progressively decreased and insulin secretion progressively increased from Lean-NL to Obese-NL to Obese-NAFLD. Fractional hepatic insulin extraction progressively decreased from Lean-NL to Obese-NL to Obese-NAFLD, whereas total hepatic insulin extraction (molar amount removed) was greater in Obese-NL and Obese-NAFLD than Lean-NL. Insulin appearance in the systemic circulation and extrahepatic insulin extraction progressively increased from Lean-NL to Obese-NL to Obese-NAFLD. Total hepatic insulin extraction plateaued at high rates of insulin delivery, whereas the relationship between systemic insulin appearance and total extrahepatic extraction was linear.

Conclusion. Hyperinsulinemia after glucose ingestion in Obese-NL and Obese-NAFLD is due […]

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ABSTRACT

Background. Insulin is a key regulator of metabolic function. The effects of excess adiposity, insulin resistance and hepatic steatosis on the complex integration of insulin secretion and hepatic and extrahepatic tissue extraction are not clear.

Methods. A hyperinsulinemic-euglycemic clamp and a 3-hour oral glucose tolerance test were used to evaluate insulin sensitivity and insulin kinetics after glucose ingestion in three groups: i) lean with normal intrahepatic triglyceride (IHTG) and glucose tolerance (Lean-NL; n=14); ii) obese with normal IHTG and glucose tolerance (Obese-NL; n=24); and iii) obese with hepatic steatosis and prediabetes (Obese-NAFLD; n=22).

Results. Insulin sensitivity progressively decreased and insulin secretion progressively increased from Lean-NL to Obese-NL to Obese-NAFLD. Fractional hepatic insulin extraction progressively decreased from Lean-NL to Obese-NL to Obese-NAFLD, whereas total hepatic insulin extraction (molar amount removed) was greater in Obese-NL and Obese-NAFLD than Lean-NL. Insulin appearance in the systemic circulation and extrahepatic insulin extraction progressively increased from Lean-NL to Obese-NL to Obese-NAFLD. Total hepatic insulin extraction plateaued at high rates of insulin delivery, whereas the relationship between systemic insulin appearance and total extrahepatic extraction was linear.

Conclusion. Hyperinsulinemia after glucose ingestion in Obese-NL and Obese-NAFLD is due to an increase in insulin secretion, without a decrease in total hepatic or extrahepatic insulin extraction. However, the liver's maximum capacity to remove insulin is limited because of a saturable extraction process. The increase in insulin delivery to the liver and extrahepatic tissues in Obese-NAFLD is unable to compensate for the increase in insulin resistance, resulting in impaired glucose homeostasis.
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INTRODUCTION

Obesity is associated with nonalcoholic fatty liver disease (NAFLD), multiorgan insulin resistance, and hyperinsulinemia, which are major risk factors for both type 2 diabetes and coronary heart disease (1-4). Although hyperinsulinemia and insulin resistance are likely involved in the pathogenesis of NAFLD (5), excess intrahepatic triglyceride (IHTG) content could also contribute to hyperinsulinemia and insulin resistance. The liver is important in regulating systemic plasma insulin concentrations because it is the major site for insulin clearance; in people who are lean and healthy, a large portion (~50%) of the insulin delivered to the liver is cleared during first pass transit and an additional 20% is cleared through subsequent passes (6, 7). The remaining 30% of insulin secreted by the pancreas is removed by extrahepatic organs, primarily the kidneys and skeletal muscle (6, 8). Increased insulin secretion and impaired hepatic insulin clearance in people with NAFLD could contribute to insulin resistance by chronically exposing insulin sensitive tissues to large amounts of insulin, which can downregulate insulin receptor binding affinity and insulin receptor number (9-12). Even 24 hours of an experimentally-induced increase in plasma insulin concentration causes hepatic and skeletal muscle insulin resistance (13) and a single dose of a pharmacological agent that decreases insulin secretion lowers 24-h plasma glucose and insulin concentrations and improves oral glucose tolerance (14) in healthy, lean adults. However, the relationship between IHTG content and insulin kinetics is not clear because of conflicting data from different studies that found insulin secretion was either increased or the same and insulin clearance was either decreased or the same in people with NAFLD than in those with normal IHTG content (15-18). The reason for the differences between studies could be related to differences in subject characteristics and the methods used to assess IHTG content and insulin metabolism.

The purpose of the present study was to provide a comprehensive evaluation of the complex interrelationship among obesity, insulin resistance, hepatic steatosis and insulin kinetics. A hyperinsulinemic-euglycemic clamp procedure (HECP), in conjunction with stable
isotopically labeled glucose tracer infusion, and a 3-hour oral glucose tolerance test (OGTT) were used to evaluate insulin sensitivity and insulin kinetics in three cohorts of people who differed in adiposity, insulin sensitivity and IHTG content: i) lean with normal IHTG content and normal fasting glucose and oral glucose tolerance (Lean-NL); ii) obese with normal IHTG content and normal fasting glucose and oral glucose tolerance (Obese-NL); and iii) obese with high IHTG content and evidence of abnormal glucose metabolism (impaired fasting glucose or oral glucose tolerance) (Obese-NAFLD). We used a recently developed modeling approach (15, 19) in conjunction with C-peptide deconvolution to assess different aspects of insulin kinetics in response to glucose ingestion, including insulin secretion rate and hepatic, extrahepatic, and whole-body insulin plasma clearance and tissue extraction rates. A better understanding of the inter-relationships among adiposity, IHTG content, insulin sensitivity and insulin kinetics can provide new insights into the mechanisms that regulate glucose homeostasis in people with obesity and those with obesity and NAFLD.

RESULTS

Body composition and metabolic characteristics

The Obese-NL and Obese-NAFLD groups were matched on BMI and percent body fat, but IHTG content was nine-fold greater in the Obese-NAFLD than the Obese-NL group, without a difference in IHTG content between the Lean-NL and Obese-NL groups (Table 1). Fasting plasma glucose concentration, plasma glucose 2 hours after glucose ingestion, and HbA1c were higher in the Obese-NAFLD group than in both the Obese-NL and Lean-NL groups, without any differences between the Obese-NL and Lean-NL groups (Table 1). Fasting plasma insulin and C-peptide concentrations increased progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group (Table 1). Both hepatic insulin sensitivity (assessed as the reciprocal of the product of basal endogenous glucose production rate and basal plasma insulin
concentration) and muscle insulin sensitivity (assessed as the glucose disposal rate relative to plasma insulin concentration during the HECP) decreased progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group (Table 1).

Plasma glucose, insulin and C-peptide responses to glucose ingestion

Both plasma glucose concentration and plasma glucose area under the curve (AUC) after glucose ingestion were greater in the Obese-NAFLD group than in the Obese-NL and Lean-NL groups, which were not significantly different from each other (Figure 1, A and B). Plasma insulin and C-peptide concentrations and AUCs after glucose ingestion increased progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group (Figure 1, C - F). Plasma insulin concentration AUC in the Obese-NAFLD group was two-fold greater than in the Obese-NL group and 3.5 times greater than in the Lean-NL group, whereas plasma C-peptide concentration AUC was only 50% greater in the Obese-NAFLD than in the Obese-NL group and two-fold greater than in the Lean-NL group.

Insulin kinetics

The kinetic model accurately described the insulin data from both the OGTT and HECP (average normalized root mean square error: 6.3 ± 3.5%) (Supplemental Figure 1). The amount of insulin delivered to the liver is comprised of both insulin secreted by β-cells and insulin that passes through the liver into the systemic circulation that is recycled back to the liver. Both the insulin secretion rate (ISR) and the rate of insulin recycled back to the liver during the 3-h OGTT increased progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group (Figure 2A). Fractional hepatic insulin extraction (i.e. the fraction of insulin delivered to the liver that is removed by the liver) decreased progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group, and was significantly lower in the Obese-NAFLD group than in both the Obese-NL and Lean-NL groups (Figure 2B). However, the rate of total hepatic insulin extraction (i.e. the
molar amount of insulin removed from plasma by the liver per minute) progressively increased from the Lean-NL to the Obese-NL to the Obese-NAFLD group, and was greater in both the Obese-NAFLD and Obese-NL groups than in Lean-NL group without a difference between the Obese-NAFLD and Obese-NL groups (Figure 2C). Although the fractional extraction of insulin by extrahepatic tissues (i.e. the fraction of insulin delivered to extrahepatic tissues that is removed) was not different in the Lean-NL (34 ± 2%), Obese-NL (28 ± 3%) and Obese-NAFLD (30 ± 2%) groups (P=0.60), the rate of total extrahepatic insulin extraction (i.e. the molar amount of insulin removed by extrahepatic tissues per minute) progressively increased from the Lean-NL to the Obese-NL to the Obese-NAFLD group and was more than double in the Obese-NAFLD than in the Obese-NL group (Figure 2D). The rate of total (whole-body) insulin extraction increased progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group because of increases in both total hepatic and extrahepatic insulin extraction rates (Figure 2E). The liver accounted for ~70% of whole-body insulin extraction in the Lean-NL and Obese-NL groups, but only ~50% in the Obese-NAFLD group; conversely, extrahepatic insulin extraction increased from ~30% of whole-body insulin extraction in the Lean-NL and Obese-NL groups to ~50% in the Obese-NAFLD group (Figure 2F). The relationship between the rate of insulin delivered to the liver (i.e. ISR and post-hepatic insulin that is not removed by extrahepatic tissues and is recycled back to the liver) and the rate of total hepatic insulin extraction demonstrated a saturable process that began to plateau with the increase in hepatic insulin delivery rates observed in the Obese-NL group, with considerable variability in the rate of hepatic insulin extraction at any given rate of insulin delivery (Figure 2G). In contrast, total extrahepatic insulin extraction rate increased linearly with increasing rates of insulin delivery into the systemic circulation (i.e. total post-hepatic insulin appearance in plasma) (Figure 2H).
Inter-relationships among insulin sensitivity, plasma insulin concentration, intrahepatic triglyceride content and insulin kinetics

Whole-body insulin clearance rate (i.e., volume of plasma cleared of insulin per minute) during the OGTT was positively correlated with insulin sensitivity (assessed as the glucose rate of disposal relative to plasma insulin concentration [glucose Rd/I] during the HECP) (Figure 3A), whereas whole-body insulin extraction rate (i.e. the molar amount of insulin removed per minute) was negatively correlated with muscle insulin sensitivity (Figure 3B). The rate of whole-body insulin clearance was negatively correlated with plasma insulin AUC during the OGTT (Figure 3C), whereas whole-body insulin extraction rate was positively correlated with plasma insulin AUC and was best described by a saturable, Michaelis-Menten relationship (20) (Figure 3D), presumably driven by the saturability of hepatic insulin extraction. There was no significant correlation between either fractional hepatic insulin extraction or total hepatic insulin extraction rate and IHTG content in the Obese-NAFLD group (Supplemental Figure 2).

Indices of β-cell function

The ISR during the OGTT was inversely correlated with muscle insulin sensitivity, and ISR increased as muscle insulin sensitivity decreased in a curvilinear fashion (Figure 4A). The β-cell function index (i.e. the incremental ISR in relation to muscle insulin sensitivity), which provides a measure of insulin secretion by β-cells in relation to insulin sensitivity, decreased progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group and was significantly lower in the Obese-NAFLD group than in the Lean-NL and Obese-NL groups (Figure 4B). Therefore, the high ISR in the Obese-NL group adequately compensated for the decrease in insulin sensitivity needed to maintain normal oral glucose tolerance. However, even the very high ISR in the Obese-NAFLD was not adequate to compensate for the further decrease in insulin sensitivity in the Obese-NAFLD group, resulting in abnormal glucose tolerance (Figure 1A).
DISCUSSION

We conducted an OGTT and a HECP in three carefully characterized cohorts of participants who were either lean with normal glucose tolerance and normal IHTG content, obese with normal glucose tolerance and normal IHTG content, or obese with prediabetes and NAFLD to help dissect the effects of adiposity, insulin resistance and hepatic steatosis on insulin kinetics. Based on the assessment of hepatic and muscle insulin sensitivity measured during the HECP, these groups represented a progressive deterioration in insulin sensitivity from the Lean-NL to the Obese-NL to the Obese-NAFLD group. A recently developed modeling approach (15, 19) and C-peptide deconvolution were used to provide a comprehensive analysis of insulin kinetics in response to glucose ingestion, including insulin secretion by β-cells and hepatic, extrahepatic, and whole-body insulin plasma clearance and tissue extraction rates. The major findings from our study are: i) ISR in response to glucose ingestion progressively increased from the Lean-NL to the Obese-NL to the Obese-NAFLD groups, but β-cell function, assessed as the increase in ISR in relation to insulin sensitivity, was lower in Obese-NAFLD than in Lean-NL and Obese-NL groups; ii) hepatic steatosis does not impair the rate of hepatic insulin extraction (molar amount of insulin removed from plasma per unit of time), and total hepatic insulin extraction rates were greater in the Obese-NL and Obese-NAFLD groups than in the Lean-NL group, but not different between Obese-NAFLD and Obese-NL; iii) the rate of total extrahepatic insulin extraction progressively increased from the Lean-NL to the Obese-NL to the Obese-NAFLD group; iv) total hepatic insulin extraction rate plateaus when hepatic insulin delivery (from newly secreted and recycled insulin) is high, whereas total extrahepatic insulin extraction rate increases linearly with increasing delivery of insulin into the systemic circulation; and v) whole-body insulin clearance rate (volume of plasma cleared of insulin) is positively correlated, whereas whole-body insulin extraction rate (the molar amount of insulin removed by all tissues) is negatively correlated, with muscle insulin sensitivity. Our data demonstrate that the greater increase in plasma insulin concentrations in response to an oral glucose challenge
in the Obese-NL and Obese-NAFLD than in the Lean-NL groups is due to an increase in insulin secretion, not a reduction in total insulin extraction by the liver or extrahepatic tissues. However, the liver’s capacity to remove insulin is a saturable process that reaches maximum capacity when hepatic insulin delivery is high, which occurred after glucose ingestion in our Obese-NL and Obese-NAFLD groups. In contrast, extrahepatic insulin extraction after glucose ingestion increases linearly with increases in insulin delivery into the systemic circulation. Nonetheless, the marked increases in hepatic and extrahepatic insulin delivery and extraction in the Obese-NAFLD group were not adequate to compensate for the decrease in insulin sensitivity, resulting in impaired glucose homeostasis.

Systemic plasma insulin concentration is determined by the rate of insulin secretion by β-cells and the rate of insulin removal by the liver and extrahepatic tissues. The results from our study provide an integrated assessment of insulin kinetics in response to an oral glucose challenge, including rates of insulin secretion, hepatic and extrahepatic insulin extraction and recycling of post-hepatic insulin back to the liver, in distinct cohorts of people who differed in adiposity, IHTG content, and hepatic and muscle insulin sensitivity (Figure 5). The data demonstrate that both insulin secreted by the pancreas and insulin recycled from the systemic circulation progressively increase the total delivery of insulin to the liver (pre-hepatic insulin) from Lean-NL to Obese-NL to Obese-NAFLD. The liver’s ability to increase the rate of insulin extraction when insulin delivery to the liver is increased, as in the Obese-NL and Obese-NAFLD groups, is limited presumably because of a saturable hepatic insulin transport system (21-23). Therefore, an increase in the delivery of insulin to the liver is associated with a decrease in fractional hepatic insulin extraction, and more insulin passes through the liver into the systemic circulation. Most of the insulin that enters the systemic circulation (post-hepatic insulin) is recycled back to the liver but a progressively increasing amount of insulin is removed by extrahepatic tissues (primarily the kidneys and skeletal muscle (6, 8)) in the Lean-NL, Obese-NL and Obese-NAFLD groups. In all groups, >99% of insulin secreted by β-cells was removed by
hepatic and extrahepatic tissues during the 180-min OGTT. However, small differences between the rate of insulin secretion and removal among the three groups resulted in marked differences in plasma insulin concentration at the 180-minute timepoint (60 ± 22, 158 ± 38, and 532 ± 80 pmol/L in the Lean-NL, Obese-NL, and Obese-NALFD groups, respectively) (Figure 1C). These results demonstrate that the major factor responsible for hyperinsulinemia in people with obesity who have insulin resistance and NAFLD is β-cell hypersecretion in conjunction with a saturable insulin extraction process in the liver.

Although there was a large range in IHTG content in the Obese-NAFLD group, there was no correlation between either fractional hepatic insulin extraction or the rate of total hepatic insulin extraction and the severity of steatosis. In addition, total hepatic insulin extraction rate was not significantly different in the Obese-NAFLD and Obese-NL groups. These results challenge the notion that NAFLD per se impairs hepatic insulin extraction. However, we also found considerable variability in hepatic insulin extraction rate at any given rate of insulin delivery to the liver in the Obese-NL and Obese-NAFLD groups. The reasons responsible for the heterogeneity in the rate of total hepatic insulin extraction are not clear, but could be related to individual subject variability in some of the assumed values the kinetic model uses, such as hepatic blood flow and C-peptide kinetics, differences in the expression of insulin receptors, and differences in the content of intrahepatic proteins involved in insulin degradation, namely hepatic carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) and insulin-degrading enzyme (IDE) (24).

In people with normal glucose tolerance, there is a hyperbolic relationship between insulin sensitivity and the increase in plasma insulin concentration in response to an oral or intravenous glucose challenge; the product of these two variables is known as the disposition index (DI) (25-27). Accordingly, DI values are maintained when a decrease or increase in insulin sensitivity is compensated by a corresponding increase or decrease, respectively, in the plasma insulin response to a glucose load (28-30). However, the prevailing plasma insulin concentration
is a function of both the rate of insulin secretion and the rate of insulin removal. Therefore, the DI concept implies that β-cells and the liver are somehow able to sense changes in whole-body insulin sensitivity and adjust the rate of insulin secretion and removal as needed to prevent hypoglycemia, while increasing circulating insulin to compensate for insulin resistance. The data from our study suggest insulin secretion drives this process, whereas hepatic insulin extraction is likely a passive function of insulin delivery that becomes saturated at high insulin delivery rates. Despite the very high ISR and plasma insulin concentrations after glucose ingestion in the Obese-NAFLD group, postprandial plasma glucose concentrations were much higher in the Obese-NAFLD than the Obese-NL and Lean-NL groups. Therefore, the increased β-cell response and increase in plasma insulin concentrations in the Obese-NAFLD group were unable to compensate for the increase in insulin resistance, which is consistent with the observed lower β-cell function index (i.e. incremental insulin secretion in relation to insulin sensitivity) in the Obese-NAFLD group than in the Lean-NL and Obese-NL groups.

Our study is not able to determine whether the increased ISRs in the Obese-NL and Obese-NAFLD groups are a cause or a consequence of insulin resistance, or possibly both. The increased ISRs could be due to a β-cell compensatory response to insulin resistance as proposed by others (31), which is consistent with the inverse correlation between ISR and whole-body insulin sensitivity observed among our entire cohort of subjects. However, the mechanism responsible for the β-cell’s ability to “sense” insulin resistance in other tissues has not been identified. Increased insulin secretion can also be caused by intrinsic β-cell hyper-reactivity to substrate, hormonal and neural stimuli and even environmental pollutants (32, 33). In addition, the normal feedback suppression of insulin secretion by circulating insulin is blunted in people with obesity (34). Accordingly, hyperinsulinemia in people with obesity could lead to a “vicious insulin cycle,” in which increased insulin secretion causes insulin resistance, which in turn stimulates increased insulin secretion. The high rate of insulin secretion and plasma insulin concentrations can have adverse long-term clinical consequences because high ISR is a risk
factor for developing type 2 diabetes (29, 32). These findings suggest the most effective approach for preventing prediabetes and T2D in people with obesity should include interventions that decrease demand for insulin secretion and result in lower plasma concentrations (35, 36).

Several limitations of our study should be considered. First, differences in insulin secretion and clearance rates have been reported among different racial/ethnic groups (37-39), so the results from our study, which was conducted primarily in Caucasian (65%) and African American (27%) participants, might not apply to other racial/ethnic populations. In an effort to reduce the potential confounding effect of race, we included race as a covariate in our statistical analyses. In addition, we performed additional analyses that evaluated the data from the Caucasian and African American participants separately. All significant differences between Lean-NL, Obese-NL, and Obese-NAFLD groups and significant correlations between outcome measures were maintained when evaluating Caucasian participants only. The same pattern in differences in outcomes between groups and the correlations between outcomes were maintained in the African American participants, but some of these assessments did not achieve statistical significance because of inadequate sample size in this subgroup. Second, the model used to assess insulin kinetics includes estimated values for C-peptide kinetic parameters and hepatic blood flow that are based on standard estimates that do not fully account for inter-individual variability and are assumed to be the same during the oral glucose tolerance test and the clamp procedure. Third, our study is a cross-sectional analysis of weight-stable participants, so we cannot exclude the possibility that changes in insulin kinetics occur over time or in response to changes in diet or body weight.

In summary, the large increase in plasma insulin concentrations in response to an oral glucose challenge that is commonly observed in people with obesity and further exacerbated in people with obesity who have NAFLD and greater insulin resistance is driven by increased insulin secretion, without an intrinsic defect in hepatic or extrahepatic insulin extraction.
Therefore, the progressive decrease in whole-body insulin clearance rates (volume of plasma cleared of insulin per unit of time) from Lean-NL to Obese-NL to Obese-NAFLD is likely a consequence, rather than a cause, of hyperinsulinemia. The rate of insulin extraction by the liver, but not by extrahepatic tissues, becomes saturable when the postprandial delivery of insulin to the liver is high. In people with severe insulin resistance, the increased delivery of insulin to the liver and extrahepatic tissues is unable to compensate for the decrease in insulin sensitivity, resulting in impaired glucose homeostasis.

**METHODS**

**Subjects**

A total of 60 men and women participated in this study. The subject flow is shown in Supplemental Figure 3. 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 procedures for this study were conducted in the Clinical Translational Research Unit (CTRU) and Center for Clinical Imaging Research (CCIR) at Washington University School of Medicine. Potential subjects completed an initial evaluation that included a medical history and physical examination, standard blood tests, a 3-h OGTT and an assessment of body composition including IHTG content. Subjects were enrolled if they met the criteria for inclusion into one of three groups: i) Lean-NL, defined as having a body mass index (BMI) of 18.5-24.9 kg/m\(^2\), and normal fasting plasma glucose (<100 mg/dL), oral glucose tolerance (2-h glucose <140 mg/dL) and IHTG content (≤5%) (n=14; 36 ± 2 yrs old; 7 men and 7 women; 9 Caucasian, 1 African American and 4 Asian); Obese-NL, defined as having a BMI of 30.0-49.9 kg/m\(^2\) and normal fasting plasma glucose, oral glucose tolerance and IHTG content (n=24; 39 ± 2 yrs old; 3 men, 21 women; 13 Caucasian and 11 African American); and Obese-NAFLD, defined as having a BMI of 30.0-49.9 kg/m\(^2\), impaired fasting glucose or oral glucose
tolerance and high IHTG content (≥10%) (n=22; 42 ± 2 yrs old; 6 men and 16 women; 17 Caucasian, 4 African American and 1 Pacific Islander). No subject had evidence of diabetes, serious illnesses other than NAFLD, was taking medications that could interfere with insulin action or secretion, consumed excessive alcohol (>14 drinks/week for women and >21 drinks/week for men) or smoked tobacco products.

Experimental Procedures

Body composition analyses. Total body fat and fat-free masses were determined by using dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Madison, WI) and IHTG content was determined by using magnetic resonance imaging (3.0-T superconducting magnet; Siemens, Iselin, NJ) (5).

Oral glucose tolerance test. Subjects were admitted to the CTRU at Washington University School of Medicine at 0700 h after subjects fasted for ~11 h overnight at home. An intravenous catheter was inserted into an antecubital or hand vein for serial blood sampling. Plasma glucose, insulin and C-peptide concentrations were determined at 15, 10, and 5 minutes before, and 10, 20, 30, 60, 90, 120, 150, and 180 minutes after consuming a 75 g glucose beverage. The average of the 3 baseline samples (i.e., -15, -10 and -5 minutes before consuming the 75 g glucose beverage) was used as the t=0 glucose, insulin and plasma C-peptide concentrations.

Hyperinsulinemic-euglycemic clamp procedure. Subjects were admitted to the CTRU at 1800 h for ~48 hours. Subjects were given standard meals containing one-third of their estimated energy requirements (40) upon admission (day 0) and at 0700 h, 1300 h, and 1900 h on day 1. The HECP was performed on Day 2, after subjects fasted overnight. At 0700 h, a primed (8.0 µmol/kg) continuous (0.08 µmol/kg/min) infusion of [U-13C]glucose (Cambridge Isotope Laboratories Inc., Andover, MA) was started. After the infusion of glucose tracer for 3.5 h (basal period), insulin was infused at a rate of 50 mU/m²/min (initiated with a two-step priming
dose of 200 mU/m²/min for 5 min followed by 100 mU/m²/min for 5 min) and euglycemia (~100 mg/dl) was maintained by variable rate infusion of a 20% dextrose solution that was enriched to ~1% with [U-¹³C]glucose. The infusion of [U-¹³C]glucose was stopped during insulin infusion because of the expected decrease in hepatic glucose production. Blood samples were obtained before beginning the glucose tracer infusion and every 6-7 minutes during the last 20 min of the basal and insulin infusion stages to determine glucose, C-peptide and insulin concentrations and glucose kinetics.

Sample analyses and calculations. Blood samples were collected in chilled tubes containing EDTA or heparin and placed in ice. Plasma was separated by centrifugation within 30 min of collection and then stored at -80 °C until final analyses. Plasma glucose concentration was determined by using the glucose oxidase method (YSI, Inc., Yellow Springs, Ohio) and plasma insulin and C-peptide concentrations were determined by using electrochemiluminescence assays (Elecsys 2010, Roche Diagnostics, Indianapolis, IN). Plasma glucose tracer-to-tracee ratio was determined by using gas-chromatography/mass-spectrometry as described previously (41).

Plasma glucose, insulin and C-peptide concentration areas under the curve (AUC) during the OGTT were calculated by using the trapezoidal method (42). Hepatic insulin sensitivity was calculated as the reciprocal of the product of basal endogenous glucose production rate (in µmol/kg fat-free mass/min) and basal plasma insulin concentration (in µU/mL) (1). Total glucose rate of disappearance (Rd) during insulin infusion was assumed to be equal to the sum of endogenous glucose rate of appearance into the bloodstream and the rate of infused glucose during the last 20 min of the HECP (1). An index of muscle insulin sensitivity was calculated as glucose Rd expressed per kg fat-free mass divided by the plasma insulin concentration (glucose Rd/I) during the final 20 minutes of the HECP. Insulin secretion rates were calculated by using C-peptide deconvolution (43). Insulin secretion in relationship to insulin sensitivity was used to provide an index of β-cell function, and calculated as the product of the
incremental AUC in insulin secretion rate (ISR) above time 0 from 0 to 180 min of the OGTT and insulin sensitivity assessed during the HECP (ΔISR0-180 x Glucose Rd/I).

Whole-body insulin clearance rate (i.e. volume of plasma cleared of insulin per minute) was calculated by using a one-compartment model for plasma insulin: (AUC ISR/AUC I) - V×(I180-I0)/AUC I, where V is the distribution volume for insulin estimated as 141 mL/kg (19), and I0 and I180 are the plasma insulin concentrations at time 0 (baseline) and 180 min, respectively during the OGTT. A recently developed, mathematical modeling approach that involves the use of plasma insulin concentration and ISR data from both the OGTT and HECP (15, 19) was used to provide a comprehensive assessment of the kinetics of hepatic and extrahepatic insulin removal from plasma during the OGTT. In this model, hepatic insulin clearance for each subject was modeled by using either a linear or saturable model, and the model that provided the better fit was used for that subject. In addition, extrahepatic insulin clearance was assumed to be linear; this assumption was confirmed by testing a saturable model for extrahepatic insulin clearance and finding a linear model provided the best fit of the data for all subjects. The following measures of insulin kinetics were determined: i) fractional hepatic insulin extraction (i.e. the fraction of insulin delivered to the liver that is removed by the liver); ii) total hepatic insulin extraction rate (i.e. molar amount of insulin removed from plasma by the liver per minute); iii) rate of insulin recycled from the systemic circulation back to the liver (i.e., insulin that passes through the liver into the systemic circulation that is not removed by extrahepatic tissues and is recycled back to the liver); iv) rate of extrahepatic insulin extraction (i.e. molar amount of insulin removed from plasma by extrahepatic tissues per unit of time); and v) whole-body insulin extraction rate (i.e. sum of the hepatic and extrahepatic insulin extraction rates).

Statistical analysis

A one-way analysis of variance (ANOVA) was used to compare subject characteristics among Lean-NL, Obese-NL and Obese-NAFLD groups. Between-group differences in insulin
secretion rate, clearance rate, total extraction rate and fractional extraction were assessed by using analysis of covariance (ANCOVA) with race and sex as covariates. Where appropriate, post-hoc analyses were used to locate significant mean differences. Modeled and measured plasma insulin concentration profiles were compared by using the normalized root mean square error, as previously described (15, 19). The significance of the relationships among outcome measures were evaluated by using either linear or nonlinear regression. Relationships that involved IHTG content were analyzed separately for subjects with normal IHTG content (Lean-NL and Obese-NL groups) and high IHTG content (Obese-NAFLD group) because there was no continuum in IHTG content per study design. The relationship between whole-body insulin extraction rate and plasma insulin concentration AUC during the OGTT was assessed by using Michaelis-Menten kinetics to determine whether rate of insulin extraction could be explained by saturable, receptor-mediated insulin uptake. Statistical significance was defined as a P value <0.05. Statistical analyses were performed by using SPSS (version 25, IBM, Armonk, NY). Data are reported as means ± SEM.

Based on the inter-individual variability in fractional hepatic insulin extraction we previously reported (15), we estimated that 15 subjects in each group would be needed to detect between-group differences in fractional hepatic insulin extraction rates of 20% by using a two-sided test with a β-value of 0.9 and an α-value of 0.05. These computations were performed by using G*Power 3.1.9.2 (44).

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

GIS, MY and MLK conducted the studies. DCP performed the insulin kinetic modeling. BWP supervised the sample analyses. GIS, DCP, BWP, BM and SK analyzed the data, performed the statistical analyses and wrote the manuscript. SK designed and supervised the studies and obtained funding for the work. SK is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors critically reviewed and edited the manuscript.

ACKNOWLEDGEMENTS

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REFERENCES


4. Magkos, F, Fabbrini, E, Mohammed, BS, Patterson, BW, Klein, S. Increased whole-body adiposity without a concomitant increase in liver fat is not associated with augmented metabolic dysfunction. *Obesity (Silver Spring)*. 2010;18(8):1510-1515.


<table>
<thead>
<tr>
<th></th>
<th>Lean-NL (n=14)</th>
<th>Obese-NL (n=24)</th>
<th>Obese-NAFLD (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>65 ± 2</td>
<td>106 ± 4*</td>
<td>116 ± 4*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23 ± 1</td>
<td>38 ± 1*</td>
<td>40 ± 1*</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>46 ± 2</td>
<td>55 ± 2*</td>
<td>59 ± 2*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>28 ± 2</td>
<td>47 ± 1*</td>
<td>48 ± 1*</td>
</tr>
<tr>
<td>IHTG content, %</td>
<td>1.6 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>21.0 ± 1.4*†</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.0 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.7 ± 0.1*†</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.7 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>5.6 ± 0.1*†</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>36 ± 3</td>
<td>84 ± 7*</td>
<td>196 ± 23*†</td>
</tr>
<tr>
<td>Fasting C-peptide, pmol/L</td>
<td>487 ± 34</td>
<td>826 ± 34*</td>
<td>1,491 ± 94*†</td>
</tr>
<tr>
<td>OGTT 2-h glucose, mmol/L</td>
<td>5.3 ± 0.3</td>
<td>6.0 ± 0.2</td>
<td>9.4 ± 0.3*†</td>
</tr>
<tr>
<td>HISI, 100/(μmol/kg FFM/min · μU/mL)</td>
<td>1.13 ± 0.12</td>
<td>0.53 ± 0.04*</td>
<td>0.30 ± 0.03*†</td>
</tr>
<tr>
<td>Glucose Rd/Insulin, (nmol/kg FFM/min)/(pmol/L)</td>
<td>89 ± 6</td>
<td>56 ± 5*</td>
<td>30 ± 2*†</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Abbreviations: FFM=fat-free mass; HbA1c=hemoglobin A1c; HISI=hepatic insulin sensitivity index; IHTG=intrahepatic triglyceride; NAFLD=nonalcoholic fatty liver disease; NL=normal; OGTT=oral glucose tolerance test; Rd=rate of disappearance. One-way analysis of variance (ANOVA) and post-hoc testing where appropriate was used to identify significant mean differences among groups. *Value significantly different from the corresponding value in the Lean-NL group, P <0.05.

†Value significantly different from the corresponding value in the Obese-NL group, P <0.05.
Figure 1. Plasma glucose, insulin, and C-peptide responses to glucose ingestion. Plasma glucose, insulin and C-peptide concentrations before and for 3 hours after ingesting a 75 g glucose drink (A,C,E) and plasma glucose, insulin and C-peptide 3-h concentration areas under the curve (AUC) (B,D,F) in the Lean-normal (NL), Obese-NL and Obese-nonalcoholic fatty liver disease (NAFLD) groups. White, gray and black circles in panels A, C and E represent the Lean-NL (n=14), Obese-NL (n=24) and Obese-NAFLD (n=22) groups, respectively. Values are means ± SEM. In panels B, D and F one-way analysis of variance (ANOVA) and post-hoc testing where appropriate were used to identify significant mean differences between groups when appropriate. *Value significantly different from the Lean-NL value, P<0.05. †Value significantly different from the Obese-NL value, P<0.05.
Figure 2. Insulin kinetics after glucose ingestion. Rate of total insulin delivered to the liver, comprised of the rate of insulin secreted from β-cells (white bars) and the rate of insulin recycled from the systemic circulation back to the liver (gray bars) (A), fractional hepatic insulin extraction (B), rate of total hepatic insulin extraction (C), rate of total extrahepatic insulin extraction (D), absolute contribution of hepatic (white bars) and extrahepatic (gray bars) insulin extraction to total rate of whole body insulin extraction (E), and relative contribution of hepatic (white bars) and extrahepatic (gray bars) insulin extraction to total rate of whole-body insulin extraction (F) in the Lean-normal (NL) (n=14), Obese-NL (n=23) and Obese-nonalcoholic fatty liver disease (NAFLD) (n=21) groups. Values are means ± SEM and represent averages for 3 hours after glucose ingestion. One-way analysis of covariance (ANCOVA) with race and sex as covariates and post-hoc testing where appropriate were used to identify significant mean differences between groups. *Value significantly different from the Lean-NL value, \( P < 0.05 \). †Value significantly different from the Obese-NL value, \( P < 0.05 \). Relationship between insulin delivery to the liver and rate of total hepatic insulin extraction (G) and relationship between insulin delivery rate into the systemic circulation and rate of total extrahepatic insulin extraction (H) in Lean-NL (white circles; n=14), Obese-NL (gray circles; n=23) and Obese-NAFLD (black circles; n=21) participants. Logarithmic and linear regression analyses were used to determine the line of best-fit to the data in panels G and H, respectively.
Figure 3. Relationships among insulin sensitivity and insulin concentration after glucose ingestion and whole-body insulin clearance and extraction rates. Relationships among whole-body insulin clearance and extraction rates assessed for 3 hours after ingesting a 75 g glucose drink and muscle insulin sensitivity, assessed as glucose rate of disposal (Rd in nmol/kg fat-free mass/min) divided by plasma insulin (I) concentration (in pmol/L) during a hyperinsulinemic-euglycemic clamp procedure (A, B), and plasma insulin concentration area under the curve (AUC) (C, D). White, gray and black circles represent participants in the Lean-normal (NL) (n=14), Obese-NL (n=24 in panels A and C and n=23 in panels B and D) and Obese-nonalcoholic fatty liver disease (NAFLD) (n=22 in panels A and C and n=21 in panels B and D) groups, respectively. Logarithmic regression analysis was used to determine lines of best-fit to the data in panels A-C with Michaelis-Menten kinetics used to describe the line of best-fit in panel D.
Figure 4. Indices of β-cell function. (A) Relationship between muscle insulin sensitivity, assessed as glucose rate of disposal (Rd, in nmol/kg fat-free mass/min) divided by plasma insulin (I) concentration (in pmol/L) during a hyperinsulinemic-euglycemic clamp procedure and mean insulin secretion rate, assessed for 3 hours after ingesting a 75 g glucose drink in Lean-normal (NL) (white circles; n=14), Obese-NL (gray circles; n=24) and Obese-nonalcoholic fatty liver disease (NAFLD) (black circles; n=22) participants. Logarithmic regression analysis was used to determine the line of best-fit to the data. (B) β-cell function, assessed as the product of the incremental insulin secretion rate (in nmol • min) for 3 hours after glucose ingestion ($\Delta$ISR$_{0-180}$) and muscle insulin sensitivity. Values are means ± SEM. One-way analysis of covariance (ANCOVA) with race and sex as covariates and post-hoc testing where appropriate was used to identify significant mean differences between groups. *Value significantly different from the Lean-NL value, $P < 0.05$. †Value significantly different from the Obese-NL value, $P < 0.05$. 
Figure 5. Integrated summary of insulin kinetics after glucose ingestion. Values are mean rates (in pmol/min) for β-cell insulin secretion, tissue insulin extraction, and insulin accumulation in the systemic circulation assessed for 3 hours after ingesting a 75 g glucose drink. Insulin secretion by the pancreas into the portal circulation increases progressively from the Lean-normal (NL) to the Obese-NL to the Obese-nonalcoholic fatty liver disease (NAFLD) group. In addition, a large portion of insulin that enters the portal circulation is not immediately removed by the liver and extrahepatic tissues and is recycled back to the liver via the portal vein and hepatic artery, so the total amount of insulin delivered to the liver (newly secreted and recycled insulin) also increases progressively from the Lean-NL to the Obese-NL to the Obese-NAFLD group. Although the fractional hepatic extraction of delivered insulin progressively decreases, the rate of total hepatic insulin extraction progressively increases, from the Lean-NL to the Obese-NL to the Obese-NAFLD group. However, the rate of hepatic insulin extraction plateaus when the delivery of insulin to the liver is high, as in the Obese-NL and Obese-NAFLD groups, because of a saturable hepatic insulin transport system. Most of the insulin that passes through the liver and enters the systemic circulation is recycled back to the liver, and a progressively increasing amount of insulin is removed by extrahepatic tissues (primarily the kidneys and skeletal muscle) in Lean-NL, Obese-NL and Obese-NAFLD groups. A small portion of insulin that enters the systemic circulation (post-hepatic insulin) is not removed by 180 min after glucose ingestion and is responsible for the increase in plasma insulin concentration above baseline at the 180-min timepoint.