Iatrogenic Hyperinsulinemia, Not Hyperglycemia, Drives Insulin Resistance in Type 1 Diabetes as Revealed by Comparison With GCK-MODY (MODY2)

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Although insulin resistance consistently occurs with type 1 diabetes, its predominant driver is uncertain. We therefore determined the relative contributions of hyperglycemia and iatrogenic hyperinsulinemia to insulin resistance using hyperinsulinemic-euglycemic clamps in three participant groups (n = 10/group) with differing insulinemia and glycemia: healthy control subjects (euinsulinemia and euglycemia), glucokinase–maturity-onset diabetes of the young (GCK-MODY; euinsulinemia and hyperglycemia), and type 1 diabetes (hyperinsulinemia and hyperglycemia matching GCK-MODY). We assessed the contribution of hyperglycemia by comparing insulin sensitivity in control and GCK-MODY and the contribution of hyperinsulinemia by comparing GCK-MODY and type 1 diabetes. Hemoglobin A_{1c} was normal in control subjects and similarly elevated for type 1 diabetes and GCK-MODY. Basal insulin levels in control subjects and GCK-MODY were nearly equal but were 2.5-fold higher in type 1 diabetes. Low-dose insulin infusion suppressed endogenous glucose production similarly in all groups and suppressed nonesterified fatty acids similarly between control subjects and GCK-MODY, but to a lesser extent for type 1 diabetes. High-dose insulin infusion stimulated glucose disposal similarly in control subjects and GCK-MODY but was 29% and 22% less effective in type 1 diabetes, respectively. Multivariable linear regression showed that insulinemia—but not glycemia—was significantly associated with muscle insulin sensitivity. These data suggest that iatrogenic hyperinsulinemia predominates in driving insulin resistance in type 1 diabetes.

Insulin resistance consistently occurs in type 1 diabetes, even among patients who lack traditional insulin resistance risk factors (1–5). Individuals with type 1 diabetes typically have 35–55% lower insulin sensitivity than matched control subjects (1–6). Because insulin resistance is strongly correlated with macrovascular disease in this condition (6–9), a better understanding of its root cause is needed. Early investigations attributed type 1 diabetes insulin resistance to hyperglycemia (10–15); however, more recent studies show little correlation between hyperglycemia and insulin resistance (3,4,6,16,17). Thus, the magnitude of hyperglycemia's contribution to insulin resistance in type 1 diabetes is uncertain, suggesting other factors may predominate.

Insulin resistance in type 1 diabetes can be alternatively hypothesized to be a homeostatic response to iatrogenic peripheral hyperinsulinemia. In the physiologic state, the liver clears ~50% of secreted insulin before it reaches the peripheral circulation. As a result, insulin levels at the liver are approximately two- to threefold higher

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than insulin levels at peripheral tissues. By contrast, in type 1 diabetes, the insulin injected into subcutaneous tissue is directly absorbed into the peripheral circulation. Thus, patients with type 1 diabetes have insulin concentrations that are higher in the peripheral circulation and lower in the hepatic portal blood compared with individuals without diabetes (18–20). This chronic peripheral circulation hyperinsulinemia could be the predominant contributor to insulin resistance and is an abnormality that could be remedied by hepatopreferential insulin analogs or intraperitoneal insulin delivery.

To distinguish the relative contributions of hyperinsulinemia versus hyperglycemia to type 1 diabetes insulin resistance, we quantified tissue-specific insulin sensitivity using a two-step hyperinsulinemic-euglycemic clamp technique in a cross-section of three groups of age- and BMI-matched participants (n = 10/group): *1*) control subjects without diabetes, *2*) subjects with type 1 diabetes, and *3*) individuals with glucokinase mutations causing glucokinase–maturity-onset diabetes of the young (GCK-MODY, also known as MODY2). The three groups possess key differences and similarities in glycemia and insulin distribution, allowing us to assess each factor's contribution to insulin resistance (Fig. 1*A*). Individuals with GCK-MODY retain pancreatic insulin secretion, but their GCK mutation raises their glycemic "set point," resulting in mild hyperglycemia (fasting plasma glucose 104–137 mg/dL, hemoglobin A_{1c} [HbA_{1c}] 5.8–7.6% [40–60 mmol/mol]) (21). Thus, whereas the group with GCK-MODY and the control group both have normal and similar portal-to-peripheral insulin distributions, hyperglycemia in the group with GCK-MODY is greater than in the control subjects, thereby potentially reducing insulin sensitivity in the subjects with GCK-MODY. We also recruited participants with type 1 diabetes with glycemia matching that of the group with GCK-MODY. Because glycemia was matched between groups, the presence of iatrogenic hyperinsulinemia in subjects with type 1 diabetes is the key difference affecting insulin sensitivity between the two groups. Our study exploited these key between-group differences to test the hypothesis that iatrogenic hyperinsulinemia plays a larger role than hyperglycemia in driving insulin resistance.

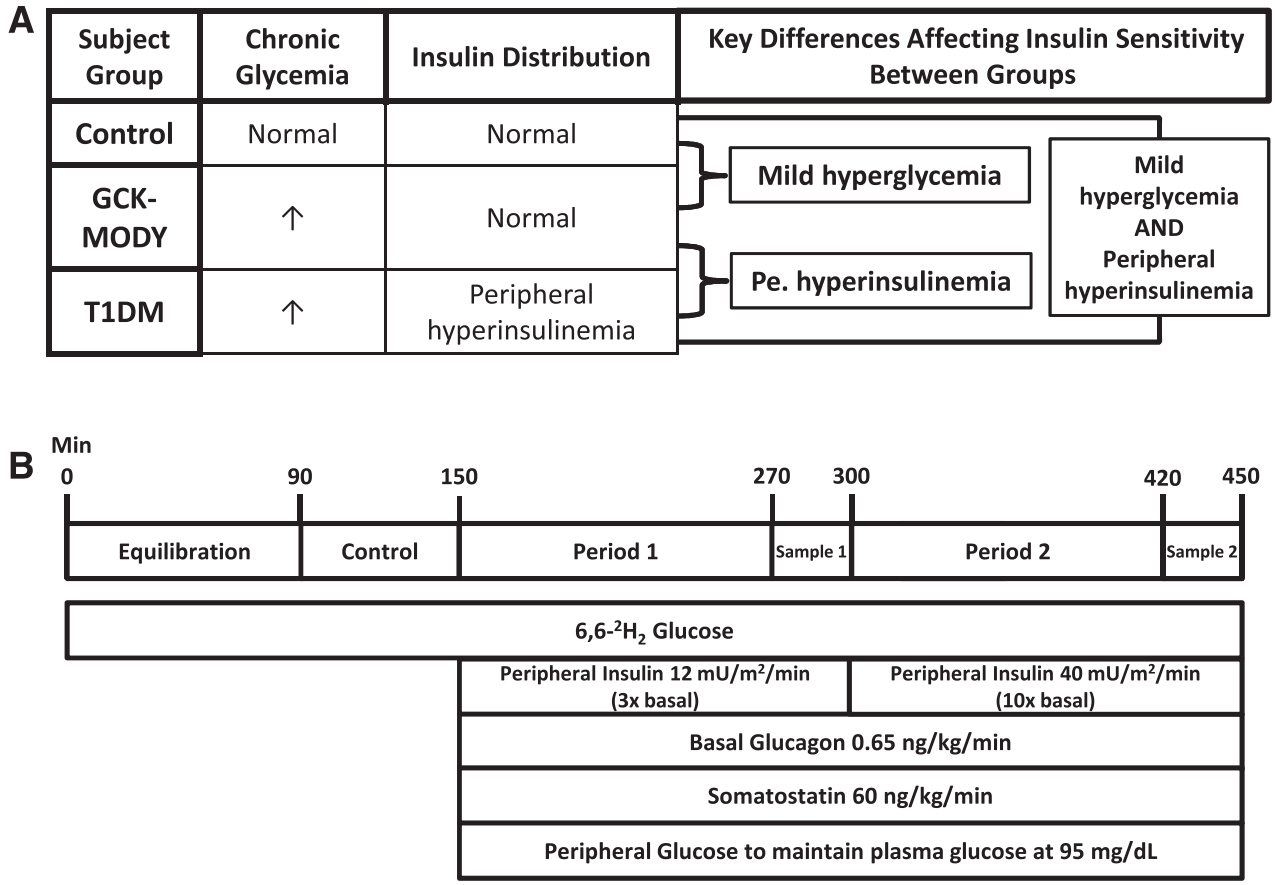


Figure 1—*A*: Key differences and similarities in chronic glycemia and insulin distribution affecting insulin sensitivity between participant groups. *B*: Hyperinsulinemic-euglycemic clamp protocol. Insulin infusion rates were intended to cause a 3- and 10-fold rise in plasma insulin levels in control subjects. In a 70 kg, 1.73 m^2 individual, 12 mU/m^2/min would approximately equal 0.3 mU/kg/min, and 40 mU/m^2/min would approximately equal 1.0 mU/kg/min. Pe. hyperinsulinemia, peripheral hyperinsulinemia; T1DM, type 1 diabetes.

RESEARCH DESIGN AND METHODS

Participants

Supplementary Table 1 details inclusion and exclusion criteria for study participants. In brief, volunteers were between ages 13 and 51 years, were nonobese, had no recent episodes of severe hypoglycemia or diabetes comorbidities, were taking no medications affecting insulin sensitivity, had reached Tanner stage 5, and were not pregnant. Participants with type 1 diabetes and GCK-MODY were required to have an HbA_{1c} between 5.9 and 7.5% (41–58 mmol/mol). Participants were recruited from the Vanderbilt Eskind Diabetes Clinic and from The University of Chicago Monogenic Diabetes Registry (http://monogenicdiabetes.uchicago.edu) (22). The study team recruited volunteers with type 1 diabetes to match volunteers with GCK-MODY within an HbA_{1c} ± 0.3% (3.3 mmol/mol), age ± 5 years, and BMI ± 1.5 kg/m^2. Control volunteers were recruited to match volunteers with GCK-MODY within a BMI of ±1.5 kg/m^2 and age ± 5 years.

Screening Visit

Potential participants fasted overnight and then reported to the Vanderbilt Clinical Research Center (CRC) for a screening visit to determine whether each individual met inclusion criteria. To quantify potential covariates differentially affecting insulin sensitivity between groups, the study team also measured several metabolic parameters. These factors included resting energy expenditure (REE), body composition, reactive hyperemia–peripheral artery tonometry (RH-PAT) score, VO_{2max}, and fasting blood concentrations of lipids, HbA_{1c}, insulin, and C-peptide.

The research team elicited each participant's clinical history, conducted a physical exam, and made anthropometric measurements. REE was determined using a metabolic cart system (TrueOne 2400; ParvoMedics, Sandy, UT) under thermoneutral conditions (23). REE was calculated using the Weir equation (24). RH-PAT score was measured to assess risk of endothelial dysfunction (Endo-PAT; Itamar Medical Ltd.) (25,26). Body composition was measured using DEXA (Lunar Prodigy, enCore software version 10.5; GE Medical Systems). Finally, the team determined VO_{2max} by measuring respiratory gas exchange during treadmill exercise per the Bruce protocol (Ultima CardiO2 gas exchange analysis system; MG Diagnostics, St. Paul, MN) (27).

Participants avoided strenuous exercise and consumed a caloric intake equaling 1.2 × REE over the 3 days preceding their clamp study. Individuals with type 1 diabetes and three individuals with GCK-MODY also monitored and recorded blood glucose eight times daily over these 3 days.

Hyperinsulinemic-Euglycemic Clamp Studies

Participants returned to the CRC within 1 month of the screening visit on the evening prior to their clamp study. At 10:00 P.M., all participants began an overnight fast. Upon beginning the overnight fast, participants with type 1 diabetes received an intravenous infusion of regular human insulin according to the protocol of Goldberg et al. (28), modified for use in healthy patients with type 1 diabetes (see Supplementary Data). The protocol targeted a plasma glucose concentration of 90–120 mg/dL by the next morning. Patients taking long-acting basal insulin used either insulin glargine or detemir, which was last given 24 h prior to beginning the intravenous insulin infusion. Patients taking continuous subcutaneous insulin infusions suspended and disconnected their pumps 15–30 min before starting the intravenous insulin infusion. Participants with GCK-MODY required no overnight insulin, but CRC staff monitored glucose every 2 h overnight. Female participants were studied on day 2–10 of their menstrual cycle.

Each clamp study commenced at 7:30 A.M. Experiments consisted of a 90-min equilibration period for [6,6-$^2H_2$]-glucose tracer infusion, a 60-min basal sampling period, and then two consecutive, 150-min experimental periods (Fig. 1*B*).

Insulin was infused intravenously at 12 mU/m^2/min in the first experimental period (period 1) and at 40 mU/m^2/min in the second (period 2). These rates were chosen to partially suppress lipolysis and hepatic glucose production at the end of period 1 and to completely inhibit lipolysis and hepatic glucose production while near-maximally stimulating muscle glucose uptake in period 2. In both experimental periods, somatostatin and glucagon were infused intravenously at 60 ng/kg/min and 0.65 ng/kg/min, rates selected to ensure glucagon remained at basal levels and equal between participants. A glucose tracer solution was prepared by dissolving 2.19 g (12.0 mmol) of [6,6-$^2H_2$]-glucose (Cambridge Isotope Laboratories, Tewksbury, MA) in 60 mL of isotonic saline. Participants received a [6,6-$^2H_2$]glucose priming dose of 22 μmol/kg over the first 10 min of the equilibration period, followed by infusion at 0.22 μmol/kg/min through the end of the basal sampling period. This rate was lowered to 0.11 μmol/kg/min during period 1 and discontinued during period 2. A 0.5-mL aliquot of arterialized blood was drawn and centrifuged every 10 min during period 1 and every 5 min during period 2 to sample plasma glucose and adjust a 20% dextrose solution infusion to maintain plasma glucose between 95 and 100 mg/dL. The 20% dextrose solution was spiked with glucose tracer by adding 6.9 g (37.8 mmol) of [6,6-$^2H_2$]glucose to 1,500 mL of stock 20% dextrose solution.

Research staff drew blood to assess metabolic and hormonal parameters three times during each of three 30-min steady-state sampling periods: during the basal sampling period and during the last 30 min of both 150-min experimental periods.

Analytical Procedures

The research team drew each arterialized venous blood sample from the upper extremity and immediately added the blood into tubes containing potassium EDTA. Blood

concentrations of lactate, alanine, and glycerol were determined using a fluorometric method of Lloyd et al. (29) modified for the Packard Multiprobe II (Meriden, CT) (30). Plasma nonesterified fatty acid (NEFA) concentrations were quantified using a colorimetry kit (Wako Life Sciences, Mountain View, CA) modified for the Packard Multiprobe II. Plasma catecholamine concentrations were measured using high-performance liquid chromatography (31). Plasma concentrations of insulin, glucagon, C-peptide, and cortisol were determined using radioimmunoassay (MilliporeSigma, Burlington, MA) (30). Plasma glucose concentrations were measured by the glucose oxidase method (YSI 2300 Stat Plus; YSI Life Sciences, Yellow Springs, OH). To obtain a measure of [6,6-$^2H_2$]glucose enrichment, plasma samples were derivatized to obtain a di-*O*-isopropylidene propionate derivative of glucose for gas chromatography/mass spectrometry analysis, as previously described (32). A custom Microsoft Excel macro was then used to correct for the theoretical natural abundance of isotopes to determine the plasma fractional enrichment of M+2 glucose.

Calculations

Plasma enrichment of [6,6-$^2H_2$]glucose was steady during each sampling period (Supplementary Fig. 1), and glucose turnover was calculated using the steady-state assumption for glucose tracer and tracee. Under these conditions,

$$R_a = \frac{R_a^*}{a}$$

and $R_a = R_d$, where R_a is the glucose appearance rate in the plasma, R_d is the glucose utilization rate, R_a^* is the infusion rate of [6,6-$^2H_2$]glucose tracer, and a is the plasma enrichment of tracer (i.e., the [6,6-$^2H_2$]glucose isotopomer fraction of total plasma glucose). Endogenous glucose production (EGP) was calculated by subtracting the unlabeled glucose infusion rate and the small but finite [6,6-$^2H_2$]glucose tracer rate from R_a. Glucose turnover was normalized for fat-free mass (FFM) to account for sex-related differences in fat mass.

To quantify insulin's ability to suppress whole-body EGP (ΔEGP), i.e., the net suppressive effect of insulin directly at liver and indirectly in the periphery by restraining mobilization of gluconeogenic substrates and NEFA, we subtracted each participant's mean EGP at the end of period 1 (when insulin partially suppressed EGP) from mean EGP during the basal period. Similarly, to assess insulin sensitivity at fat tissue, we subtracted the mean levels for NEFA and glycerol at period 1 from mean NEFA and glycerol levels at baseline. Insulin-dependent R_d is largely (~90%) reflective of glucose uptake by skeletal muscle during hyperinsulinemia (i.e., the net effect of insulin to facilitate muscle glucose disposal via microvascular, interstitial, intracellular, and neural mechanisms) (33). Thus, we subtracted mean R_d at the end of period 1 from mean R_d at the end of period 2 to quantify each participant's muscle insulin sensitivity.

Statistics

The sample size in this cross-sectional study design (10 per group) was calculated to detect a 40% difference in mean R_d between subjects with GCK-MODY and type 1 diabetes during period 2 with a two-sided α-level of 5% and 80% statistical power. The R_d variance and R_d for well-controlled individuals with type 1 diabetes used in sample size calculations were taken from Bergman et al. (3), where a 55% difference in R_d was seen between the group with type 1 diabetes and the control group.

The research team collected and managed study data using REDCap electronic data capture tools hosted at Vanderbilt University (34). Statistical analyses were conducted using SPSS 25 (IBM Corp., Armonk, NY). Statistically significant differences in continuous data were assessed using an independent-samples Student *t* test. A two-tailed *P* value of <0.05 was considered significant. HbA_{1c} was used to quantify glycemia and mean fasting basal plasma insulin concentration before the clamp was used to quantify insulinemia. Two separate bivariate linear regression analyses quantified the effect of each of these two independent variables on the dependent variable for muscle insulin sensitivity, mean R_d at the end of period 2. Then we used standard multivariable linear regression analysis to determine each independent variable's effect on muscle insulin sensitivity adjusted for one another. Data are summarized as means ± SD unless otherwise indicated.

Study Approval

Prior to participation, adult volunteers provided written, informed consent and adolescent volunteers provided written, informed assent with both parents providing parental consent. The Institutional Review Board of Vanderbilt University approved the study protocol. The U.S. Food and Drug Administration approved the use of somatostatin (IND 132209). ClinicalTrials.gov registered the study under NCT02971202.

RESULTS

Participant Characteristics

Potential confounders of insulin resistance between cohorts (n = 10 per cohort) measured at screening were well matched between cohorts (Fig. 2, Supplementary Fig. 2, and Supplementary Table 2). HbA_{1c} was 4.8 ± 0.4% (29 ± 4.4 mmol/mol), 6.2 ± 0.3% (44 ± 3.3 mmol/mol), and 6.6 ± 0.5% (49 ± 5.5 mmol/mol) in the control cohort and cohorts with GCK-MODY and type 1 diabetes, respectively (Fig. 2*A*). Study participants included seven females in the control group, nine females in the group with GCK-MODY, and six females in the group with type 1 diabetes. Subjects with type 1 diabetes had a mean disease duration of 9.4 ± 5.1 years. Supplementary Table 3 lists the GCK mutations affecting each participant with GCK-MODY.

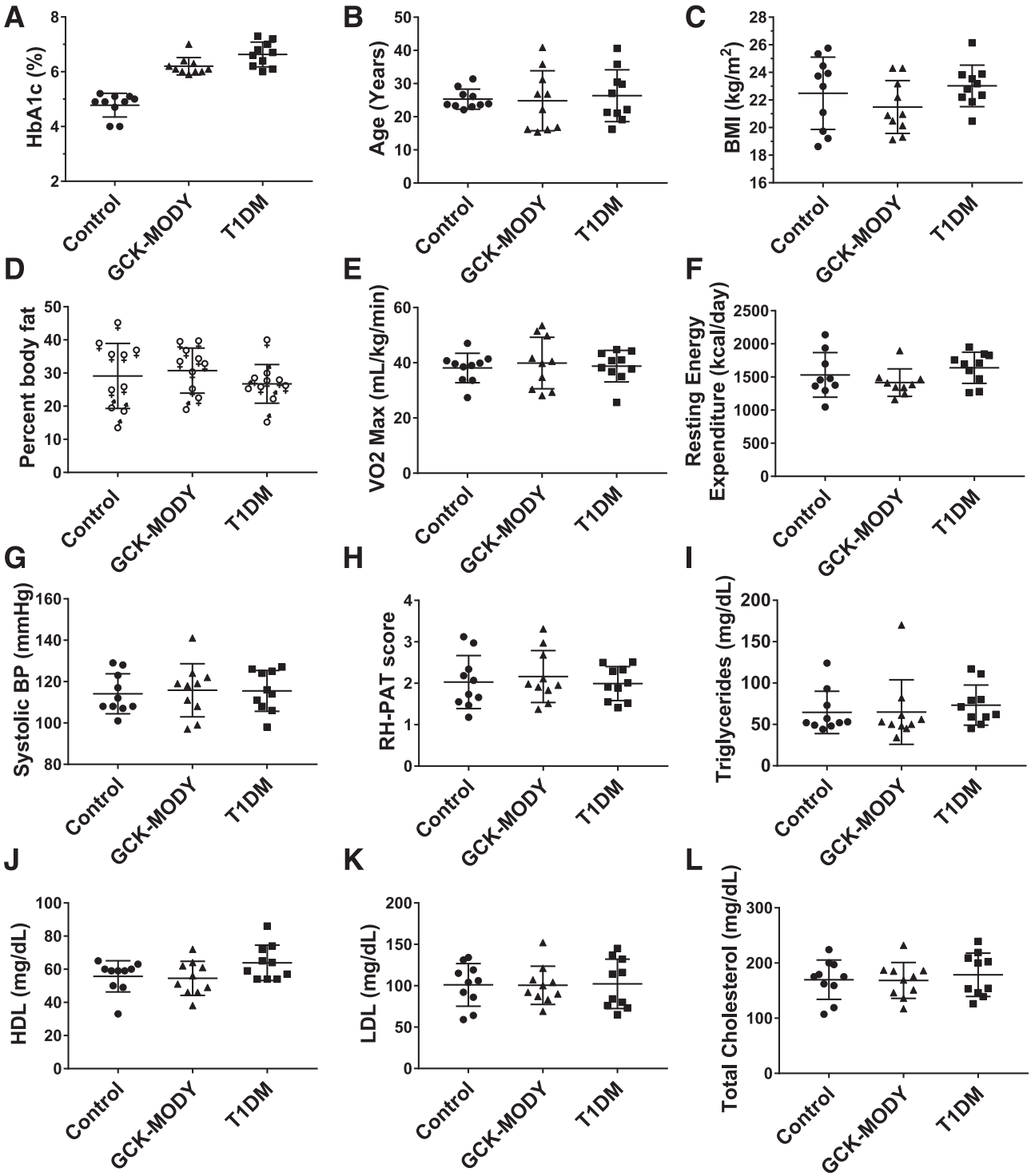


Figure 2—Baseline values for key factors affecting insulin sensitivity between cohorts: HbA_{1c} (*A*), age (*B*), BMI (*C*), percent body fat (*D*), VO_{2max} (*E*), resting energy expenditure (*F*), systolic BP (*G*), RH-PAT score (*H*), and blood concentrations of triglycerides (*I*), HDL (*J*), LDL (*K*), and total cholesterol (*L*). Data were collected during the screening visit after an overnight fast. Graphs depict mean values and SD. BP, blood pressure; T1DM, type 1 diabetes.

Hyperinsulinemic-Euglycemic Clamp Studies

Glycemia Prior to Clamp Studies

Supplementary Table 4 characterizes the insulin regimen of participants with type 1 diabetes, and Supplementary Fig. 3 summarizes their self-monitored blood glucose over the 3 days prior to the clamp study. Sixty-eight percent of glucose readings were between 70 and 180 mg/dL. Among the 10 participants with type 1 diabetes, 6 experienced a total of 18 episodes of a blood glucose <70 mg/dL and 4 experienced none over the 3 days prior to the clamp study. Two

episodes of a blood glucose <50 mg/dL occurred. Supplementary Fig. 4 depicts hourly plasma glucose concentrations and insulin infusion rates for participants with type 1 diabetes overnight before the clamp study.

Hormone and Glucose Concentrations

Basal plasma insulin concentrations (Fig. 3*A* and *B*) were virtually identical between the control cohort and the cohort with GCK-MODY (8.7 ± 2.9 vs. 8.5 ± 4.6 μU/mL) and 2.5-fold higher in the cohort with type 1 diabetes (21.2 ± 10.5 μU/mL). Plasma insulin concentrations in the control group and groups with GCK-MODY and type 1 diabetes rose to 21.1 ± 4.5, 20.5 ± 4.3, and 28.1 ± 7.4 μU/mL, respectively, in period 1 and to 80.5 ± 17.3, 74.2 ± 11.9, and 79.0 ± 18.0 μU/mL in period 2. Basal C-peptide levels in the control group and group with GCK-MODY suppressed to the lower limit of detection during the clamp (owing to the somatostatin infusion) and for the group with type 1 diabetes remained at the lower limit of detection throughout the study (Fig. 3*C*). Plasma glucagon concentrations (Fig. 3*D*) remained at basal levels in all three groups throughout the study (as a consequence of the somatostatin and glucagon infusions).

Basal glucose concentrations immediately prior to the clamp study for the control cohort and cohorts with GCK-MODY and type 1 diabetes were 89.7 ± 7.7, 119.7 ± 8.0, and 112.4 ± 12.4 mg/dL, respectively (Fig. 3*E*). Each subject required intravenous glucose to maintain plasma glucose between 95 and 100 mg/dL during the clamp, except one participant with GCK-MODY during period 1. Plasma concentrations of cortisol, epinephrine, and norepinephrine remained at basal levels throughout the study in all groups (Fig. 3*F*–*H*).

Metabolite Response

Blood concentrations of lactate rose minimally from basal to the end of period 1 and then rose in all three groups in period 2 (Fig. 4*A*). Blood alanine levels changed negligibly in each group throughout the study (Fig. 4*B*). For the control cohort and cohort with GCK-MODY, the lower insulin infusion used in period 1 suppressed both NEFA and glycerol levels dramatically, whereas the group with type 1 diabetes saw only a modest decrease in these levels (Fig. 4*C*–*F*). When the insulin infusion rate increased during period 2, NEFA and glycerol in all groups became fully suppressed.

Glucose Turnover

Basal EGP was modestly higher for GCK-MODY compared with the other two groups (Fig. 5*A*). The lower insulin infusion used in period 1 suppressed EGP similarly for the control group and the groups with GCK-MODY and type 1 diabetes, decreasing from the basal period by 1.7 (95% CI 1.4, 2.0), 2.1 (95% CI 1.7, 2.4), and 1.9 mg/kg FFM/min (95% CI 1.5, 2.2), respectively (Fig. 5*A* and *B*). All participants had near-complete suppression of EGP during the higher insulin infusion of period 2. Fractional plasma enrichment of [6,6-$^2H_2$]glucose is shown in Supplementary Fig. 1.

R_d for all groups was similar basally and increased only slightly during period 1 (Fig. 5*C*). The increase in R_d during period 2 (Fig. 5*D*) was similar in the control group and the group with GCK-MODY (12.1 [95% CI 10.3, 14.0] vs. 11.0 mg/kg FFM/min [95% CI 9.1, 13.0], respectively; difference = 1.1 [95% CI −1.5, 3.6], P = 0.39). R_d for the group with type 1 diabetes was stimulated to a lesser extent (8.5 mg/kg FFM/min [95% CI 6.1, 10.9]; difference vs. GCK-MODY = 2.5 mg/kg FFM/min [95% CI −0.4, 5.4], P = 0.086; difference vs. control = 3.6 mg/kg FFM/min [95% CI 0.7, 6.4], P = 0.018). The coefficient of variation for self-monitored blood glucose among the 10 participants with type 1 diabetes and 3 participants with GCK-MODY who submitted a glucose log ranged from 0.17 to 0.51, yet had virtually no association with period 2 R_d (Supplementary Fig. 5*A*). Likewise, the coefficient of variation of blood glucose among participants with GCK-MODY and type 1 diabetes overnight before the clamp had no appreciable association with period 2 R_d (Supplementary Fig. 5*B*).

Bivariate analyses of the effect of glycemia (HbA_{1c}) or insulinemia (mean basal insulin concentration) on R_d revealed coefficients of determination (R^2) of 0.093 and 0.356, respectively (Fig. 5*E* and *F*). When the effect of both glycemia and insulinemia on R_d was examined using multivariable linear regression analysis, R^2 was 0.356 (Fig. 5*G*). To assess colinearity between insulinemia and a series of potential factors that would cause both hyperinsulinemia and insulin resistance, we considered a series of adjusted multivariable linear models, but none appreciably altered the relationship between insulinemia and R_d (Supplementary Table 5).

DISCUSSION

These results support the hypothesis that iatrogenic peripheral hyperinsulinemia contributes substantially more to local-tissue insulin resistance than hyperglycemia. To our knowledge, this study is the first to simultaneously compare the contribution of both factors to insulin resistance in populations with type 1 diabetes and GCK-MODY. Insulin resistance has been closely linked with macrovascular disease risk in type 1 diabetes (6,7,35). Thus, these data imply that therapeutic approaches to modify hyperinsulinemia-mediated insulin resistance could mitigate macrovascular disease in type 1 diabetes.

Our data suggest that chronic exposure of insulin-sensitive tissues to iatrogenic hyperinsulinemia leads to insulin resistance in those tissues. Further, clinical chronic hyperglycemia had little if any association with insulin resistance at any tissue. In assessing muscle tissue insulin sensitivity between groups, R_d in subjects with type 1 diabetes during period 2 was 22% lower than GCK-MODY and 29% lower than control subjects. Linear regression analysis showed that insulinemia alone explained 36% of the variance in R_d, a factor that was virtually unchanged with the addition of glycemia in multivariable linear regression analysis. Hyperglycemia also seemed to have little effect on insulin sensitivity in fat tissue, as evidenced by the control group and group with GCK-MODY having

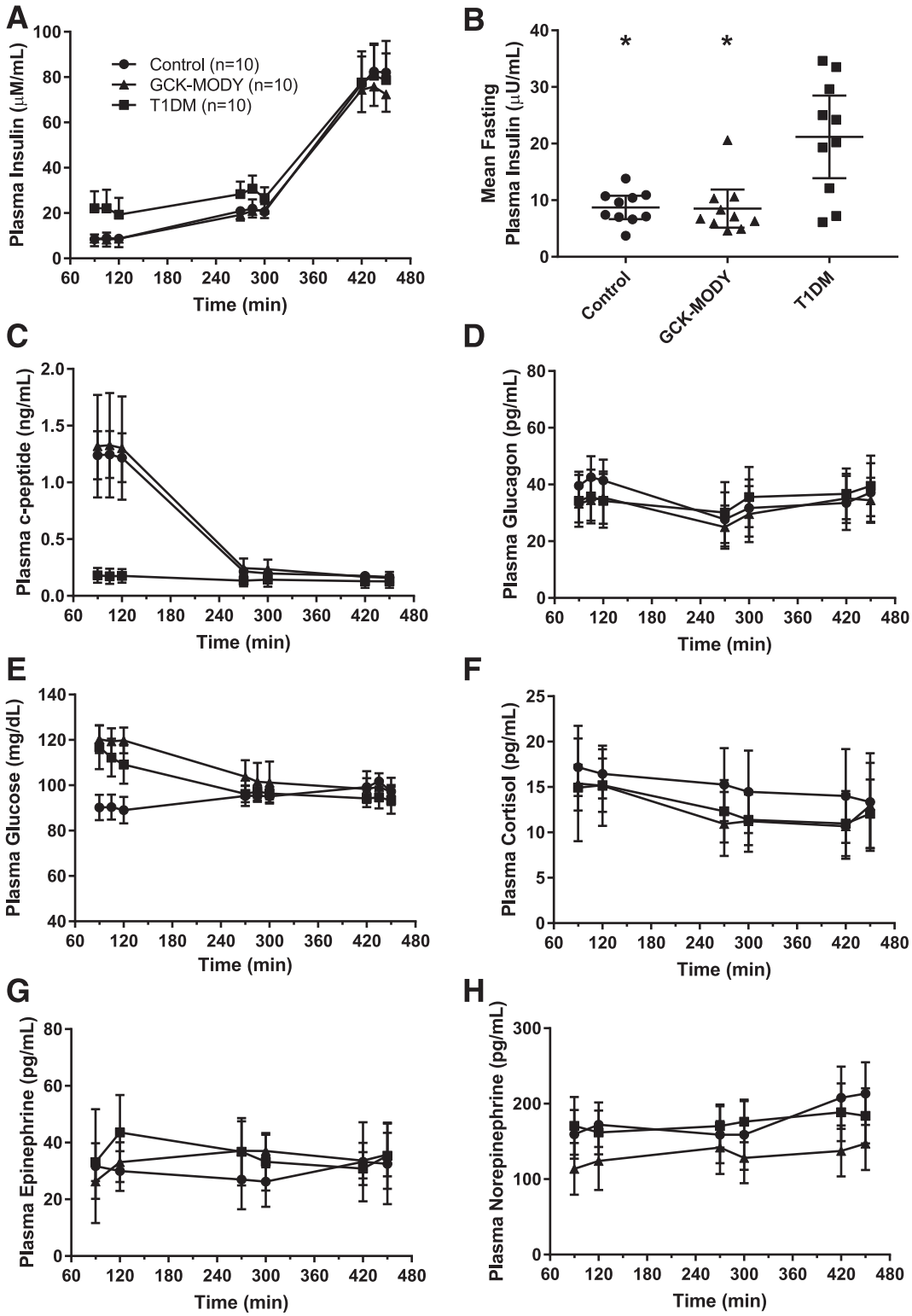


Figure 3—Arterialized plasma concentrations of insulin (*A*), each participant's mean basal insulin concentration grouped by cohort (*B*), C-peptide (*C*), glucagon (*D*), glucose (*E*), cortisol (*F*), epinephrine (*G*), and norepinephrine (*H*). Graphs depict mean values and the 95% CI. *$P < 0.05$ vs. T1DM. T1DM, type 1 diabetes.

nearly identical and complete insulin-mediated suppression of NEFA and glycerol during period 1. By contrast, the group with type 1 diabetes had only partial suppression of NEFA and glycerol during period 1, suggesting that hyperinsulinemia was the key element driving insulin resistance in fat tissue.

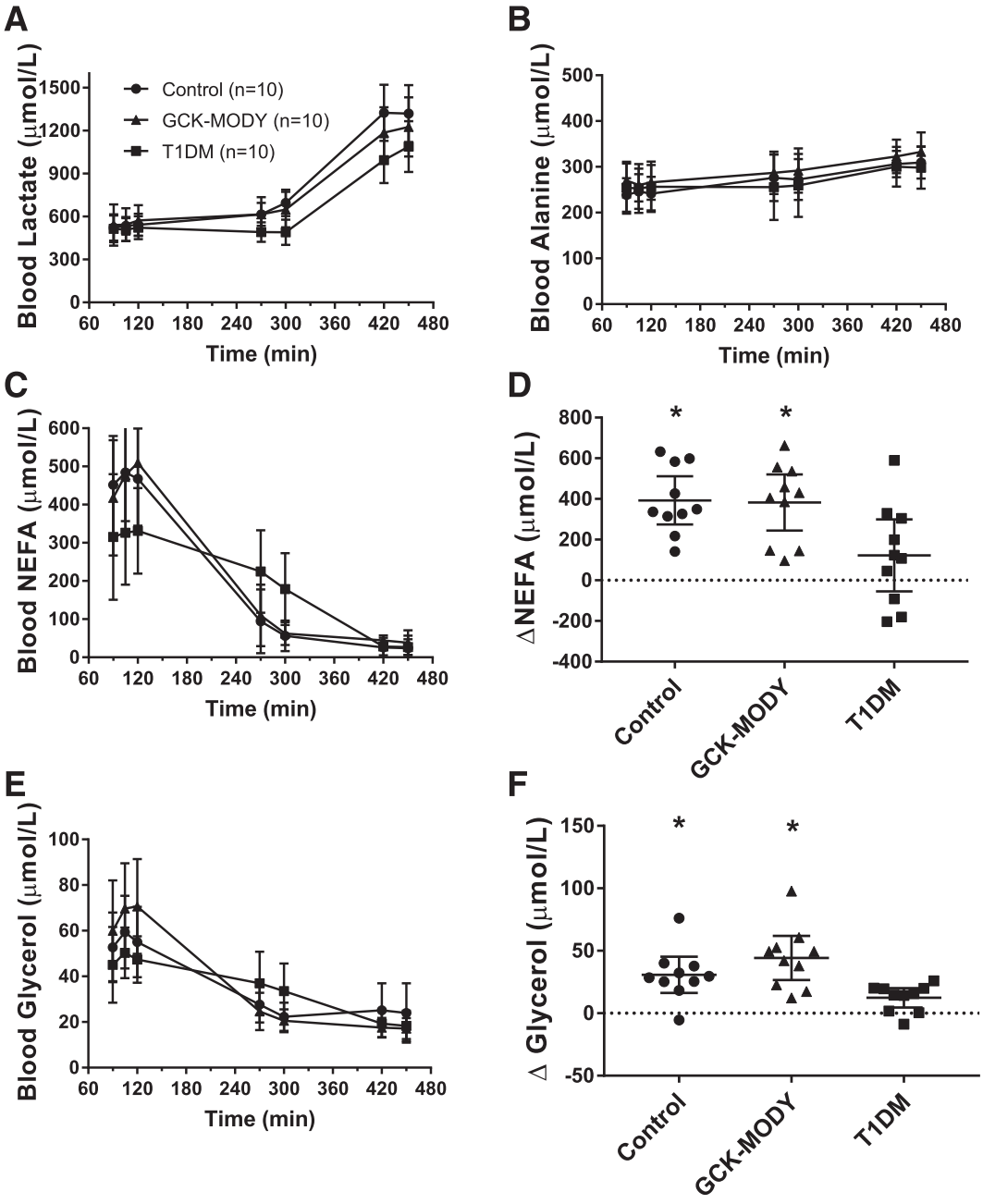
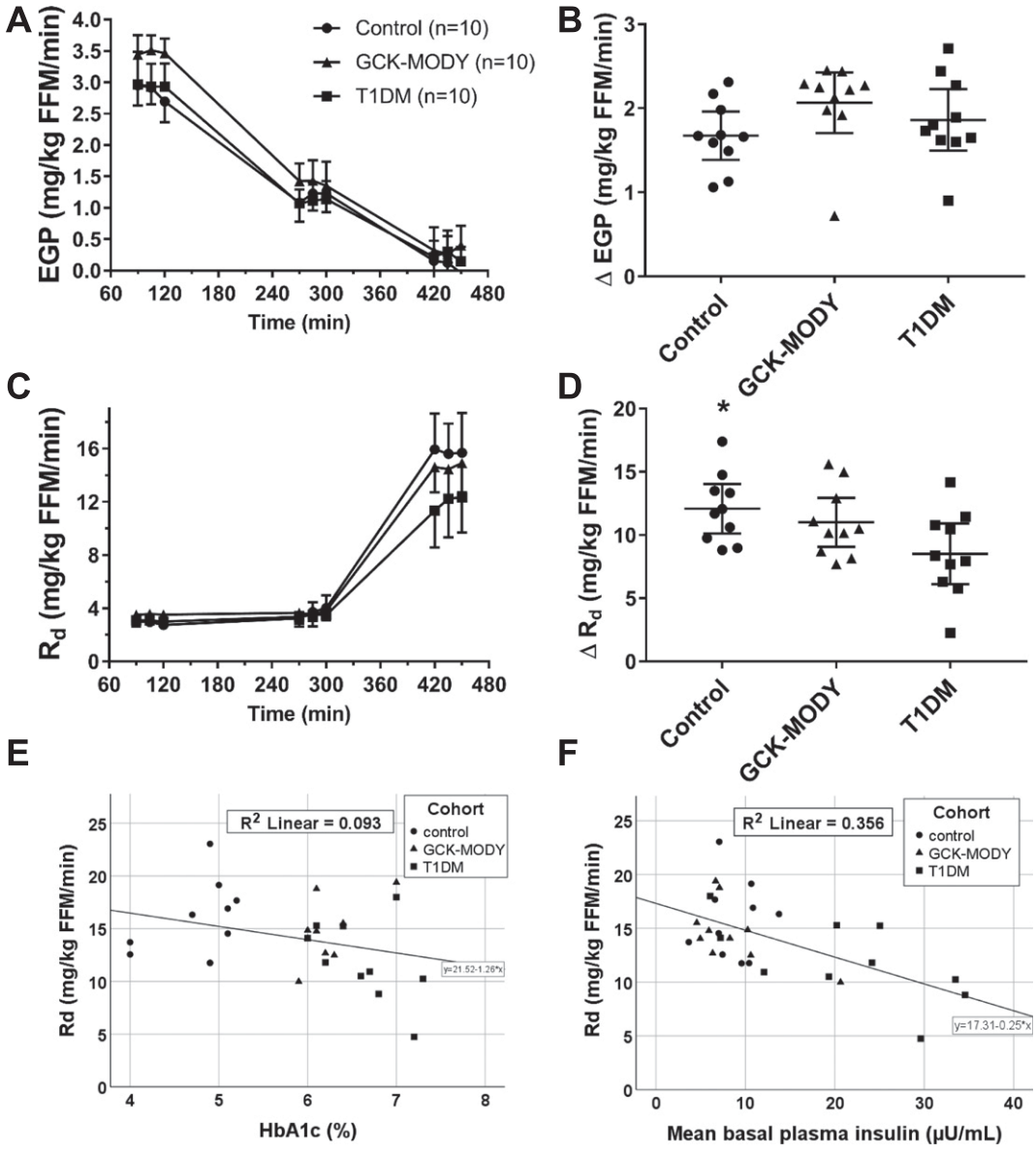


Figure 4—Arterialized blood concentrations of lactate (*A*), alanine (*B*), NEFA (*C*), insulin-mediated NEFA suppression (each participant's mean period 1 NEFA level minus mean basal NEFA level) (*D*), glycerol (*E*), and insulin-mediated glycerol suppression (each participant's mean period 1 glycerol level minus mean basal glycerol level) (*F*). Graphs depict mean values and the 95% CI. *$P < 0.05$ vs. T1DM. T1DM, type 1 diabetes.

Although insulin sensitivity at muscle and fat were lower in the group with type 1 diabetes compared with the other two groups, insulin-mediated suppression of whole-body EGP was nearly the same between each cohort regardless of glycemic and insulinemic status. One plausible explanation for this observation is that none of the groups experience chronic hepatic sinusoidal hyperinsulinemia. Based on previous studies (20,36), when insulin enters the circulation via the portal vein, the hepatic sinusoidal insulin concentration is on average 2.7-fold higher than the arterial insulin concentration. On the other hand, when insulin enters the circulation via peripheral insulin delivery, hepatic sinusoidal insulin concentrations are on average 16% lower than arterial insulin levels. Thus, for the mean basal insulin concentrations of 8.7, 8.5, and 21.1 μU/mL seen in the control group and groups with GCK-MODY and type 1 diabetes, the corresponding estimated hepatic sinusoidal insulin concentrations are 23.5, 23.0, and 17.7 μU/mL.



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Linear regression model	Independent Variable(s)	p-value	Standardized β-coefficients	R^2
Bivariate	Glycemia (HbA1c, %)	0.101		0.093
Bivariate	Insulinemia (μU/mL)	0.001		0.356
Multivariable	Glycemia (HbA1c, %) Insulinemia (μU/mL)	0.867 0.003	-0.025 -0.584	0.356

Figure 5— Glucose turnover data. *A*: EGP. *B*: Insulin-mediated EGP suppression (each participant's mean period 1 EGP level minus mean basal EGP level. *C*: Glucose utilization (R_d). *D*: Increase in mean R_d for each individual from period 1 to period 2. *E*: Scatterplot depicting bivariate analysis of the effect of glycemia (i.e., HbA_{1c}) on muscle insulin sensitivity (mean R_d during period 2). *F*: Scatterplot depicting bivariate analysis of the effect of insulinemia (i.e., mean basal insulin concentration) on muscle insulin sensitivity (mean R_d during period 2). *G*: Linear regression analyses assessing the effect of the independent variables for glycemia and insulinemia on the dependent variable for muscle insulin sensitivity (mean R_d during period 2). Figures depict mean values and the 95% CI. *$P < 0.05$ vs. type 1 diabetes. T1DM, type 1 diabetes.

These data clarify uncertainty regarding the degree to which hyperglycemia drives whole-body insulin resistance in type 1 diabetes and GCK-MODY. Multiple investigations of type 1 diabetes have shown an inverse relationship between hyperglycemia and insulin sensitivity when hyperglycemia was reduced (37,38), induced (11,12), or partially resolved during the "honeymoon phase." (10) Likewise, a study investigating insulin sensitivity in GCK-MODY also attributed decreased insulin sensitivity to hyperglycemia (39). By contrast, the primacy of hyperglycemia in driving type 1 diabetes insulin resistance was most strongly challenged by a series of studies that consistently showed little to no correlation between glycemia and whole-body insulin resistance (3,4,6,17). Interestingly, in some of the studies linking improved glycemia and improved insulin sensitivity, a reduction in hyperglycemia was also accompanied by reduced insulin doses (10,37) or levels (11).

Whether iatrogenic hyperinsulinemia is a primary or secondary cause of insulin resistance among patients with type 1 diabetes who are otherwise healthy has been a matter of significant debate. Does hyperinsulinemia per se initiate and sustain insulin resistance or does another factor cause insulin resistance, which then leads to higher insulin levels (40)? Although an observational study with a limited sample size cannot completely exclude the possibility that an unmeasured, unknown confounder caused insulin resistance that necessitated hyperinsulinemia, our analysis supports iatrogenic hyperinsulinemia as a primary driver. First, numerous potential confounding covariables were quantified and well balanced between cohorts. We considered the possibility that differences in glycemic variability between participants with type 1 diabetes and the other two groups could influence insulin sensitivity such as through divergent hormonal responses. We did not find any changes in the counter-regulatory hormones we measured during the study. In addition, bivariate analyses examining subjects with recorded prestudy blood glucose monitoring revealed there was essentially no relationship between glycemic variability and R_d (Supplementary Fig. 5). Although we were not able to monitor a long duration of prestudy glucose values, the variability of glycemia within our well-controlled subjects with type 1 diabetes likely reflects their overall pattern of control and did not account for changes in R_d. Second, when several multivariable linear regression models were tested, none of the potential confounders diminished the effect of insulinemia on R_d. These potential confounders included BMI, REE, age, body composition parameters, lipid levels, and baseline NEFA concentrations. Because the distribution of age was different between control and the other groups due to restrictions on testing healthy adolescent control subjects, we accounted for the known effect of age (41) on insulin sensitivity using multivariable linear regression. Age did not alter the relationship between insulinemia and R_d, and the association between age and R_d did not reach statistical significance. Our analysis aligns with other studies in which investigators tested hyperinsulinemia as a primary driver. Transfecting mice with extra copies of the human insulin gene led to euglycemic hyperinsulinemia, which was associated with diminished insulin sensitivity during oral glucose and intravenous insulin tolerance tests (40,42). A 50% increase in basal insulinemia induced by 28 days of intraportal insulin infusion in the dog led to only a slight fall in fasting glucose but a 39% decrease in muscle insulin sensitivity (43). In two separate studies, investigators infused intravenous insulin for 40 (44) and 72 h (45) to raise basal insulin concentrations in healthy volunteers to levels typically seen in fasting, euglycemic patients with type 1 diabetes. The mild hyperinsulinemia led to 16% and 20% reductions in muscle insulin sensitivity. In another study, euglycemic patients with type 1 diabetes who were recipients of a kidney-pancreas transplant with anastomosis into the systemic circulation had twofold higher basal insulin concentrations and 24% lower muscle insulin sensitivity than recipients with anastomosis into the portal circulation (46). Thus, in addition to addressing the primary question of whether iatrogenic hyperinsulinemia or hyperglycemia has a greater association with insulin resistance, the unique study design addresses this long-debated question for patients with type 1 diabetes. By matching numerous potential confounders between groups and by showing that the potential confounders did not diminish the linkage between hyperinsulinemia and R_d in multivariable linear regression modeling, this approach strengthens the case that iatrogenic hyperinsulinemia per se principally drives insulin resistance rather than an unmeasured or unknown covariable.

These findings underscore the importance of iatrogenic hyperinsulinemia as a potentially modifiable contributor to insulin resistance, a factor known to promote macrovascular disease in this population (4,7,35,47). Although hyperglycemia reduction remains profoundly important to reduce macrovascular disease risk (48), patients with an $HbA_{1c} \leq 6.9\%$ (52 mmol/mol) still have a nearly threefold risk for macrovascular disease death compared with matched control subjects (49). By contrast, in the largest study of vascular disease risk in adults with GCK-MODY ($n = 99$), the median HbA_{1c} was 6.9% (52 mmol/mol), yet the prevalence of macrovascular complications was no different from control subjects (50). We suggest that the differences in macrovascular disease between the population with type 1 diabetes with $HbA_{1c} \leq 6.9\%$ (52 mmol/mol) and the population with GCK-MODY may be related to the differences in insulin sensitivities as identified in the current study.

In conclusion, our results indicate that iatrogenic hyperinsulinemia plays a much larger role in driving insulin resistance at muscle and fat than hyperglycemia in type 1 diabetes. We propose that therapies designed to restore the physiologic distribution of insulin between the liver and periphery (e.g., hepatopreferential insulin analogs and intraperitoneal insulin delivery) will mitigate

insulin resistance significantly and improve long-term outcomes across the life span in type 1 diabetes.

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Author Contributions. J.M.G. designed the study, conducted experiments, gathered and analyzed data, and wrote the manuscript. T.J.S. conducted experiments, gathered data, and analyzed data. J.C.S. assisted with the study design and statistical analysis. H.R.M. conducted experiments. C.C.H. conducted isotopic tracer analyses. M.S.S. performed hormonal and metabolite assays. B.K., S.A.W.G., L.H.P., R.N.N., and L.R.L. facilitated participant recruitment and conducted genotyping. N.N.A., A.D.C., and D.J.M. helped design the study, analyze the data, and write the manuscript. All authors critically reviewed the manuscript. J.M.G. 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.

Data and Resource Availability. The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

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