Lipid-Induced Insulin Resistance Is Not Mediated by Impaired Transcapillary Transport of Insulin and Glucose in Humans

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Increased lipid availability reduces insulin-stimulated glucose disposal in skeletal muscle, which is generally explained by fatty acid–mediated inhibition of insulin signaling. It remains unclear whether lipids also impair transcapillary transport of insulin and glucose, which could become rate controlling for glucose disposal. We hypothesized that lipid-induced insulin resistance is induced by inhibiting myocellular glucose uptake and not by interfering with the delivery of insulin or glucose. We measured changes in interstitial glucose and insulin in skeletal muscle of healthy volunteers during intravenous administration of triglycerides plus heparin or glycerol during physiologic and supraphysiologic hyperinsulinemia, by combining microdialysis with oral glucose tolerance tests and euglycemic-hyperinsulinemic clamps. Lipid infusion reduced insulin-stimulated glucose disposal by ~70% ($P < 0.05$) during clamps and dynamic insulin sensitivity by ~12% ($P < 0.05$) during oral glucose loading. Dialysate insulin and glucose levels were unchanged or even transiently higher ($P < 0.05$) during lipid than during glycerol infusion, whereas regional blood flow remained unchanged. These results demonstrate that short-term elevation of free fatty acids (FFAs) induces insulin resistance, which in skeletal muscle occurs primarily at the cellular level, without impairment of local perfusion or transcapillary transport of insulin and glucose. Thus, vascular effects of FFAs are not rate controlling for muscle insulin-stimulated glucose disposal. ***Diabetes*** **61:3176–3180, 2012**

Skeletal muscle accounts for the majority of glucose uptake after a meal and almost all glucose disposal during hyperinsulinemic-euglycemic clamps (1). In type 2 diabetes (T2DM), muscle insulin resistance predicts postprandial hyperglycemia, but the underlying mechanisms are unclear. Insulin-resistant humans frequently present with increased plasma free fatty acids (FFAs) (2), which can give rise to myocellular diacylglycerols or ceramides and impair insulin signaling (3–5). Insulin increases muscle microvascular perfusion and facilitates delivery of nutrients and hormones to the interstitium (6). Animal models of lipid-induced insulin resistance suggest that insulin-mediated microvascular perfusion is already reduced in prediabetic states and relates to impaired insulin action (7,8). Preventing the access of glucose and insulin to myocytes could contribute to lower glucose disposal and place abnormal microvascular insulin action as an early event in the development of T2DM.

We hypothesized that lipid-induced insulin resistance results from myocellular glucose uptake, but not from impaired delivery of insulin or glucose to the interstitium. We monitored changes of interstitial insulin and glucose in muscle of humans during intravenous triglycerides or glycerol administration under physiologic dynamic (oral glucose tolerance test [OGTT]) and supraphysiologic constant hyperinsulinemic (clamp) conditions.

RESEARCH DESIGN AND METHODS

The study was registered at www.clinicaltrials.gov (NCT01482455) and approved by the local ethics committee in accordance with the most recent version of the Helsinki Declaration. Exclusion criteria comprised family history of T2DM or dyslipidemia, glucose intolerance, <19 years of age, BMI >27 kg/m^2, fasting triglycerides >140 mg/dL, total cholesterol >200 mg/dL, serum C-reactive protein >1 mg/dL, clotting disorders, hypersensitivity against study medication, any medication within 2 weeks before the study, alcohol consumption >40 g/d, or smoking.

Eight healthy, male volunteers randomly underwent four studies separated by ≥1 week. After 12 h overnight fasting, three plastic cannulas (Venflon) were inserted into antecubital veins of both forearms for infusions and blood drawing. On day 1 (LIPc), a triglyceride/heparin infusion was administered (0–360 min: Intralipid 20% [Pharmacia AB, Stockholm, Sweden], 90 mL/h; heparin "Immuno," Immuno AG, bolus: 200 IU, 0.2 IU · kg^{-1} · min^{-1}), and a hyperinsulinemic-euglycemic clamp test (Actrapid [Novo Nordisk, Bagsvaerd, Denmark]; 40 mU · m^{-2} body surface area · min^{-1}) was performed (120–360 min). Plasma glucose was maintained at ~5.4 mmol/L using a variable glucose infusion. On day 2 (GLYc), glycerol (in 0.9% saline, 0.7 mg · kg^{-1} · min^{-1}) was infused instead of triglyceride/heparin. Triglyceride/heparin was infused on day 3 (LIPo) and glycerol on day 4 (GLYo) (0–420 min), each followed by a 75-g OGTT (Glucodrink [Roche Diagnostica]; 240–420 min).

Microdialysis. As previously described, a microdialysis probe (cutoff, 20 kDa; shaft length, 70 mm; membrane length, 16 mm; outer diameter, 0.5 mm [CMA, Stockholm, Sweden]) was perfused with Ringer's solution + 5% human albumin (flow rate, 1.5 μL/min) for sampling of interstitial glucose (9). For sampling of interstitial insulin, another probe (cutoff, 100 kDa; shaft length, 70 mm; membrane length, 16 mm; outer diameter, 0.5 mm [CMA]) was perfused with bidistilled water + 5% human albumin (flow rate, 3 μL/min). The microdialysis system was perfused using a microinfusion pump (Precidor; Infors-AG, Basel, Switzerland).

For most analytes, the equilibrium between the respective concentrations in interstitial fluid and perfusion medium is incomplete, so that the measured dialysate concentrations underestimate the absolute interstitial concentrations (10,11). As we aimed to monitor dynamic changes rather than absolute interstitial levels, we report the relative changes of interstitial glucose and insulin of dialysate concentrations compared with baseline (fasting). This avoided time-consuming calibration experiments in addition to the already exhaustive protocols.

Regional blood flow. Muscle blood flow was measured by the laser Doppler flow technique (LDF; Moor Instruments, Devon, U.K.) (12) using a fiber LDF

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probe (total diameter, 0.5 mm), inserted 5 cm into m. vastus medialis, 5 cm distant from the microdialysis probe.

Laboratory analyses. Glucose was measured in plasma by a Beckman II glucose analyzer (Beckman Coulter) and in dialysate by a CMA 600 MD analyzer (CMA, Uppsala, Sweden). Serum and dialysate insulin were measured using an ultrasensitive insulin immunoassay (Mercodia, Uppsala, Sweden). Plasma FFAs were assayed microfluorimetrically (Wako USA, Richmond, VA) after collecting blood into orlistat-containing vials (13).

Data analysis. Areas under the plasma glucose and insulin curves ($AUC_{120–360min\text{-}serum}$ for clamps; $AUC_{240–420min\text{-}serum}$ for OGTT) were calculated using the trapezoidal rule. Fasting insulin sensitivity was assessed from fasting plasma insulin (FPI) and glucose (FPG) as QUICKI (1/[logFPI + logFPG]). During clamps, whole-body insulin sensitivity was calculated as M-value (13). During OGTT, dynamic insulin sensitivity was assessed from the oral glucose insulin sensitivity index (OGIS), a validated index of glucose clearance (14). β-Cell function was determined in relation to glycemia (insulinogenic index) and to insulin sensitivity (disposition index) (14).

Statistical analysis. Data (text, means ± SD; figures, means ± SEM) were analyzed using SPSS 11.0 software (SPSS, Chicago, IL). Statistical comparisons between LIP and GLY were performed using two-tailed, paired Student *t* tests. Changes of sequential data within experiments were evaluated by repeated-measures one-way ANOVA with post hoc Tukey correction. Linear correlations are Pearson product-moment correlations. $P < 0.05$ was considered to indicate significant differences.

RESULTS

Supraphysiologic hyperinsulinemia

Plasma parameters and insulin sensitivity. Plasma FFAs were similar on both days during fasting (GLYc vs. LIPc, 0.36 ± 0.19 vs. 0.37 ± 0.19 mmol/L), increased within 2 h, and remained elevated during LIPc ($P = 5.10^{-9}$) (Fig. 1*A*). Plasma glucose was comparable on both days during fasting and clamps (5.3 ± 0.2 vs. 5.4 ± 0.9 mmol/L) (Fig. 1*C*). Plasma insulin was also not different at fasting and during clamps (426 ± 155 vs. 514 ± 75 pmol/L) (Fig. 1*E*). The M-value was ~70% lower during LIPc (180–210 min, $P = 0.007$; 210–240 min, $P = 0.011$) (Fig. 2).

Interstitial parameters and muscle blood flow. The change of interstitial glucose was 35% greater in LIPc vs. GLYc (180–240 min, 1.37 ± 0.21 vs. 1.06 ± 0.28, $P < 0.05$)

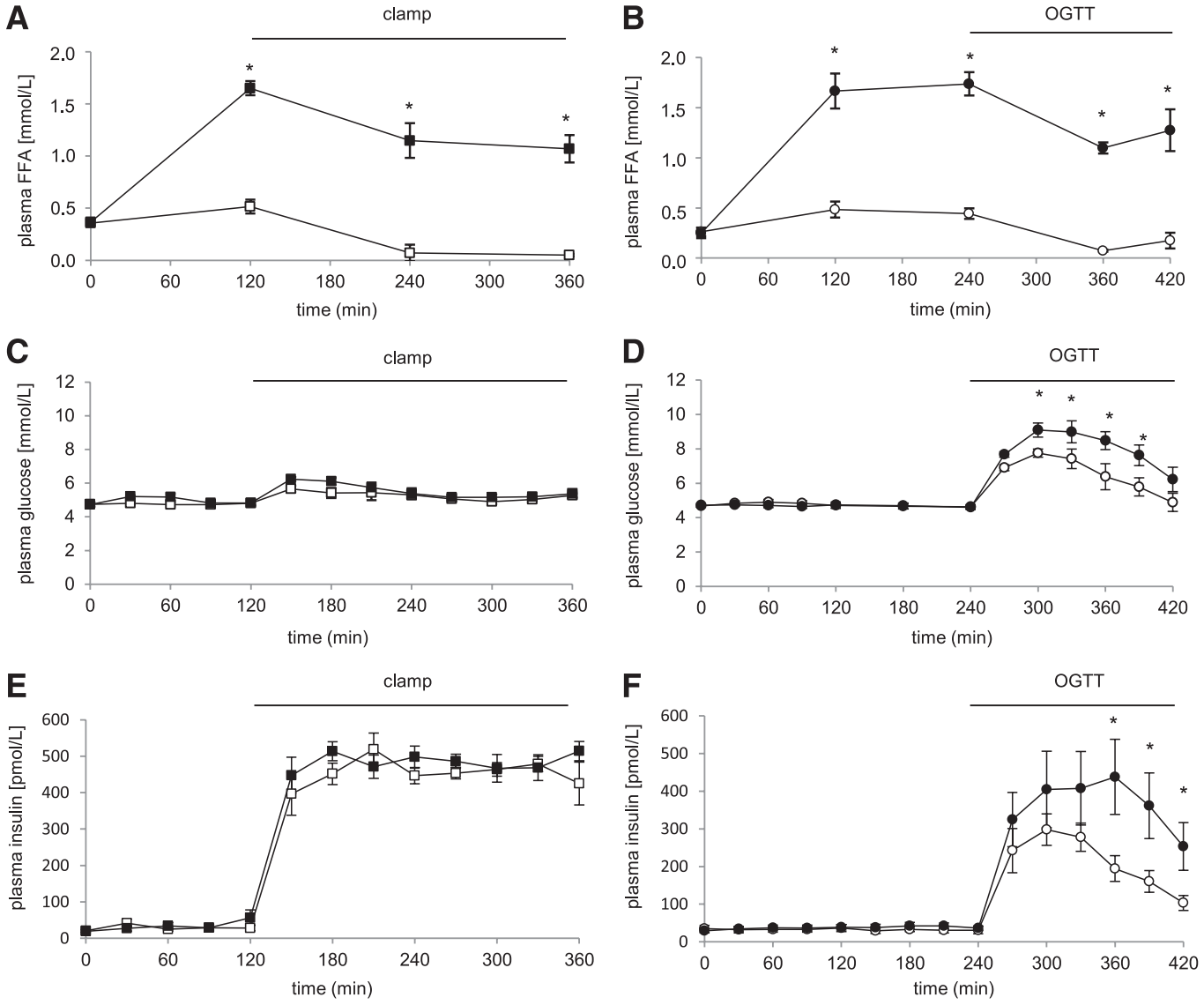

FIG. 1. Time course of plasma concentrations (means ± SEM) of FFAs during hyperinsulinemic-euglycemic clamps (*A*) and during OGTT (*B*), of glucose during hyperinsulinemic-euglycemic clamps (*C*) and during OGTT (*D*), and of insulin hyperinsulinemic-euglycemic clamp (*E*) and during OGTT (*F*). Young, healthy participants received lipid infusion (black symbols) or glycerol infusion (white symbols). Differences in lipid vs. glycerol infusion: *$P < 0.05$.

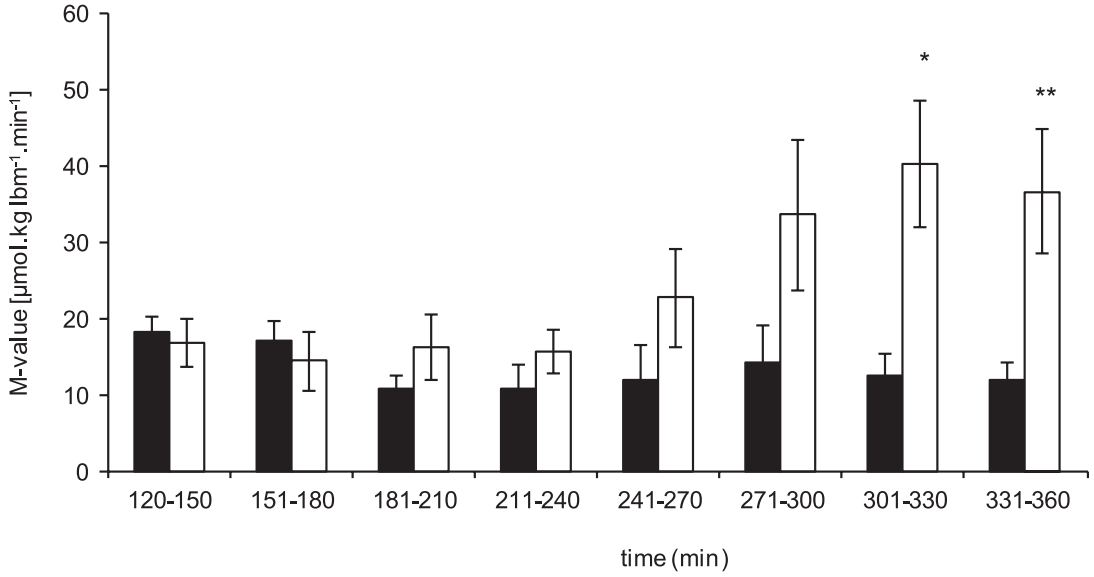

FIG. 2. Whole-body glucose disposal (means ± SEM) during hyperinsulinemic-euglycemic clamps in the presence of lipid infusion (■) and glycerol infusion (□). Differences in lipid vs. glycerol studies: *$P < 0.05$; **$P < 0.01$.

(Fig. 3*A*). The relative increase of interstitial insulin tended to be greater during LIPc compared with GLYc (~1.7- vs. ~1.2-fold, $P = 0.1$) within 60 min into the clamp (Fig. 3*C*). Regional blood flow tended ($P = 0.06$) to be higher during LIPc compared with GLYc without changing above baseline (Fig. 3*E*).

Physiologic hyperinsulinemia

Plasma parameters, insulin sensitivity, and secretion. Plasma FFAs were similar during fasting (0.26 ± 0.12 vs. 0.25 ± 0.14 mmol/L), increased within 2 h of LIPo (0.49 ± 0.23 vs. 1.67 ± 0.50 mmol/L, $P = 3.10^{-5}$), and decreased during OGTT in GLYo ($P = 0.0002$) (Fig. 1*B*). Plasma glucose was comparable during fasting (4.4 ± 0.3 vs. 4.4 ± 0.3 mmol/L) but higher after lipid infusion during OGTT (Fig. 1*D*). Fasting plasma C-peptide (1.27 ± 0.17 vs. 1.21 ± 0.16 ng/mL) and insulin did not differ between interventions (34 ± 27 vs. 29 ± 16 pmol/L). Plasma insulin was higher after 4 h of LIPo, reflecting glucose-induced stimulation of insulin secretion due to lipid-induced insulin resistance (Fig. 1*F*). Fasting insulin sensitivity did not differ (Table 1), whereas dynamic insulin sensitivity was ~12% lower during LIPo. β-Cell sensitivity to glucose and to insulin sensitivity was not different between interventions (Table 1).

Interstitial parameters and muscle blood flow. The relative increase in interstitial glucose was ~64% higher in LIPo during OGTT (300–420 min, 2.4 ± 0.2– vs. 1.4 ± 0.1–fold increase, $P < 0.05$) (Fig. 3*B*). The relative increase of interstitial insulin was ~17% ($P < 0.05$) during lipid infusion and did not change in GLYo (Fig. 3*D*). Regional blood flow relative to baseline did not differ between the interventions (Fig. 3*F*).

DISCUSSION

This study demonstrates that transcapillary transport of insulin and glucose is not blunted during the onset of FFA-induced insulin resistance under conditions of dynamic and constant hyperinsulinemia.

We did not observe increased blood flow under insulin-stimulated conditions, which is in line with studies in healthy humans at comparable and ~20-fold higher plasma insulin concentrations (15). Of note, other studies suggested that meal-induced increases in insulin have hemodynamic effects, thereby enhancing its access and that of nutrients to myocellular metabolism (16,17). Here, we report that lipid infusion induced whole-body insulin resistance, while blood flow tended to be greater during constant hyperinsulinemia, indicating increased rather than reduced blood flow. Similarly, FFA elevation markedly raised retinal and skin blood flow in humans, although the underlying mechanisms remain unclear (18). Nevertheless, obese humans have a reduced activation of lower-limb blood flow in response to insulin (16,17) and impaired postprandial microvascular recruitment (19). Of note, insulin mainly increases the contribution of the nutritive versus the nonnutritive flow route to microvascular blood flow within muscle (6). Thus, in insulin-resistant states, impaired redirection of blood toward the nutritive flow into the capillary bed could lead to bypass of hormones and substrates away from myocytes.

In the absence of lipid or glycerol infusions, interstitial insulin concentrations can rise 10-fold during clamps or OGTT in healthy humans (20). In our lipid infusion study, dialysate insulin increased slightly above baseline only during the clamp, but not during OGTT-induced splanchnic insulin secretion, which is likely due to its substantial removal by the liver. In dogs, measurement of plasma and lymph insulin, as a surrogate of the interstitial compartment, during euglycemic-hyperinsulinemic clamps revealed a tight relationship between lymph insulin and whole-body glucose uptake (21,22). After lipid infusion or high-fat diet, they had increased venous, but lower lymphatic, insulin concentrations upon intramuscular insulin injections (7,8). The authors concluded that these conditions prevent insulin from diffusing through the interstitium, maybe due to endothelial dysfunction. Our study suggests that both FFA and glycerol, which is also a component of triglycerides, could have been responsible for the absent rise in interstitial insulin in both lipid and control studies. But as insulin resistance was seen only during lipid infusion, we conclude that lipids may affect transcapillary transport of insulin into and/or out of the interstitial space but without being rate controlling for insulin action in lean humans.

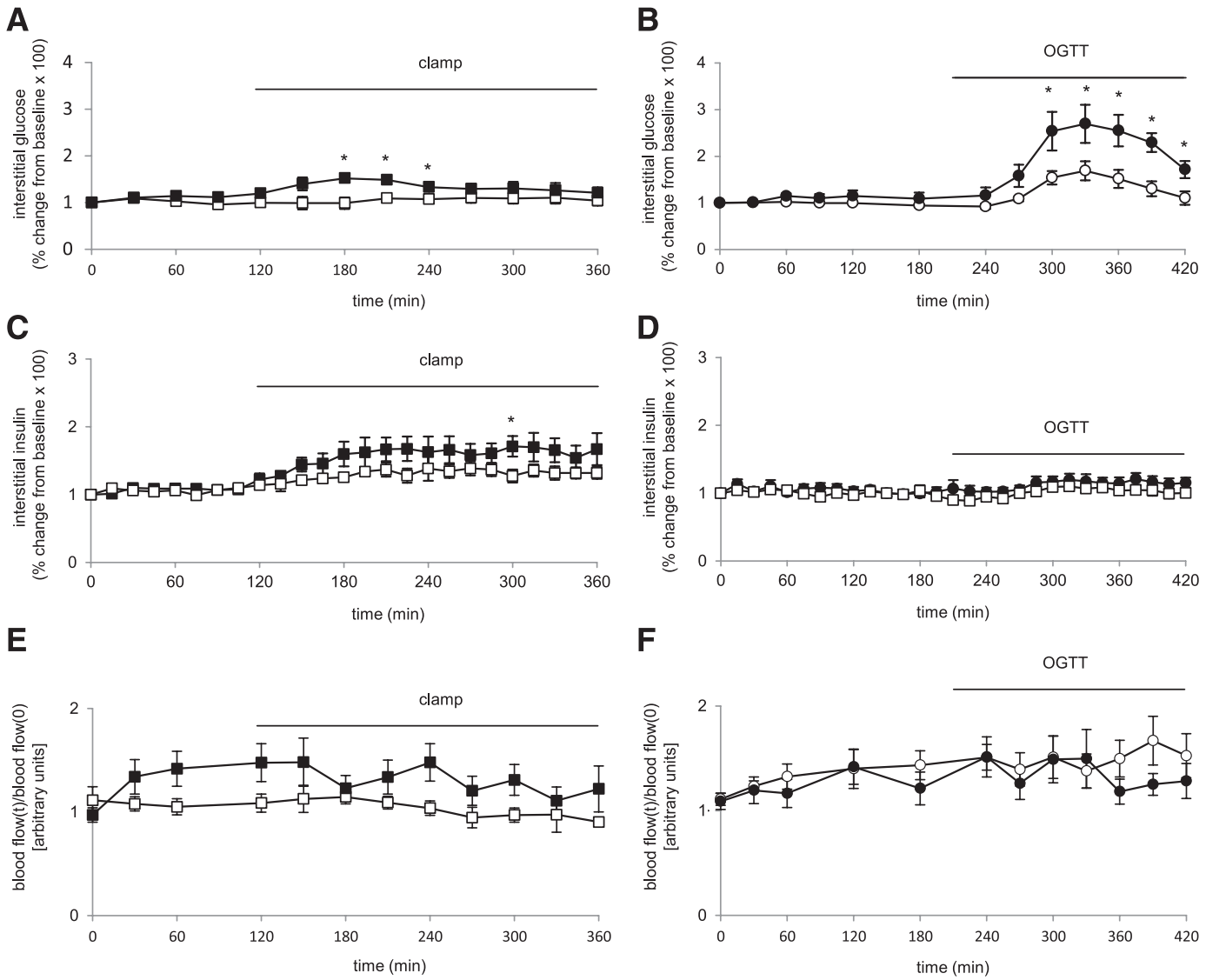

FIG. 3. Time course of changes vs. baseline (means ± SEM) of interstitial glucose during hyperinsulinemic-euglycemic clamps (*A*) and during OGTT (*B*), and changes of interstitial insulin vs. baseline during hyperinsulinemic-euglycemic clamps (*C*) and during OGTT (*D*). Regional blood flow relative to baseline (means ± SEM) during the hyperinsulinemic-euglycemic clamps (*E*) and during OGTT (*F*). Young, healthy participants received lipid infusion (black symbols) or glycerol infusion (white symbols). Differences in lipid vs. glycerol infusion: *$P < 0.05$.

Here we confirm that lipid infusion decreased both M-value and OGIS (23). Plasma FFAs correlate inversely with muscle insulin sensitivity (2), and their elevation impairs muscle glucose transport/phosphorylation within 2 h (5,23). This is likely due to myocellular accumulation of diacylglycerols, which interfere with insulin signaling, at least in rat muscle (4), and supports the evidence for direct myocellular FFA effects.

Increased lipid concentrations can also directly interfere with β-cells and affect insulin secretion. Short-term plasma FFA elevation increases rather than decreases insulin secretion, leading to gradually higher peripheral venous

TABLE 1
AUCs of glucose and insulin as well as surrogate parameters (means ± SD) of dynamic insulin sensitivity and β-cell function during the OGTT mimicking physiologic hyperinsulinemia in the presence of glycerol (GLYo) or lipid (LIPo) infusion

	GLYo	LIPo	*P* value
Plasma glucose (AUC, mmol · L^{-1} · min^{-1})	1,170 ± 189	2,057 ± 465	$P = 0.0002$
Plasma insulin (AUC, pmol · L^{-1} · min^{-1})	37,339 ± 35,986	62,669 ± 36,374	$P = 0.08$
QUICKI	0.52 ± 0.03	0.48 ± 0.02	NS
OGIS (mL · min^{-1} · m^{-2})	416 ± 17	365 ± 21	$P = 0.007$
Insulinogenic index ($\times 10^{-6}$)	73.75 ± 14.67	101 ± 25	NS
Disposition index (L · min^{-2} · 10^{-3})	36.35 ± 5.72	46 ± 10.42	NS

P compares lipid vs. glycerol infusion.

insulin concentrations (24). In the current study, plasma insulin and glucose concentrations were not different between GLY and LIP during equilibration, indicating no relevant stimulation of insulin secretion. Likewise, dynamic β-cell function as assessed from sensitivity to glucose (insulinogenic index) and to insulin sensitivity (disposition index) did not differ between GLY and LIP. Thus, the higher plasma insulin concentrations during the OGTT in the lipid/heparin study most likely reflect glucose-induced insulin secretion in response to the FFA-mediated peripheral insulin resistance.

Here we monitored the time course of dialysate glucose and insulin, which allowed the assessment of the effectiveness of transcapillary substrate transport independent of differences of muscle perfusion. Nevertheless, this study also has limitations. First, we cannot report absolute interstitial glucose and insulin concentrations without calibration procedures (20), which, however, does not affect our conclusions based on the time-dependent relative changes. It has also been shown that the ratio between interstitial and dialysate analyte concentrations is comparable between various insulin-resistant states and insulin-sensitive humans (10). Second, lipids can interfere with analyte measurements using microdialysis (25). To prevent this phenomenon, we added human albumin to the perfusion medium and used probes with low-molecular-weight cutoff membranes (25). Of note, despite increased plasma FFA or glycerol levels, dialysate glucose and insulin concentrations were stable during the equilibration periods, arguing against any interference of lipids with glucose or insulin measurements. Third, M-value and OGIS are composite measures of whole-body insulin sensitivity so that other tissues, particularly the liver, could have contributed to the observed FFA-induced insulin resistance (23), but skeletal muscle accounts for the vast majority of glucose uptake during such hyperinsulinemic conditions (1). Finally, we did not obtain biopsies to measure FFA effects on insulin signaling because these were demonstrated before (23) and the study design was already demanding.

In conclusion, short-term FFA elevation induces muscle insulin resistance primarily at the cellular level, without impairment of local perfusion or transcapillary transport of insulin and glucose. However, this does not exclude that microvascular alterations contribute to insulin resistance in long-term dyslipidemia and overt T2DM.

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J.S. wrote the manuscript and analyzed data. M.F. researched data, contributed to discussion, and reviewed and edited the manuscript. N.K., C.B., O.W., G.P., and P.N. researched data and reviewed and edited the manuscript. J.D. analyzed data and reviewed and edited the manuscript. M.M. designed the study, contributed to discussion, and reviewed and edited the manuscript. M.R. designed the study, analyzed data, contributed to discussion, and wrote, reviewed, and edited the manuscript. M.R. 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.

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