

Athletes feature greater rates of muscle glucose transport and glycogen synthesis during lipid infusion

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Clinical Medicine

Metabolism

Muscle biology

Insulin resistance results from impaired skeletal muscle glucose transport/phosphorylation, linked to augmented lipid availability. Despite greater intramuscular lipids, athletes are highly insulin sensitive, which could result from higher rates of insulin-stimulated glycogen synthesis or glucose transport/phosphorylation and oxidation. Thus, we examined the time course of muscle glycogen and glucose-6-phosphate concentrations during low and high systemic lipid availability.

Eight endurance-trained and 9 sedentary humans (VO_2 peak: 56 ± 2 vs. 33 ± 2 mL/kg/min, $P < 0.05$) underwent 6-hour hyperinsulinemic-isoglycemic clamp tests with infusions of triglycerides or saline in a randomized crossover design. Glycogen and glucose-6-phosphate concentrations were monitored in vastus lateralis muscles using $^{13}\text{C}/^{31}\text{P}$ magnetic resonance spectroscopy.

Athletes displayed a 25% greater ($P < 0.05$) insulin-stimulated glucose disposal rate (Rd) than sedentary participants. During Intralipid infusion, insulin sensitivity remained higher in the athletes (ΔRd : 25 ± 3 vs. 17 ± 3 $\mu\text{mol}/\text{kg}/\text{min}$, $P < 0.05$), supported by higher glucose transporter type 4 protein expression than in sedentary humans. Compared to saline infusion, AUC of glucose-6-phosphate remained unchanged during Intralipid infusion in athletes (1.6 ± 0.2 mmol/L vs. 1.4 ± 0.2 [mmol/L] \times h, $P = \text{n.s.}$) but tended to [...]

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Athletes feature greater rates of muscle glucose transport and glycogen synthesis during lipid infusion

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BACKGROUND. Insulin resistance results from impaired skeletal muscle glucose transport/phosphorylation, linked to augmented lipid availability. Despite greater intramuscular lipids, athletes are highly insulin sensitive, which could result from higher rates of insulin-stimulated glycogen synthesis or glucose transport/phosphorylation and oxidation. Thus, we examined the time course of muscle glycogen and glucose-6-phosphate concentrations during low and high systemic lipid availability.

METHODS. Eight endurance-trained and 9 sedentary humans ($\text{VO}_2\text{ peak}$: 56 ± 2 vs. 33 ± 2 mL/kg/min, $P < 0.05$) underwent 6-hour hyperinsulinemic-isoglycemic clamp tests with infusions of triglycerides or saline in a randomized crossover design. Glycogen and glucose-6-phosphate concentrations were monitored in vastus lateralis muscles using $^{13}\text{C}/^{31}\text{P}$ magnetic resonance spectroscopy.

RESULTS. Athletes displayed a 25% greater ($P < 0.05$) insulin-stimulated glucose disposal rate (Rd) than sedentary participants. During Intralipid infusion, insulin sensitivity remained higher in the athletes (ΔRd : 25 ± 3 vs. 17 ± 3 $\mu\text{mol}/\text{kg}/\text{min}$, $P < 0.05$), supported by higher glucose transporter type 4 protein expression than in sedentary humans. Compared to saline infusion, AUC of glucose-6-phosphate remained unchanged during Intralipid infusion in athletes (1.6 ± 0.2 mmol/L vs. 1.4 ± 0.2 [mmol/L] $\times h$, $P = \text{n.s.}$) but tended to decrease by 36% in sedentary humans (1.7 ± 0.4 vs. 1.1 ± 0.1 [mmol/L] $\times h$, $P < 0.059$). This drop was accompanied by a 72% higher rate of net glycogen synthesis in the athletes upon Intralipid infusion (47 ± 9 vs. 13 ± 3 $\mu\text{mol}/\text{kg}/\text{min}$, $P < 0.05$).

CONCLUSION. Athletes feature higher skeletal muscle glucose disposal and glycogen synthesis during increased lipid availability, which primarily results from maintained insulin-stimulated glucose transport with increased myocellular glucose-6-phosphate levels for subsequent glycogen synthesis.

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Introduction

Insulin resistance characterizes obesity, type 2 diabetes (T2DM) (1), and nonalcoholic fatty liver disease (2) and strongly correlates with intramyocellular lipid content (IMLC) in sedentary individuals (3–5). Likewise, elevation of circulating free fatty acids rapidly induces peripheral insulin resistance in sedentary humans (6–9). Using noninvasive monitoring of muscle glucose-6-phosphate (G6P) and glycogen concentrations with $^{31}\text{P}/^{13}\text{C}$ magnetic resonance spectroscopy (MRS), we demonstrated that lipid-induced insulin resistance is preceded by attenuated increases in muscle G6P reflecting inhibition of glucose transport/phosphorylation in sedentary humans (9, 10). This is followed by slower rates of nonoxidative glucose

disposal (NOGD) reflecting lower muscle glycogen synthesis. Similar reductions in muscle glucose transport/phosphorylation are typical for chronic insulin-resistant states such as obesity and T2DM (11–13).

In contrast to sedentary humans, endurance-trained athletes generally present with higher IMLC content in the face of higher peripheral insulin sensitivity (14). We previously reported that endurance-trained insulin-sensitive humans could be at least partially protected from lipid-induced insulin resistance (15). Upon lipid infusion, these athletes exhibited an only minor decrease in peripheral insulin sensitivity compared to untrained humans. However, this study did not allow us to clarify whether greater muscle glucose transport/phosphorylation or glycogen synthesis is mainly responsible for the protection from lipid-induced insulin resistance in trained skeletal muscle. Noninvasive ¹³C MRS is the state-of-the-art technique to directly monitor the time course of intramuscular G6P and glycogen concentrations and to measure net rates of muscle glycogen synthesis. This method overcomes artifacts resulting from ex vivo glycogen breakdown in muscle biopsies; from steady state issues and extramuscular glucose metabolism, which affect both glucose disappearance rate (R_d) and glucose appearance rate (R_a) in isotopic dilution studies; and from the intraindividual variability of indirect calorimetry, which affects the assessment of nonoxidative glucose metabolism (16).

Skeletal muscle glucose uptake is regulated by glucose transporter type 4 (GLUT4) abundance as well as by translocation from the cytosol toward the sarcolemmal membrane, which is independently stimulated by exercise and insulin. The insulin-mediated translocation of GLUT4 to the sarcolemmal membrane is impaired in patients with T2DM (17, 18) and results in lower intramuscular glucose and in turn lower G6P concentrations (19). Intravenous intralipid infusion reduces both intramuscular glucose and G6P concentrations, preceding the development of lipid-induced insulin resistance (20). Total GLUT4 protein and GLUT4 mRNA content also rise over time with chronic endurance training (21–23) as well as within 3 hours of exercising (24). This may potentiate the higher insulin-stimulated glucose uptake and glycogen storage in skeletal muscle after exercise. Interestingly, a single session of exercise preceding intralipid infusion completely prevented lipid-induced insulin resistance (25). This could partly be explained by postexercise high insulin-stimulated IRS-1-associated PI3K activity, responsible for GLUT4 translocation (25). Several studies provided evidence for a link between acute lipid-induced insulin resistance and defective insulin signaling (26–29). Moreover, a recent study indicated that activation of the DAG/protein kinase C-θ (PKC-θ) pathway during intralipid infusion inhibits muscle insulin signaling in sedentary humans (30); however, its role in athletes remains controversial (31). To our knowledge, no studies reported on GLUT4 protein levels in the vicinity of increased free fatty acids via the infusion of Intralipid in sedentary individuals or in endurance athletes.

This study therefore tested the hypothesis that endurance-trained athletes maintain greater rates of insulin-stimulated muscle glycogen synthesis *in vivo* during lipid infusion than sedentary humans. In addition, this study examined whether glucose transport/phosphorylation is rate controlling in athletes under insulin-stimulated conditions.

Results

Participants' characteristics. By design, athletes had higher VO_2 peak values (Table 1). Athletes also showed slightly lower fasting plasma insulin concentrations ($P = 0.07$). Fasting plasma glucose, triglycerides (TGs), free fatty acids (FFAs), and glycerol concentrations at baseline were comparable between groups (Table 1). Fasting glucagon concentrations were not different between athletes and sedentary participants (Table 1).

Plasma lipids. During saline infusion, insulin similarly suppressed circulating FFA concentrations in both groups (Figure 1A), while plasma FFA concentrations increased in both groups during the clamp with Intralipid infusion, with a tendency toward higher FFA levels in sedentary participants at the end of the infusion period ($P = 0.07$) (Figure 1B). Serum TGs (32) increased upon Intralipid versus saline infusion, with no difference between athletes and sedentary participants (TG during Intralipid 402 ± 66 mg/dL in sedentary and 312 ± 49 mg/dL in athletes vs. TG during saline 48 ± 9 mg/dL in sedentary vs. 35 ± 6 mg/dL in athletes; $P < 0.05$ for Intralipid vs. saline; $P = \text{n.s.}$ between sedentary humans and athletes). Likewise, plasma glycerol concentrations were higher upon Intralipid versus saline infusion without a difference between groups (glycerol during Intralipid 76 ± 11 mmol/L in sedentary and 62 ± 11 mmol/L in athletes vs. glycerol during saline 14 ± 1 mmol/L in sedentary and 15 ± 1 mmol/L in athletes; $P < 0.05$ for Intralipid vs. saline; $P = \text{n.s.}$ between sedentary and athletes).

Whole-body and hepatic glucose metabolism. On saline infusion, insulin-stimulated ΔR_d was higher in athletes than in sedentary participants at $t = 210\text{--}240$ minutes (Figure 1C) and $t = 330\text{--}360$ minutes (Figure 1D, both $P < 0.05$). Upon Intralipid infusions, ΔR_d dropped in athletes as well as in sedentary

Table 1. Participants' characteristics

	Sedentary (n = 9)	Athletes (n = 8)
Age (yr)	23.4 ± 1.1	26.6 ± 1.4
Body weight (kg)	71.7 ± 3.0	68.7 ± 3.3
Body mass index (kg/m²)	24.0 ± 0.6	21.5 ± 0.5 ^A
VO₂ peak (mL/kg/min)	33.2 ± 1.9	56.0 ± 1.5 ^A
Fasting glucose (mmol/L)	4.2 ± 0.1	4.1 ± 0.1
Fasting insulin (mU/L)	6.3 ± 1.1	4.6 ± 0.5
Fasting free fatty acids (mol/L)	378 ± 47	361 ± 53
Fasting triglycerides (mg/dL)	94 ± 18	78 ± 11
Fasting glycerol (mmol/L)	23 ± 1	22 ± 2
Fasting glucagon (pg/mL)	83 ± 13	105 ± 17

Data are mean ± SEM. ^AP < 0.05 vs. sedentary participants.

participants at $t = 210$ –240 minutes (Figure 1C) and $t = 330$ –360 minutes (both $P < 0.05$) (Figure 1D). At 330–360 minutes of Intralipid infusion, ΔR_d was approximately 57% and approximately 62% lower (both $P < 0.05$) in athletes and sedentary participants, respectively. Average baseline rates for endogenous glucose production (EGP) (before the start of infusions) were slightly higher in athletes compared with sedentary participants ($13.2 \pm 0.5 \mu\text{mol}/\text{kg}/\text{min}$ vs. $11.8 \pm 0.6 \mu\text{mol}/\text{kg}/\text{min}$, $P < 0.05$). At 210–240 minutes Intralipid infusion, hepatic insulin sensitivity was similar between sedentary participants and athletes ($P = \text{n.s.}$, Figure 1E), but suppression of EGP was higher in athletes at 330–360 minutes (–76% vs. –63% in sedentary, $P < 0.05$, Figure 1F). Within sedentary participants, at 330–360 minutes, hepatic insulin sensitivity was also lower during Intralipid versus saline condition (–63% vs. –96%, $P < 0.05$, Figure 1F). At this time, hepatic insulin sensitivity remained comparable between both conditions for athletes (–76% vs. –86%, $P = \text{n.s.}$, Figure 1F).

Time course of muscle glucose flux. The time course of the glucose infusion rates (GIRs) throughout saline and Intralipid infusion studies in sedentary participants and in athletes is shown in Figure 2, A and B, respectively. The AUC of GIR was about 30% higher under saline-infused conditions in athletes ($0.99 \pm 0.06 \mu\text{mol}/\text{kg}$) than in sedentary participants ($0.70 \pm 0.06 \mu\text{mol}/\text{kg}$, $P < 0.05$, Figure 2C).

To examine tissue-specific glucose flux in skeletal muscle, we monitored *in vivo* glycogen and G6P concentrations. At baseline, average muscle glycogen concentration was 2-fold higher in athletes compared with sedentary participants (110 ± 17 vs. $54 \pm 2 \text{ mmol/L}$, $P < 0.05$). The time course of insulin-stimulated glycogen concentrations, given by the difference between the clamp and baseline (Δ glycogen), were monitored for sedentary participants (Figure 2D) and athletes (Figure 2E). During saline conditions, the AUC of Δ glycogen was higher in athletes ($92 \pm 13 [\text{mmol/L}] \times \text{h}$) than in sedentary participants ($46 \pm 8 [\text{mmol/L}] \times \text{h}$, $P < 0.01$, Figure 2E). During Intralipid infusion, the AUC of Δ glycogen was higher in the athletes ($69 \pm 14 [\text{mmol/L}] \times \text{h}$) compared with the sedentary participants ($24 \pm 5 [\text{mmol/L}] \times \text{h}$, $P < 0.01$, Figure 2F). Within the athletes, the AUC was only nominally, but not significantly ($P = 0.23$), lower upon Intralipid than upon saline infusion (Figure 2F). Within the group of sedentary participants, the AUC fell by approximately 49% upon Intralipid compared with saline infusion ($P < 0.01$). From the glycogen concentrations, we calculated the net muscle glycogen synthesis rate (GSR) for the time intervals 0–180 minutes and 180–360 minutes (Figure 3) as well as for the entire period (0–360 minutes). Initially (0–180 minutes), the GSR was approximately 64% higher in the athletes than in sedentary participants under saline conditions ($P < 0.05$) and approximately 72% higher under lipid-infused conditions ($P < 0.05$). From 180 minutes to 360 minutes, the GSR did not differ between athletes and sedentary participants under saline-infused conditions ($P = \text{n.s.}$, Figure 3B). The GSR over the entire period from 0 to 360 minutes during saline infusion was approximately 46% higher in athletes versus sedentary participants ($P < 0.001$, Figure 3C). However, Intralipid infusion completely inhibited GSR between 180 and 360 minutes in the sedentary participants ($P < 0.05$, Figure 3B) in contrast to the athletes. The GSR calculated over the entire period (0–360 minutes) did not differ between Intralipid and saline infusion in the athletes ($P = \text{n.s.}$, Figure 3C) but decreased by 72% ($P < 0.01$, Figure 3C) in the sedentary participants during Intralipid compared with saline infusion.

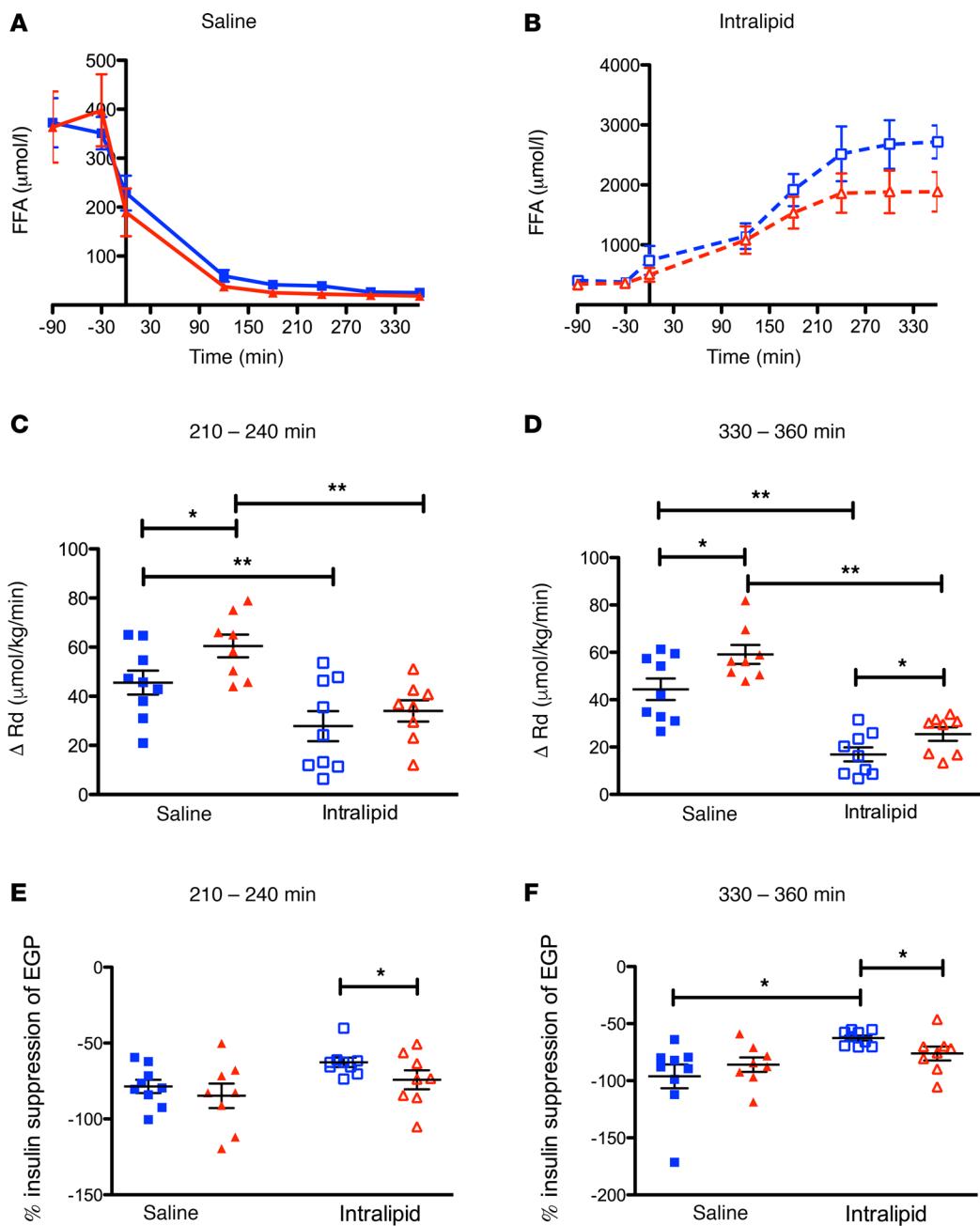


Figure 1. Plasma FFA and insulin sensitivity during saline or Intralipid infusion. Nine sedentary humans (blue squares) and 8 endurance-trained athletes (red triangles) underwent 2 hyperinsulinemic-isoglycemic clamp tests with either saline (full symbols and lines) or Intralipid infusion (empty symbols and dashed lines). The time course of plasma FFA (A and B) and whole-body (expressed as ΔRd vs. baseline; C and D) and hepatic (expressed as percentage of insulin-suppressed EGP vs. baseline; E and F) insulin sensitivity is shown. Insulin sensitivity was calculated for 2 steady state time intervals, 210–240 minutes (C and E) and 330–360 minutes (D and F). Data expressed as mean \pm SEM; * $P < 0.05$ and ** $P < 0.01$. FFA, free fatty acid; EGP, endogenous glucose production; ΔRd , delta rate of glucose disappearance during insulin stimulation minus baseline.

The higher Aglycogen and GSR of the athletes during Intralipid infusion were paralleled by the time course of intramyocellular G6P concentrations (Figure 2G). The AUC of G6P in athletes upon Intralipid infusion did not differ from the AUC of G6P concentrations during saline infusion (athletes: AUC Intralipid 1.4 ± 0.2 vs. AUC saline 1.6 ± 0.2 [$\text{mmol/L} \times \text{h}$], $P = \text{n.s.}$, Figure 2I). Conversely, sedentary participants showed a strong trend toward lower AUC of G6P during Intralipid than during saline infusion (sedentary: 1.1 ± 0.1 vs. 1.7 ± 0.4 [$\text{mmol/L} \times \text{h}$], $P = 0.059$, Figure 2H). These data indicate that glucose transport is

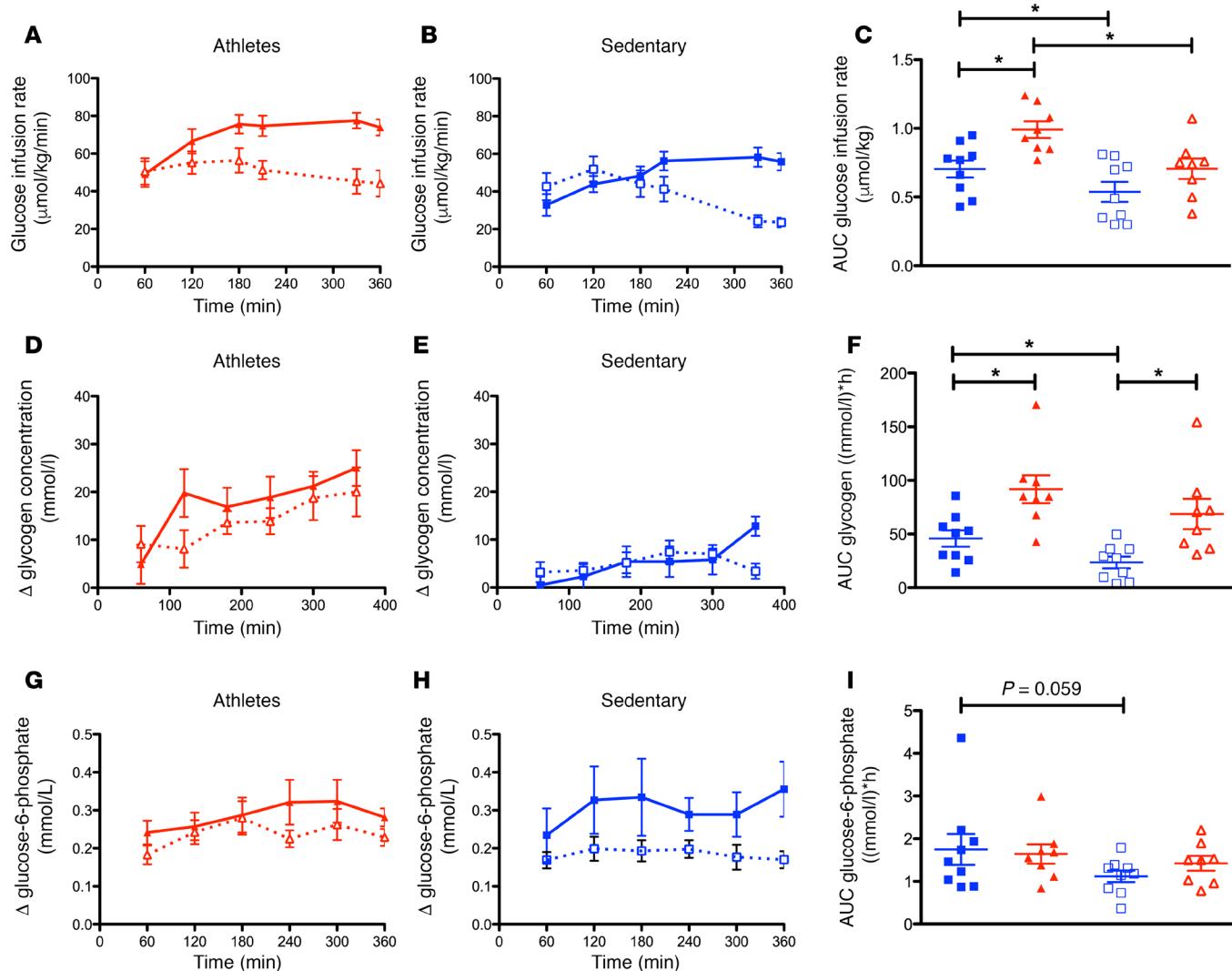


Figure 2. Time course of changes in GİRs, intramuscular glycogen, and intramuscular G6P during saline or Intralipid infusion. Nine sedentary humans (blue squares) and 8 endurance-trained athletes (red triangles) underwent 2 hyperinsulinemic-isoglycemic clamp tests with either saline (full symbols and lines) or Intralipid infusion (empty symbols and dashed lines). Infusion rates of exogenous glucose are shown for sedentary participants (**A**) and for athletes (**B**), as well as the AUC (**C**). Changes in insulin-stimulated muscle glycogen concentrations (measured with in vivo ¹³C-MRS and expressed as Δ glycogen vs. baseline) as well as the AUC (**D-F**) and G6P (measured with in vivo ³¹P-MRS and expressed as Δ G6P) and the AUC (**G-I**) during the clamps are depicted. Data are expressed as mean \pm SEM; * P < 0.05 and ** P < 0.01.

reduced upon Intralipid infusion in sedentary participants but not in athletes. This reduction in glucose transport in sedentary people could result from reduced GSR. In athletes, however, the GSR did not account for reduced glucose transport during Intralipid infusion.

Within the athlete group, we performed a subgroup analysis of the differences of AUCs of Δ glycogen and Δ G6P between the saline- and Intralipid-infused conditions for runners ($n = 5$) compared with cyclers ($n = 3$). The AUC for Δ glycogen under saline versus Intralipid did not differ for runners (AUC Δ glycogen: saline 79 ± 9 vs. Intralipid 54 ± 9 [mmol/L] \times h, $P = \text{n.s.}$) or for cyclers (Δ glycogen: saline 113 ± 18 vs. Intralipid 94 ± 19 [mmol/L] \times h, $P = \text{n.s.}$). Also, for Δ G6P no differences were found between runners (AUC Δ G6P: saline 1.7 ± 0.3 vs. Intralipid 1.4 ± 0.2 [mmol/L] \times h, $P = \text{n.s.}$) and cyclers (AUC Δ G6P: saline 1.5 ± 0.1 vs. Intralipid 1.6 ± 0.2 [mmol/L] \times h, $P = \text{n.s.}$). Differences between runners and cyclers with the sedentary group remained similar to results presented for the whole athlete group.

Muscle protein expression. Muscle glucose uptake depends on the amount of GLUT4 in the muscle cell membrane. In sedentary individuals, membrane-bound GLUT4 was 28% lower during Intralipid-insulin than during insulin and saline-insulin infusions (1.23 ± 0.10 AU vs. 0.87 ± 0.11 AU, $P < 0.05$).

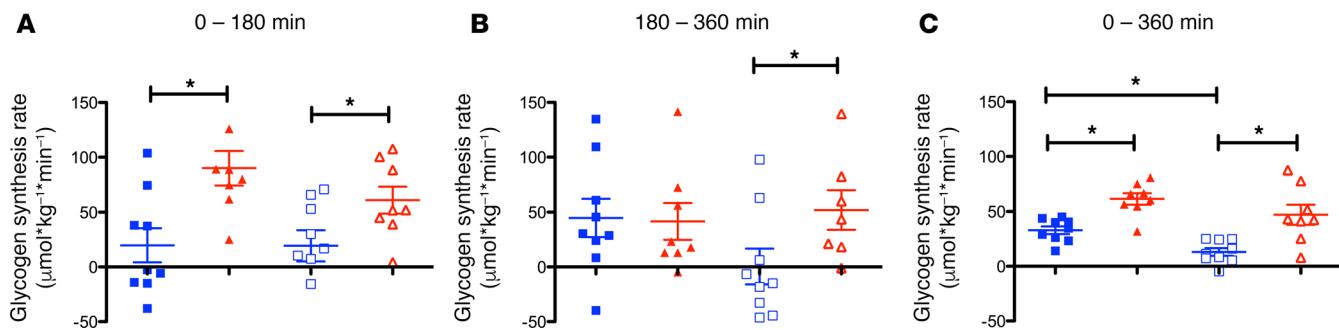


Figure 3. Rates of intramuscular glycogen synthesis during saline or Intralipid infusion. Nine sedentary humans (blue squares) and 8 endurance-trained athletes (red triangles) underwent 2 hyperinsulinemic-isoglycemic clamp tests with either saline (full symbols) or intralipid infusion (empty symbols). Rates of muscle glycogen synthesis are shown for the time intervals 0–180 minutes (A), 180–360 minutes (B), and 0–360 minutes (C) of the clamps. Data expressed as mean \pm SEM; * P < 0.05.

In athletes, however, membrane-bound GLUT4 upon insulin stimulation was similar during the concomitant Intralipid or saline infusions (0.93 ± 0.18 AU vs. 0.85 ± 0.11 AU, $P = \text{n.s.}$, Figure 4).

Discussion

This study reports for the first time to our knowledge the *in vivo* time course of intramyocellular G6P and glycogen concentrations in endurance-trained athletes under both low and high lipid availability. Although augmented lipid availability rapidly induces insulin resistance in sedentary humans at approximately 120 minutes — as reported before (8, 9, 20, 33–35), endurance-trained athletes develop insulin resistance at approximately 180 minutes and thus are largely prevented from developing lipid-induced muscle insulin resistance by maintaining transmembrane muscle glucose transport.

By *in vivo* monitoring of glucose flux using multinuclei MRS, this study found that the lipid-induced insulin resistance in athletes is at least partly restored when compared with sedentary people. In line, muscle G6P levels in the athletes did not drop upon Intralipid infusion, and insulin-stimulated GSRs were maintained despite Intralipid infusion. This finding indicates that endurance training can protect against the inhibitory effect of fatty acids on glucose transport/phosphorylation with concomitant chronically high glycogen synthesis.

Muscle glycogen synthesis reflects NOGD and represents the major fraction of whole-body glucose disposal during insulin stimulation (13). The partial prevention of lipid-induced insulin resistance in the athletes seems mainly due to preserved NOGD. This finding extends our previous findings of higher whole-body NOGD in athletes than in sedentary humans during increased lipid availability (15). The remaining high NOGD supports the notion that myocellular glycogen synthesis could be the predominant route of whole-body insulin-stimulated NOGD in athletes also, as examined in the present study. The maintenance of high rates of glycogen synthesis could result either (a) from greater activity of glycogen synthase despite lower availability of G6P (resulting from impaired glucose transport/phosphorylation as reported for sedentary humans) or (b) from maintained glucose transport/phosphorylation resulting in constant myocellular G6P levels, sufficient to stimulate glycogen synthesis. Monitoring of myocellular G6P concentrations with ^{31}P MRS revealed marked differences between saline and Intralipid infusion. The decrease in G6P observed in the sedentary participants under lipid-infused conditions was consistent with the original report of lipid-induced effects on muscle glucose flux (9). In athletes, the absence of any decrease in G6P along with the constantly high GSRs despite high lipid availability clearly indicates that exercise training preserves myocellular glucose transport/phosphorylation under these conditions. These data are supported by a previous study demonstrating that severe insulin-resistant offspring of parents diagnosed with T2DM, featuring blunted insulin-stimulated increase of muscle G6P and reduced glycogen, exhibit a marked improvement of insulin-stimulated muscle G6P and glycogen synthesis upon chronic exercise training (36). Nevertheless, these studies show that exercise controls insulin-stimulated muscle glucose transport/phosphorylation and glycogen synthesis, thereby increasing insulin sensitivity. Of note, the improvement in insulin-stimulated whole-body glucose uptake after 12 weeks of exercise training seemed to be exclusively due to oxidative glucose metabolism, at least in T2DM and obese humans (37).

The athletes featured higher basal glycogen concentrations compared with the sedentary participants. By design, all participants refrained from exercise 3 days preceding the clamp tests. Actually, the

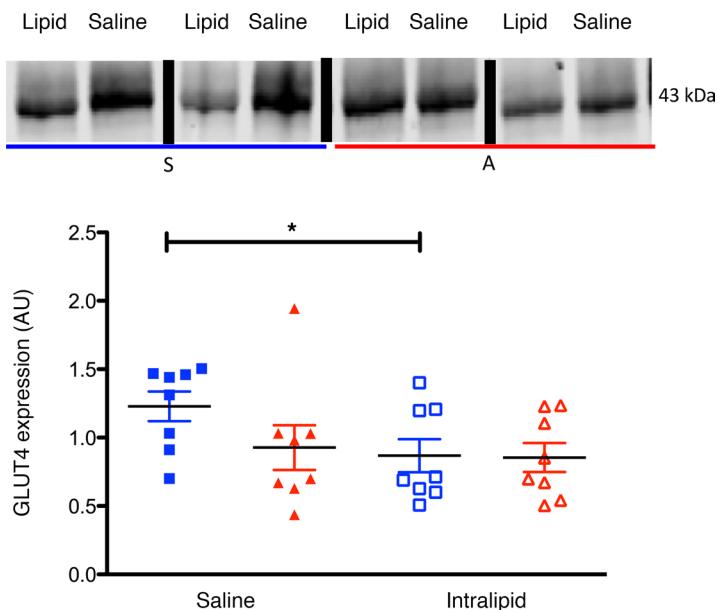


Figure 4. Expression of GLUT4 in skeletal muscle during saline or Intralipid infusion. Eight sedentary humans (blue squares) and 8 endurance-trained athletes (red triangles) underwent 2 hyperinsulinemic-isoglycemic clamp tests with either saline (full symbols) or Intralipid infusion (empty symbols). Biopsies were obtained from the vastus lateralis muscle at the end of the clamps. Lanes represent GLUT4 expression of 4 randomly chosen sedentary participants and athletes each, determined with Western blotting run on the same gel. The thick lines indicate noncontiguous lanes. Data expressed as mean \pm SEM; * $P < 0.05$.

exercise constraint could explain the higher amount of glycogen stores because normally these glycogen stores would deplete upon exercise, depending on the exercise intensity and duration (38). However, lower glycogen content would stimulate activation of glycogen synthase and thereby drive muscle glycogen synthesis (39–41). In other words, the activity of glycogen synthase has been shown to be greater when glycogen is heavily depleted. Previously, we reported that glycogen synthase phosphorylation before the start of the clamp tests is similar between athletes and sedentary participants (15). The higher baseline muscle glycogen levels in the athletes would therefore have not resulted in higher rates of glycogen synthesis than in the sedentary participants.

The maintained rates of muscle glucose uptake, as reflected by constant myocellular G6P, could have resulted from differences in myocellular lipid handling between athletes and sedentary participants. Our previous muscle biopsy study addressed myocellular TGs in vastus lateralis muscle (15). Briefly, only in type I, but not in type II fibers, the mean area fraction of TGs was increased exclusively in untrained persons upon exactly the same lipid infusion protocol as presented here. This is in line with previous results (42). In another study — although without an intralipid infusion protocol — trained athletes displayed lipid storage in a higher number of droplets in the intramyofibrillar region of type I fibers when compared with patients with T2DM, who store lipids predominately in larger droplets in the subsarcolemmal region of type II fibers (43). Together, these findings imply that lipid droplet location and distribution contribute to skeletal muscle insulin sensitivity.

The maintained rates of muscle glucose uptake in athletes could have resulted from greater glucose transport via GLUT4. Lipid infusion did not decrease membrane-bound GLUT4 content during insulin stimulation in athletes as compared to sedentary persons, exhibiting clearly compromised membrane-bound GLUT4 levels during Intralipid infusion. This likely contributes to the higher levels of muscle glucose transport in the athletes. Interestingly, one previous study found no alterations of insulin receptor signaling and AKT levels in athletes during lipid infusion (15). Muscle insulin resistance due to lipid infusion, obesity, or T2DM most likely occurs via activation of the DAG/PKC pathway, which leads to inhibitory serine phosphorylation of IRS-1 and subsequent reductions in AKT and glucose transport (30, 33). Consequently, the athletes' partial protection from insulin resistance may be the result of alteration in cellular lipid intermediates. A recent study provided supporting evidence by reporting a significant positive relationship

between muscle PKC-ε and sarcolemmal 1,2 DAG C16:0/C18:2 in insulin-resistant groups but not in athletes (31). In addition, lipid metabolites in other compartments may contribute to the metabolic changes: mitochondrial DAG accumulation has been hypothesized to promote increased mitochondrial morphology and function and thereby affect metabolism in athletes (44).

Previously, some studies reported that Intralipid infusion diminishes insulin-stimulated microvascular recruitment, which may contribute to reduced muscle glucose flux. Rodent studies support this concept by showing inhibition of insulin-mediated capillary recruitment by acute elevation of FFA (45). Studies in humans revealed inconsistent data. We found that vascular effects of Intralipid infusion are not rate controlling for human muscle insulin-stimulated glucose disposal (46), while others reported reduced insulin-stimulated microvascular recruitment upon lipid infusion (47, 48). The present study cannot rule out that the endurance-trained athletes were protected against this lipid-hampering of insulin-stimulated microvascular recruitment and in turn diminished glucose uptake.

In the present study, athletes featured a slightly higher basal rate of EGP than sedentary humans. The higher EGP may have contributed to the higher insulin-suppressed EGP percentage, which could have led to overestimation of the effect of exercise training on hepatic insulin sensitivity. Of note, knowledge on fasting and even postexercise hepatic glucose metabolism in endurance-trained athletes is still scarce. The current evidence indicates that — in contrast to skeletal muscle — liver glycogen concentrations as well glycogenolysis is lower in athletes than in untrained humans during moderate-to-high exercise (49). In the present study, both groups followed a carbohydrate-rich diet for 3 days preceding the clamp days, which would render such effects unlikely. Interestingly, prolonged low-carbohydrate diets may lead to lower EGP and glycogenolysis during exercise, when compared with a normal, mixed diet (50). One may speculate that the high basal EGP in athletes may result in increased responsiveness of the adrenal medulla, in turn favoring higher production and lower clearance of glucose (51). The design of the present study cannot rule out the operation of such a mechanism underlying the higher basal EGP values in chronically trained humans.

In this study, the athlete group consisted of both runners and cyclers. Although electromyography recording showed that both types of physical activity train the vastus lateralis muscle (52–54), some studies do report differences in the efficiency of the vastus lateralis muscle between runners and cyclers (55, 56). Separate analysis of glucose flux in runners and cyclers, however, revealed no differences between runners and cyclers.

Taken together, athletes exhibit higher muscle glucose disposal and glycogen synthesis during increased lipid availability primarily resulting from maintained insulin-stimulated glucose transport with increased myocellular G6P levels for subsequent glycogen synthesis.

Methods

Participants. Nine healthy, sedentary young humans (6 males/3 females) and 8 chronically endurance-trained humans (5 males/3 females), matched for age, were included (Table 1 and Supplemental Figure 1; supplemental material available online with this article; <https://doi.org/10.1172/jci.insight.127928DS1>). Medication use, family history of diabetes, and unstable dietary habits were exclusion criteria. All participants underwent an incremental aerobic cycling test until exhaustion (57). By design, endurance-trained humans had to participate in endurance exercise activities, such as running and/or cycling, thrice weekly for at least 2 years, with or without combination with other sports. Untrained participants had a sedentary lifestyle and did not participate in any regular physical exercise. Participants were defined as athletes if maximal oxygen uptake (VO_2 peak) was more than 55 mL/kg/min for men and more than 50 mL/kg/min for women and if insulin-stimulated Rd was more than 60 $\mu\text{mol}/\text{kg}/\text{min}$. Participants were defined as sedentary when VO_2 peak was less than 45 mL/kg/min for both sexes. In total, 26 humans were screened, of whom 9 sedentary participants and 8 athletes fulfilled the inclusion criteria. All measurements were conducted at the research facility of the German Diabetes Center in Düsseldorf.

Hyperinsulinemic-euglycemic clamp. Three days before the clamp tests, all participants refrained from any exercise. Also, participants consumed a carbohydrate-rich meal the evening before the test day, i.e., fresh pasta with a minimum of approximately 115 g carbohydrates. On the test day, a baseline biopsy was taken from the vastus lateralis muscle before the clamp under local anesthesia (2% lidocaine) (30, 58). Each of the participants underwent two 6-hour hyperinsulinemic (40 mU/m²/min) to euglycemic clamps with a primed, continuous (0.04 mg/kg/min) intravenous infusion of [6,6-²H]₂glucose. Participants received an intravenous

infusion of either 0.9% saline (1.32 mL/min as control condition) or heparinized (0.2 U/kg/min) long-chain triacylglycerols (1.32 mL/min; Intralipid, Braun, Melsungen, Germany) in random order (9, 15). Immediately after the clamp another muscle biopsy was taken.

MRS. The clamp tests were performed within a 3-T whole-body MRI scanner (Achieva Philips Healthcare, Best, the Netherlands). Participants were situated feet first into the magnet in the supine position, and the left leg was placed in an MRI-compatible foot brace, which was secured firmly with straps for the *in vivo* detection of G6P and glycogen concentrations within the vastus lateralis muscle. Real-time monitoring of G6P and glycogen concentrations was conducted at baseline, and during $t = 60, 120, 180, 240, 300, 360$ minutes of the clamp, via individual 7-cm ^{31}P and ^{13}C coils equipped with 12-cm quadrature ^1H coils for decoupling, shimming, and imaging (PulseTeq, Cobham, United Kingdom). All MRS spectra were processed within the NUTS software package (Acorn NMR Inc., Livermore, California, USA). ^{31}P -MRS proton-decoupled pulse-acquired measurements (repetition time [TR]: 3500 ms, bandwidth [BW]: 2048 kHz, number of averages [NSA]: 100, decoupling: WALTZ-4, time: 6 minutes) were conducted with an excitation block pulse calibrated to adjust for coil loading via multiple flip angle measurements of an internal H_3PO_4 reference to provide 90° excitation at 2 cm. Differences in G6P spectra (effective line broadening: 10 Hz, zero filling: 32k) were detected through integration of the region between 6.83 and 7.43 parts per million (ppm) (12) after the subtraction of spectra from baseline measurements and baseline correction. Concentrations of G6P were determined through comparing the resonance areas with the β -ATP resonance from a fully relaxed (TR: 15 seconds) baseline spectrum, and a concentration of 5.5 mmol/kg muscle, as assumed for resting ATP concentration (59) ^{13}C -MRS proton-decoupled pulse-acquired measurements (TR: 197 ms, BW: 8 kHz, NSA: 4000, data points: 256, decoupling: continuous wave, time: 13 minutes) were acquired with a 4.992-ms rapid half-passage adiabatic excitation pulse. Coil loading was corrected via integration of the right-most peak of a ^{13}C -enriched sample of formic acid (MilliporeSigma, Milwaukee, Wisconsin, USA) placed within the coil housing (60). C1-glycogen peak areas were determined through the integration of the region between 85 and 115 ppm after signal conditioning (effective line broadening: 20 Hz, zero filling: 8k) and baseline correction. Absolute concentrations of glycogen within the vastus lateralis muscle were determined from 1-L aqueous phantom replacement measurements (KCl: 40 mM, sodium azide: 0.05%, phosphocreatine: 40 mM, glycogen: 30, 60, and 90 mM, MilliporeSigma, Milwaukee, Wisconsin, USA), and correction for B1-field penetration depth (0–30 mm) via agar gel spacers (KCl: 70 mM, agar: 2% weight/volume) was used to simulate subcutaneous adipose tissue.

Blood analyses. Blood samples were immediately cooled and centrifuged and supernatants stored at –80°C. Blood glucose was measured using the glucose oxidase method (EKF biosen C-Line glucose analyzer, EKF Diagnostics GmbH, Barleben, Germany) (61). Serum TGs were analyzed enzymatically (Hitachi analyzer, Roche Diagnostics, Mannheim, Germany) and FFAs microfluorimetrically (Wako, Neuss, Germany) after prevention of lipolysis using orlistat (62). Plasma glycerol was measured enzymatically (R-Biopharm, Darmstadt, Germany). Isotopic enrichments of plasma glucose were measured as previously described (63).

Western blotting. For Western blot analysis, equal amounts of protein were loaded and protein concentration was directly controlled and analyzed on the nitrocellulose blots by the REVERT Total Protein Stain Kit (LI-COR Biosciences, Westburg, Leusden, the Netherlands). For GLUT4 content, muscle membrane fractions and total muscle protein fractions were prepared as described previously (64). Blots for GLUT4 were incubated with a goat polyclonal GLUT4 antibody (sc1608; Santa Cruz Biotechnology, BioConnect, Huissen, the Netherlands). An appropriate near-infrared-tagged secondary antibody (IRDye, LI-COR Biosciences, Westburg, Leusden, the Netherlands) was used to visualize and quantify the appreciated GLUT4 band with the Odyssey near-infrared imaging system (LI-COR Biosciences, Westburg, Leusden, the Netherlands). Because of technical issues, the GLUT4 expression analysis was performed in $n = 8$ athletes and $n = 6$ sedentary humans. See the full, uncut blots in the online supplemental materials.

Calculations. Ra and Rd were calculated for non-steady state conditions according to Steele (65). Peripheral insulin sensitivity was expressed as the ΔRd , which represents the difference between insulin-stimulated and baseline Rd. EGP was calculated from the difference between Ra and mean GIRs. Hepatic insulin sensitivity was calculated as percentage of EGP suppression by insulin during clamp steady state versus baseline (EGP). Net GSR was calculated from the slope of the linear fit to the glycogen concentration time curve during the periods 0–180 minutes and 180–360 minutes (9). The development of intralipid-induced insulin resistance occurs between 120 and 180 minutes in sedentary people (9, 34, 35), which forms the

rationale for splitting the analysis of GSR into these 2 time intervals. For the athletes so far, no information on the time course of lipid-induced insulin resistance has been reported. The GSR, however, was also calculated over the entire period (0–360 minutes). For the GIR and both the time courses of Δ glycogen and Δ G6P concentrations, the AUC was calculated using the trapezoid method. Test days were randomized by the first authors of this study using <https://www.randomizer.org>.

Statistics. Statistical analyses were performed using the statistics computer program SPSS 18 for Mac OS X. Differences between groups and between conditions were analyzed by 2-way ANOVA, in which comparisons between groups for repeated measures were computed with Tukey's post hoc correction. Pearson's linear regression analysis was performed to identify correlations between variables. Differences were considered significant at $P < 0.05$.

Study approval. The institutional ethics board of Heinrich-Heine University approved the study, and all participants gave their informed consent in writing.

Author contributions

EP, MR, MKCH, and PS designed the study. EP, PB, SG, AB, EK, and GS contributed to the data collection and/or analysis. EP, PB, and SG performed the statistical analysis. All authors contributed to the interpretation of the results. EP and MR wrote the manuscript. All authors revised it critically for important intellectual content and approved the submission of the final version.

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