

# Exercise-Stimulated Glucose Turnover in the Rat Is Impaired by Glucosamine Infusion

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The infusion of glucosamine causes insulin resistance, presumably by entering the hexosamine biosynthetic pathway; it has been proposed that this pathway plays a role in hyperglycemia-induced insulin resistance. This study was undertaken to determine if glucosamine infusion could influence exercise-stimulated glucose uptake. Male SD rats were infused with glucosamine at  $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (low-GlcN group),  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (high-GlcN group), or saline (control group) for 6.5 h and exercised on a treadmill for 30 min (17 m/min) at the end of the infusion period. Glucosamine infusion caused a modest increase in basal glycemia in both experimental groups, with no change in tracer-determined basal glucose turnover. During exercise, glucose turnover increased ~2.2-fold from  $46 \pm 2$  to  $101 \pm 5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the control group. Glucose turnover increased to a lesser extent in the glucosamine groups and was limited to 88% of control in the low-GlcN group ( $47 \pm 2$  to  $90 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.01$ ) and 72% of control in the high-GlcN group ( $43 \pm 1$  to  $73 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.01$ ). Similarly, the metabolic clearance rate (MCR) in the control group increased 72% from  $6.1 \pm 0.2$  to  $10.5 \pm 0.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in response to exercise. However, the increase in MCR was only 83% of control in the low-GlcN group ( $5.2 \pm 0.5$  to  $8.7 \pm 0.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.01$ ) and 59% of control in the high-GlcN group ( $4.5 \pm 0.2$  to  $6.2 \pm 0.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.01$ ). Neither glucosamine infusion nor exercise significantly affected plasma insulin or free fatty acid (FFA) concentrations. In conclusion, the infusion of glucosamine, which is known to cause insulin resistance, also impaired exercise-induced glucose uptake. This inhibition was independent of hyperglycemia and FFA levels. *Diabetes* 50:139–142, 2001

**C**hronic hyperglycemia is the cardinal metabolic feature of diabetes and can adversely affect glucose homeostasis by interfering with insulin action and/or  $\beta$ -cell function (1). Studies in humans have shown that experimental hyperglycemia or diabetes-associated hyperglycemia can cause insulin resistance or

exacerbate an underlying insulin-resistant state (2). This finding is supported by animal and in vitro studies that directly show that hyperglycemia can cause impaired insulin action in vitro and insulin resistance in vivo (1,3,4).

The mechanism by which hyperglycemia causes insulin resistance remains incompletely understood, but several studies have implicated the hexosamine biosynthesis pathway (5–8). This hypothesis holds that hyperglycemia, by way of mass action, leads to increased flux of glucose carbons through the hexosamine pathway (9). This occurs by glutamine:fructose-6-phosphate amidotransferase (GFAT)-mediated conversion of fructose-6-phosphate (F6P) to glucosamine-6-phosphate (G6P), with subsequent metabolism of G6P through the hexosamine pathway. Distal metabolic products of this pathway in some way may lead to decreased insulin-stimulated GLUT4 translocation and subsequent reduction in glucose metabolism (10). Glucosamine also increases flux through the hexosamine pathway; however, it bypasses GFAT and enters the pathway as G6P. Indeed, the infusion of glucosamine has been shown to produce insulin resistance in rats (6,11), which is not additive to the effect of hyperglycemia (6). Furthermore, mice who overexpress GFAT are insulin resistant (12).

In addition to insulin, exercise increases the uptake of glucose into contracting muscle by stimulating the movement of GLUT4 transporter-containing vesicles to the plasma membrane and transverse tubules (13). Despite being insulin-resistant, obese rats have normal increases in peripheral glucose uptake in response to moderate exercise (14,15), and similar findings have been found in obese type 2 diabetic subjects (16). These findings are not unexpected, considering it is generally accepted that exercise and insulin use different intracellular signaling pathways and recruit distinct GLUT4 transporters from different intracellular vesicular pools (13).

Because exercise and insulin use distinct pathways to augment glucose transport, it is not known whether stimulation of the hexosamine pathway can influence exercise-stimulated glucose uptake. To examine this question, we studied the effects of glucosamine infusion on exercise-stimulated glucose disposal in male SD rats.

## RESEARCH DESIGN AND METHODS

**Animals and training.** Male SD rats (Harlan, Indianapolis, IN) weighing 200–224 g were housed individually under controlled light/dark (12/12 h) and temperature conditions and had free access to food and water. The animals were trained daily for 4 days to run on a treadmill (in-house design and construction) for 30 min at 17 m/min and 0% slope. The animals then underwent surgery during which catheters were implanted. Experiments were performed on the animals 4–5 days after surgery with a single training bout in between. All procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health and approved by the Animal Subjects Committee of the University of California, San Diego.

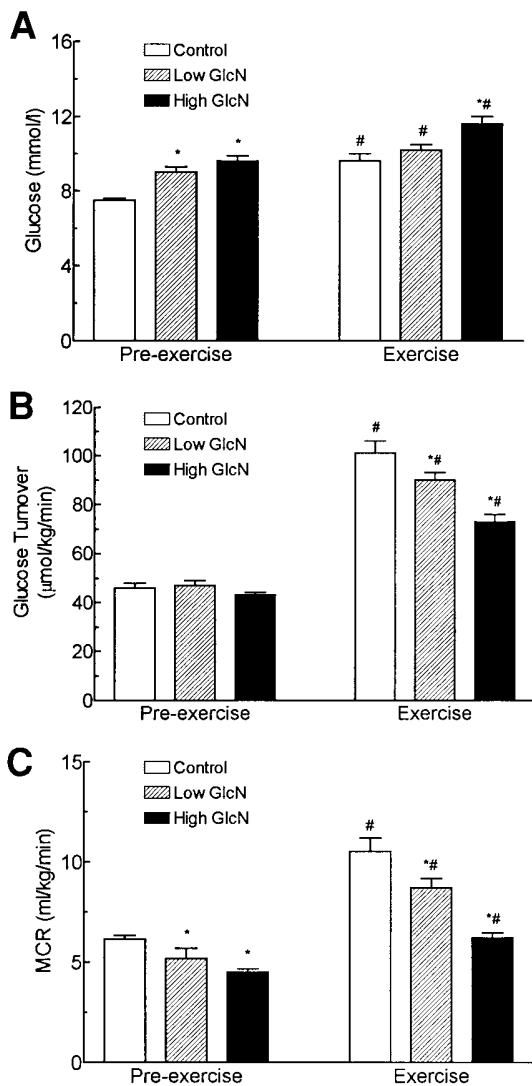
**Surgery.** One catheter was placed in the left jugular vein (Micro-Renathane MRE-033, 0.033 in OD and 0.014 in ID; Braintree Scientific, Braintree, MA) and

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F6P, fructose-6-phosphate; FFA, free fatty acid; G6P, glucosamine-6-phosphate; GDR, glucose disposal rate; GFAT, glutamine:fructose-6-phosphate amidotransferase; HGP, hepatic glucose production; MCR, metabolic clearance rate.



**FIG. 1.** The effect of glucosamine infusion (low-GlcN group,  $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; high-GlcN group,  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and saline infusion (control group) on plasma glucose (A), glucose turnover (B), and MCR (C) before and during exercise. Pre-exercise values were measured after 6 h of glucosamine infusion, and exercise values were measured 30 min into exercise and after 6.5 h of glucosamine infusion. Data are means  $\pm$  SE. \*Significantly different from control group ( $P < 0.05$ ); #significantly different from the corresponding pre-exercise group ( $P < 0.05$ ).

another was placed in the left carotid artery under general anesthesia. The anesthetic cocktail consisted of ketamine HCl (50 mg/kg) (Aveco, Fort Dodge, IA), acepromazine maleate (1 mg/kg) (Aveco), and xylazine (4.8 mg/kg) (Lloyd, Shenandoah, IA) given intramuscularly. Catheters were tunneled subcutaneously, exteriorized at the back of the neck, filled with heparinized saline, and flushed every other day to maintain patency. Ampicillin (1 mg/kg) (Aveco) was given prophylactically at the time of surgery. The jugular and carotid catheters were used for infusion and blood sampling, respectively, during the subsequent experiment.

**Exercise experiment.** The jugular line was connected to an infusion pump via swivel 4–5 days after catheter placement, allowing the animals to move freely in a circular container. The animals were infused (0.5 ml/h) for 6.5 h with saline or glucosamine ( $0.1$  or  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Glucose turnover during exercise was assessed with the infusion of tritiated glucose. A priming injection ( $2.5 \text{ } \mu\text{Ci}/0.5 \text{ ml}$ ) and constant infusion ( $0.04 \text{ } \mu\text{Ci}/\text{min}$ ) of  $\text{D-}[3\text{-}^3\text{H}]\text{glucose}$  (DuPont-NEN, Boston, MA) was started 5 h into the saline or glucosamine infusion. Tracer glucose was diluted to  $5 \text{ } \mu\text{Ci}/\text{ml}$  in saline containing  $100 \text{ mg}/\text{dl}$  unlabeled  $\text{D-glucose}$  (Mallinckrodt, Paris, KY) as carrier and  $200 \text{ mg}/\text{dl}$  sodium benzoate (Mallinckrodt) as preservative. The animals were

then transferred onto the treadmill. After a 1-h tracer equilibration period at hour 6, the animals were run for 30 min at  $17 \text{ m}/\text{min}$  and  $0\%$  slope. Blood samples (0.5 ml) were collected in heparinized microtubes immediately before exercise and at the end of exercise for determination of plasma glucose, glucose-specific activity, insulin, and free fatty acid (FFA).

All blood samples were immediately stored at  $4^\circ\text{C}$ . Blood was centrifuged within 20 min of collection and the resultant plasma was stored at  $-20^\circ\text{C}$ .

**Assays.** Plasma glucose concentration was measured with a YSI 23A glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma for determination of  $[3\text{-}^3\text{H}]\text{glucose}$  was deproteinized with perchloric acid and assayed as previously described (2). Insulin was measured by radioimmunoassay with a double-antibody immunoprecipitation technique as previously described (17). FFA was assayed according to standardized technique (18).

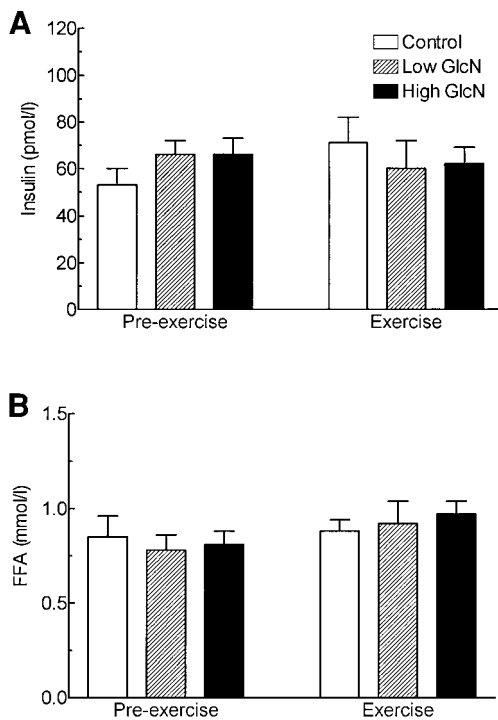
**Calculations.** Pre-exercise and exercise glucose turnover was calculated using the Steele equation for steady-state conditions (19). Specifically, hepatic glucose production (HGP) and glucose disposal rate (GDR) were assumed to be equal by 30 min of exercise (see APPENDIX for the validation study). The metabolic clearance rate (MCR) was calculated by normalizing the glucose turnover with the glucose concentration (20). Data are means  $\pm$  SE. Statistical analysis was performed using a two-way analysis of variance for unbalanced data and significance was assumed at  $P < 0.05$ .

## RESULTS

We studied three groups of exercised animals: 1) saline infused rats (control group;  $n = 9$ ), 2) rats infused with  $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  glucosamine (low-GlcN group;  $n = 6$ ), and 3) rats infused with  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  glucosamine (high-GlcN group;  $n = 11$ ). The weights of the control ( $237 \pm 9 \text{ g}$ ), low-GlcN ( $239 \pm 5 \text{ g}$ ), and high-GlcN ( $245 \pm 6 \text{ g}$ ) groups were all similar (NS).

**Pre-exercise period.** As seen in Fig. 1A, the infusion of glucosamine for 6 h caused a modest increase in plasma glucose concentration in the low-GlcN ( $9.0 \pm 0.3 \text{ mmol}/\text{l}$ ;  $P < 0.01$ ) and high-GlcN ( $9.6 \pm 0.3 \text{ mmol}/\text{l}$ ;  $P < 0.01$ ) groups compared with the saline-infused control group ( $7.5 \pm 0.1 \text{ mmol}/\text{l}$ ). Glucosamine administration did not significantly alter tracer-determined glucose turnover compared with the control group value of  $46 \pm 2 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (Fig. 1B). However, the higher plasma glucose level can increase glucose disposal by mass action. Therefore, when considering the prevailing glucose concentration, MCR (Fig. 1C) decreased in both the low-GlcN ( $5.2 \pm 0.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.05$ ) and high-GlcN ( $4.5 \pm 0.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.01$ ) groups in comparison with the control group ( $6.1 \pm 0.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). The glucosamine infusions had no significant effects on plasma insulin or FFA concentrations (Fig. 2).

**End of exercise period.** The 30 min of exercise led to a marked increase in plasma glucose in the control group ( $7.5 \pm 0.1$  to  $9.6 \pm 0.4 \text{ mmol}/\text{l}$ ;  $P < 0.001$ ), and further exacerbated the hyperglycemia in the low-GlcN group ( $9.0 \pm 0.3$  to  $10.2 \pm 0.3 \text{ mmol}/\text{l}$ ;  $P < 0.05$ ) and high-GlcN group ( $9.6 \pm 0.3$  to  $11.6 \pm 0.4 \text{ mmol}/\text{l}$ ;  $P < 0.01$ ) groups (Fig. 1A). As expected, glucose turnover (Fig. 1B) increased 2.2-fold in response to exercise, from  $46 \pm 2$  to  $101 \pm 5 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the control group. On the other hand, the effect of exercise in stimulating GDR was blunted in the glucosamine-infused animals. Thus, in the low-GlcN group, GDR was only  $90 \pm 3 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , which was 12% less ( $P < 0.05$ ) than in the control group, and was only  $73 \pm 3 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the high-GlcN group, which was 28% less ( $P < 0.01$ ) than in the control group. Similarly, as seen in Fig. 1C, the MCR in the control group increased markedly from  $6.1 \pm 0.2$  to  $10.5 \pm 0.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in response to exercise, whereas this effect was blunted in the glucosamine groups. MCR values were  $8.7 \pm 0.5$  ( $P < 0.01$ ) and  $6.2 \pm 0.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P < 0.01$ ) in the low- and high-GlcN groups, respectively. The glucosamine-induced inhibition of



**FIG. 2.** The effect of glucosamine infusion (low-GlcN group,  $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; high-GlcN group,  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and saline infusion (control group) on plasma insulin (**A**) and FFA (**B**) before and during exercise. Pre-exercise values were measured after 6 h of glucosamine infusion, and exercise values were measured 30 min into exercise and after 6.5 h of glucosamine infusion. Data are means  $\pm$  SE. \*Significantly different from control group ( $P < 0.05$ ); #significantly different from corresponding pre-exercise group ( $P < 0.05$ ).

GDR was even more pronounced if one examines only the exercise-induced increment in GDR. With this analysis, the exercise-induced increments in MCR were decreased by 21 and 61% in the low- and high-GlcN groups, respectively. Exercise and the combination of exercise and glucosamine infusion had no effect on plasma insulin or FFA concentrations (Fig. 2).

## DISCUSSION

Hyperglycemia can cause a secondary state of insulin resistance in vitro (1), in animals (3,4), and in humans (2); it has been proposed that one mechanism underlying this phenomenon involves the hexosamine pathway (5–8). According to this hypothesis, hyperglycemia prevents increased glucose uptake by increasing flux through the hexosamine pathway with the production of distal hexosaminoglycan products, which impair insulin signaling. Glucosamine enters this pathway distal to the GFAT enzymatic step, and administration of glucosamine to animals causes in vivo insulin resistance, reportedly by inhibiting early steps in insulin-stimulated glucose transport (10). Acute exercise stimulates GLUT4 translocation and glucose transport by mechanisms that are independent of the insulin-signaling pathway (13). In previous studies, we found that glucosamine infusion markedly inhibited insulin-stimulated GDR in rats (11), and in the current study we found that glucosamine administration has a similar effect on impairing the effects of acute exercise to enhance GDR.

From these results, several conclusions and speculations are possible. First, if glucosamine causes insulin resistance

by interfering with the initial steps of insulin action, then, because exercise stimulates GDR by a separate pathway, one would not expect glucosamine infusions to inhibit the effect of exercise on GDR. Therefore, glucosamine must either have two separate and independent effects, one in the insulin and the other in the exercise pathway, or work at a distal step in glucose transport stimulation common to both. In this latter regard, it has been shown that glucosamine can cause cellular ATP depletion, which inhibits distal steps of GLUT4 trafficking (21). Such an effect of glucosamine is unlikely to be representative of the mechanisms by which hyperglycemia causes insulin resistance.

It is well known that obese type 2 diabetic patients are insulin resistant (22,23), and it has also been shown that the acute effects of exercise to augment GDR are normal in these patients (16). In other words, they are insulin resistant, but not exercise resistant, with respect to stimulation of GDR. Because glucosamine inhibits the effects of insulin and exercise on GDR, insofar as glucosamine mimics activation of the hexosamine pathway, our results suggest that increased flux through the hexosamine pathway is probably not a major mechanism of in vivo hyperglycemia-induced insulin resistance.

Circulating FFAs were not a factor in the impairment of exercise-stimulated glucose turnover. FFAs can inhibit glucose metabolism via the glucose/fatty acid cycle (24), but, as seen in Fig. 2, they did not change with glucosamine infusion or exercise in the current study.

Our results compare well with those of previous studies in which the influence of glucosamine on glucose metabolism was examined. In this study, glucosamine was infused at 0.1 and  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and saline infusion served as the control. Rossetti et al. (6) previously demonstrated that these infusion rates represent the minimal and maximal effective dosages that induce insulin resistance, respectively, and thus they were chosen in this study. Although we did not directly measure insulin sensitivity, the significant decline seen in pre-exercise MCR, for which MCR is a measure of glucose turnover normalized for plasma glucose, was evidence of insulin resistance at both dosages. In our hands, the infusion of glucosamine at 0.1 and  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  resulted in a 17 and 41% reduction in exercise-stimulated MCR, respectively, compared with the rates in saline-infused animals. These impairments of MCR are comparable with those previously observed with insulin-stimulated glucose uptake, when glucose uptake was reduced by 18% in animals infused with  $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  glucosamine and 30% in those infused with  $6.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  glucosamine (6).

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## APPENDIX

This study was undertaken to determine when glucose turnover reaches steady state during exercise and whether a single blood sample taken during this period can be used to measure it. Animals were handled as described above with the following differences. The animals were fasted for 6 h before exercise rather than being infused with saline or glucosamine.

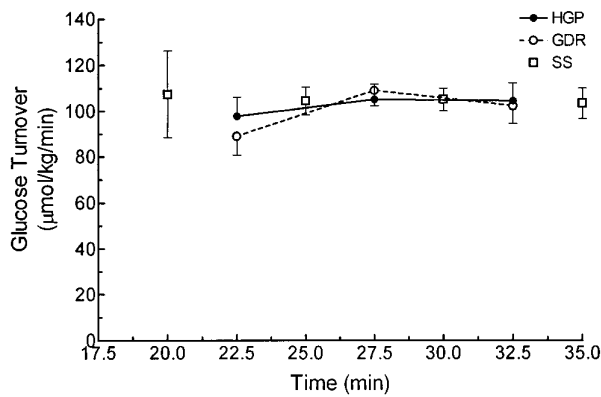


FIG. 3. A comparison of glucose turnover in response to moderate exercise estimated using Steele's equation for non-steady-state conditions (HGP, GDR) and steady state (SS) conditions, which assumes HGP and GDR are equal. A total of six animals were tested.

Blood samples were collected at 20, 25, 30, and 35 min during exercise for determination of plasma glucose and glucose-specific activity. Glucose concentration (G) and specific activity (SA) were smoothed using the optimal segments program (25) for the purpose of estimating  $dSA/dt$  and  $dG/dt$ . HGP and GDR were calculated using the Steele Equation for non-steady-state conditions (19). Glucose turnover was calculated using the Steele equation for steady-state conditions (19), which assumes HGP and GDR are equal.

A total of six animals completed the study. As seen in Fig. 3, HGP and GDR were equal and remained constant toward the end of the exercise period; that is, glucose turnover was in steady state by the end of exercise. Not surprisingly, the calculation of glucose turnover for non-steady-state conditions and assuming steady-state conditions ( $dSA/dt$  and  $dG/dt = 0$ ) produced similar values. Consequently, the estimation of glucose turnover using a single blood sample taken 25–35 min into moderate exercise (17 m/s, 0% slope) is valid.

It should be noted, however, that these validation experiments were successfully completed in only 6 of 17 animals tested. In 66% of the animals, multiple blood sampling during exercise was not possible, or the action of drawing blood caused the animal to repeatedly stop running. Therefore, we settled on a single blood sample collected at 30 min of exercise to estimate glucose turnover.

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