Prevailing hyperglycemia is critical in the regulation of glucose metabolism during exercise in poorly controlled alloxan-diabetic dogs

Michael J. Christopher,1,2 Christian Rantzau,1,2 Glenn McConell,3 Bruce E. Kemp,4 and Frank P. Alford1,2

1Departments of Endocrinology and Diabetes, St. Vincent’s Hospital Melbourne, Fitzroy; Departments of 2Medicine and 3Physics, University of Melbourne, Parkville; and 4St. Vincent’s Institute of Medical Research, Fitzroy, Victoria, Australia

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Christopher, Michael J., Christian Rantzau, Glenn McConell, Bruce E. Kemp, and Frank P. Alford. Prevailing hyperglycemia is critical in the regulation of glucose metabolism during exercise in poorly controlled alloxan-diabetic dogs. J Appl Physiol 98: 930–939, 2005; doi:10.1152/japplphysiol.00687.2004.—The separate impacts of the chronic diabetic state and the prevailing hyperglycemia on plasma substrates and hormones, in vivo glucose turnover, and exercise skeletal muscle (SkM) during exercise were examined in the same six dogs before alloxan-induced diabetes (prealloxan) and after 4–5 wk of poorly controlled hyperglycemic diabetes (HGD) in the absence and presence of ~300-min phlorizin-induced (glycosuria mediated) normoglycemia (NGD). For each treatment state, the ~15-h-fasted dog underwent a primed continuous 150-min infusion of [3-3H]glucose, followed by a 30-min treadmill exercise test (~65% maximal oxygen capacity), with SkM biopsies taken from the thigh (vastus lateralis) before and after exercise. In the HGD and NGD states, preexercise hepatic glucose production rose by 130 and 160%, and the metabolic clearance rate of glucose (MCRg) fell by 70 and 37%, respectively, compared with the corresponding prealloxan state, but the rates of glucose uptake into peripheral tissues (Rdtissue) and plasma free fatty acid, stepwise regression analysis revealed that an increment in hepatic glucose production, Rdtissue, and plasma-derived GF were severely blunted by ~30–50% in the NGD state, but increments in MCRg remained markedly reduced by ~70–75% in both diabetic states. SkM intracellular glucose concentrations were significantly elevated only in the HGD state. Although Rdtissue during exercise in the diabetic states correlated positively with preexercise plasma glucose and insulin and GF and negatively with preexercise plasma free fatty acid, stepwise regression analysis revealed that an individual’s preexercise glucose and GF accounted for 88% of Rdtissue during exercise. In conclusion, the prevailing hyperglycemia in poorly controlled diabetes is critical in maintaining a sufficient supply of plasma glucose for SkM glucose uptake during exercise. During phlorizin-induced NGD, increments in both Rdtissue and GF are impaired due to a diminished fuel supply from plasma glucose and a sustained reduction in increments of MCRg.

glucose uptake; plasma-derived glycolysis; skeletal muscle; free fatty acids; metabolic clearance rate of glucose; phlorizin-induced normoglycemia

THE REGULATION OF GLUCOSE METABOLISM in skeletal muscle (SkM) during exercise occurs predominantly via an insulin-independent pathway (13, 16, 22) and involves the recruitment of SkM GLUT4 transporters from a distinct insulin-independent GLUT-4 pool (13). It is known that the prior insulin sensitivity of an individual is important in determining the rate of glucose uptake into SkM (Rdtissue) in response to acute exercise (28). Furthermore, the intensity and duration of the exercise are also critical in determining the relative importance of carbohydrate and fat substrates as prime sources of oxidative energy and whether the fuel substrates are derived from plasma or from within the exercising muscle itself (36, 49). During acute, moderately intense exercise at ~55–65% of maximal oxygen capacity (V˙O2 max) in humans, the total energy expenditure of normal exercising SkM increases ~12-fold, with the rates of carbohydrate and fat oxidation increasing by ~14- and ~10-fold, respectively, compared with the resting state (36, 49). At this exercise intensity, the major fuel substrates carbohydrate (derived from SkM glycogen and plasma glucose) and fat (derived from plasma free fatty acids (FFA) and other fat sources) contribute equally to the total energy expenditure (36, 49). To meet this rapidly increasing demand for oxidative energy (ATP production), there are marked enhancements in the translocation of SkM GLUT4 transporters to the cell surface membrane (16, 22), the transformation of SkM pyruvate dehydrogenase (PDH) complex into its active form (33), and the activation of SkM carnitine palmitoyltransferase-1 (34, 39).

Poorly controlled Type 1 (26, 54), Type 2 (27, 47), and experimentally induced (3, 23) diabetes are characterized by chronic hyperglycemia, absolute and/or relative hypoinsulinemia, raised hepatic glucose production (HGP), elevated circulating FFA and glucagon levels, and severe insulin resistance, particularly in SkM (48, 54). Moreover, in response to acute mild to moderate exercise (~40–60% V˙O2 max), suboptimally controlled hypoinsulinemic diabetic subjects exhibit metabolic abnormalities, including reduced exercise-stimulated Rdtissue [Rdtissue(ex)] and oxidation rates from both plasma glucose and SkM glycogen (29, 34, 57), and increased exercise-stimulated oxidation rates from both plasma FFA and SkM fat (34, 51). In such diabetic individuals, the shift from carbohydrate to fat oxidation during exercise in diabetic subjects has been attributed to a combination of factors, including downregulation of GLUT4 transporters (12, 14), inadequate plasma insulin concentrations (41, 52, 53), decreased PDH activity (18), inadequate glycogenolysis due to a reduced SkM glycogen storage pool (1, 34), enhanced exercise-induced lipolysis (17, 53), and/or a chronically altered oxidative status in SkM due to the diabetic state itself (10).

Hyperglycemia per se has a clearly defined impact on whole body glucose turnover, the partitioning of intracellular (IC) glucose into the total glycolytic (GF) and glucose storage pathways, and SkM glucose metabolism (6, 47, 48, 55). It is also well established that the actions of hyperglycemia in the
resting, poorly controlled diabetic state involve both compensatory (48, 55) and glucose toxic (38, 39) effects. However, little is known about the metabolic role of hyperglycemia per se on the regulation of exercise-stimulated whole body and SkM glucose metabolism in poorly controlled diabetes. Previous exercise studies in hypoinsulinemic diabetes, either produced experimentally (14, 41, 52, 53) or in Type 1 diabetic subjects (29, 34, 51, 57), have provided conflicting data. Some studies observed normal Rdissue (ex) with moderate exercise in diabetic models employing either fixed low (51), normal (14), or several-fold increased supplemental insulin (57). Other investigators found reduced Rdissue (ex) during moderate exercise in totally insulin-deficient (52, 53), hypoinsulinemic (41), normoinsulinemic (34), or hyperinsulinemic (29) diabetes. Unfortunately, interpretation of the role of hyperglycemia per se on the regulation of exercise-stimulated in vivo whole body and IC glucose metabolism in these diabetic exercise studies is made difficult by the levels of glycemia, which were not well controlled, varying from normoglycemic to markedly hyperglycemic levels and the varying insulentic states.

In fact, only two groups have specifically examined the impact of hyperglycemia per se (at “basal” insulinsina) on the exercise-induced increment (d) in Rdissue (dRdissue) and glucose metabolism. In the first study, nondiabetic dogs infused with somatostatin and constant basal insulin (~14 μU/l) were exercised (~40% VO2 max) at various glycemic steady states (56). They showed that dRdissue, both in the whole body and across the leg, rose in direct proportion to the increasing circulating glucose concentration, thereby resulting in an unchanged exercise-induced increment in the metabolic clearance rate of glucose (dMCRg). These authors concluded that hyperglycemia profoundly enhances exercise-induced glucose uptake and glucose metabolism, which may be critical in diabetes (56). In contrast, Fisher et al. (14) performed exercise studies (also at ~40% VO2 max) in alloxan-induced chronic diabetic dogs at their prevailing fasting hypoinsulinemia (~4 μU/l) and hyperglycemia (~22 mM). These workers found that dRdissue in the untreated alloxan-induced diabetic dogs was normal, but dMCRg was reduced approximately fourfold compared with the nondiabetic dogs. However, when relative normoglycemia (NGD; ~7 mM) was restored in the alloxan-induced hyperglycemic diabetic dogs by an acute infusion of the insulin-independent glycosuric agent, phlorizin, dRdissue remained normal, and dMCRg was normalized (14). Therefore, Fisher et al. (14) concluded that the markedly reduced MCRg in suboptimally controlled hyperglycemic diabetes (HGD) protects resting and working SkM against the potentially deleterious effects of excessive glucose uptake. Thus two opposing views exist as to the role that hyperglycemia plays in the glucose metabolic response of SkM to exercise in insulin-resistant states at basal insulinemia and that hyperglycemia directly enhances glucose uptake and metabolism during exercise (56) or that hyperglycemia is potentially harmful to working SkM but is protected by the coexisting reduced MCRg (14). Unfortunately, neither of these glucose turnover studies (14, 56) examined ex vivo SkM glucose metabolism at rest and during exercise.

We recently reported that the preexercise activity of SkM AMP-activated protein kinase (AMPK), a key regulator and monitor of SkM IC energy balance, was chronically elevated in the low-dose alloxan-induced diabetic dogs after 4- to 5-wk poorly controlled (low-dose insulin treated) HGD. When the same diabetic dogs underwent acute phlorizin infusion to restore NGD, preexercise AMPK α1- and α2-isoform activities remained elevated compared with the prealloxan state (5). However, AMPK isoform activities did not increase further with moderate treadmill exercise (~65% VO2 max) in either diabetic state. We also noted a ~50% reduction in dRdissue in the NGD state (5).

Therefore, the aims of the present study were, first, to examine the separate impacts of the alloxan-induced, poorly controlled chronic diabetic state itself and the levels of glycemia (hyperglycemia vs. phlorizin-induced NGD) on plasma substrates (glucose, FFA, and lactate) and hormones (insulin and glucagon), in vivo HGP, Rdissue, MCRg, and GF, and ex vivo SkM glucose metabolism [total and IC glucose, glucose 6-phosphate (G-6-P), lactate, and glycogen concentrations]. Second, we wished to determine the impacts of these two diabetic states on the exercise-induced whole body and SkM glucose metabolic responses to 30 min of moderate treadmill exercise (~65% VO2 max) and whether hyperglycemia plays a primary role in the supply of fuel during exercise. In particular, we wished to identify which preexercise parameters were important in the regulation of Rdissue (ex) in the diabetic states compared with the prealloxan state and whether there was a reduction in the utilization of carbohydrates as a major fuel source during exercise in the HGD and/or NGD states.

We hypothesized that, in the chronic HGD state, normal basal and exercise-stimulated Rdissue and plasma-derived GF are maintained by compensatory alterations in glucose turnover, in particular HGP and MCRg, and SkM IC glucose metabolism. However, when the mass-action effect of the hyperglycemia in diabetes is removed by acute phlorizin infusion, independently of insulin, both exercise-induced Rdissue and plasma-derived GF are impaired due to an insufficient fuel supply from plasma glucose to meet the increased energy demand and a sustained defect in exercise-induced MCRg.

MATERIALS AND METHODS

**Animals.** Studies were carried out on six male dogs of mixed breed (18–26 kg body wt), with the permission of the Experimental Medical and Surgical Research Ethics Committee at St. Vincent’s Hospital, Melbourne, Australia. Surgical preparation of an arteriovenous shunt for arterialized blood sampling and catheterization of a jugular vein, connected by tubing to a subcutaneously fastened stainless-steel port, for infusion of solutions was performed at least 14 days before the first study, as previously described (6, 7). Exercise, training, and health monitoring of dogs was as previously described (6, 7). In brief, the daily diet given to the prealloxan dogs consisted of ~2,800 calories/day (28% energy from protein, 30% from fat, 42% from carbohydrate). Dogs were acclimatized to the treadmill stepwise exercise program for at least 2 wk before the first study and underwent a standard 3-h intravenous (iv) glucose tolerance test (IVGTT), commencing with an iv bolus of 50% glucose (0.3 g/kg) and 50 μCi highly purified tritiated water (3H2O) (NEN Life Science Products, Boston, MA) given over 30 s, with frequent blood samples taken over the next 3 h for measurement of plasma glucose and total insulin, and 3H2O-specific activity. These data were used to calculate glucose tolerance, the acute incremental insulin response to the glucose bolus from 0 to 10 min and from 0 to 40 min (24), and total body water content (11). After this study, a recovery period of at least 9 days was allowed before the prealloxan exercise study was performed.

Dogs were rendered diabetic (after a 24-h fast) by iv injection of low-dose alloxan monohydrate (35 mg/kg) (Sigma, St. Louis, MO), as previously described (5). Within 2–3 days, the diabetic dogs began...
receiving twice daily subcutaneous injections of low-dose, long-acting (duration: 15–20 h) insulin (human Monotard, Novo Nordisk, Sydney, Australia) with their food for 4–5 wk, aimed at producing chronic hyperglycemia [premeal blood glucose concentrations were 14.6 ± 0.4 mM (mean ± SE) and 18.3 ± 0.3 mM in the morning and afternoon, respectively]. The diabetic dogs were given a weight-maintaining [diabetic: 21.3 ± 1.2 vs. prealloxan: 22.1 ± 1.2 kg; P = not significant (NS)] diet, which consisted of an ~40% increase in dog chow per day to compensate for the urinary loss of calories due to glycosuria (5). The diabetic dogs required 10 ± 1 units of Monotard per day. The HGD dogs underwent a 3-h IVGTT at their prevailing fasting hyperglycemia, with a constant “basal” iv infusion rate (9.3 ± 0.9 μU·kg⁻¹·h⁻¹) of insulin (human Actrapid, Novo Nordisk; in isotonic saline containing 10% Haemaccel for plasma substitution) commenced 35 min before and continued throughout the IVGTT. The infusion rate of insulin given to each HGD dog was chosen to match the plasma insulin level seen in the same fasting prealloxan dog.

**Experiments.** Dogs were fasted for at least 15 h before each study and had free access to water at all times. Before fasting, the diabetic dogs received a 20–30% lower dose of insulin (that is, the dogs received an average of 3–4 U Monotard) with their usual food ration to reduce the risk of insulin-induced nocturnal fasting hypoglycemia. On the morning of each study, an 18-gauge blood sampling catheter was inserted in the dog’s foreleg arteriovenous fistula, and a 19-gauge infusion set was inserted in the subcutaneous venous access port connected to the jugular vein (5–7). After a rest period of at least 30 min, fasting blood samples were taken for measurement of plasma glucose, total insulin, FFA, lactate, glucagon, tritiated glucose ([3-3H]glucose), and H2O.

The prealloxan exercise study involved a 150-min fasting preexercise equilibration period, employing a primed (20 μCi)-continuous infusion (10 μCi/h) of highly purified [3-3H]glucose (NEM Life Science Products), as previously described (5–7), with the 30-min exercise test performed from 150 to 180 min. The paired diabetic exercise studies were randomized. In one exercise study, the HGD dog underwent a 150-min fasting preexercise equilibration period, commencing with a primed (30–50 μCi) iv bolus of [3-3H]glucose [adjusted in proportion to the prevailing fasting hyperglycemia to adequately label the glucose pool (6, 20)] followed by a continuous infusion (10 μCi/h) of [3-3H]glucose, with the same exercise test performed from 150 to 180 min. In the other exercise study, the same fasting diabetic dog underwent an ~330-min constant iv infusion (50 μg·kg⁻¹·min⁻¹) of phlorizin (phlorotin-2,1-ß-D-glucoside; Sigma) to induce a normoglycemic state (NGD). Phlorizin inhibits sodium-dependent glucose cotransport in the renal tubules, leading to inhibition of renal glucose reabsorption and profound glycosuria-induced NGD independent of insulin (14, 39). After phlorizin had been infused for ~150 min, a 150-min primed (25 μCi)-continuous infusion (20 μCi/h) of [3-3H]glucose was commenced, with the same exercise test performed from 300 to 330 min.

The 30-min treadmill exercise test protocol has been described previously (5) and represents an exercise load of ~65% V̇O₂max for dogs (9). During each exercise test, additional saline (3–4 ml/min) was infused to ensure adequate hydration of the dogs (14), and the [3-3H]glucose infusion rate was increased stepwise by 3.0-, 2.0-, and 1.5-fold during exercise in the prealloxan, HGD, and NGD groups, respectively, to minimize the change in specific activity obtained due to exercise-induced increments in SKM Rd(g) (14, 36, 49).

**Collection of blood, urine, and SKM biopsy samples.** For the exercise studies, regular blood samples were collected 10–15 min apart during the preexercise periods and every 5 min during the exercise test, placed into tubes containing appropriate anticoagulants and preservatives, and centrifuged at 4°C within 2 h, and the separated plasma was stored frozen at ~20°C until assayed for plasma glucose, total insulin, FFA, lactate, glucagon, [3-3H]glucose, and 3H2O, as previously described (7). In the diabetic studies, the bladder was emptied (using a urinary catheter coated with 1% lignocaine gel) immediately before administering the [3-3H]glucose bolus, just before the commencement of exercise, and at the completion of exercise. The total volume of urine produced throughout each period was measured, and the urine analyzed for both glucose concentration and specific activity of [3-3H]glucose to precisely quantify urinary glucose and [3-3H]glucose loss (UrGloss) throughout the preexercise and exercise periods (14). Preparation, collection, and storage of SKM biopsy samples from the thigh (vastus lateralis) at the completion of the basal (preexercise) and exercise periods (on separate days) were performed as previously described (5). Contracting SKM biopsy samples were taken under anesthesia within 2–4 min of the completion of exercise (5). SKM biopsy samples were analyzed for total glucose and IC glucose, G-6-P, lactate, and glycogen concentrations (8, 19, 47).

**Laboratory analyses and calculations.** Plasma levels of glucose, total insulin, FFA, lactate, and glucagon, and specific activities of [3-3H]glucose in plasma and urine samples were measured as previously described (6, 7). The rates of total glucose appearance (Ratotal) and total glucose disposal (Rdtotal) during the 150-min preexercise periods were determined from plasma [3-3H]glucose-specific activities at steady state over the last 30 min, as previously defined (6, 20), or if not at steady state by employing Steele’s non-steady-state equations (45), assuming a glucose pool fraction of 0.65 and a glucose volume of distribution of 200 ml/kg (7). During the exercise period, Rdtotal and Rdstatic were calculated, employing Steele’s non-steady-state equations and the same assumptions detailed above (45). Any errors in the calculation of Rdstatic and Rdtotal using these assumptions are minimized by the regularity of sampling (5 min) and the lack of exercise-induced changes in plasma glucose levels and [3-3H]glucose-specific activities (due to the increasing [3-3H]glucose infusion rates). The rate of UrGloss was subtracted from Rdtotal in the diabetic studies to reflect the actual Rdstatic (5–7). The measurement of the rate of in vivo GF during the preexercise period from extracellularly derived glucose due to the generation of plasma H2O from [3-3H]glucose infusion was estimated from the formula described previously (6, 37). The accumulation of plasma [3-3H]H2O was linear [r² (adjusted) = 94.3 ± 0.7%] from 30 to 150 min in all preexercise periods. During the exercise period, a combination of the increased muscle capillary blood flow (31) and non-steady-state conditions does not make it possible to precisely measure GF using this method. However, given that published data have shown that virtually all plasma glucose that enters exercising muscle during moderate exercise is oxidized (36, 49), we can assume that the exercise-induced increment in plasma-derived GF (dGF) is the difference between Rdstatic(ex) and preexercise GF. Preexercise MCRg was calculated by averaging the four values of Rdstatic determined over the last 30 min of the preexercise period and dividing this number by the average plasma glucose value obtained at the corresponding time points. For the exercise period, MCRg was calculated by averaging the two values of Rdstatic determined over the last 5 min of exercise, and dividing this number by the average plasma glucose value obtained at the corresponding time points.

The concentrations of SKM G-6-P and IC glucose were determined in an ~15- to 20-mg wet weight muscle sample using a fluorometric-coupled enzymatic assay, as previously outlined from our laboratory (8, 19), as modified from the method of Schalin-Jantti et al. (40). For the remaining assays, ~20–30 mg wet weight of muscle was freeze dried, reweighed, crushed into a powder, and separated from any connective tissue. Approximately 1.5 mg of this freeze-dried muscle was hydrolyzed in 375 μl of 2 M HCl at 95–100°C (with frequent agitation) for 2 h, neutralized with 1.125 μl of 0.667 M NaOH, and stored at ~70°C until analysis (25). SKM glycogen content was determined from 10 μl of neutralized extract measured in triplicate using a fluorometric enzymatic assay, as previously described by our laboratory (5, 8, 19). Of the remaining free-dried muscle, ~2.0 mg was homogenized in 250 μl precooled 0.5 M perchloric acid/1 mM EDTA, frequently vortexed for 10 min, and centrifuged at 14,000 rpm (2°C) for 2 min. Then, 50 μl of precooled 2.1 M KHC03 was added to exactly 200 μl of the supernatant, vortexed, allowed to stand for 5
RESULTS

**IVGTT studies.** The HGD dogs were studied at their prevailing fasting hyperglycemia (pre-IVGTT: 18.5 ± 2.4 vs. prealloxan: 5.0 ± 0.1 mM; \( P < 0.05 \)) and constant basal insulinemia (pre-IVGTT: 9.4 ± 1.2 vs. prealloxan: 9.6 ± 1.5 mU/l; \( P = \text{NS} \)). Glucose tolerance (not corrected for UrGloss) was severely impaired (0.6 ± 0.1 vs. prealloxan: 4.7 ± 0.4 min\(^{-1}\) × 10\(^{-2} \); \( P < 0.05 \)), there was no acute endogenous insulin secretion (acute incremental insulin response to the glucose bolus from 0 to 10 min: −0.2 ± 0.2 vs. prealloxan: 37.0 ± 7.0 mU/l; \( P < 0.05 \)) or delayed endogenous insulin secretion (acute incremental insulin response to the glucose bolus from 0 to 40 min: 0.0 ± 0.2 vs. prealloxan: 17.8 ± 4.3 mU/l; \( P < 0.05 \)), and there was no change in total body water content (59.0 ± 1.9 vs. prealloxan: 59.3 ± 2.0%; \( P = \text{NS} \)). Therefore, these alloxan-induced HGD dogs represent a model of chronic (30 ± 2 days) hyperglycemic and hypoinsulinemic diabetes under poor control (low-dose insulin therapy) (3, 5).

**Exercise studies: plasma substrate and hormone levels.** As previously reported (5), the HGD dogs exhibited marked fasting (preexercise) hyperglycemia (17.1 ± 1.0 vs. prealloxan: 5.1 ± 0.1 mM; \( P < 0.05 \)) and modest hypoinsulinemia (6.6 ± 2.1 vs. prealloxan: 9.2 ± 1.4 mU/l; \( P = \text{NS} \)). However, in the 300-min phlorizin-infused diabetic dogs (NGD), both preexercise plasma glucose (7.7 ± 0.4 mM) and total insulin (3.4 ± 0.8 mU/l) fell significantly (\( P < 0.05 \) for both vs. corresponding prealloxan and HGD states). Importantly, this fall in total insulin in the NGD state (−3 mU/l) was similar to that observed when the same HGD dogs were fasted for 5 h (data not shown). During exercise, there was no significant alteration in plasma glucose (preexercise: 5.0 ± 0.1 vs. HGD: 16.5 ± 1.1 vs. NGD: 8.1 ± 0.3 mM) or total insulin (vs. prealloxan: 10.9 ± 2.6 vs. HGD: 6.9 ± 2.3 vs. NGD: 4.2 ± 0.8 mU/l) in any treatment group. The mean percent coefficients of variation obtained for plasma glucose, total insulin, and [3-\(^{3}H\)]glucose-specific activities during the last 30 min of the preexercise period, respectively, in the three treatment groups were prealloxan: 2.8 ± 0.7, 14.7 ± 1.7, and 3.5 ± 0.3%; HGD: 1.8 ± 0.4, 13.5 ± 3.3, and 1.7 ± 0.3%; and NGD: 3.6 ± 1.2, 16.2 ± 2.9, and 4.4 ± 1.2%. In addition, the mean specific activities of plasma [3-\(^{3}H\)]glucose obtained during the last 30 min of the preexercise period and at the completion of exercise, respectively, in the three treatment groups were kept constant within each group (prealloxan: 1,469 ± 69 and 1,754 ± 106 dpm/\( \mu \)mol; HGD: 761 ± 82 and 842 ± 91 dpm/\( \mu \)mol; and NGD: 1,184 ± 144 and 1,336 ± 145 dpm/\( \mu \)mol). In 16 of the 18 exercise studies, the plasma [3-\(^{3}H\)]glucose-specific activity at the completion of exercise was within the prescribed 30% of the value obtained immediately before exercise (20).

As previously reported (5), preexercise plasma FFA levels were significantly elevated in the NGD state compared with both the prealloxan and HGD states (Fig. 1A). The absolute rise in FFA induced by exercise was similar in the three treatment states, and therefore the FFA levels obtained with exercise in the NGD state were significantly higher than the corresponding prealloxan and HGD values. Preexercise plasma lactate was similar in the three treatment states, but the increment in lactate induced by exercise in the NGD dogs was significantly higher than that observed in the other two states (Fig. 1B). In both the HGD and NGD states, preexercise glucagon levels were significantly elevated compared with the prealloxan state and were −50% higher (but not significantly) in the NGD state.

**Statistical analysis.** Data are presented as means ± SE. One-way analysis of variance was used for repeated measures, with differences within and/or between groups determined by using Wilcoxon’s matched-pairs signed-rank test. Correlation analyses were performed by using the Spearman rank correlation coefficient (\( r \) values). Stepwise (default), best-subsets, and multiple-regression analyses were performed using the statistical analysis program Minitab (Minitab, State College, PA).

Fig. 1. Plasma concentrations of free fatty acids (FFA; A), lactate (B), and glucagon (C) before (open bars) and after 30 min of treadmill exercise at −65% maximal oxygen consumption (\( \dot{V}O_2 \text{max} \); hatched bars) in the same 6 dogs as in the prealloxan state and after 4–5 wk of suboptimally controlled diabetes in the absence (hyperglycemia) and presence of acute phlorizin-induced normoglycemia. Values are means ± SE. *Significant difference vs. corresponding preexercise value (\( P < 0.05 \)). ‡Significant difference vs. corresponding prealloxan value (\( P < 0.05 \)). †Significant difference vs. corresponding hyperglycemic diabetic value by Wilcoxon matched-pairs signed-rank test (\( P < 0.05 \)).

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compared with the HGD state (Fig. 1C). With exercise, there was a significant rise in glucagon in both diabetic states but not in the prealloxan state. Similar to that seen with plasma lactate, the increment in glucagon induced by exercise (dglucagon) in the NGD dogs was significantly higher than that observed in the other two states.

In vivo HGP, Rdissue, GF, UrGloss, and MCRg in response to diabetes, NGD, and exercise. As shown in Fig. 2A, preexercise HGP was significantly elevated by ~130 and ~160% in the HGD and NGD states, respectively, compared with the corresponding prealloxan state. It is important to note that, in three of the six NGD studies, a variable infusion rate of exogenous 10% glucose prelabeled with 2.0 μCi/g [3-3H]glucose (GINF) was required to maintain NGD (blood glucose ~5.5 mM) during the preexercise period. In addition, a constant infusion rate of GINF, equal to the rate of GINF required to maintain NGD immediately before exercise, was employed during the exercise period. The mean rate of GINF required during the exercise period and during the exercise period for the six NGD studies was 7.9 ± 4.6 and 8.4 ± 4.8 μmol·kg⁻¹·min⁻¹, respectively. Therefore, the mean preexercise and exercise-induced values of HGP obtained for the six NGD studies (calculated by subtracting the GINF rate from the corresponding value of Rd(total)) were 30.9 ± 4.8 and 40.3 ± 6.5 μmol·kg⁻¹·min⁻¹, respectively, resulting in an exercise-induced increment in HGP (dHGP) of 9.4 ± 1.7 μmol·kg⁻¹·min⁻¹. This value was significantly lower than that seen in the other two states (prealloxan: 20.4 ± 2.2 μmol·kg⁻¹·min⁻¹).

1.9 and HGD: 14.9 ± 1.8 μmol·kg⁻¹·min⁻¹; P < 0.05 for both) (Fig. 2A). However, in the three NGD dogs that required GINF, the preexercise HGP and dHGP values averaged 22.1 ± 2.6 and 6.6 ± 1.1 μmol·kg⁻¹·min⁻¹, respectively. In contrast, in the three NGD dogs that did not require GINF, the preexercise HGP and dHGP values averaged 39.6 ± 5.7 and 12.2 ± 2.2 μmol·kg⁻¹·min⁻¹, respectively. Therefore, the presence of GINF in the NGD dogs resulted in a 45% reduction in preexercise HGP and a 50% reduction in dHGP. However, the GINF infusion did not influence either preexercise or exercise-induced peripheral glucose metabolism (namely Rdissue, MCRg, and GF) in the NGD state (data not shown). Preexercise HGP was positively correlated with preexercise glucagon levels for the combined groups (r = 0.83, P = 0.001; n = 18 studies) and for the two diabetic states (r = 0.71, P = 0.02; n = 12 studies) but showed no relationship with preexercise FFA.

As previously reported (5), preexercise Rdissue was remarkably similar in the three treatment groups, regardless of the glycemic status and degree of glycosuria observed (Fig. 2, B and C), but both Rdissue(ex) and dRdissue were significantly blunted by ~25–30% and ~40–50%, respectively, in the NGD state compared with the prealloxan and HGD states (Fig. 2B). Preexercise UrGloss accounted for 58 and 74% of Rd(total) in the HGD and NGD states, respectively (Fig. 2C). With exercise, the absolute rate of UrGloss did not change in either diabetic state. Preexercise MCRg was markedly reduced by 70% in the HGD state compared with the prealloxan state (Fig. 3). Although phlorizin-induced NGD in the diabetic dogs improved preexercise MCRg, it was still significantly lower (by 37%) compared with the prealloxan state but may reflect the raised plasma glucose level obtained in the NGD state compared with the prealloxan state. However, dMCRg was markedly reduced by ~70–75% in both diabetic states compared with the prealloxan state (Fig. 3, inset). Preexercise GF was similar in all three treatment groups (prealloxan: 9.5 ± 1.1 vs. HGD: 10.3 ± 2.1 vs. NGD: 7.7 ± 2.0 μmol·kg⁻¹·min⁻¹), although it was reduced by ~25% in the NGD group compared with the HGD state (P = non significant). However, dGF was significantly lower by ~30–40% in the NGD state compared with the other treatment group states (Fig. 4).
First, we performed nonparametric utilization of fuel sources. The exercise protocol employed. HGD state was significant. Preexercise SkM glycogen content correlations to determine which preexercise metabolic param-

However, the absence and presence of acute phlorizin-induced normoglycemia. Values are means ± SE. *Significant difference vs. corresponding prealloxan value (P < 0.05). †Significant difference vs. corresponding hyperglycemic diabetic value with Wilcoxon matched-pairs signed-rank test (P < 0.05).

Ex vivo SkM glucose processing in response to diabetes, NGD, and exercise. As shown in Table 1, preexercise SkM total glucose and IC glucose concentrations were significantly raised in the HGD state compared with the prealloxan state but were normalized in the NGD state. There was no significant change in either SkM total or IC glucose levels with exercise in any group, but the absolute values obtained during exercise in the HGD and NGD states were no longer significantly different. Preexercise SkM G-6-P concentrations were similar in all groups and rose modestly (but not significantly) with exercise. Preexercise SkM lactate concentrations were similar in all three groups and rose modestly (by ~30–40%) but not significantly with exercise in the prealloxan and NGD groups. However, the ~75% rise in SkM lactate with exercise in the HGD state was significant. Preexercise SkM glycogen content was similar in all groups and did not decrease significantly with the exercise protocol employed.

Determinants of the metabolic responses to exercise and utilization of fuel sources. First, we performed nonparametric correlations to determine which preexercise metabolic parameters were associated with the altered $R_{d\text{issue}}(ex)$ and dGF obtained in the HGD and NGD states compared with the prealloxan state and which fuel sources (carbohydrate vs. fat) were utilized during exercise. We found that preexercise plasma glucose showed significant positive correlations with $R_{d\text{issue}}(ex)$ and dGF for the two diabetic states (n = 12 studies) but not for the combined groups (n = 18 studies) (Table 2). Preexercise plasma total insulin correlated positively with $R_{d\text{issue}}(ex)$ for both the combined and diabetic states and negatively with preexercise plasma FFA for the combined (r = −0.66, P = 0.003) and diabetic (r = −0.64, P = 0.024) groups. Preexercise plasma FFA showed a negative correlation with $R_{d\text{issue}}(ex)$ for both the combined and diabetic groups (Table 2). In addition, FFA with exercise negatively correlated with $R_{d\text{issue}}(ex)$ for the combined (r = −0.67, P = 0.002) and diabetic (r = −0.59, P = 0.045) groups. Preexercise GF showed a positive correlation with $R_{d\text{issue}}(ex)$ for both the combined and diabetic groups and a negative correlation with both preexercise FFA and FFA with exercise for the combined (r = −0.65, P = 0.004; and r = −0.51, P = 0.030) and diabetic (r = −0.68, P = 0.015; and r = −0.65, P = 0.022) groups.

We also found that both preexercise plasma glucose and SkM IC glucose showed significant positive correlations with $R_{d\text{issue}}(ex)$ for the two diabetic states (r = 0.81, P = 0.001; and r = 0.59, P = 0.045, respectively). Both d$R_{d\text{issue}}$ and dGF negatively correlated with $dglucagon$ (r = −0.61, P = 0.009; and r = −0.63, P = 0.006, respectively) for the combined groups only, and dGF showed a positive correlation with the increment in SkM lactate with exercise (dSKM lactate) for the two diabetic states only (r = 0.60, P = 0.039).

Second, we examined in greater detail the preexercise metabolic parameters that could account for the majority of the abnormal $R_{d\text{issue}}(ex)$ observed in the diabetic states. We found that $R_{d\text{issue}}(ex)$ significantly correlated with four preexercise metabolic parameters, namely plasma glucose, total insulin, FFA, and GF, using nonparametric analysis (Table 2). To more closely examine these four basal variables and their relationship with $R_{d\text{issue}}(ex)$, we confirmed that each variable was normally distributed and that each parameter fit the simple linear regression model with $R_{d\text{issue}}(ex)$ (19). These four basal variables were then combined against $R_{d\text{issue}}(ex)$ using multiple linear regression, yielding an $r^2$ (adjusted) value of 86% (r = 0.93). We then performed stepwise (default) regression analysis to select the independent predictors that could account for the majority of $R_{d\text{issue}}(ex)$ in the two diabetic states and

Table 1. Skeletal muscle concentrations of substrates before and after 30 min of treadmill exercise at ~65% $V_{O2\text{max}}$ in the same 6 dogs as in the prealloxan state and after 4–5 wk of suboptimally controlled diabetes in the absence and presence of acute phlorizin-induced normoglycemia

<table>
<thead>
<tr>
<th>SkM Substrate</th>
<th>Prealloxan</th>
<th>Exercise</th>
<th>Hyperglycemia</th>
<th>Prealloxan</th>
<th>Exercise</th>
<th>Phlorizin-induced normoglycemia</th>
<th>Prealloxan</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total glucose</td>
<td>3.07±0.42</td>
<td>3.32±0.71</td>
<td>9.51±1.79*</td>
<td>9.18±1.13*</td>
<td>4.26±0.62†</td>
<td>6.33±1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC glucose</td>
<td>0.54±0.15</td>
<td>0.64±0.25</td>
<td>1.73±0.46*</td>
<td>1.40±0.43*</td>
<td>0.72±0.20†</td>
<td>1.37±0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-6-P</td>
<td>3.59±1.15</td>
<td>4.37±1.22</td>
<td>2.34±0.42</td>
<td>3.79±0.91</td>
<td>2.67±0.79†</td>
<td>3.84±1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>2.90±0.92</td>
<td>3.97±0.73</td>
<td>2.76±0.44</td>
<td>4.73±0.55‡</td>
<td>3.09±0.62</td>
<td>4.00±0.55†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>273±23</td>
<td>264±23</td>
<td>270±31</td>
<td>251±11</td>
<td>288±19</td>
<td>254±18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. $V_{O2\text{max}}$, maximal oxygen consumption; SkM, skeletal muscle; IC, intracellular; G-6-P, glucose 6-phosphate. Concentrations of SkM total glucose, G-6-P, and glycogen are expressed as mmol/kg dry wt muscle. Concentrations of SkM IC glucose and lactate are expressed as mM IC water, assuming an extracellular water content in the biopsies of 0.3 l/kg dry wt and an IC water content in the biopsies of 2.8 l/kg dry wt. *P < 0.05 vs. corresponding prealloxan value. †P < 0.05 vs. corresponding hyperglycemic diabetic value. ‡P < 0.05 vs. corresponding preexercise value.

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found that only two of the predictors, namely preexercise plasma glucose and GF, combined to fit the model, yielding an $r^2$ (adjusted) value of 88% ($r = 0.94$). The regression equation is $R_{d\text{tissue}}(ex) = 11.2 + 0.506(\text{preexercise plasma glucose}) + 0.840(\text{preexercise GF})$.

**DISCUSSION**

In the present study, we examined the separate impacts of low-dose, alloxan-induced, chronic, poorly controlled diabetes itself and the glycemic state (hyperglycemia vs. phlorizin-induced NGD) on basal and exercise-stimulated, in vivo, whole body glucose turnover and IC glucose metabolism and ex vivo SkM glucose metabolism. Our major findings were that the prevailing hyperglycemia in poorly controlled diabetes was critical in ensuring normal preexercise and exercise-induced $R_{d\text{tissue}}$ and GF and involved a compensatory increase in preexercise HGP and SkM total glucose and IC glucose levels but a decrease in preexercise $M_{C\text{rg}}$ and $dM_{C\text{rg}}$. When the hyperglycemia was corrected by acute infusion of the glycosemic agent phlorizin (5), the response of the NGD dog to exercise was compromised and included a marked reduction in $dR_{\text{tissue}}$ and GF, and a sustained decrease in $dM_{C\text{rg}}$, which was accompanied by excessive dglucagon and absolute FFA levels. In addition, despite the apparent normalisation of preexercise SkM IC glucose levels with phlorizin, the mean SkM IC glucose level almost doubled ($P = \text{nonsignificant}$) with exercise in the NGD dogs, and, importantly, this value was similar to the value obtained in the exercising HGD state.

Therefore, the degree of hyperglycemia in the severely insulin-resistant hypoinsulinemic diabetic dogs is precisely balanced by the raised HGP and ongoing UrGloss and plays an important compensatory metabolic role in maintaining normal preexercise and exercise-induced $R_{d\text{tissue}}$ and plasma-derived GF. When the same diabetic dogs underwent acute phlorizin-induced NGD, the importance of the precise balance between preexercise $R_{d\text{total}}$, UrGloss, and $R_{d\text{tissue}}$ was reinforced. Under these conditions, both $R_{d\text{total}}$ and UrGloss increased significantly by a further $\sim 10 \text{ mol per kg}^{-1} \text{min}^{-1}$ but still resulted in normal preexercise $R_{d\text{tissue}}$ and GF. However, with the extra stress of exercise during phlorizin infusion, both $dR_{d\text{tissue}}$ and $dGF$ were severely reduced in the NGD state. Interestingly, we still found a strong positive correlation between preexercise glucagon and HGP levels for both the combined and diabetic states, even when glucose was infused in three of the six NGD studies, which supports the role of glucagon in regulating preexercise plasma glucose and HGP (2).

Importantly, our matched studies in the same dogs revealed that both preexercise $M_{C\text{rg}}$ and $dM_{C\text{rg}}$ were significantly reduced in both diabetic states compared with the prealloxan state, although an improvement in preexercise $M_{C\text{rg}}$ did occur in the NGD state. These data imply that the chronic hyperglycememic and insulin-resistant diabetic state itself may lead to an underlying chronic glucose-toxic state (12, 38, 39). Fisher et al. (14) argued that the impaired $M_{C\text{rg}}$ and $dM_{C\text{rg}}$ in the hyperglycemic diabetic dogs indicated reduced SkM transmembrane glucose transport and GLUT4 availability. However, when the exercise intensity surpasses $\sim 40\% \text{V}^{\text{O}}_2_{\text{max}}$, IC glucose processing itself becomes rate determining for SkM $R_{d\text{tissue}}$ (15, 36, 49) and presumably for dMCRg, a fact acknowledged by Zinker et al. (56). Given that the activation of SkM hexokinase II is critical to $R_{d\text{tissue}}(ex)$ (15), the reduced activation of SkM hexokinase II and PDH that occurs in states of chronic diabetes (18, 50) would limit the supply of pyruvate (i.e., reduced GF) (30) and necessitate an increased availability of FFA (17, 53). This situation would lead to a rise in SkM IC glucose, a scenario that was observed in our HGD dogs both before and during moderate exercise (55–65% $\text{V}^{\text{O}}_2_{\text{max}}$) and in the same phlorizin-induced NGD dogs during the extra stress of exercise. These observations are also consistent with our failure to observe a significant improvement in dMCRg in the NGD state. Our findings of impaired dMCRg and $dR_{d\text{tissue}}$ in our NGD dogs contrast with those of Fisher et al. (14) and may reflect differences in the intensity of exercise (55–65% $\text{V}^{\text{O}}_2_{\text{max}}$), the metabolic status of the animals, and the availability of GLUT4 receptors at the onset of exercise. It also cautions against overemphasizing the change or lack of change in dMCRg as a marker of GLUT4 availability in diabetic states, because the postmembrane defects in SkM glucose metabolism that characterize the diabetic state itself (e.g., impaired activation of hexokinase II and/or PDH) may persist and impact on dMCRg and $dR_{d\text{tissue}}$ (15), despite the restoration of near-NGD with phlorizin. Therefore, although lowering plasma glucose by various methods (e.g., by increasing insulin secretion or decreasing glucose production) is important in reducing glucose toxicity, our data indicate that glucose toxicity cannot account for all of the abnormalities of diabetes. However, regardless of the difficulties in interpreting the pathophysiological significance of MCRg and dMCRg, our findings clearly support an important compensatory role for the hyperglycemia per se in poorly controlled diabetes in maintaining a sufficient supply of plasma glucose to the working muscle. This supports the original conclusion of Wahren et al. (51) in hyperglycemic Type 1 diabetic subjects.

The other important aim of the present study was to determine whether the source of energy for the working SkM is altered by phlorizin-induced NGD compared with the HGD state. During moderate exercise (55–65% $\text{V}^{\text{O}}_2_{\text{max}}$), ATP in working SkM is generated from glucose and fat oxidation, with carbohydrate and fat substrates contributing approximately equally to the total energy expenditure (36, 49). At this exercise intensity, the supply of glucose from SkM glycogen and plasma glucose for oxidation in SkM occurs at a ratio of 3 to 1 (36, 49). We found that preexercise GF (derived from plasma glucose and HGP).
IC glucose levels to be $0.7 \pm 0.2$, $1.0 \pm 0.3$, and $2.3 \pm 0.4$ mM IC water, respectively (Christopher MJ, Rantzau C, and Alford F, unpublished observations). These results confirm the findings of Katz et al. (21) and indicate that our measurements of SkM IC glucose concentrations appear to reflect the IC pathophysiological metabolic conditions present in SkM at rest and under conditions of hyperglycemia.

Finally, when the two diabetic states were combined, we found that $R_d^{\text{tissue(ex)}}$ significantly correlated with four preexercise metabolic parameters, namely plasma glucose, total insulin, total FFA, and GF, using nonparametric analysis. However, we confirmed that the only two independent predictors, namely preexercise GF and plasma glucose, combined to fit the stepwise regression model, yielding an $r^2$ (adjusted) value of 88.1% ($r = 0.94$). In an earlier paper (5), our laboratory reported that preexercise SkM AMPK$\alpha_1$ and AMPK$\alpha_2$ isomeric activities and site-specific phosphorylation of acetyl-CoA carboxylase were markedly increased in both the HGD and NGD states. Whether the elevated levels of these enzymes play a permissive role with the mass action of glucose to normalize the metabolic response to moderate exercise in the HGD state remains speculative (5).

In conclusion, in low-dose, alloxan-induced, 4- to 5-wk poorly controlled DHG dogs, the prevailing hyperglycemia plays a critical metabolic role in ensuring normal preexercise and moderate-exercise-induced rates of $R_d^{\text{tissue}}$ and GF, despite a markedly reduced $dMCR_g$. When the mass-action effect of glucose is removed by acute phlorizin-induced (glycosuria mediated) NGD, the metabolic response to exercise is compromised, resulting in marked reductions in $R_d^{\text{tissue}}$ and dGF, and a sustained decrease in $dMCR_g$, in the presence of partially normalized SkM IC glucose levels and increased circulating FFA. Although $R_d^{\text{tissue(ex)}}$ in the diabetic states significantly correlated with four preexercise metabolic parameters, namely plasma glucose, total insulin, total FFA, and GF, subsequent stepwise regression analysis revealed that only preexercise plasma glucose and GF combined to fit the model, accounting for 88% of $R_d^{\text{tissue(ex)}}$. Whether the impaired dGF in the DNG dogs can be directly attributed to the reduced availability of plasma-derived glucose (combined with the sustained reduction of $dMCR_g$) or the increased availability of FFA remains to be determined. Therefore, the prevailing hyperglycemia in poorly controlled diabetes plays a critical compensatory role in maintaining a sufficient fuel supply from plasma glucose to meet the increased energy demands in working SkM.

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