Regulation of glucose turnover during exercise in pancreatectomized, totally insulin-deficient dogs. Effects of beta-adrenergic blockade.

O Bjorkman, … , L Lickley, M Vranic

Research Article

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Regulation of Glucose Turnover during Exercise in Pancreatectomized, Totally Insulin-deficient Dogs

Effects of β-Adrenergic Blockade

Ola Bjorkman, Philip Miles, David Wasserman, Lavina Lickley, and Mladen Vranic
Departments of Physiology, Medicine and Surgery, and Women's College Hospital, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Abstract

To examine whether glucose metabolic clearance increases and whether catecholamines influence glucose turnover during exercise in total insulin deficiency, 24-h fasted and insulin-deprived pancreatectomized dogs were studied before and during exercise (60 min; 100 m/min; 10% slope) with ($n = 8$) and without ($n = 8$) propranolol infusion (PI, 5 μg/kg-min). Exercise with or without PI was accompanied by four and fivefold increments in norepinephrine and epinephrine respectively, while glucagon (extrapancreatic) fell slightly. Basal plasma glucose and FFA concentrations and rates of tracer-determined (3[^{3}H]glucose) hepatic glucose production (R_a) and total glucose clearance (including urinary glucose loss) were 459±24 mg/dl, 1.7±0.5 mmol/liter, 7.8±0.9 mg/kg-min and 1.6±0.1 ml/kg-min, respectively. When corrected for urinary glucose excretion, basal glucose metabolic clearance rate (MCR) was 0.7±0.1 ml/kg-min and rose twofold ($P < 0.0001$) during exercise. Despite lower lactate (3.3±0.6 vs. 6.6±1.3 mmol/liter; $P < 0.005$) and FFA levels (1.1±0.2 vs. 2.2±0.2 mmol/liter; $P < 0.0001$) with PI, PI failed to influence MCR during exercise. R_a rose by 3.7±1.7 mg/kg-min during exercise ($P < 0.02$) while with PI the increase was only 1.9±0.7 mg/kg-min ($P < 0.002$). Glucose levels remained unchanged during exercise alone but fell slightly with PI ($P < 0.0001$). Therefore, in total insulin deficiency, MCR increases marginally with exercise (13% of normal); the beta adrenergic effects of catecholamines that stimulate both FFA mobilization and muscle glycogenolysis do not regulate muscle glucose uptake. The exercise-induced rise in hepatic glucose production does not require an increase in glucagon levels, but is mediated partially by catecholamines. Present and previous data in normal and alloxan-diabetic dogs, suggest that (*a*) in total insulin deficiency, control of hepatic glucose production during exercise is shifted from glucagon to catecholamines and that this may involve catecholamine-induced mobilization of peripheral substrates for gluconeogenesis and/or hepatic insensitivity to glucagon, and (*b*) insulin is not essential for a small exercise-induced increase in muscle glucose uptake, but normal insulin levels are required for the full response. Furthermore, the catecholamines appear to regulate muscle glucose uptake during exercise only when sufficient insulin is available to prevent markedly elevated FFA levels. We speculate that the main role of insulin is not to regulate glucose uptake by the contracting muscle directly, but to restrain lipolysis and thereby also FFA oxidation in the muscle.

Introduction

Physical exercise involving large muscle groups is accompanied by a rise in total body glucose uptake up to fourfold (1–4), reflecting a severalfold increase in glucose uptake by the working muscle (5). Simultaneously, catecholamine and glucagon levels increase while the insulin concentration falls (2, 4, 6). In partially insulin-deficient, poorly controlled, alloxan-diabetic dogs, exercise is accompanied by an exaggerated rise in catecholamine levels, higher FFA concentration and a lower rate of metabolic clearance of glucose as compared to normal dogs (7). In these animals, beta adrenergic blockade significantly reduced FFA and lactate levels and virtually normalized glucose metabolic clearance rate during exercise. These findings indicate that the exercise-induced rise in catecholamines, directly or indirectly (via increased FFA levels and/or stimulation of muscle glycogenolysis), contribute markedly to the suppressed muscle glucose clearance under conditions of partial insulin deficiency.

Recent in vitro studies in isolated rat muscle have demonstrated that the presence of insulin is not essential for an increased glucose uptake in response to muscle contraction (8, 9). It should be noted that this was observed under experimental conditions with no potentially inhibitory factors present such as FFA or catecholamines. Previous data from in vivo experiments have indicated that there is some increase in glucose clearance in response to exercise in insulin deficient pancreatectomized dogs (10). However, the exercise-induced rise in glucose clearance is smaller than in normal animals or may even be absent during mild work (11). One problem with all previous studies in depancreatized exercising dogs was that glycosuria was not measured and, therefore, changes in total glucose clearance reflected the effects of exercise on both glycosuria and glucose metabolic clearance rate. These effects could counterbalance each other thereby masking the true metabolic effects. Whether this impairment in glucose clearance is a consequence of (*a*) insulin deficiency, per se, (*b*) the exaggerated catecholamine response that accompanies exercise under conditions of insulin deficiency, or (*c*) the elevated FFA levels associated with insulin deficiency and catecholamine excess, remains to be determined. In view of recent reports demonstrating an inhibitory influence of epinephrine

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on insulin-stimulated but not basal glucose uptake by muscle in vitro (12, 13), epinephrine-insulin interactions during insulin deficient states should be considered separately from normal physiology.

The interaction of insulin with glucagon represents the key regulator of hepatic glucose production during exercise in both normal and alloxan diabetic dogs (1, 14) as indicated by a highly significant positive correlation between the glucagon/insulin molar ratio and hepatic glucose production during exercise. On the other hand, circulating catecholamines, in the presence of insulin and glucagon, appear to play only a minor role (7). Suppression of glucagon effectively inhibits basal hepatic glucose production in resting normal dogs (15, 16) and in pancreatectomized dogs acutely deprived of insulin (17). In contrast, in depancreatized dogs deprived of insulin for 3 d (18), glucagon suppression does not decrease plasma glucose concentration or glucose production suggesting that with prolonged insulin lack, the liver may lose its sensitivity to the effects of changes in plasma glucagon. It is thus conceivable that glucagon is relatively unimportant in the regulation of hepatic glucose production during exercise in the total absence of insulin and that catecholamines may play a greater role.

The present study was therefore undertaken to examine whether during exercise, under conditions of total insulin deficiency: (a) the rate of metabolic clearance of glucose increases, (b) catecholamines, by direct or indirect β-adrenergic mechanisms influence muscle glucose uptake, are regulators of hepatic glucose production. Therefore, we studied totally insulin-deficient, pancreatectomized dogs before, during and after exercise, with and without propranolol infusion. The use of the dog as a model for this purpose offers the following unique advantages: (a) The entire pancreas can be removed. (b) The dog has abundant amounts of extrapancreatic glucagon (IRG 3500), with identical immunochemical and biological properties as pancreatic glucagon (19, 20), so that the depancreatized dog is a model of selective insulin deficiency. (c) Dogs injected with procine insulin do not develop insulin antibodies for at least 3 mo (2). The latter point is particularly important since small amounts of insulin will always be present if an animal or man develops antibodies against the injected insulin. (d) Exercise does not represent stress in trained dogs as it often does in other animal models, and (e) continuous monitoring of glycosuria makes it possible, for the first time, to study glucose metabolic clearance during exercise under severely hyperglycemic conditions.

In addition, this investigation and previous studies in alloxan-diabetic and normal dogs provide the opportunity to compare the responses to exercise under different conditions (total insulin deficiency, partial insulin deficiency, and normoinsulinemia, respectively). Such comparisons provide new information regarding the complex, multifactorial control of glucose kinetics during exercise.

Methods

Preparation of experimental animals. Eight mongrel dogs (body wt 20-29 kg) underwent a 2- to 3-wk exercise program comprised of treadmill running at gradually increasing workloads until they managed to run comfortably for at least 60 min at a slope of 10%. After exercise conditioning, and a 16-20-h fast, laparotomy was performed under general anesthesia (halothane, nitrous oxide) and the entire pancreas was carefully removed. Once the pancreatectomy was completed,

two catheters (silastic, 0.75 mm i.d. or tygon, 1.00 mm i.d.) were placed in the superior vena cava via a jugular vein for the purpose of infusion. One catheter (silastic, 1.00 mm i.d. or tygon, 1.25 mm i.d.) was inserted into the aortic arch via a carotid artery for blood sampling.

Feeding was resumed and insulin treatment started 1-2 d after surgery. The amounts of food and insulin were then gradually increased. A combination of intermediate acting (NPH; 15-30 U) and short acting (regular 10-30 U) pork insulin (Eli Lilly Co., Indianapolis, IN) was injected subcutaneously once daily at the time of the morning feeding to keep glucosuria below 1%. The dogs were given a high protein diet consisting of 300-350 g of dog chow (Ralston Purina Canada, Mississauga, Ontario) and 400 g of beef chunks (Romar Pet Supply, Toronto, Ontario) supplemented by tablets containing pancreatic enzymes and raw beef pancreas.

Before the first experiment in the postoperative period as well as between experiments, the dogs were not engaged in strenuous exercise but received only occasional runs at low speeds for 5-15 min to maintain familiarity with treadmill running. Dogs were treated with intramuscular injection of antibiotics (Penlong or Pen Di Strep; Rogar STB, Montreal, Quebec) in the postoperative period.

Experimental protocol. Each dog participated in two to three experiments, the first at least 10 d after surgery. Each study was separated by at least 7 d. The last injection of intermediate and short acting insulin was given 48 and 22 h, respectively, before an experiment. The regular insulin was injected at the time of the morning feeding.

In the morning after a 22-h fast, a primed infusion of 3-[^{3}H]glucose was started and continued throughout the experiment. The first 140 min of tracer infusion served as an equilibrium period. After the equilibration period (after 24 h of fasting), blood samples were collected at intervals from the arterial catheter from 140 to 210 min at rest, from 210 to 270 min during exercise (60 min; 100 m/min, 10% slope) and from 270 to 330 min after exercise.

Two exercise protocols were applied. In one ($n = 8$; six with and two without infusion of glucose tracer), blood samples were collected before, during, and after exercise. In the second protocol ($n = 8$; six with and two without infusion of glucose tracer), an intravenous infusion of propranolol (Inderal; Ayerst Laboratories, Montreal, Canada) at a rate of 5 μg/kg per min was started 40 min before exercise (at 170 min) and continued throughout the exercise and postexercise periods. This design allowed us to examine the effects of propranolol infusion on glucose turnover at rest as well as the effects of beta adrenergic blockade on the response to exercise. A third protocol was carried out to examine the influence of a prolonged propranolol infusion in the resting state ($n = 5$; three with and two without infusion of glucose tracer). The animals were studied in the resting state before (140-170 min) and during propranolol infusion (170-270 min).

Tracer methods. In all experiments, 3[^{3}H]glucose was infused at a constant rate (0.47 μCi/min) throughout the study. A priming dose equivalent to the amount infused during the equilibrium period was given at the beginning of the tracer infusion. Smoothing of the plasma glucose concentration and specific activities were performed according to the optimal segments technique (21). The rate of appearance $(R_a)^1$ and disappearance (R_d) for glucose in the resting period was calculated separately from that of the exercise and postexercise periods by using the formula for nonsteady state conditions developed by Steele (22) and subsequently validated by Radziuk et al. (23). Urinary glucose loss was determined in 10 experiments by inserting a catheter into the urinary bladder. The catheter was secured and connected to a tube to allow gravity-assisted free urine flow into a plastic collection bag. Collection of urine was made at 30-40-min intervals for the measurements of volume and glucose concentration. In the resting state, a significant relationship between mean glucose concentration and urinary glucose excretion was observed (Fig. 1). The threshold for urinary
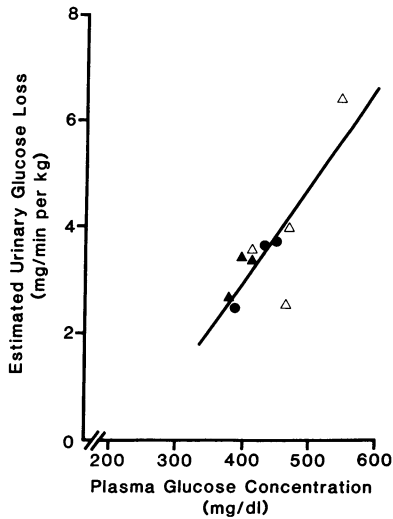

Figure 1. Relationship between mean basal plasma glucose concentration and urinary glucose loss in 10 experiments (exercise control: △, exercise propranolol: ▲ and rest propranolol: ●). Linear regression: $y = 0.018x - 4.4$, $r = 0.81$, $P < 0.01$.

glucose loss (as estimated from the intercept at zero urinary glucose loss) was at a plasma glucose level of ~ 240 mg/dl. To estimate urinary glucose loss, in the resting and basal state, the regression line depicted in Fig. 1 was applied in all 21 experiments. In seven studies in four dogs, it was possible to obtain estimations for urinary glucose losses during exercise and these demonstrated there was a 39±3% drop in urinary glucose excretion that was applied to the measurements in all exercise experiments. Glucose metabolic clearance rate (MCR) was calculated by dividing R_d (corrected for urinary glucose excretion) by the prevailing plasma glucose concentration. MCR represents an estimate of glucose utilization, partially corrected for the mass action effects of glucose. The value and meaning of this measurement has recently been reviewed (24).

Laboratory methods. Blood samples for measurements of labeled and unlabeled glucose, immunoreactive insulin (IRI) and cortisol were collected in dried heparinized tubes with sodium fluoride as preservative. Plasma for tracer glucose determination was deproteinized by equal volumes of 5% zinc sulfate and 0.3 N barium hydroxide. Deproteinized samples were evaporated to remove any tritiated water and then redissolved in distilled water. Samples were counted in Aquasol II (New England Nuclear, Lachine, Quebec) by liquid scintillation spectrometry. For each experiment, an aliquot of the infused tracer solution was treated and analyzed in the same way as plasma samples and used as the standard. Plasma glucose concentrations were determined by the glucose oxidase method (glucose autoanalyzer; Beckman Instruments, Fullerton, CA). Plasma IRI and cortisol were assayed as previously described (1). Lactate, glycerol, alanine, and beta hydroxybutyrate were analyzed by fluorometric methods (25).

Blood samples for determination of immunoreactive glucagon (IRG) and plasma FFA were collected in 0.1 ml aprotinin (Trasylol; FBA Pharmaceuticals, New York) and 0.1 ml EDTA (24 mg/ml). Plasma IRG and FFA levels were measured as previously described (1). For the determination of norepinephrine and epinephrine, blood samples were collected in polyethylene tubes containing 2.5 mg glutathione. To each sample, 10 μl of EGTA was added as anticoagulant. Within an hour of blood sampling, the blood samples were centrifuged and the plasma removed and deproteinized with 2 N HClO₄ and stored at −70 C. Analysis was performed by a radioenzymatic assay (1, 26).

Statistical analysis. Values were presented as mean±SEM. Statistical analysis was performed using a two-way analysis of variance (Statistical Analysis System; SAS Institute Inc., Carey, NC). Responses to the two to four treatments given within a protocol were assessed with a factorial design using the Proc GLM (General Linear Models) procedure, which allows for multiple comparisons using the contrast option. Comparisons among the three different protocols were also assessed using a similar factorial model taking into account the different sources

of variation that apply in interprotocol as opposed to intraprotocol comparisons.

Results

Arterial glucose concentration and glucose turnover in pancreatectomized dogs

Resting state. Basal plasma glucose concentration in 21 experiments (exercise control; $n = 8$, exercise during propranolol infusion (PI); $n = 8$, PI at rest; $n = 5$) ranged from 322 to 595 mg/dl. R_a in 15 experiments (exercise control; $n = 6$, exercise during PI; $n = 6$, and PI at rest; $n = 3$) ranged from 4.8 to 10.1 mg/kg-min, respectively. There was a significant correlation
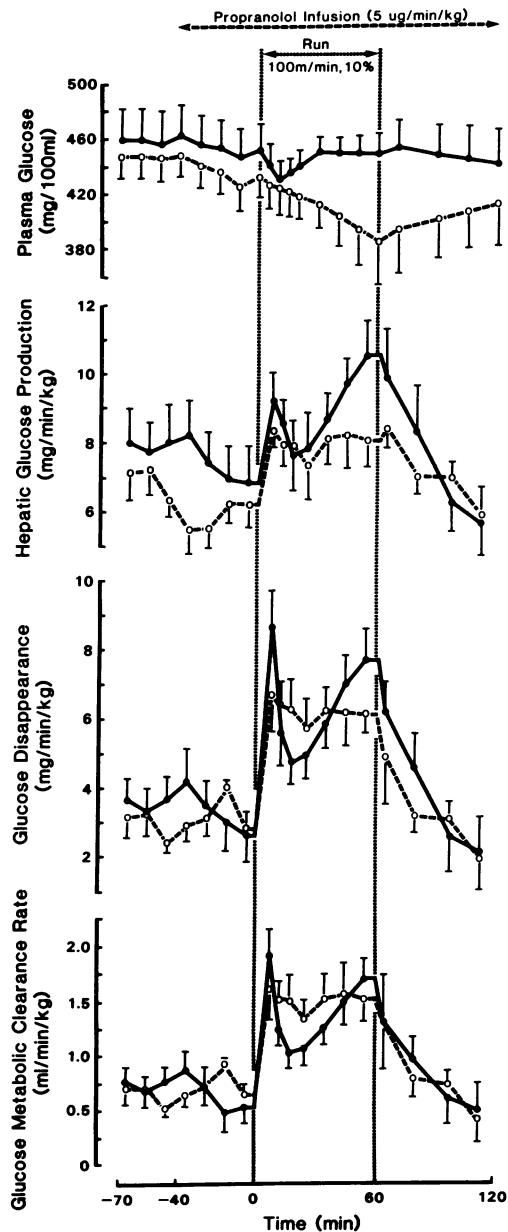

Figure 2. Changes in plasma glucose concentration, glucose production, and glucose metabolic clearance rate before, during, and after exercise with (- - -) and without (———) concomitant propranolol infusion.

Table I. Plasma Glucose Concentrations and Rates of Appearance (R_a), Disappearance (R_d) and Metabolic Clearance (MCR) of Glucose during Prolonged Infusion of Propranolol at Rest in Insulin-deprived Depancreatized Dogs

Time (min)	−30	−20	−10	0	10	20	30	40	45	50	55	60	70	80	90	100	110	130	145	160
Plasma glucose concentration																				
Mean (mg/dl)	400	400	397	403	396	393	393	383	381	373	373	373	373	372	371	369	369	369	369	369
±SEM (n = 5)	33	33	33	33	31	29	30	26	26	25	24	22	20	21	21	21	20	18	17	16
R_a																				
Mean (mg/kg·min)	6.9	6.8	6.8	6.6	6.5	6.3	5.7	5.3		5.9	6.4	6.6	6.3	5.9	5.8	6.3	6.6	5.8	5.1	5.1
±SEM (n = 3)	1.3	1.3	1.3	1.2	1.3	1.2	0.9	0.6		0.9	0.8	0.7	0.9	1.2	1.0	0.3	0.2	0.7	1.1	1.0
R_d*																				
Mean (mg/kg·min)	3.1	3.2	3.2	2.8	4.1	4.0	3.4	4.3		4.2	3.7	3.9	3.7	3.3	3.2	3.7	4.6	3.5	2.8	2.8
±SEM (n = 3)	1.0	1.0	1.0	0.8	0.9	0.8	0.4	1.3		1.0	0.5	0.3	0.7	1.3	1.0	0.4	0.3	0.8	1.1	1.0
MCR*																				
Mean (ml/kg·min)	0.8	0.7	0.7	0.7	0.9	0.9	0.8	1.1		1.0	0.9	1.0	1.0	0.8	0.8	0.9	1.2	0.9	0.7	0.7
±SEM (n = 3)	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.3		0.2	0.1	0.1	0.1	0.3	0.2	0.1	0.1	0.2	0.3	0.2

* Corrected for measured urinary glucose loss.

between the glucose concentrations and R_a in the basal state (linear regression; $y = 0.028x - 5.7$; $P < 0.001$).

In control studies, as shown in Fig. 2 ($n = 6$), plasma glucose concentrations were 451±19 mg/dl at the onset of exercise. R_a was 6.8±1.1 mg/kg·min and R_d and MCR (both corrected for measured urinary glucose loss) were 2.6±0.7 mg/kg·min and 0.5±0.1 ml/kg·min, respectively. PI for 40 min at rest did not significantly affect plasma glucose concentrations, R_a, R_d or MCR.

Response to exercise. As seen in Fig. 2, during exercise alone, R_a rose from 6.8±1.1 to 10.5±1.0 mg/kg·min ($P < 0.01$). Both R_d and MCR (corrected for measured urinary glucose loss) also rose ($P < 0.0001$), thus plasma glucose levels remained stable. Propranolol infusion resulted in a diminished exercise-induced increase in R_a (from 6.2±0.6 to 8.0±0.7 mg/kg·min; $P < 0.0001$). R_a ($P < 0.05$) and plasma glucose levels ($P < 0.0003$) were lower when PI was given during exercise and plasma glucose concentrations fell from 432±15 to 384±30 mg/dl ($P < 0.001$). Corrected R_d rose from 2.8±0.4 to 6.1±0.5 mg/kg·min ($P < 0.0001$) and MCR from 0.7±0.1 to 1.5±0.2 mg/kg·min ($P < 0.0001$). These increments in R_d and MCR were not affected by propranolol.

Postexercise. In the postexercise period, R_a, R_d and MCR returned gradually to values at or below the basal level with both protocols while the glucose concentration did not change significantly after exercise (Fig. 2).

Prolonged propranolol infusion without exercise. Propranolol infusion was accompanied by a slight but significant drop in plasma glucose ($P < 0.0001$) and R_a ($P < 0.01$) during prolonged PI in the resting state (Table I). R_d did not change significantly but MCR increased slightly ($P < 0.04$).

Substrates and hormones in pancreatectomized dogs

Resting state and response to exercise. Epinephrine and norepinephrine concentrations both rose four to fivefold in response to exercise with and without propranolol infusion ($P < 0.0001$; Fig. 3). Dopamine values were 69±16 pg/ml at rest, rose gradually to 135±27 pg/ml during exercise and returned to basal after exercise. Plasma glucagon concentration fell during exercise both with and without ($P < 0.0001$) PI (Fig. 3) and returned toward basal after exercise. Plasma cortisol levels also rose significantly both during exercise alone ($P < 0.04$) and during exercise with PI ($P < 0.004$) and then returned to the basal level by 60 min after exercise (Fig. 3). Propranolol did not affect these hormonal responses. In control studies, FFA (Fig. 4) rose during exercise alone ($P < 0.006$). When PI was given during rest FFA levels fell slightly and there was a further significant drop during exercise + PI ($P < 0.0005$). Thus, when propranolol was infused during exercise, the FFA concentrations were 40–50% lower than in the controls ($P < 0.0001$).

The arterial glycerol concentration rose threefold in response to exercise alone ($P < 0.0001$, Fig. 4). Propranolol did not influence the glycerol concentration at rest but the exercise-induced rise was blunted by 30–40% ($P < 0.0001$). After exercise, glycerol levels were no longer different from those in the control group.

Exercise did not affect beta hydroxybutyrate concentrations. However, there was a small but significant decrease ($P < 0.0001$) when PI was given before or during exercise. The plasma alanine concentration rose by twofold both with and without PI ($P < 0.004$, Fig. 4). Exercise alone resulted in a gradual sixfold rise in lactate levels ($P < 0.0003$, Fig. 4). Pro-
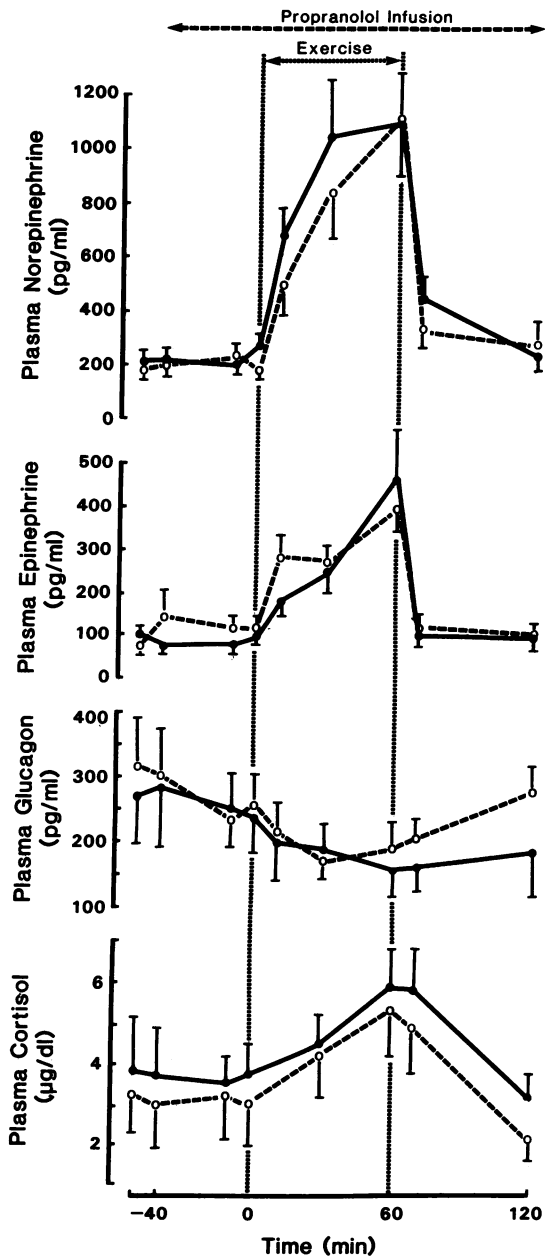
Figure 3. Plasma concentrations of norepinephrine, epinephrine, glucagon, and cortisol before, during and after exercise with (- - -) and without (———) concomitant propranolol infusion (n = 8 in both groups).
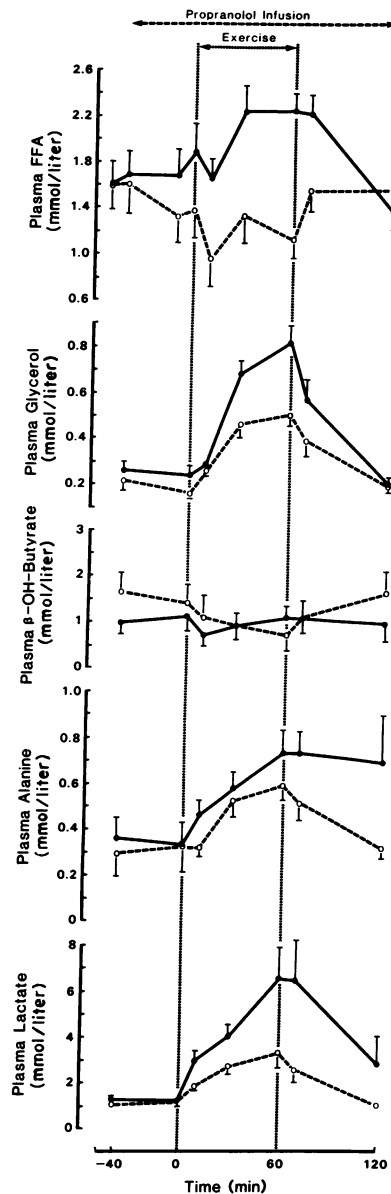

Figure 4. Plasma concentrations of FFA, glycerol, B-OH butyrate, alanine, and lactate before, during and after exercise with (- - -) and without (———) concomitant propranolol infusion (n = 8 in both groups).

pranolol attenuated this exercise-induced rise by 50% both during and after exercise ($P < 0.005$).

Prolonged propranolol infusion without exercise. In resting animals, propranolol caused a slight but significant fall in plasma glucagon ($P < 0.03$), but the other hormones measured did not change significantly (Table II). FFA ($P < 0.001$), lactate ($P < 0.001$), alanine ($P < 0.001$) and glycerol ($P < 0.01$) levels all fell slightly during the prolonged PI (Table III).

Discussion

Glucose metabolic clearance. Glucose uptake (R_d) is determined not only by active mechanisms for glucose transport but is also dependent on the mass action effect of glucose concentration. Based on the assumption that glucose uptake is proportional to glucose concentration, a measure of glucose uptake that is relatively independent of the glucose concentration can be obtained either directly from tracer concentration in plasma (24) or indirectly by dividing R_d by the glucose concentration (MCR). When changes in MCR were compared under conditions of widely varying glucose concentration it has been shown that glucose clearance is not fully independent of the glucose concentration but falls progressively as the glucose level increases (27, 28). This fall in glucose clearance has been attributed to the failure of glucose uptake to increase in insulin independent tissues only. Therefore, we feel that our measurements of exercise-induced increments in MCR reflecting augmented rates of glucose uptake by muscle should be relatively independent of glycemia allowing us to compare MCR in conditions with different glucose levels.

In this study, we have combined measurements of tracer-determined total glucose turnover with those of urinary glu-

Table II. Plasma Concentrations of Norepinephrine, Epinephrine, Glucagon, and Cortisol during Prolonged Infusion of Propanolol at Rest in Insulin-deprived, Depancreatized Dogs (n = 5)

Time (min)	−10	0	30	40	50	70	100	110	160
Norepinephrine									
Mean (pg/ml)	181	225	162	164	113	205	219	—	240
±SEM	24	52	21	23	50	70	56	—	74
Epinephrine									
Mean (pg/ml)	100	72	94	69	61	96	118	—	76
±SEM	29	16	19	17	36	23	55	—	12
Glucagon									
Mean (pg/ml)	320	338	170	219	251	255	306	304	326
±SEM	98	93	42	64	85	90	115	161	126
Cortisol									
Mean (μg/dl)	2.8	2.7	3.5	3.1	—	3.9	3.0	3.4	2.6
±SEM	0.8	0.7	1.5	1.6	—	1.6	0.5	0.7	0.5

cose loss at rest and during exercise in order to assess that portion of glucose clearance that is related to glucose uptake into tissues, i.e., the metabolic clearance of glucose. Under conditions of total insulin deficiency, exercise resulted in a small but significant rise in MCR. These findings are consistent with previous results demonstrating an increase in total glucose clearance in pancreatectomized running dogs (10). The current results and those obtained previously in alloxan diabetic (7) and normal dogs (1) using the same exercise model provide the opportunity to compare MCR during exercise under widely varying conditions of insulinization. Such comparisons reveal that the exercise induced rise in MCR in pancreatectomized dogs (1.1 ml/min per kg) is markedly inhibited by total insulin deficiency since MCR amounted to only 13% of that in normal dogs (8.7 ml/min per kg) (1) and 48% of that in partially insulin deficient alloxan diabetic dogs (2.3 ml/min

per kg) (7). These data indicate that the presence of normoinsulinemia is necessary for a normal exercise-induced rise in glucose uptake by muscle.

Studies in vitro have indicated that experimental diabetes (streptozotocin) in rats is accompanied by a reduced maximal capacity for glucose transport in muscle (29) possibly in association with a reduced number of glucose transporters (30). It is, therefore, conceivable that the blunted exercise-induced rise in glucose uptake in our diabetic dogs may, at least in part, be due to a reduced capacity for glucose transport in association with prolonged insulin deficiency. The other possibility is that the presence of insulin, by direct or indirect mechanisms, is necessary for the moment to moment control of muscle glucose uptake during exercise. Support for the latter notion is found in our previous work on pancreatectomized dogs (2). These studies demonstrated that insulin-infused (via the portal vein)

Table III. Plasma Concentrations of FFA, Plasma Glycerol, Plasma B-OH-Butyrate, Plasma Alanine, and Plasma Lactate during Prolonged Infusion of Propanolol at Rest in Insulin-deprived, Depancreatized Dogs (n = 5)

Time (min)	−10	0	30	40	50	70	100	110	160
FFA									
Mean (mmol/liter)	1,696	1,492	1,093	1,183	1,175	1,225	1,337	1,357	1,425
±SEM	607	213	198	167	201	187	202	284	218
Glycerol									
Mean (mmol/liter)	—	222	—	182	—	165	171	170	209
±SEM	—	13	—	34	—	23	25	27	36
Plasma B-OH-Butyrate									
Mean (mmol/liter)	—	1,424	—	1,322	—	1,268	1,272	1,327	1,423
±SEM	—	761	—	780	—	779	759	776	780
Plasma alanine									
Mean (mmol/liter)	—	455	—	421	—	366	336	329	277
±SEM	—	198	—	182	—	148	142	136	109
Plasma lactate									
Mean (mmol/liter)	—	1,014	—	912	—	908	870	831	851
±SEM	—	73	—	56	—	67	62	80	102

dogs displayed a normal exercise-induced rise in glucose uptake and maintained glucose levels. Under this condition of normal capacity for glucose uptake, acute insulin deficiency before exercise was accompanied by increasing glucose levels and only a marginal rise in glucose clearance. Regardless of whether the role of insulin is to maintain the capacity for glucose transport or whether insulin is involved in the exercise-induced stimulation of glucose uptake by muscle these findings indicate that a normal response to exercise requires the presence of normal amounts of insulin.

It is well established that catecholamines exert an inhibitory influence on muscle glucose uptake via beta adrenergic mechanisms (13, 31). This effect may involve both inhibition of glucose transport (32) as well as indirect mechanisms related to an increase in lipid-oxidation (33–35) and muscle glycogenolysis (12, 13). In the present study, propranolol infusion failed to influence glucose metabolic clearance rate despite pronounced suppression of FFA (Fig. 4) and lactate (Fig. 4) levels, the latter probably a reflection of suppressed muscle glycogenolysis (1). This is in sharp contrast to the augmented glucose uptake observed during exercise when FFA and lactate levels were suppressed by propranolol in partially insulin-deficient, alloxan diabetic dogs. Fig. 5 shows the relationship between FFA levels and glucose metabolic clearance rate during exercise under five different conditions (described in the legend). This plot demonstrates an inverse relationship between FFA levels and glucose metabolic clearance rate but this is only observed when FFA concentrations are below ~ 1.5 mmol/liter. This observation may explain, at least in part, the failure of propranolol infusion to stimulate glucose uptake during exercise in our pancreatectomized running dogs. Thus, despite a 50% suppression of FFA levels by propranolol infusion, the resultant FFA levels during exercise exceeded the apparent threshold for this relationship. The current results therefore suggest that even in the absence of a catecholamine-induced increase in FFA levels during exercise, the already elevated FFA levels resulting from total insulin deficiency may be sufficient to exert a maximal inhibitory effect on glucose clearance. It is also possible that FFA suppression requires a longer period of time to exert its effects on the metabolic clear-

ance of glucose. However, in the present study, there was a fairly well established steady state with respect to FFA levels and glucose metabolic clearance between 30 and 60 min of exercise with propanolol. Thus it is unlikely that a more prolonged exposure to the lower FFA levels would have induced a late rise in glucose clearance. Also in the other studies summarized in Fig. 5, the inverse relationship between FFA levels and rates of glucose metabolic clearance was always apparent by between 30 and 60 min of exercise. It appears therefore, that catecholamines interfere with muscle glucose uptake during exercise in the diabetic state only if sufficient insulin is available to prevent markedly elevated FFA levels. Thus, the main role of insulin during exercise may be related to its strong antilipolytic effect on the adipocytes, which acts to restrain fat oxidation in muscle, rather than a direct effect on glucose transport into muscle, although it is entirely possible that insulin can exert both effects.

The other potential indirect mechanism for a catecholamine-induced inhibition of glucose uptake is via an increased muscle glycogenolysis as may be reflected by a rise in lactate levels. Propranolol infusion markedly suppressed the exercise-induced rise in lactate levels thus suggesting decreased muscle glycogenolysis. However, despite this response, there was no augmentation of the exercise-induced rise in glucose metabolic clearance. Based on the present findings, we conclude that under conditions of total insulin deficiency, the diminished increase in glucose metabolic clearance rate during exercise is not due to a beta adrenergic, catecholamine-induced stimulation of muscle glycogenolysis.

Studies in vitro (in the absence of FFA), have indicated that the inhibitory effects of the catecholamines on muscle glucose uptake are mediated by an accelerated rate of glycogenolysis resulting in elevated glucose-6-phosphate levels which in turn suppress glucose phosphorylation by hexokinase (12, 13). Interestingly, it appears that the catecholamines exert their inhibitory effect exclusively on insulin-mediated increases in glucose utilization; no effect is seen at low or basal insulin concentrations associated with low rates of glucose phosphorylation (12, 13). These findings are consistent with the present results indicating that catecholamines fail to inter-
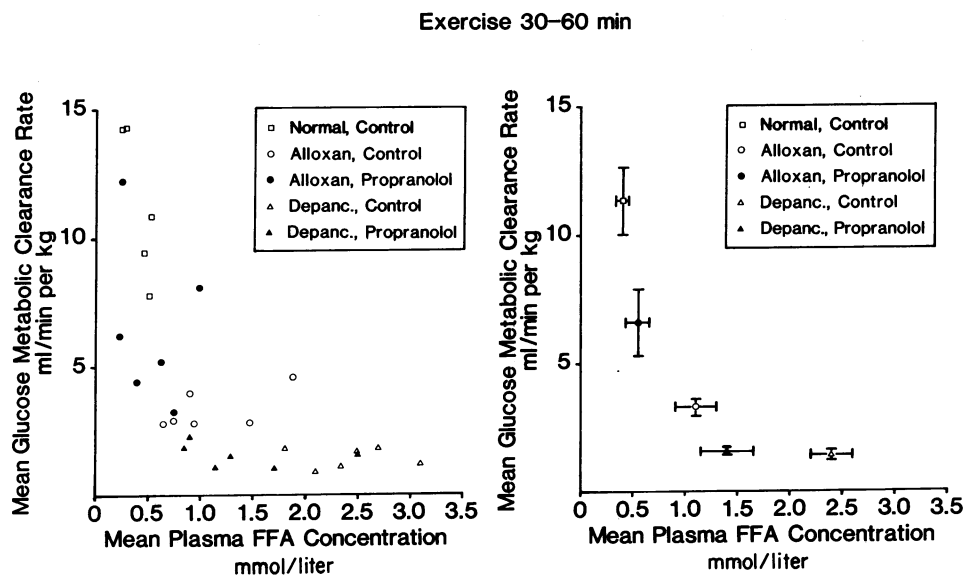
Exercise 30–60 min

Figure 5. Relationship between mean FFA and mean glucose metabolic clearance rate during 30–60 min of running exercise in dogs under five different conditions (1, exercise control, normal dogs; 2, exercise control; and 3, during propranolol infusion in partially insulin-deficient, alloxan diabetic dogs; and 4, exercise control; and 5, during propranolol infusion in totally insulin-deficient, pancreatectomized dogs). Data on alloxan diabetic and normal dogs have previously been published (1, 7). The left panel shows individual values and the right panel depicts mean values±SEM.

fere with muscle glucose uptake during exercise under conditions of total insulin deficiency and a relatively low rate of glucose utilization.

Hepatic glucose production. In normal (1) and alloxan diabetic dogs (7), glucagon levels increase in response to exercise. Suppression of glucagon during exercise is accompanied by an inhibition of heaptic glucose production (1, 14) indicating that glucagon is an important regulator of hepatic glucose production during exercise in dogs.

In pancreatectomized dogs, the source for pancreatic glucagon secretion is removed but in contrast to other species, dogs produce substantial amounts of glucagon (IRG 3500) of extrapancreatic origin (19, 20). This glucagon has been shown to possess normal biological activity with respect to stimulation of hepatic glucose production in vivo (17) as well as in vitro (20). In this study, basal glucagon values were in the same range as those observed in normal (1) animals. This was not surprising since very high levels of IRG in plasma have been noted only during a more prolonged period of insulin withdrawal (19). However, in sharp contrast to the rise in glucagon levels observed in response to exercise in normal and alloxan diabetic dogs (1, 7), in the pancreatectomized dogs, glucagon levels did not rise but actually fell during exercise indicating that extrapancreatic glucagon secretion fails to respond normally to exercise. These findings are consistent with the attenuated epinephrine-induced rise in glucagon levels previously observed in pancreatectomized dogs (36). The mechanism(s) behind the differences in pancreatic and extrapancreatic glucagon secretion (or, more unlikely, degradation) in response to exercise remains to be defined.

The exercise-induced absolute rise in hepatic glucose production was similar to that observed in normal dogs (1), although the 50% increase in R_a was small compared to the 400% increase seen in normal dogs. This was observed despite the absence of increase in glucagon levels indicating that under conditions of total insulin deficiency, increased glucagon levels are not essential for a normal rise in glucose production in response to exercise. This corresponds to previously observed loss of liver sensitivity to changes in plasma IRG levels in resting depancreatized dogs (18).

In the present study, propranolol infusion inhibited both basal and the exercise-induced rise in hepatic glucose production and glucose concentrations fell (Fig. 4). These results suggest that circulating catecholamines stimulate both basal and the exercise-induced rise in glucose production under these conditions. The mechanism(s) for this effect may involve a direct stimulation of hepatic glycogenolysis and gluconeogenesis or an increased supply of gluconeogenic precursors. The documented catecholamine-mediated increase in lactate and alanine presumably derived from muscle glycogen, as well as stimulation of glycerol release from increased lipolysis could augment the hepatic availability of gluconeogenic precursors for gluconeogenesis. The effects of decreasing gluconeogenic substrate load, as occurs with beta blockade, may be particularly profound in depancreatized dogs as insulin deficiency has been shown to result in a greater reliance on gluconeogenesis (37).

Previous studies have shown that poorly controlled alloxan-diabetic dogs exhibited an exaggerated catecholamine response to exercise (7). In the current study, the exercise induced increase in catecholamine levels were similar to those in the alloxan-diabetic dogs. Since a further deterioration of

metabolic control brought about by total insulin deficiency failed to further exaggerate the response of catecholamines to exercise, it appears that the metabolic consequences of partial insulin deficiency in exercise are sufficient to allow a maximal response.

In conclusion, this study and our previous work in normal and alloxan diabetic dogs, suggest that the presence of insulin is not essential for a small exercise-induced increase in muscle glucose uptake, but that normal amounts of insulin are required for the full response. Catecholamines appear to interfere with muscle glucose uptake during exercise only when sufficient insulin is available to prevent markedly elevated FFA levels. We speculate that the main role of insulin in exercise is not to control glucose transport in muscle directly, but to restrain lipolysis. Thus, even when catecholamines increased during exercise, the availability of FFA in muscle is controlled by insulin's antilipolytic effect so that glucose transport and oxidation can remain adequate. Under conditions of total insulin deficiency, it seems that control of glucose production in exercise is shifted from glucagon to the catecholamines. This may involve catecholamine-induced mobilization of peripheral substrates for gluconeogenesis and/or hepatic resistance to glucagon.

Acknowledgments

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