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Research Article

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The Essentiality of Insulin and the Role of Glucagon in Regulating Glucose Utilization and Production during Strenuous Exercise in Dogs

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ABSTRACT In order to elucidate the role of insulin and glucagon during strenuous exercise (100 m/min, slope 10-12°), we have determined the rates of production (R_a), utilization (R_d), and metabolic clearance (M) of glucose in normal dogs before pancreatectomy and 2 wk after total pancreatectomy (*a*) when they were being maintained on constant intraportal basal insulin infusion, (245 μU/kg-min) and (*b*) when insulin supply had been withheld before and during exercise. Such an intense exercise induced in normal dogs a prompt decrease in mean immunoreactive serum insulin (IRI) from 20±3 to 11±2 μU/ml. In depancreatized insulin-infused dogs serum IRI during rest and exercise was between 14±1 and 12±2 μU/ml. In the third group, after cessation of insulin infusion, IRI decreased by 76% (from 17±5 to 4±1) and did not decrease further during exercise. During exercise, serum immunoreactive glucagon (IRG) increased threefold in normal dogs. In depancreatized dogs serum IRG was the same as in normal resting dogs (indicating a nonpancreatic source of the hormone) but it did not increase during exercise. In normal dogs exercise induced proportional increases in R_a, R_d, and M (threefold) and normoglycemia was maintained. Changes in glucose turnover in depancreatized insulin-infused dogs were similar to those seen in normal dogs suggesting that a decrease in insulin secretion and a rise in IRG are not essential to prevent hypoglycemia in diabetic dogs. With the cessation of insulin infusion in resting depancreatized dogs, R_a increased, M decreased, and hyperglycemia ensued. During exercise, R_a continued to rise, but M did not increase significantly. Conclusions: (*a*) Regulation of glucose production by the liver during exercise is multifactorial. A decrease in IRI and an increase in IRG are not the only factors which can promote delivery of glucose to the peripheral tissues. The insulin glucagon molar ratio was found not to be an essential metabolic functional unit in regulating glucose metabolism during exercise. (*b*) It is hypothesized that increases in blood flow and capillary surface area can lead to an increase in the amount of insulin delivered to the muscle even when serum levels of IRI are reduced during exercise. It is suggested that small, but adequate amounts of insulin (as found in normal and depancreatized insulin-infused dogs) are essential in regulating glucose uptake in the working muscle. (*c*) Since totally depancreatized dogs had normal serum levels of IRG (originating presumably from the gastrointestinal tract), the question of essentiality of basal glucagon activity in glucose homeostasis during exercise could not be resolved by these experiments. It appears, however, that regulation of secretion of nonpancreatic glucagon differs from that of pancreatic glucagon.

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INTRODUCTION

Increased energy demands of the working muscle are met by an increased uptake and metabolism of a variety of substrates; an increased supply of circulating glucose to the muscle is essential during exercise (1). In well-trained animals and human subjects the liver can supply the muscle with increased amounts of glucose essentially without any changes in circulating levels of glucose be-

cause the increased rate of glucose production by the liver (R_a)[1] and the increased rate of glucose utilization (R_d) by the muscle are synchronous and proportional (2, 3).

The aim of the present experiments was to investigate the role of insulin and glucagon in the regulation of glucose turnover during strenuous exercise in normal and in diabetic dogs. Glucose turnover and serum levels of immunoreactive insulin (IRI) and glucagon (IRG) were measured during periods of rest and exercise in normal dogs before pancreatectomy and after total pancreatectomy (*a*) when they were receiving constant basal intraportal infusions of insulin, and (*b*) when they were acutely deprived of insulin supply. Measurements of recycling of labeled glucose were also made to estimate the contribution of glucose-lactate cycle to the newly formed glucose.

Answers were sought to the following questions pertaining to metabolic alterations which occur during strenuous exercise: (*a*) Are the decreases in insulin secretion (4, 5) and the increases in secretion of glucagon (6) which occur in normal dogs essential for the increases in hepatic glucose production? (*b*) Does the insulin glucagon molar ratio (I/G) serve as a metabolic functional unit in the regulation of glucose fluxes? (*c*) Is insulin an essential hormone in the regulation of the uptake of glucose by the muscle during exercise?

METHODS

Animals and surgical procedures. Experiments were carried out in five male mongrel dogs weighing 13–19 kg that were selected on the basis of their ability to run well on the treadmill. For periods of 3–4 wk before the experiments they were trained to lie quietly or to run on the treadmill for 75 min at a rate of 100 m/min at a slope of 10–12°. As reported by Issekutz et al., an exercise of this magnitude increases the overall oxygen uptake by five- to sevenfold and is considered to be a strenuous effort in the dogs (4). When measured during training, the oxygen uptake had also increased five- to sevenfold in two of our dogs. The dogs were given a high protein diet consisting of 30 g of liver chunks (Society Brand, Toronto, Ontario), 10 g of dog chow (Ralston Purina Ltd., Mississagi, Ontario), and 10 g of raw pancreas per kg body weight per day in two equal portions. Raw pancreas was also given during the prepancreatectomy period in order to maintain the dietary composition the same as that given after pancreatectomy. After pancreatectomy the adequacy of the supply of pancreatic enzymes was further ensured by administering 12 enteric-coated tablets/day of digestive enzymes and bile salts (Festal, Hoechst Pharmaceuticals Ltd., Montreal, Quebec).

On the day of the prepancreatectomy experiment in the 24-h fasted dogs, two vascular connections were established under local anesthesia (1% lidocaine). For sampling of blood a vinyl cannula was inserted through the jugular vein into the right atrium. The cannula for the infusion of [1-^{14}C]glucose was placed into a saphenous vein and advanced until its tip was located in the inferior vena cava. This cannulation procedure was employed also for the postpancreatectomy experiments.

2 wk after the prepancreatectomy experiment, total pancreatectomy was performed under general anesthesia on each dog. At the time of surgery a vinyl cannula filled with a dilute heparin solution (200 IU/ml) was inserted into a tributary of the splenic vein and was advanced until its tip was located at the junction of the splenic and the portal veins. This cannula was used at a later time for the infusion of insulin during the experiments described below (7).

After pancreatectomy, for a period of 12 days, the dogs were treated with porcine insulin (Connaught Laboratories, Toronto, Canada), which has an amino acid sequence identical to that of canine insulin. Isophane insulin (NPH), 8–12 U were administered subcutaneously each morning. In addition, crystalline insulin, 5–12 U, was given subcutaneously twice daily after each feeding. The dose of insulin was adjusted as needed to keep the animals as free of glycosuria as possible. As estimated by Clinitest tablets (Ames Co., Div. of Miles Lab., Inc., Elkhart, Ind.), the urinary loss of glucose was usually less than 0.1 g/kg per day. During this period of insulin treatment, ketone bodies (Labstix, Ames Co.) were not detected in the urine; and the mean body weight decreased by less than 4%. The training program was resumed 2–3 days after the operation. 10–12 days after pancreatectomy, NPH insulin was withheld for 48 h and food for 24 h in preparation for the first postpancreatectomy experiment. Injections of crystalline insulin were continued until 15 h before the beginning of an experiment. At this time point the fasting plasma levels of glucose were between 250 and 400 mg/100 ml. Regular insulin was infused intraportally for 2–3 h at a rate of 2,000–3,000 μU/kg-min until plasma glucose decreased to normal levels (estimated using Dextrostix and a reflectance meter, Ames Co.). Thereafter plasma glucose was maintained at a near normal steady-state level by intraportal insulin infused at a rate of 227–270 μU/kg-min, which will be referred to henceforth as the basal rate.

After completion of the first postpancreatectomy exercise experiment, the feeding was resumed and a single injection of NPH insulin was administered on that day. During the following 2 days the dogs were given subcutaneous injections of crystalline insulin. On the 3rd day, 24 h after the last meal and 15 h after the last insulin injection, the second postpancreatectomy experiment was performed during which glucose was again normalized by intraportal infusion of insulin. Thereafter, the animals were sacrificed. At autopsy the patency and location of all catheter tips were verified; no pancreatic tissue was found in any dog, and there was no evidence of infection.

Experimental design. Three experiments were performed in each dog. The prepancreatectomy experiment consisted of an initial rest period of 100 min, followed by a 75-min period of strenous exercise, and a postexercise rest period of 75 min. The protocols of the two postpancreatectomy experiments which were performed in random sequence were as follows: (*a*) An intraportal infusion of insulin was started. As soon as the basal rate of insulin was established an experimental protocol identical to that of the prepancreatectomy experiment was followed. The basal infusion of insulin continued throughout the entire experimental period. (*b*) The intraportal infusion was started and the basal rate of insulin was established. The basal infusion was continued during the initial 100-min rest period and

[1] *Abbreviations used in this paper:* I/G, insulin glucagon molar ratio; IRG, immunoreactive glucagon; IRI, immunoreactive insulin; M, metabolic clearance; R_a, rate of production; R_d, rate of utilization.

then terminated. The rest period was then extended for another 60 min in the absence of insulin. The dogs did not receive any insulin during the 75-min exercise and the 75-min postexercise rest periods which followed.

Tracer methods and calculations. In all experiments a priming dose (37 μCi) of [1-^{14}C]glucose (New England Nuclear, Boston, Mass.) was injected intravenously at the beginning of the initial rest period ($t=0$) and a tracer dose (2 μCi/ml isotonic saline) of the labeled glucose was infused throughout the entire experimental period at a rate of 0.167 ml/min. The specific activity of plasma glucose did not reach a plateau until approximately $t=60$ min; therefore only the data from the last 40 min ($t=60$–100 min) of the initial rest period were used for the calculation of the base-line turnover values.

The rates of production (rate of appearance, R_a) and utilization (rate of disappearance, R_d) of endogenous glucose were determined by the method of primed tracer infusion (8), as modified previously (9, 10). We have demonstrated recently in dogs that using these infusion tracer methods the turnover rates of inulin (11) and glucose (12) can be predicted with accuracy under both steady-state and rapidly changing nonsteady-state conditions. The theoretical and practical aspects of the tracer methodology have been recently reviewed (13).

The rate of glucose utilization is governed both by glucose concentration in plasma (mass effect) and by factors which can accelerate or inhibit the glucose uptake at a given concentration of glucose in plasma. To discern the effects of such promoting and inhibiting factors, it is necessary to normalize R_d for the mass action of glucose. This is achieved by dividing R_d with the prevailing plasma glucose concentration (g) at that time. This ratio R_d/g is called metabolic clearance of glucose (M). The derivation and mathematical aspects of such a ratio were given by Riggs (14). We feel that under experimental conditions when glucose concentration in plasma changes, it is necessary to calculate M in addition to R_d in order to assess whether an increment in R_d is related solely to changes in glucose concentration or also to some other regulating factors. Under most physiological conditions, the factor which regulates M is insulin. We have previously shown that in resting depancreatized dogs, when plasma insulin concentrations were kept constant by intraportal infusions of insulin, the metabolic clearance of glucose did not change, despite an increase in plasma glucose induced by glucagon (15). However, when insulin concentration in plasma increased, a simple equation described and predicted accurately the relationship between plasma insulin and M under steady and nonsteady conditions (11, 15, 16). The values for the specific activity of glucose in plasma were corrected for recirculation of labeled tricarbon metabolites of glucose back into glucose (17), and all turnover rates were calculated from these corrected specific activities. The difference between turnover rates corrected for recycling and the rates not corrected for recycling gives an estimate of the contribution of recycled tricarbon metabolites (mainly via glucose-lactate-glucose cycle) to glucose production. However these estimates represent approximations, because the minimal rather than absolute rates of conversion of metabolites to glucose can be determined by the method employed (18).

Processing of blood samples. Blood samples were obtained from the jugular venous cannula at the time points indicated in figures. Portions of each sample were placed into an heparinized tube that also contained sodium fluoride and a plain tube. The plasma was separated by centrifugation and then deproteinized with a mixture of equal volumes of zinc sulfate (5%) and barium hydroxide (0.3 N). The supernate was passed through an ion exchange resin (Ag-2X8, Bio-Rad Laboratories, Richmond, Calif.) to isolate radioactive glucose from its metabolites (17). The concentration of labeled glucose in the samples was measured by liquid scintillation counting procedures and the concentration of unlabeled glucose was determined using the glucase oxidase method as previously described (9). Specific activity of plasma glucose (disintegrations per minute/microgram) was calculated as a ratio between concentrations of labeled (disintegrations per minute/microliter) and unlabeled glucose (microgram/microliter).

The blood samples in the plain tubes were chilled and allowed to clot. Serum was separated by centrifugation at 4°C. Three 200-μl portions of serum were placed in polystyrene incubation tubes to which 100 μl (1,000 KI-U) Trasylol (FBA Pharmaceuticals, Inc., New York) had been added. These portions were used for the measurement of immunoreactive glucagon (IRG). The remainder of the serum was used for the measurement of immunoreactive insulin (IRI). All serum samples were stored at −20°C until the time of the immunoassays. Immunoreactive insulin was determined in triplicate by the method of Hales and Randle (19) using the Amersham-Searle Kit (Amersham/Searle Corp., Arlington Heights, Ill.). The limit of sensitivity of this assay system is around 7 μU/ml. In the insulin-deprived depancreatized dogs, serum IRI was measured also by a separate "double-antibody" assay which has a sensitivity as low as 0.20±0.09 (mean±2 SD, $N=61$) μU/ml (7). Random samples obtained from each dog after the 2-wk period of insulin treatment were screened for possible presence of insulin antibodies by the method of hydrodynamic flow cellulose paper chromatoelectrophoresis (20) after they had been incubated for 5 days with ^{131}I-insulin. No endogenous insulin antibodies were found in any of the dogs during any of the experimental periods. Immunoreactive glucagon was measured by a "double-antibody" method using the rabbit antibovine-porcine glucagon serum G9-I. The immunoglobulins of the antiserum G9-I react with the carboxyl terminal of the glucagon molecule; they do not react with glucagon-related compounds such as gastric inhibitory polypeptide (GIP), secretin, somatostatin, and the threonine-phenylalanine-threonine-serine tetrapeptide, nor with gastrin, insulin, or proinsulin. The affinity of the immunoglobulins to glucagon-like compounds present in a purified extract of porcine intestinal mucosa ("MUC-101," Novo Research Institute, Copenhagen) is 2–5% of their affinity to pancreatic glucagon (7). The sensitivity of the assay is 3.6±1.7 (mean±2 SD, $N=72$) pg/ml.

RESULTS

Intact dogs. During strenuous exercise endogenous serum IRI decreased rapidly (−44%) ($P<0.01$) so that within 6 min after the beginning of exercise a low steady level was reached (Fig. 1). When the treadmill run was discontinued, serum IRI returned rapidly to levels slightly above those observed during the rest period but decreased again after a short period of time. This apparent short-lived "rebound" in IRI which occurred after strenuous exercise may be the result of sudden and transient changes in blood supply to various tissues affecting the secretion, disposal, or distribution of insulin.

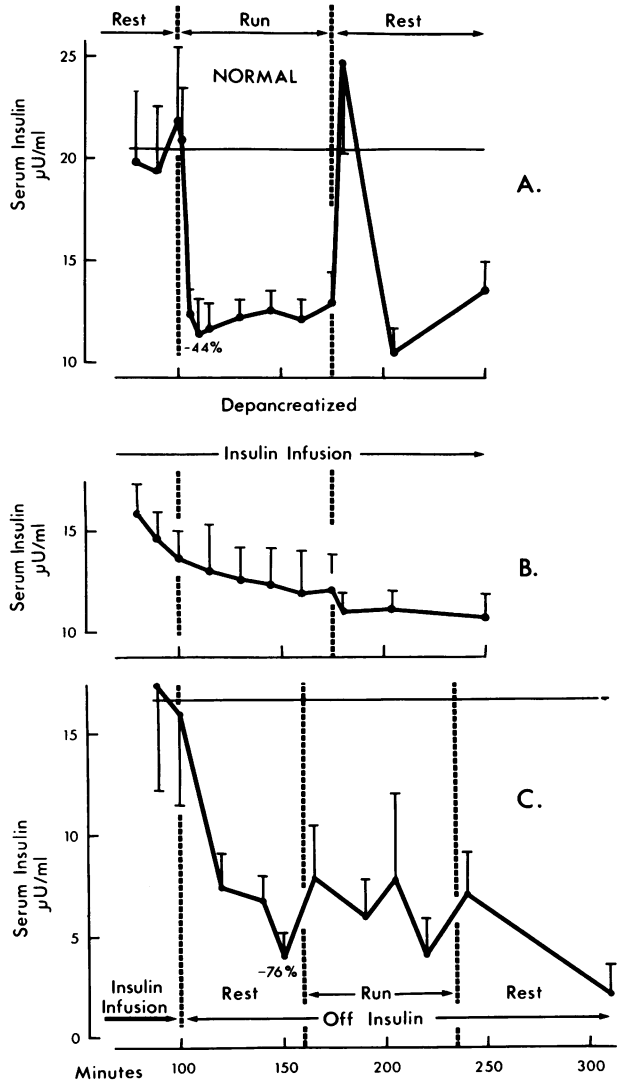

FIGURE 1 Serum levels of immunoreactive insulin (mean ±SEM) during rest and exercise (treadmill run, 100 m/min, slope 10–12°) in dogs (A) before pancreatectomy, (B) after pancreatectomy during infusions of insulin (220–270 μU/kg-min), and (C) after pancreatectomy when the basal insulin infusion was discontinued after the initial 100-min rest period.

At the time that serum IRI had decreased during exercise, serum levels of IRG increased above the basal levels by approximately threefold (Fig. 2). (I/G) decreased rapidly from resting values of 5.7±1.7 to 2.6± 0.6 at 6 min after beginning of strenuous exercise; at the end of the run it was 1.2±0.4 ($P < 0.05$) and remained at 2.1±0.6 during the rest period. There was a significant negative correlation between the rate of glucose production and the logarithm of I/G ($r = -0.593$, $P < 0.01$).

The specific activity of glucose was at a steady state during the control period, but declined gradually during exercise (Fig. 3A). It started to increase again in the postrun rest period. Glucose production as well as glucose utilization increased during exercise (Fig. 3C and D). These increases in R_a and R_d of glucose were synchronous and of similar magnitudes, so that the plasma levels of glucose remained remarkably constant (Fig. 3B). The increments in glucose production varied from dog to dog, yet in each dog at all time intervals the rates of production and utilization were precisely matched. The rate of recycling of glucose increased during exercise, and decreased in the postrun rest period (Fig. 4). The contribution of recycled tricarbon fragments to R_a was 8, 13, and 13% during the initial rest, exercise, and postrun rest periods, respectively.

Depancreatized dogs maintained on basal infusion of insulin. After pancreatectomy at a time when the dogs were being maintained on constant intraportal infusions of insulin, the mean serum IRI decreased slightly during the rest period and during the early part of the exercise period; however, this decline in IRI was not statistically significant (Fig. 1B). With insulin infusions the resting levels of IRI were lower than those observed in the intact state ($P < 0.001$) but during exercise the mean serum IRI was similar in both groups, approximately 12 μU/ml.

Despite total pancreatectomy, serum levels of IRG were within the normal range (Fig. 2), suggesting the existence of an extrapancreatic source for glucagon. Unlike the intact animals, during exercise, serum IRG failed to rise in the depancreatized dogs. The I/G was

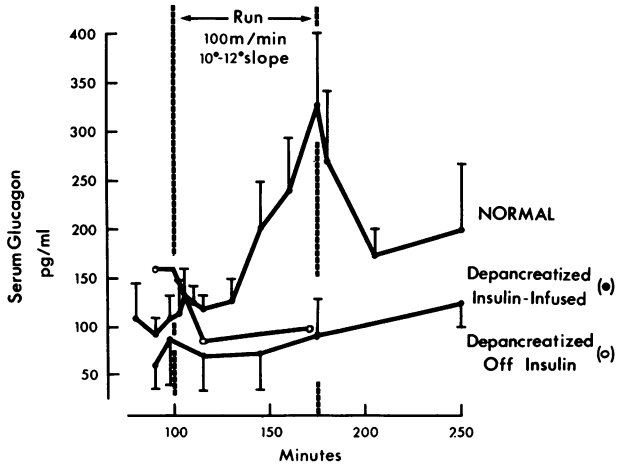

FIGURE 2 Serum levels of immunoreactive glucagon (IRG) (mean±SEM) during rest and exercise in five dogs before pancreatectomy (normal), and after pancreatectomy during basal infusions of insulin. Also shown is average serum IRG for three depancreatized dogs in which insulin infusion had been discontinued 60 min before the start of exercise.

11.2±4.8 during the rest period and did not change significantly during exercise.

In these depancreatized dogs which were receiving basal infusions of insulin but not of glucagon, the kinetics of glucose homeostasis during rest and strenuous exercise resembled closely the patterns found in the intact dogs (Fig. 5). During exercise, plasma levels of glucose did not change at the time R_a and R_d had increased proportionally. The mean increase in glucose production during exercise was by 24% less than that seen in the intact dogs, but this difference was not statistically significant.

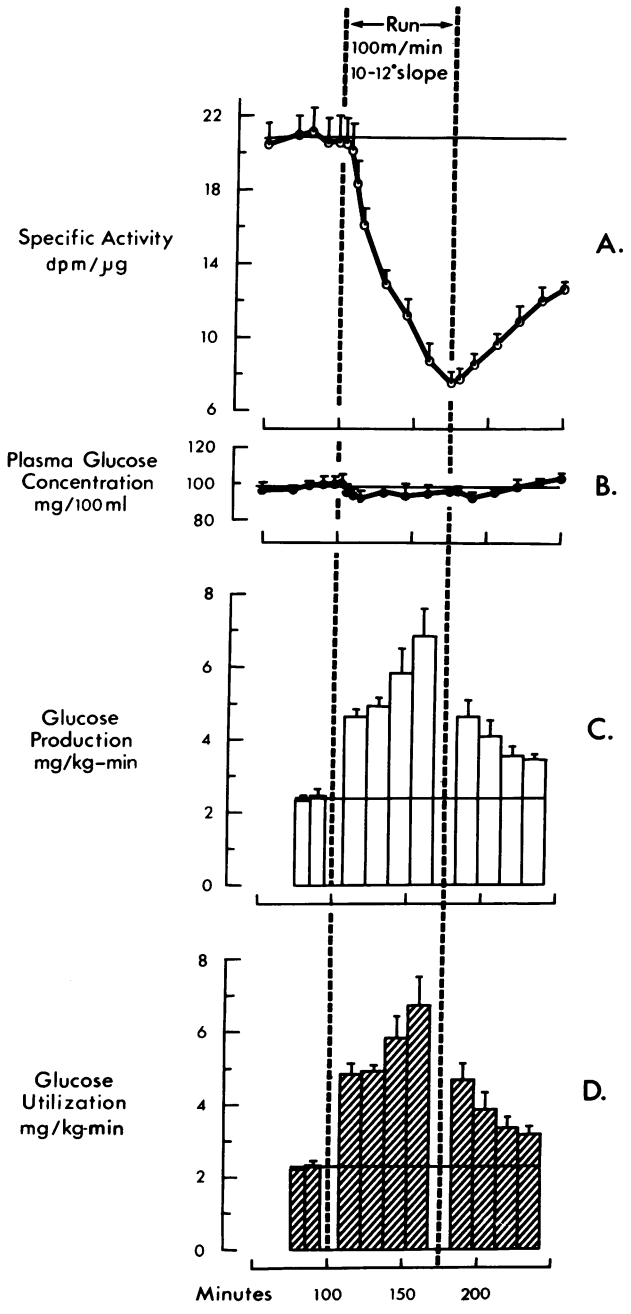

FIGURE 3 The effects of exercise on mean (±SEM) (A) specific activity (corrected for recycling), (B) plasma levels, (C) rates of production, and (D) utilization of glucose in five normal dogs. All values of R_a and R_d were significantly elevated during exercise ($P < 0.001$) as well as during the postrun rest period ($P < 0.005$).

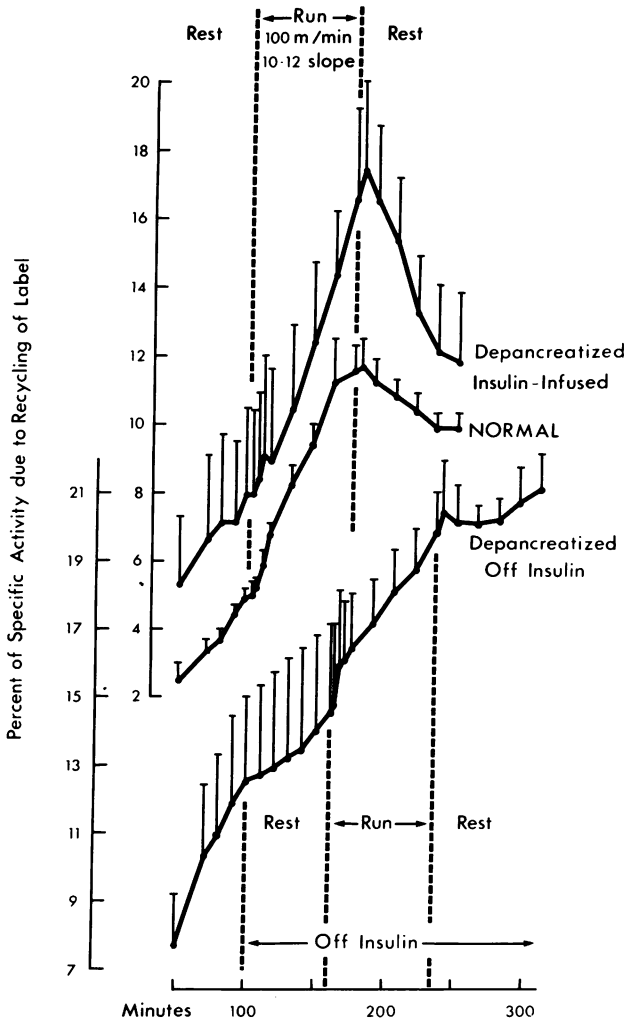

FIGURE 4 Mean (±SEM) percent of total specific activity of plasma glucose contributed by recycling of the labeled tricarbon fragments back to glucose in five dogs in the normal state, and after pancreatectomy with and without basal infusions of insulin.

Although the basal infusion of insulin was sufficient to maintain normal plasma levels and turnover of glucose, the recycling of glucose through the tri-carbon fragments was greater than that seen in the intact dogs ($P < 0.025$) (Fig. 4). Based on the difference between R_a corrected for recycling and R_a not corrected for recycling, the contribution of tricarbon fragments to glu-

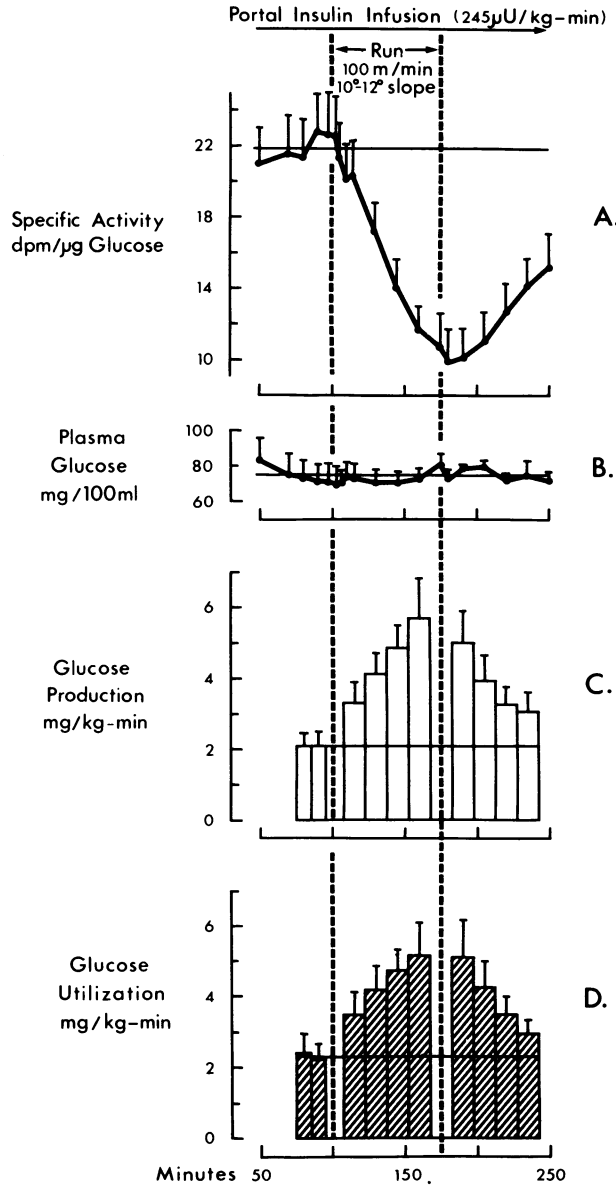

FIGURE 5 The effects of exercise on mean (±SEM) (A) specific activity (corrected for recycling), (B) plasma levels, (C) rates of production, and (D) utilization of glucose in five depancreatized dogs maintained throughout the experiment on constant basal portal fusion of insulin (220–270 μU/kg-min). R_a and R_d increased significantly within 23 min after start of exercise ($P < 0.025$) and remained significantly elevated until the midpoint of the postrun rest period.

cose production was 17, 24, and 18% during the initial rest, exercise, and postrun rest periods, respectively.

Depancreatized dogs deprived of insulin. These experiments were conducted in animals which initially had received basal infusions of insulin, but were deprived acutely of insulin at the onset of the experimental period. Upon cessation of intraportal infusion of insulin serum, IRI decreased rapidly from a mean level of 17.1± 4.9 to 7.2±1.7 μU/ml ($P < 0.025$) within 20 min and to 4.0±1.2 ($P < 0.005$) before the beginning of the exercise period (Fig. 1C). At the end of the postrun rest period the mean serum IRI had reached the lowest level observed (2.1±1.5 μU/ml). During exercise, serum levels of IRI were significantly lower in the insulin-deprived dogs than in either normal or insulin-infused depancreatized dogs (paired analyses $P < 0.05$). However, despite complete cessation of insulin supply, low levels of insulin continued to circulate in the blood of depancreatized dogs during the period of strenuous exercise.

Serum IRG was measured only in three dogs. During the rest period 1 h after cessation of insulin infusion, the average value was 159 pg/ml (50-326 pg/ml) (Fig. 2). At the end of the exercise period, serum IRG was 98 pg/ml (61-160 pg/ml). Thus in these insulin-deprived depancreatized dogs serum IRG failed to increase with exercise, an observation which is similar to that made in insulin-infused depancreatized dogs and which contrasts with the increases seen in the intact dogs.

During the initial rest period with the constant infusion of insulin, plasma levels and specific activity of glucose were stable and within the normal range (Fig. 6A-C). Upon termination of the insulin infusion, plasma glucose started to rise and the specific activity of glucose decreased, reflecting the accompanying increases in the production of glucose (Fig. 6A, C, D). The utilization of glucose did not change (Fig. 6E). The plasma concentration of [^{14}C]glucose increased and corresponded to the reduction in the metabolic clearance of glucose (Fig. 6B, F) (16).

As insulin deprivation continued, exercise brought forward a further R_a increase, so that by the end of the exercise period R_a was 2.5-fold above normal. It is concluded that exercise in addition to insulin, induced such an R_a increase, because in resting depancreatized dogs insulin deprivation induced a much slower rise in R_a; a 2.5-fold increase was observed only after 48 h of insulin deprivation (21). In contrast, in the exercising dogs such an increment in R_a occurred within 160 min after cessation of insulin infusion. As shown in Fig. 6C, glucose concentration rose during exercise because the increase in R_a exceeded that of R_d. The specific activity of glucose decreased further. The concentration of [^{14}C]glucose remained constant and the metabolic clearance of glucose increased only slightly and insignificantly. The failure of the metabolic clearance to increase during exercise in these insulin-deprived dogs contrasts with the observations made in normal and in depancreatized insulin-infused dogs in which the clearance of glucose increased threefold by the end of the

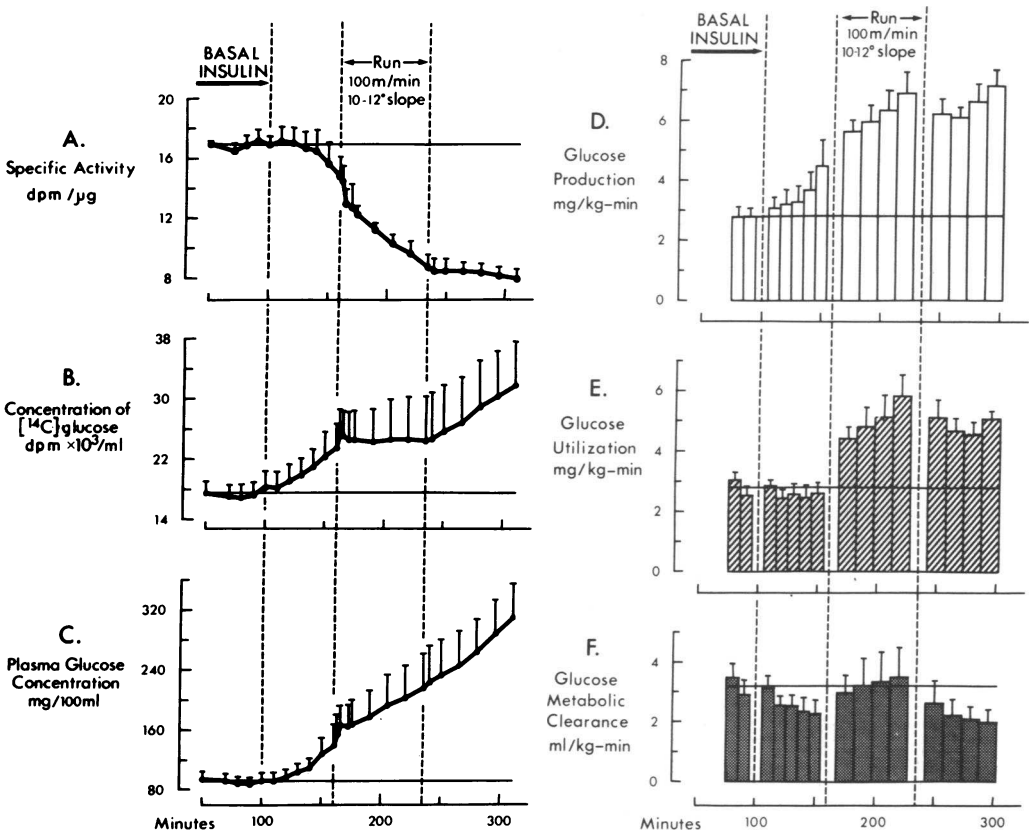

FIGURE 6 The effects of insulin withdrawal and exercise on mean (±SEM) (A) specific activity, (B) plasma levels of labeled and (C) unlabeled glucose, (D) the rates of production, (E) utilization and (F) metabolic clearance of glucose in five depancreatized dogs. Plasma levels and turnover of glucose were maintained near normal levels during the initial 100 min of rest by basal infusions of insulin. Insulin infusions were discontinued thereafter and experiments were continued for an additional 60-min rest, 75-min run, and 75-min postrun rest periods in the complete absence of insulin supply.

exercise period.[2] In other words, a specific effect of exercise on R_d is revealed in normal and depancreatized insulin-infused dogs because R_d increased threefold while glucose concentrations remained unchanged (Figs. 3 and 5). In insulin-deprived dogs it appears that an increase in glucose concentration induced an increase in R_d merely by the mass effect of glucose, because the concentration and uptake of glucose rose proportionately. This is conveniently depicted by the unchanged rate of metabolic clearance of glucose, (calculated as a ratio between R_d and glucose concentration). If other factors than glucose had contributed to the R_d increase, M would have increased during exercise, as it did in normal and depancreatized insulin-infused dogs.

After the exercise period, as insulin was still being withheld, plasma glucose rose progressively. The specific activity of glucose remained constant at a level much lower than that seen during the period of basal insulin infusion. The R_a of glucose was sustained at a high level, a finding which is characteristic of insulin-deprived diabetic dogs (2). The R_d of glucose also remained high but was considerably lower in magnitude than the R_a. The metabolic clearance of glucose decreased to levels which were significantly lower than those observed either at rest during insulin infusion ($P < 0.001$) or during exercise in absence of insulin ($P < 0.025$). The lowering in M was evident despite the fact that during this period of the experiments marked hyperglycemia had resulted in considerable glycosuria and consequently 20-30% of glucose was being cleared by the kidneys, leading to an overestimation of M (2).

The recycling of glucose contributed to 16-20% of the R_a of glucose during the insulin-deprived periods of initial rest and exercise at a rate similar to that seen in the

[2] In normal and in insulin-infused depancreatized dogs, in the absence of any changes in plasma glucose, M equaled R_d; therefore separate data on M were not given.

insulin-infused depancreatized dogs (Fig. 4). However, during the postrun rest period, in the absence of insulin, the recycling continued at a high rate, while the basal insulin infusion it had decreased.

DISCUSSION

During strenuous exercise the production and utilization of glucose increased both in normal dogs, when serum IRI decreased and serum IRG increased and in depancreatized insulin-infused dogs where serum IRI and IRG did not change. The data suggest that insulin and glucagon are not the sole determinants of the increases in the production of glucose which occur during exercise. The question of whether a basal amount of glucagon is essential for continued production of glucose by the liver could not be answered because serum IRG remained within the normal range after pancreatectomy. These observations confirm or previous findings that totally depancreatized dogs are not deficient of glucagon (*7*, *22*). We have proposed that a compound or compounds immunologically indistinguishable from pancreatic glucagon (based on immunological dilution curves) are secreted from an extrapancreatic site. In depancreatized dogs, upon withdrawal in insulin, plasma IRI became undetectable while plasma IRG (measured by four different antibodies) increased progressively (*7*). High IRG values in dogs after pancreatectomy were also found by Matsuyama and Foa (*23*) and by Mashiter et al. (*24*). Near normal IRG values were found in two depancreatized patients by Müller et al. (*25*). The most likely source of the extrapancreatic glucagon is the gastrointestinal tract, in particular the fundus and corpus of the stomach (*7*, *26*, *27*). The failure of IRG to increase in response to infusions of arginine in insulin-infused depancreatized dogs (*22*) and the altered relationship between plasma levels of glucose and IRG (*7*) had suggested that the regulation of the release of extrapancreatic glucagon differs from that of pancreatic glucagon. This is corroborated in the present experiments: as reported previously serum IRG rose during exercise in intact animals (*6*, *28*, *29*), but not in the depancreatized dogs. These dissimilar IRG responses to exercise may be due to differences in the innervation and/or anatomical distribution of the glucagon-producing cells. The fact that α and β cells are juxtaposed in the pancreatic islets but not in the gut may play a role as well. The currently available information suggests that the metabolic consequences of feeding or exercise affect the release of the pancreatic, but not the gastrointestinal glucagon. The following evidence indicates that gut glucagon has biological activity similar to that of pancreatic glucagon with respect to regulation of glucose production: (*a*) In the resting dog, acute deficiency of insulin and of glucagon induced by somatostatin is accompanied by decreases in the production and plasma levels of glucose (*30*). On the other hand, in the depancreatized dogs reported in this study at the time of acute insulin deficiency glucagon levels had not decreased (Fig. 2). This glucagon from extrapancreatic source must have contributed to the increases in the production and plasma levels of glucose (Fig. 6). (*b*) R_a increments induced by arginine occur concomitant with increases in serum IRG in the intact animals and in insulin-deprived depancreatized dogs (G. Ross, L. Lickley, and M. Vranic, unpublished observations). (*c*) Sasaki et al. indicated that purified stomach extracts stimulate the production of glucose by the perfused liver (*27*). In the depancreatized insulin-infused dogs other factors in addition to insulin and glucagon must be responsible for R_a increments which occur despite the lack of decreases in serum IRI and of increases in IRG. Factors controlling R_a during exercise could include autonomic innervation, a variety of glucoregulatory hormones and an increased supply of gluconeogenic substrates. Gollnick et al. have shown that exercise-induced glycogenolysis proceeds unaltered when a variety of known regulatory systems had been inactivated by pharmacological or surgical means (*31*), indicating that a reduced activity of one or more inducers of glucose production can be compensated for readily by others.

During exercise, gluconeogenesis contributes to the increases in the hepatic production of glucose. In our dogs the rate of gluconeogenesis was not determined. However our data on the rate of recycling of glucose reflect a portion of the gluconeogenic process (glucose-lactate-glucose cycle). In normal and in depancreatized dogs the rates of recycling and of production of glucose increased proportionately. The fraction of R_a due to recycling was higher in depancreatized dogs (20%) than in normal dogs (10%). Wahren et al. have found that also in diabetic patients the splanchnic glucose output increases normally during exercise (*3*). On the basis of splanchnic arterio-venous differences they concluded that in their patients the net uptake of gluconeogenic substrates accounted for a greater proportion of net splanchnic glucose output than in normal subjects. Their data on the percent contribution of lactate to splanchnic glucose output corresponds closely to our results.

In contrast to the multifactorial regulation of glucose production, it is known that in resting dogs the utilization of glucose is governed mainly by the prevailing plasma levels of insulin and glucose (*11*, *15*, *16*). In our intact and depancreatized insulin-infused dogs, R_d increased markedly during exercise despite the low serum IRI, at a time plasma glucose had not increased. A possible explanation for this discordance could be that during exercise an increase in serum IRI may not be

required for increased delivery of insulin to the muscle. The circulatory adaptation to exercise leads to preferentially increased blood flow to the working muscle, which would allow for increased insulin delivery even if the concentration of insulin is low. During strenuous exercise, blood flow through the exercising leg increases eightfold (3) in human subjects. Thus in our normal dogs the amount of insulin reaching the muscle per unit time would have increased four- to fivefold despite the fact that serum IRI had decreased by 40%. Krogh estimated that at rest the capillary surface area in the muscle amounts to only a fraction of that during exercise (32). The exercise-induced increases in blood flow and capillary surface area within the muscle tissue could provide important regulatory mechanisms for the enhanced delivery of insulin to the exercising muscle and the same mechanism could also enhance the exposure of muscle cells to glucose. Low serum IRI with other factors would promote glucose production in the liver, while the circulatory changes in the working muscle could ensure that insulin supply is adequate.

R_d of glucose increased also in the insulin-deprived depancreatized dogs even though serum IRI was considerably lower than that in the other two groups. R_d increased proportionally to glucose concentration in plasma. This indicates that the R_d increments were due to the mass effect of glucose and not to a specific effect of exercise. When the disappearance data are expressed as the metabolic clearance of glucose thus making adjustments for the changes in plasma glucose, the deficiency in R_d regulation becomes evident. The minimal increases in the metabolic clearance in these dogs contrast with the threefold increases which occurred in the intact or insulin-infused depancreatized dogs. Since the only difference between the two groups of depancreatized dogs is the cessation of insulin infusion in the latter, we have concluded that the failure of M to increase is due to insulinopenia. This could indicate that a small amount of insulin in circulating blood (as seen in normal and depancreatized insulin-infused dogs) is essential for the adequate utilization of glucose by peripheral tissues. Wahren et al., reported that in diabetic patients 24 h after injection of long-acting insulin, exercise increased peripheral glucose uptake, but glucose was not elevated (3). It is feasible that their patients were not totally deprived of insulin. In a manner similar to that observed in another study, strenuous exercise can enhance the release of long-acting insulin from a previously injected subcutaneous site into the circulation (33). Alternatively insulin bound to endogenous antibodies may dissociate at an accelerated rate due to altered kinetics which may occur during exercise.

In the experiments being reported here, the depancreatized insulin-infused dogs had been subjected to strenuous exercise; R_a and R_d increased proportionately so that plasma glucose remained constant. These results differ from those reported previously when the dogs were exercised only moderately: R_a increments were not accompanied by comparable R_d increments; therefore plasma glucose increased (2). Because in both groups of experiments serum IRI was maintained at similar levels by insulin infusions, other factors have to be considered to explain why glucose uptake increased with strenuous but not with moderate exercise. The increases in blood flow and capillary bed surface are greater with strenuous than with moderate exercise so that differences in the magnitude of the delivery of insulin to the working muscle could be partly responsible. The glucose uptake during strenuous exercise could be further enhanced in the presence of insulin by "muscular activity factors" which were found only during strong muscular stimulations (34-36).

Unger and Lefebvre have suggested that the molar ratio of insulin to glucagon (I/G) is an important factor in controlling glucose fluxes in health and disease (37). However, we have previously indicated that the concept of I/G ratio as a metabolic functional unit does not hold under a variety of conditions (15, 22). Although insulin and glucagon at certain concentrations may modify the effects of one another upon the liver, at the peripheral level glucagon did not interfere with the effects of insulin on glucose uptake in depancreatized insulin-infused dogs (15, 38). In our exercising intact dogs a significant negative correlation between I/G and R_a increments was observed. However, the R_a increments in depancreatized insulin-infused dogs were similar to those in the intact dogs when the I/G ratio did not change. The hazards in the interpretation of the I/G can be exemplified further by comparing the effects of exercise to those of starvation. In the former, a decrease in I/G is associated with a threefold increase in glucose turnover (mainly glycogenolysis); in the latter, glucose turnover decreases by approximately 50% (39) and glucose is produced through gluconeogenesis. Thus a role for I/G ratio as a metabolic functional unit in the regulation of glucose fluxes cannot be demonstrated under most physiological conditions; absolute levels of each hormone should be considered separately.

Our conclusions on the role of insulin and glucagon in regulating glucose turnover during strenuous exercise are based on data obtained in normal and depancreatized dogs. However, because of analogies between diabetic patients and the depancreatized dogs we believe that our observations may broaden the perspectives regarding the role of insulin and pancreatic or extrapancreatic glucagon in the regulation of glucose homeostasis during exercise in health and disease. A more complete knowledge about the rates and metabolic roles of these

hormones may eventually lead to a more rational management of diabetic patients.

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