Stimulation of splanchnic glucose production during exercise in humans contains a glucagon-independent component

ROBERT H. COKER,1 LENE SIMONSEN,3 JENS BÜLOW,3 DAVID H. WASSERMAN,2 AND MICHAEL KJÆR4
1Division of Exercise Science, University of Mississippi, University, Mississippi 38677; 2Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232; and 3Department of Clinical Physiology and 4Sports Medicine Research Unit, Bispebjerg Hospital, DK-2400 Copenhagen, Denmark

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Coker, Robert H., Lene Simonsen, Jens Bülow, David H. Wasserman, and Michael Kjæer. Stimulation of splanchnic glucose production during exercise in humans contains a glucagon-independent component. Am J Physiol Endocrinol Metab 280: E918–E927, 2001.—To determine the importance of basal glucagon to the stimulation of net splanchnic glucose output (NSGO) during exercise, seven healthy males performed cycle exercise during a pancreatic islet cell clamp. In one group (BG), glucagon was replaced at basal levels and insulin was adjusted to achieve euglycemia. In another group (GD), only insulin was replaced at the identical rate used in BG, and basal glucagon was not replaced. Exogenous glucose infusion was necessary to maintain euglycemia during exercise in BG and during rest and exercise in GD. Arterial glucagon was at least twofold greater in BG than in GD throughout the pancreatic islet cell clamp. Although basal NSGO remained stable in BG (2.5 ± 0.5 mg·kg⁻¹·min⁻¹), basal NSGO dropped by 70% in GD (0.7 ± 0.3 mg·kg⁻¹·min⁻¹). NSGO was also greater in BG than in GD at 10 min of moderate exercise, most likely due to the residual effect of basal glucagon replacement. However, NSGO increased slightly and remained similar throughout the remainder of moderate and heavy exercise in BG and GD. Therefore, a mechanism independent of changes in pancreatic hormones and/or the level of glycemia contributes toward modest stimulation of NSGO during moderate and heavy exercise.

islet cell clamp; hormone replacement

STIMULATION OF HEPATIC GLUCOSE PRODUCTION (Ra) is essential for the maintenance of glucose homeostasis during exercise. Exercise-induced increases in portal vein glucagon, which are threefold greater than increments in arterial blood, support the efficacy of glucagon as a primary mediator of Ra (37). The importance of exercise-induced changes in pancreatic hormone in the stimulation of Ra are further supported by studies conducted in dogs that utilize pancreatic hormone suppression and their portal venous replacement under euglycemic conditions (38, 42). Although these studies clearly described the importance of exercise-induced changes in glucagon and insulin in the stimulation of Ra, exercise-induced changes in the peripheral concentrations of these glucoregulatory hormones are known to be relatively minor in humans, and the important levels, those in the portal vein, are unknown (16). Even so, studies that have utilized the pancreatic clamp technique in humans have demonstrated a diminished Ra response to exercise (20, 25, 45). However, arterial glucagon was allowed to fall during exercise and complicates the interpretation of the data (30). Therefore, the factors responsible for the precise control of glucoregulation during moderate and heavy exercise are not completely understood.

The present study was designed to determine the importance of basal glucagon levels for the stimulation of net splanchnic glucose output (NSGO) during moderate and heavy exercise in humans under euglycemic conditions. Pancreatic hormone suppression and replacement procedures were used, which either replaced basal glucagon or left the subjects glucagon deficient. Furthermore, a glucose clamp was employed to maintain the subjects at a euglycemic level. This design provided us with a method in which the exogenous glucose infusion as well as the NSGO response to exercise could be used to delineate the relative importance of basal glucagon for the maintenance of glucose homeostasis.

METHODS

Subjects. Seven healthy young males (24 ± 1 yr old, 174 ± 5 cm in height, and 73 ± 3 kg in weight) gave their informed consent to participate in the study. The protocols for the study were approved by the Ethical Committee of Copenhagen. All of the subjects were healthy, and none was presently taking any medication or had a family history of metabolic disorders or any known allergy. Maximal O2 uptake (V̇O2 max) was determined on a semi-supine bicycle ergometer ≥1 wk before the experiments. Semi-supine bicycling has been shown to yield peak O2 uptake (V̇O2 peak) measurements equivalent to 91–93% of V̇O2 max obtained on an upright bicycle.

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bicycle ergometer (29). All seven subjects participated in two experiments each. In one subject, the data were incomplete due to displacement of the hepatic vein catheter during exercise, and thus data reported are from six individuals.

**Procedures.** All subjects arrived at the laboratory at 0800 and were 10 h fasted. Subjects were advised to abstain from physical training and intake of alcohol and tobacco use 24 h before experiments. Upon a subject's arrival, a cannula (1.0 mm ID) was inserted into the left brachial artery for blood sampling. A separate venous line was inserted into a forearm vein for infusion of indocyanine green (ICG). A catheter was introduced into a femoral vein and was advanced, under fluoroscopy, into a right-side hepatic vein to ~3–4 cm from the wedge position. Its location was verified, both during and after exercise, by use of ultrasonography and fluoroscopy, respectively. Patency of the catheter was maintained by flushing with heparinized saline solution (10 U/ml).

**Experimental procedures.** Experiments consisted of an ICG equilibration period (~130 to ~30 min), a basal period (~30 to 0 min), a moderate-intensity exercise period (0–40 min; 50% \(\text{VO}_{2\text{ max}}\), and a heavy exercise period (40–70 min; 70% \(\text{VO}_{2\text{ max}}\)). The primed/constant rate of ICG infusion was started at ~130 min and continued throughout the remainder of the study. ICG was used to measure splanchnic blood flow. A pancreatic islet cell clamp was utilized throughout both experimental protocols and was initiated ~2 h before exercise. Octreotide (a somatostatin analog) was infused into a peripheral vein at a constant rate of 30 ng-kg\(^{-1}\)min\(^{-1}\). In the first series of experiments (BG), basal levels of glucagon were maintained by infusion of glucagon (Novo-Nordisk, Bagsvaerd, Denmark) into a peripheral vein at 1 ng-kg\(^{-1}\)min\(^{-1}\). Human insulin (Actrapid, Novo-Nordisk) was also infused into the same peripheral vein, and the rate of infusion was adjusted to achieve euglycemia. The insulin infusion rate was adjusted according to plasma glucose samples taken every 5 min and was determined immediately on an automatic glucose analyzer (YSI 23AM, Yellow Springs Instrument). After the last adjustment in the insulin infusion rate, the rate was held constant throughout the remainder of the experiment. The islet cell clamp was considered successful when the difference in multiple glucose samples was less than ~4 mg/dl and remained stable. Exogenous glucose infusion was initiated at the onset of exercise in BG. In the second series of experiments [glucose deficiency (GD)], only insulin was replaced into a peripheral vein at the identical rate previously used in BG. Basal glucagon was not replaced in GD, and the exogenous glucose infusion was initiated in the basal period to maintain euglycemia and adjusted during exercise for the same purpose.

**Blood sample collection and processing.** Arterial and hepatic vein blood samples were collected at ~150 and ~120 min before the basal period, at ~30 and 0 min during the basal period, and every 10 min during the exercise period. Samples were immediately placed into chilled glass tubes and centrifuged at 4°C. Heparinized blood was collected for the determination of glucose, lactate, glycerol, free fatty acids (FFA), growth hormone (GH), cortisol, and hematocrit (Hct). Lactate, glycerol, and FFA were determined by enzymatic fluorometric methods (29). Hct was measured by the microhematocrit method. Insulin, glucagon, GH, and cortisol were determined by radioimmunoassay, as previously described (17, 18). Blood for the determination of catecholamines was collected in chilled tubes containing EDTA and glutathione and was centrifuged at 4°C; plasma was stored at ~70°C for subsequent HPLC analysis. Catecholamine concentrations were calculated on the basis of a linear regression with dihydroxybenzylamine as an internal standard. The interassay coefficients of variation with this method were 5 and 7% for norepinephrine and epinephrine, respectively.

**Splanchnic blood flow and net splanchnic glucose output.** Splanchnic plasma flow (SPF) was estimated by the ICG dye-extraction method (32). This technique involves a primed (1.0 mg), constant (200 μg/min) infusion of ICG (prepared in a 5% solution of human serum albumin in isotonic saline), with an equilibration period of ~45 min before blood sampling. Arterial and hepatic venous blood was sampled at ~150, ~120, ~30, and 0 min, and every 10 min during exercise. Plasma concentrations of ICG were determined spectrophotometrically (805 nm) in duplicate, with correction for plasma turbidity measured at 900 nm, and Hct was measured with the microhematocrit method. Plasma glucose concentrations were converted to whole blood concentrations on the basis of arterial (a)-hepatic vein (h) Hct values determined throughout the experiment. The calculation for this conversion was blood glucose = plasma glucose (1 – 0.30 × Hct). SPF was estimated according to a modification for non-steady-state conditions:

\[
I - \frac{C_{a2} - C_{v2}(t_2 - t_1)}{C_{a1} + C_{v1}/2 - (C_{a2} + C_{v2})/2}
\]

where \(I\) is the infusion rate of ICG, \(t\) is the time interval, \(V_{a}\) is the volume of distribution of ICG approximated to 5% of body weight (32), \(C_{a1}\) and \(C_{v1}\) represent the previous arterial and venous ICG concentrations, and \(C_{a2}\) and \(C_{v2}\) are the current sample concentrations. Thus SPF equals \(S_{BF}/(1 - Hct)\). NSGO was calculated as the product of SPF and a-hv glucose concentration differences expressed in milligrams per kilogram per minute, with kilogram referring to body weight. This method has been demonstrated to give values similar to tracer-determined \(R_{n}\) during exercise under postabsorptive conditions (3).

**Statistical analysis.** SuperAnova (Abacus Concepts, Berkeley, CA) software installed on a Macintosh Power PC was used to perform statistical analyses. Statistical comparisons between groups and over time were made using ANOVA designed to account for repeated measures. Specific time points were examined for significance by use of contrasts solved by univariate repeated measures. Statistics are reported in the corresponding table or figure legend for each variable. Data are presented as means ± SE. Statistical significance was defined as \(P < 0.05\).

**RESULTS**

**Arterial and hepatic vein plasma insulin and glucagon.** Arterial insulin was not different (\(P > 0.05\)) between groups during the preclamp period and rose (\(P < 0.05\)) to similar levels in the basal period in BG (11 ± 3 μU/ml) and GD (11 ± 1 μU/ml). There was an additional increase (\(P < 0.05\)) in arterial insulin during the heavy exercise period in both groups (Fig. 1). Hepatic vein insulin was higher (\(P < 0.05\)) in BG (7 ± 2 μU/ml) compared with GD (5 ± 1 μU/ml) during the preclamp period. However, hepatic vein insulin was not different (\(P > 0.05\)) between groups during the basal period and increased (\(P < 0.05\)) similarly during moderate and heavy exercise (Fig. 1). Although arterial glucagon was similar in the basal and exercise groups during the preclamp period, basal arterial glucagon was more than twofold higher in BG (75 ± 5 pg/ml) compared with GD (32 ± 6 pg/ml). Arterial glucagon was also twofold greater (\(P < 0.05\)) in BG compared
Epinephrine was not different (P > 0.05) in BG and GD during the basal period, although arterial plasma epinephrine was greater (P < 0.05) in GD (14 ± 6 mIU/l in GD by 30 min of heavy exercise (Fig. 3). Basal arterial plasma norepinephrine values were similar (P > 0.05) in BG and GD. Even though norepinephrine increased (P < 0.05) in both groups during moderate and heavy exercise, norepinephrine levels were consistently greater (P < 0.05) in BG compared with GD (Fig. 4).

**Blood glucose (a-hv), NSGO, and exogenous glucose infusion.** Arterial glucose was similar (P > 0.05) in both groups during the preclamp, basal, moderate, and heavy exercise periods (Fig. 5). In addition, there were no significant differences between groups in hepatic vein glucose in the preclamp, basal, and moderate exercise periods. However, hepatic vein glucose was less (P < 0.05) in BG (115 ± 7 mg/dl) compared with GD (151 ± 19 mg/dl) by the end of the heavy exercise period (Fig. 5). During the preclamp period, NSGO was stable and not different (P > 0.05) between BG and GD. In contrast, basal NSGO was about threefold higher (P < 0.05) in BG (2.5 ± 0.5 mg·kg⁻¹·min⁻¹) compared with GD (0.7 ± 0.3 mg·kg⁻¹·min⁻¹). There was a modest difference (P < 0.05) in NSGO in BG (3.5 ± 0.9 mg·kg⁻¹·min⁻¹) compared with GD (1.7 ± 0.4 mg·kg⁻¹·min⁻¹) at 10 min of moderate exercise, which was most likely due to the residual effect of glucagon replacement during the basal period. Basal glucagon replacement was less important during the

![Fig. 1. Arterial and hepatic vein plasma insulin before the islet cell clamp (−150 to −120 min) and during the basal (−30 to 0 min), moderate exercise (10–40 min), and heavy exercise (50–70 min) periods. Data are means ± SE; n = 6 for glucagon-deficient (GD) and insulin (BG) experiments and n = 6 for glucagon replacement during the basal period. Basal insulin replacement was less important during the preclamp period. Basal insulin and glucagon levels were greater (P < 0.05) in BG compared with GD throughout the basal (71 ± 5 pg/ml in BG; 33 ± 7 pg/ml in GD at t = 40 min) and heavy (79 ± 4 pg/ml in BG; 33 ± 6 pg/ml in GD at t = 70 min) exercise (Fig. 2). Despite similar hepatic vein glucagon levels during the preclamp period, the concentration of glucagon in the hepatic vein was greater (P < 0.05) in BG compared with GD throughout the basal, moderate, and heavy exercise periods (Fig. 2).

**Arterial plasma cortisol and GH.** Arterial plasma cortisol was similar (P > 0.05) in BG (13 ± 1 mg/dl) and GD (14 ± 1 mg/dl) during the preclamp period and remained stable in both groups during the moderate exercise period. Arterial cortisol increased (P < 0.05) similarly, to 18 ± 3 mg/dl in BG and 17 ± 2 mg/dl in GD, during the heavy exercise period (Fig. 3). Arterial plasma GH was different (P > 0.05) between BG (3.6 ± 1.4 mIU/l) and GD (2.3 ± 1.4 mIU/l) during the preclamp period. During moderate exercise, arterial GH fell (P < 0.05) similarly to almost zero and then increased (P < 0.05) to 2.6 ± 0.7 mIU/l in BG and 2.5 mIU/l in GD by 30 min of heavy exercise (Fig. 3).

**Arterial plasma epinephrine and norepinephrine.** Arterial plasma epinephrine was greater (P < 0.05) in BG compared with GD during the basal period, although epinephrine was not different (P > 0.05) and increased (P < 0.05) in BG and GD during the moderate and heavy exercise periods (Fig. 4). Basal arterial plasma norepinephrine values were similar (P > 0.05) in BG and GD. Even though norepinephrine increased (P < 0.05) in both groups during moderate and heavy exercise, norepinephrine levels were consistently greater (P < 0.05) in BG compared with GD (Fig. 4).
though arterial glycerol was less in BG compared with GD before the islet cell clamp, glycerol levels fell \((P < 0.05)\) similarly during the basal period and increased \((P < 0.05)\) throughout the moderate and heavy exercise periods (Fig. 8). Furthermore, the rate of net splanchnic glycerol uptake before the islet cell clamp decreased \((P < 0.05)\) in both groups. Net splanchnic glycerol uptake increased \((P < 0.05)\) and remained similar \((P > 0.05)\) in the groups throughout the moderate and heavy exercise periods (Fig. 8). Arterial plasma FFA levels were similar \((P > 0.05)\) in BG and GD before the islet cell clamp and fell substantially \((P < 0.05)\) in both groups during the basal period. Arterial FFA increased \((P < 0.05)\) to \(241 \pm 37 \mu \text{mol/l}\) in BG at 40 min of moderate exercise but remained similar \((P > 0.05)\) to basal levels at \(179 \pm 20 \mu \text{mol/l}\) in GD. During heavy exercise, arterial FFA was similar \((P > 0.05)\) in BG and GD (Fig. 9). Net splanchnic FFA uptake was similar \((P > 0.05)\) in BG and GD before the islet cell clamp and fell substantially \((P < 0.05)\) markedly during the basal period in both groups. Although net splanchnic FFA balance remained similar throughout most of the exercise bout, it was increased \((P < 0.05)\) to an uptake of \(0.18 \pm 0.08 \mu \text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) in BG and shifted to an output of \(0.04 \pm 0.04 \mu \text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) in GD at 30 min of moderate exercise (Fig. 9).

remainder of moderate and heavy exercise, because of support by similar NSGO \((P > 0.05)\) in BG \((3.7 \pm 1.0 \text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) at \(t = 40\) min; \(4.7 \pm 1.2 \text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) at \(t = 70\) min) and GD \((3.4 \pm 0.9 \text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) at \(t = 40\) min; \(5.0 \pm 0.9 \text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) at \(t = 70\) min; Fig. 6). Exogenous glucose infusion was required during the basal period in GD, and the rate of infusion was slightly lower \((P < 0.05)\) throughout most of the exercise sessions in BG compared with GD (Fig. 6). Finally, it is important to note that the NSGO plus the exogenous glucose infusion rate (total glucose entry into the circulation) was similar \((P > 0.05)\) in the groups throughout the preclamp, basal, and moderate and heavy exercise periods (Fig. 6).

Arterial metabolite concentrations and splanchnic metabolite balances. Arterial lactate was similar \((P > 0.05)\) in the groups before the islet cell clamp and during the basal period. Arterial lactate increased similarly \((P < 0.05)\) in BG and GD during moderate and heavy exercise (Fig. 7). Splanchnic lactate balance was similar \((P > 0.05)\) during the basal period in BG \((2.6 \pm 1.5 \mu \text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1})\) and GD \((4.0 \pm 0.6 \mu \text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) and increased in a similar fashion during the moderate exercise period. Splanchnic lactate balance shifted toward significantly greater uptake during heavy exercise in BG compared with GD (Fig. 7). Al-
Heart rates, hepatosplanchnic blood flows, oxygen consumption, and ratings of perceived exertion. Heart rates were similar \((P > 0.05)\) in BG and GD during the basal, moderate, and heavy exercise periods (Table 1). Hepatosplanchnic blood flow was not different \((P > 0.05)\) between BG and GD in the basal state or during the moderate exercise period. However, hepatosplanchnic blood flow was slightly higher \((P < 0.05)\) in BG compared with GD by the end of the heavy exercise period (Table 1). The percentage of maximal oxygen consumption was not different \((P > 0.05)\) between BG and GD and rose similarly during moderate and heavy exercise in both groups (Table 2). The ratings of perceived exertion were also similar \((P > 0.05)\) in the groups during moderate and heavy exercise (Table 2).

DISCUSSION

The results of the present study demonstrate that the prevention of exercise-induced changes in glucagon and insulin as characterized by BG reduces NSGO compared with results of previous investigations conducted under similar conditions in the absence of a pancreatic clamp (3). Furthermore, BG required an exogenous glucose infusion to maintain euglycemia during the moderate and heavy exercise periods. GD reduced basal NSGO and also required an exogenous glucose infusion to maintain euglycemia, not only during rest but also during moderate and heavy exercise in humans. Despite the effect of glucagon deficiency on these parameters in GD, the NSGO response to moderate and heavy exercise was largely similar to that in BG. This is somewhat surprising, because prior studies have demonstrated that basal glucagon and the increment in glucagon are necessary for the stimulation of normal glucose production rates (37). It is important to recognize that the normal exercise-induced increment in NSGO (2-fold at 50% and 4-fold at 75% \(V_O2_{max}\)) during semi-supine cycle exercise (3) was suppressed in BG and GD, probably due to absence of an increase in glucagon during exercise. Studies in humans (20, 45) and dogs (41, 42) have demonstrated the importance of exercise-induced increments in glucagon in the stimulation of hepatic glucose production during moderate exercise. Although evidence supports the efficacy of glucagon in the stimulation of glucose production, the present study demonstrates that other mechanisms may stimulate modest increases in NSGO under basal glucagon or glucagon-deficient conditions.

**Fig. 5.** Arterial and hepatic vein plasma glucose before the islet cell clamp (%150 to %120 min) and during basal (%30 to 0 min), moderate exercise (%10–40 min), and heavy exercise (%50–70 min) periods. Data are means ± SE; \(n = 6\) for BG (●) and \(n = 6\) for GD (□) experiments. Small □, difference between BG and GD, \(P < 0.05\).

**Fig. 6.** Net splanchnic glucose output, exogenous glucose infusion, and net splanchnic glucose output + exogenous glucose infusion before the islet cell clamp (%150 to %120 min) and during basal (%30 to 0 min), moderate exercise (%10–40 min), and heavy exercise (%50–70 min) periods. Data are means ± SE; \(n = 6\) for BG (●) and \(n = 6\) for GD (□) experiments. *Difference between BG and GD and between preclamp and pancreatic hormone suppression/replacement.
Results from previous studies utilizing pancreatic hormone suppression and replacement methodology in humans may be somewhat inconclusive because of changes in the arterial glucose concentrations (20, 25). The exercise-induced increment in glucose utilization ($R_d$) in the presence of a pancreatic clamp results in a reduction in the plasma glucose concentration (20, 25). Therefore, the fall in glucose may serve as a plausible stimulus for the corresponding increase in $R_a$ (4). Studies show that very small changes in glucose can result in the modulation of $R_a$ (1, 9, 22, 40). Furthermore, carbohydrate ingestion during exercise has also been shown to inhibit $R_a$ (24).

Arterial glucose concentrations were maintained at basal levels in the present study. However, it is important to mention that a fall in portal vein glucose (decrease of 4–5 mg/dl) has been shown to occur despite stable arterial glucose concentrations (exercise-induced change <1–2 mg/dl) during heavy exercise [85% of heart rate (HR) maximum] in dogs under normal physiological conditions (11). This may be significant, because a 2.5–6.5 mg/dl reduction in arterial glucose has been demonstrated to produce a 4.0 mg·kg$^{-1}$·min$^{-1}$ increase in hepatic glucose production (2). Therefore, subtle oscillations in portal vein (responsible for 80% of liver perfusion) glucose may exist that cannot be detected in human experimentation.

Further insight into the mechanisms responsible for the stimulation of NSGO in the present study might be gained from experiments conducted during especially intense exercise. Heavy exercise is characterized by dramatic increases in circulating catecholamines, which parallel the large changes in glucose flux (5, 16, 26, 27). These occur in the presence of small increases in arterial glucagon (2, 16, 19) and reductions in arterial insulin, as supported by a fall in C-peptide levels (31, 44). Prior studies in exercising humans (~85% $VO_2\text{max}$) found that $R_a$ increased normally despite an islet cell clamp that kept insulin and glucagon at peripheral basal levels and led the authors to suggest that the catecholamines were the primary mediators of the increase in $R_a$ during heavy exercise (33). However, studies designed to directly assess the role of the catecholamines have not supported their importance as a primary mechanism for the stimulation of $R_a$ (37).

It is known that the gut extracts ~50% of the circulating catecholamines delivered to it, resulting in a proportional reduction in the portal vein epinephrine concentrations that are ~50% of the arterial epinephrine concentrations (10). Thus it seems clear that arterial epinephrine levels overestimate those at the liver. The role of the catecholamines has also been specifically investigated with a variety of experimental conditions.
methods. For example, the use of celiac ganglion anesthesia (blocks sympathetic nerve activity to the liver and adrenal medulla) in exercising human subjects had no additional effect on $R_a$ during basal (30–0 min), moderate exercise (10–40 min), and heavy exercise (50–70 min) periods. Data are means ± SE; $n = 6$ for BG (●) and $n = 6$ for GD (○). *Difference between preclamp and pancreatic hormone suppression/replacement levels, $P < 0.05$. Small □, difference between BG and GD, $P < 0.05$.

Fig. 9. Arterial free fatty acids (FFA) and net splanchnic FFA balance before the islet cell clamp (−150 to −120 min) and during basal (−30 to 0 min), moderate exercise (10–40 min), and heavy exercise (50–70 min) periods. Data are means ± SE; $n = 6$ for BG (●) and $n = 6$ for GD (○). *Difference between preclamp and pancreatic hormone suppression/replacement levels, $P < 0.05$. Small □, difference between BG and GD, $P < 0.05$.

It is important to address the limitations of the islet cell clamp in humans. In humans, pancreatic hormones must be replaced in a peripheral vein. Therefore, the peripheral replacement of glucagon and insulin does not establish the portal vein-to-peripheral gradient in islet hormone concentrations that exists in the normal physiological state. This may create some degree of portal hypoinsulinemia and hypoglucaconemia. Previous studies have demonstrated a greater than threefold increase in $R_a$ during selective portal hypoinsulinemia in dogs (34). However, these prior studies utilized excessive decrements in portal vein insulin compared with the likely levels of portal vein insulin in the present study. A reasonable estimation of portal vein levels in the present study can be drawn from the following considerations. First, insulin is secreted directly into the portal circulation in the absence of a pancreatic clamp. Second, hepatic fractional extraction of insulin removes ~50% of the insulin delivered to the liver (35). Therefore, portal vein insulin may have been threefold higher than peripheral levels (31). The present study was characterized by some degree of peripheral hyperinsulinemia, whereas portal vein insulin levels should have remained relatively similar before and during the islet cell clamp. Due to peripheral hyperinsulinemia, the total glucose requirement (NSGO + exogenous glucose infusion) to maintain euglycemia was greater than the typical amount of glucose production required in humans during exercise in the absence of a pancreatic clamp (3).

In the present study, octreotide administration resulted in an almost total blockade of GH release and a 50% reduction in immunoreactive glucagon (IRG) levels. Although octreotide caused a dramatic decrease in arterial IRG, it was still present at 50% of preclamped values. The antibody used in this assay does not dif-

### Table 1. Hemodynamic measurements

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<th>Time, min</th>
<th>Preclamp</th>
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<td>GD</td>
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Values are means ± SE for 6 healthy subjects who exercised for 40 min at a moderate intensity and 30 min at a heavy intensity under conditions of basal glucagon and insulin (BG) or basal insulin only (GD) on 2 separate occasions. *Significantly different from BG.
ferment biologically active glucagon (3,500 molecular weight) from a larger glucagon-like peptide that does not stimulate NSGO (12). The cross-reactivity of proteins other than glucagon is generally accepted, because physiological changes in IRG are thought to primarily represent changes in the level of 3,500 molecular weight glucagon. Therefore, larger peptides may represent up to 40% of the total IRG level (29). As a result, the discrepancy in IRG between groups should represent the absence of 3,500 molecular weight glucagon in GD. Moreover, the minor changes in growth hormone and cortisol during heavy exercise are most likely attributable to exercise and/or octreotide-induced alterations in hepatic blood flow, which would facilitate changes in hepatic extraction of these hormones (14, 36).

Despite the contention that portal vein insulin levels may remain relatively similar in the preclamp and octreotide infusion periods of the present experiment, elevations in the peripheral insulin levels seemed to have significant effects on lipid kinetics. For example, arterial FFA and glycerol decreased immediately in both groups after the start of the islet cell clamp, indicating that lipolysis was decreased. Although there was a reduction in the exercise-induced glycerol response compared with normal physiological conditions (15), the exercise-induced increment in glycerol was similar in both groups in the presence of the islet cell clamp. This indicates that lipolysis was increased even in the presence of peripheral hyperinsulinemia. However, there was an attenuation in the FFA response during exercise. Therefore, the discrepancy between FFA and glycerol levels suggests that FFA reesterification and/or clearance was affected during exercise (39). It is interesting to note that the modest difference in arterial FFA and net splanchnic FFA balance between BG and GD may suggest that glucagon plays a relatively minor role in the regulation of FFA metabolism as proposed by previous investigations (6).

The attenuation of splanchnic lactate uptake with heavy exercise during GD in the present study supports the results of previous experiments in which the exercise-induced increment in glucagon partially increased $R_a$ through the stimulation of gluconeogenesis (42). It has been demonstrated that the exercise-induced increment in glucagon secretion is largely responsible for the stimulation of net hepatic lactate uptake and gluconeogenesis (42). Therefore, the rise in glucagon facilitates gluconeogenesis by converting the liver to a greater lactate-consuming organ (42). Glucagon deficiency likely attenuates the splanchnic uptake of lactate and consequently reduces the amount of gluconeogenic precursor available for conversion to glucose. It is important to note that, even though glucagon may alter the efficacy of lactate as a gluconeogenic precursor, the relative impact on NSGO is minor, because hepatic glycogenolysis is largely responsible for the increment in glucose production during heavy exercise (37).

From the present study, it can only be speculated as to what factors are responsible for the modest stimulation of NSGO in the absence of glucagon during exercise. It has been shown that sleep (decrease in HR and core temperature) is associated with a decrease in glucose production and utilization despite no detectable change in glucoregulatory hormones (glucagon, insulin, and catecholamines) (8). The mechanisms by which these changes in glucose flux occur are not clearly understood but suggest that unknown endocrine mechanisms are present during exercise and are important for the stimulation of NSGO. However, it should be mentioned that small changes in the portal vein levels of glucagon and/or insulin could have a significant effect on glucose kinetics without any change in the arterial concentrations of these hormones (37).

The present study investigated the role of glucagon deficiency during moderate and heavy exercise under euglycemic conditions. This approach allowed us to investigate the importance of basal glucagon independently of changes in arterial glucose. The results of the study indicate that glucagon deficiency decreased basal NSGO. In addition, the residual effect of glucagon deficiency was associated with a modest difference in the initial exercise-induced increase in NSGO. However, glucagon deficiency or basal glucagon conditions failed to establish the normal exercise-induced increment in NSGO (3). Furthermore, glucagon deficiency may also reduce the splanchnic uptake of lactate and FFA. There are several possible mediators that may be responsible for the slight increase in NSGO, and factors such as the catecholamines and/or substances released from working muscle may have the ability to influence $R_a$.

In conclusion, glucagon deficiency compromises the ability to establish effective glucose regulation during exercise at moderate and heavy intensities. Even so, basal glucagon replacement is also insufficient to maintain euglycemia during exercise under the same conditions. These data provide additional support to the contention that exercise-induced changes in glucagon and insulin are of primary importance for the accurate regulation of hepatic glucose production. These data also support the view that other unde-

### Table 2. Percentage of maximal O$_2$ consumption and rating of perceived exertion

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Moderate Exercise</th>
<th>Heavy Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>49 ± 1</td>
<td>50 ± 1</td>
</tr>
<tr>
<td>20</td>
<td>50 ± 1</td>
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</tr>
<tr>
<td>30</td>
<td>51 ± 2</td>
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<tr>
<td>40</td>
<td>52 ± 2</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>50</td>
<td>68 ± 2</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>60</td>
<td>69 ± 1</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>70</td>
<td>72 ± 3</td>
<td>73 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 healthy subjects who exercised for 40 min at a moderate intensity and 30 min at a heavy intensity under conditions of basal glucagon and insulin (BG) or basal insulin only (GD) on 2 separate occasions. $V_{O2\text{max}}$, maximal O$_2$ consumption.
scribed factors can be responsible for the stimulation of NSGO.

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