Regulation by Insulin of Myocardial Glucose and Fatty Acid Metabolism in the Conscious Dog

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Abstract. In vivo small doses of insulin inhibit lipolysis, lower plasma FFA, and stimulate glucose disposal. Lowering of plasma FFA, either in the absence of a change in insulin or during combined hyperglycemia and hyperinsulinemia, promotes glucose uptake by heart muscle in vivo. In the isolated perfused heart, large doses of insulin directly stimulate heart glucose uptake. To assess the effect of physiological elevations of plasma insulin upon myocardial glucose and FFA uptake in vivo independent of changes in plasma substrate concentration, we measured arterial and coronary sinus concentrations of glucose, lactate, and FFA, and coronary blood flow in conscious dogs during a 30 min basal and a 2 h experimental period employing three protocols: (a) euglycemic hyperinsulinemia (insulin clamp, n = 5), (b) euglycemic hyperinsulinemia with FFA replacement (n = 5), (c) hyperglycemic euinsulinemia (hyperglycemic clamp with somatostatin, n = 5).

In group 1, hyperinsulinemia (insulin = 73±13 µU/ml) stimulated heart glucose uptake (7.3±4.4 vs. 28.2±2.8 µmol/min, P < 0.002), lowered plasma FFA levels by 80% (P < 0.05), and decreased heart FFA uptake (28.4±4 vs. 1.5±0.9, P < 0.01). When the fall in plasma FFA was prevented by FFA infusion (group 2), hyperinsulinemia (86±10 µU/ml) provoked a lesser (P < 0.05) stimulation of glucose uptake (Δ = 8.2±4.2 µmol/min) than in group 1, and there was no significant change in FFA uptake (25.3±16 vs. 16.5±4). Hyperglycemia (plasma glucose = 186±8 mg/100 ml) during somatostatin infusion resulted in only a small rise in plasma insulin (Δ = 12±7 µU/ml), and although plasma FFA tended to decline, heart glucose uptake did not rise significantly (Δ = 5.5±3.2 µmol/min, P = NS). There was no significant change in coronary blood flow during any of the three study protocols.

We conclude that, in the dog, insulin at physiologic concentrations: (a) stimulates heart glucose uptake, both directly and by suppressing the plasma FFA concentration, and (b) does not alter coronary blood flow. Hyperglycemia per se has little effect on heart glucose uptake.

Introduction

In the postabsorptive state, cardiac muscle, like skeletal muscle, utilizes FFA as the principal oxidative fuel (1). Circulating glucose and heart glycogen furnish additional fuels, which may be of particular importance when oxygen availability is limited, as may occur in acute or chronic myocardial ischemia (2). In healthy man, the arterial fatty acid concentration appears to be the principal determinant of heart fatty acid extraction (3). Two decades ago, Randle et al. (4, 5) observed that in the perfused rat heart, fatty acids inhibit the oxidation of glucose and proposed that a "glucose-fatty acid cycle" existed whereby fat oxidation inhibited the flux of carbohydrates through the glycolytic pathway. This inhibition of carbohydrate oxidation persisted even in the presence of pharmacologic doses of insulin. In contrast, in the absence of exogenous fatty acids, high concentrations of insulin greatly stimulate heart glucose uptake, glycogen synthesis, and carbohydrate oxidation (6). While abundant work in the isolated perfused heart has demonstrated that insulin at high concentrations stimulates the uptake and metabolism of glucose (7-10), evidence that insulin at physiologic concentrations regulates heart glucose uptake in vivo is indirect (11). Furthermore, since in vivo insulin lowers plasma FFA levels by inhibiting lipolysis in adipose tissue (12), assessment of insulin's direct role in regulating heart glucose metabolism requires simultaneous estimation of heart fatty acid exchange. Clarification of the
mechanisms of myocardial metabolic control are of importance with respect to further understanding of myocardial ischemia and its therapy. In addition, changes in arterial concentration of insulin, glucose, or FFA might influence myocardial extraction of fuel substrates as observed in vivo using radioactive glucose and positron-computed tomographic imaging.

In the present study, we used the insulin clamp technique (13) in combination with arterial and coronary sinus blood sampling in the conscious dog to study the effect of physiologic changes in plasma insulin on the myocardial extraction of glucose, FFA, and lactate. Circulating concentrations of FFA and glucose were altered independently by infusing heparin with triglyceride emulsion and adjusting the glucose infusion rate during the insulin clamp. The effect of hyperglycemia, independent of alterations in plasma insulin, on heart fuel substrate exchange was also assessed using the glucose clamp technique (13). High doses of insulin have been reported to increase coronary blood flow in conscious euglycemic dogs (14). Insulin-induced changes in coronary flow could alter the net myocardial uptake or release of substrate, despite a constant arterial-coronary sinus substrate concentration gradient. Therefore, in addition to measurements of substrate extraction, serial thermodilution estimates of coronary blood flow were obtained during the course of these experiments.

Methods

Animal preparation. 24–48 h before study, adult mongrel dogs of either sex weighing between 18 and 31 kg (mean = 24±2 kg) were anesthetized with barbital (Sutinal). Under aseptic conditions, an incision was made in the right side of the neck and the carotid artery and jugular vein were isolated. A heparin-filled silastic catheter (1.25 mm interior diam., Dow Corning, Midland, MI) was placed in each vessel, and the extravascular ends occluded and fixed in the subcutaneous tissue. The overlying skin was then sutured closed. On the day of study, after an overnight fast, the awake animal was gently restrained on a fluoroscopy table in a left lateral decubitus position. Following administration of lidocaine locally, the sutures were removed from the neck, and the free ends of the catheters exposed. The arterial catheter was used for obtaining arterial blood samples and for monitoring blood pressure. The second silastic catheter was used as an introducer for a triple thermistor thermodilution catheter (Webster Laboratories Inc., Altadena, CA) which was then positioned in the main portion of the coronary sinus under fluoroscopic guidance. A #7F Sones catheter was also advanced into the coronary sinus and used for sampling of coronary sinus blood. The thermodilution and sampling catheters both remained in the coronary sinus throughout the study and the catheter's position was confirmed fluoroscopically at the end of the study. Normal saline was infused through the thermodilution catheter at a rate of 32 ml/min for 30–60 s for each determination of coronary flow. An 18-gauge polyethylene catheter was placed percutaneously in the right saphenous vein and used for infusion of hormones and substrates. Patency of all catheters was maintained by a slow infusion of normal saline. Position of the coronary sinus catheters was confirmed at the beginning and end of each experiment by fluoroscopy and measurement of oxygen saturation.

Experimental protocols. Coronary sinus blood flow and arterial and coronary sinus concentrations of glucose, FFA, and lactate were measured every 10 min during a 30-min basal period and every 20 min during the 2-h experimental period. Five dogs were studied in each of three experimental protocols. In group 1, the euglycemic insulin clamp was employed. After obtaining basal samples, plasma insulin was raised ~60 μU/ml above fasting values using a primed (2 mU/min·kg × 10 min) continuous (1 mU/min·kg × 110 min) infusion of regular insulin (Eli Lilly Co., Indianapolis, IN). Plasma glucose was maintained constant at basal values by measuring plasma glucose every 5 min and adjusting the rate of infusion of a 20% dextrose solution as previously described (13).

In group 2, plasma insulin was again raised by ~60 μU/ml above basal and plasma glucose maintained at basal levels by a variable rate glucose infusion as in group 1. In addition, a soybean triglyceride emulsion (Intralipid 20%, Cutter Laboratories, Berkeley, CA) was infused (0.5 ml/min) with sodium heparin (20 U/min) to prevent the decline in plasma FFA caused by hyperinsulinenia.

In group 3, somatostatin was infused (500 μg/h) to inhibit endogenous insulin secretion, while plasma glucose was acutely raised and maintained constant at 80 mg/100 ml above basal using a variable rate glucose infusion (+80 mg/100 ml glucose clamp) as previously described (13).

Chemical determinations. Plasma glucose was measured using the glucose oxidase method and a Beckman glucose analyzer (Beckman Instruments, Inc., Palo Alto, CA). FFA were measured by a modification of the procedure of Dole (15); blood lactate and plasma insulin were assayed as described previously (16, 17). Blood pH, Po2 and PCO2 were measured using an Ilford Micro 13 blood gas analyzer (Instrumentation Laboratories, Lexington, MA).

Calculations. Coronary sinus blood flow was estimated from the thermodilution curves as described by Ganz et al. (18) and validated by Pepine et al. (19). Four separate estimates of coronary flow were obtained during the basal period and two estimates were made every 20 min thereafter, immediately prior to obtaining coronary sinus blood samples. Myocardial uptake or release of substrate was calculated from the product of the arterial-coronary sinus substrate concentration difference and the simultaneously obtained coronary blood flow. Because of differences in their intravascular distribution, for glucose and FFA fluxes plasma concentrations and coronary plasma flows [coronary blood flow × (1-hematoctrit)] were used, while for lactate whole blood concentrations and blood flows were employed. The substrate extraction ratio at any time was determined by dividing the arterial-coronary sinus substrate concentration difference by the arterial substrate concentration.

Statistical analysis of the change in myocardial substrate exchange over time and comparison among experimental protocols were performed using either paired or unpaired t test or analysis of variance with a repeated measures design (20). Data are expressed as mean±SEM.

Results

During the 30 min basal period, the myocardium of all dogs took up fatty acids (21±5 μmol/min). In the basal state, the mean extraction ratio for FFA was 30±3% and was independent of arterial FFA concentration. However, there was a strong correlation between the arterial FFA concentration and heart FFA uptake (r = 0.88, P < 0.01). There was also a significant uptake of glucose (10.6±3.5 μmol/min), but the extraction ratio (2.2±0.9%) was much less than for FFA. The
Table I. Myocardial Uptake of Glucose and FFA During the 30-Min Basal Period and During the Last Hour of the Experimental Period

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose uptake</th>
<th>FFA uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/min</td>
<td>μmol/min</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7.3±4.4</td>
<td>28.4±4.0</td>
</tr>
<tr>
<td>60–120 min</td>
<td>28.2±2.8*</td>
<td>1.5±0.9*</td>
</tr>
<tr>
<td>Δ</td>
<td>20.9±2.8</td>
<td>−26.9±4.5</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>15.2±5.1</td>
<td>25.3±16</td>
</tr>
<tr>
<td>60–120 min</td>
<td>23.4±3.3</td>
<td>16.5±4</td>
</tr>
<tr>
<td>Δ</td>
<td>8.2±4.2‡</td>
<td>−8.8±14</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7.9±7.7</td>
<td>13.5±4.9</td>
</tr>
<tr>
<td>60–120 min</td>
<td>12.4±9.8</td>
<td>3.7±2.2</td>
</tr>
<tr>
<td>Δ</td>
<td>4.5±2.8§</td>
<td>−9.8±6</td>
</tr>
</tbody>
</table>

All values are mean±SEM.
* Denotes uptake in 40–100 min period which was significantly different (P < 0.01) than basal uptake within that group.
‡‡ Denotes an increment in glucose uptake in groups II and III which were significantly (P < 0.05 and P < 0.02, respectively) less than the increment in glucose uptake in group I.

basal rates of glucose and FFA uptake by each of the three groups of dogs are given in Table I. During the basal period, there was also a net uptake of lactate by the heart (18.6±10.6 μmol/min), and a linear correlation existed between lactate uptake and blood lactate concentration (r = 0.74, P < 0.01).

The partial pressure of O₂ in coronary sinus blood averaged 13±4 mmHg, and the pH of coronary sinus blood was consistently lower (ΔpH = 0.045±0.004, P < 0.001) and PCO₂ consistently higher (ΔPCO₂ = 8.8±1.4 mmHg, P < 0.01) than arterial blood during the 30 min basal period (Table II). Mean basal coronary blood flow averaged 114±12 ml/min. There were no differences in these parameters among the three groups of dogs.

Results of the euglycemic insulin clamp (group I) are illustrated in Fig. 1. The plasma insulin concentration rose from basal levels of 10±2 μU/ml to a mean stimulated value of 73±13 μU/ml and was maintained constant throughout the study. Arterial glucose was maintained at euglycemic levels. Coronary sinus blood flow was unchanged during the insulin infusion. The arterial-corryn sinus (a-cs) concentration differences for glucose increased following the rise in plasma insulin concentration, while the plasma FFA concentration and the a-cs concentration difference for FFA declined abruptly after raising plasma insulin. Arterial blood lactate concentration during the last hour of study was significantly greater than during the basal period (450±80 vs. 173±170 μmol/liter, P < 0.05). Heart lactate uptake increased in each of five dogs, but the mean increment in lactate uptake was not statistically significant. As shown in Table II, euglycemic hyperinsulinemia led to a significant stimulation of myocardial glucose uptake (Δ = 21±3 μmol/min) and a marked depression in myocardial fatty acid removal (Δ = −27±5 μmol/min).

In these animals, we observed that the rate of total body glucose utilization, or M, was 11.5±0.4 mg/min·kg body weight. The value M during any time interval was estimated from the measured rate of infusion of exogenous glucose during that time interval and corrected for any change in the filling of body glucose space (13).

The data from group 2 dogs are shown in Fig. 2 and Table I. When the plasma insulin concentration was raised from 26±5 to 86±10 μU/ml, and plasma glucose maintained at euglycemic levels, infusion of intralipid with heparin (group 2) prevented the decline in FFA seen in group I. Neither

Table II. Arterial and Coronary Sinus pH, PO₂, and PCO₂ During the Basal Period and the Last 20 Min of the Experimental Period

<table>
<thead>
<tr>
<th>pH</th>
<th>PO₂ (mmHg)</th>
<th>PCO₂ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>100–120 min</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>7.53±0.03</td>
<td>7.54±0.03</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>7.49±0.03</td>
<td>7.49±0.03</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>7.51±0.02</td>
<td>7.49±0.02</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>7.45±0.02</td>
<td>7.45±0.02</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>7.49±0.03</td>
<td>7.46±0.03</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>7.44±0.03</td>
<td>7.41±0.03</td>
</tr>
</tbody>
</table>
myocardial FFA uptake, FFA extraction ratio, or lactate uptake changed significantly (Fig. 1). Coronary sinus blood flow was again unchanged during the 2 h of hyperinsulinemia. The higher mean basal myocardial glucose balance in group 2 dogs is attributable to one dog with a basal heart uptake of 33 μmol/min. Hyperinsulinemia did not stimulate glucose uptake in this animal, but significantly increased glucose uptake (10.7±3 vs. 22±4 μmol/min, P < 0.025) in the remaining four dogs.

During the euglycemic clamp, M averaged 6.2±0.5 mg/min·kg in the five dogs in group 2. This was significantly less (P < 0.001) than the corresponding measure (11.5±0.4 mg/min·kg) obtained in group 1.

During the hyperglycemic clamp (group III), the steady state plasma glucose concentration averaged 188±10 mg/100 ml (Fig. 3). Somatostatin infusion inhibited the rise in plasma insulin that would normally accompany marked hyperglycemia, and the mean plasma insulin during the period of hyperglycemia averaged only 12±7 μU/ml above basal (P = NS). Coronary sinus blood flow did not change from basal (Fig. 3). There was no significant change in myocardial glucose uptake (8±8 vs. 12±10 μmol/min) or in heart FFA uptake (Δ = -9.8±6 μmol/min) (Table I).

Total body glucose utilization in these dogs averaged 8.6±1.4 mg/min·kg. In these hyperglycemic animals, this estimate of total glucose utilization includes urinary glucose losses which could not be accurately estimated in these con-

Figure 1. The upper panel illustrates the plasma insulin concentration (○) and the coronary blood flow (●) during the basal period and during the 120-min insulin clamp in five dogs studied in protocol 1. The second, third, and fourth panels show the arterial (○) and coronary sinus (●) concentrations of glucose, FFA, and lactate during the basal and experimental periods. Error bars indicate ±1 SEM.

Figure 2. Illustrated are the plasma concentration, coronary blood flow, and arterial and coronary sinus concentrations for glucose, FFA, and lactate in the five dogs studied in protocol 2. Symbols are as designated in Fig. 1.

Figure 3. Coronary blood flow and plasma concentrations of insulin, glucose, FFA, and blood lactate in the artery and coronary sinus samples are shown for the five dogs studied using protocol 3. See Fig. 1 for explanation of symbols.

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Physiologic during phosphofructokinase glucose normal (group regardless of we observed in inhibition of to demonstrated that early on observation resulting and extraction removal appears average, results in plasma stimulatory izing insulin. Results significance 0.05, greater significantly in uptake greatest when group, each that glucose uptake by myocardial glucose uptake faster than that in isolated hemidiaphragm, FFA can inhibit insulin-stimulated glucose uptake and oxidation. However, studies in sartorius muscle (25), perfused hindquarters (26), and rat diaphragm (27) failed to show any effect of FFA upon glucose uptake. The more recent work of Rennie and Holloszy (28) using perfused hindquarter suggest that the inhibitory effect of oleate on glucose utilization is clearly demonstrable only when the skeletal muscle is very well oxygenated. Differences in tissue oxygenation may account for some of the apparent lack of effect of fatty acids on glucose utilization in earlier studies.

Thus, while multiple investigators have described an inhibitory effect of FFA on glucose uptake and oxidation in isolated perfused hearts, in vitro studies in skeletal muscle have yielded conflicting results. In addition to the adequacy of oxygen supply, multiple factors, including imposed work load (10), insulin concentration (5), and the rate of generation of FFA within the isolated muscle preparation, can each affect muscle carbohydrate handling; therefore, it is perhaps not surprising that a consistent effect of FFA (or ketones) on carbohydrate utilization in isolated skeletal muscle preparations has not emerged. Results of the current study in conscious animals strongly supports the hypothesis that in vivo and under physiologic conditions, the circulating concentration of fatty acids is an important mediator of glucose metabolism in both cardiac and skeletal muscle, even when the plasma insulin concentration is significantly elevated.

Lasers and colleagues (29) have clearly demonstrated that in postabsorptive man, nicotinic acid lowers the plasma FFA and increases the myocardial uptake of glucose, lactate, and pyruvate. This nicotinate-enhanced glucose uptake occurred in the absence of any rise in plasma insulin (30). The respiratory quotient also increased, indicating a shift in myocardial metabolism to aerobic glycolysis. Kajser and his associates (31) have also examined carbohydrate extraction by the human heart during exercise or glucose infusion. During exercise, plasma FFA rose markedly while insulin and glucose declined, and heart uptake of FFA but not glucose increased (31). In contrast, during glucose infusion, plasma glucose and insulin and myocardial glucose uptake rose while plasma levels and myocardial removal of FFA declined. In these latter studies, unlike our results reported here, the plasma glucose, insulin,
and FFA each changed. Therefore, it was not possible to assess separately their effects upon myocardial substrate exchange. Results of the current study are entirely consistent with the hypothesis that plasma FFA concentrations are an important modulator of cardiac substrate exchange during fasting and when plasma insulin is raised within the physiologic range. Our observation that in four of the dogs in group II, myocardial glucose uptake rose during insulin infusion even though the decline in plasma free fatty acids was prevented by lipid infusion suggests that insulin has direct stimulatory effects on heart glucose metabolism. Such a direct effect of physiologic concentrations of insulin on heart glucose uptake may be a reflection of the well-known stimulatory effect of pharmacologic doses of insulin on glucose metabolism in the isolated perfused heart. In contrast to the important role of alterations of plasma FFA and of plasma insulin on heart metabolism, elevations of plasma glucose in the absence of a significant rise in insulin (group III) cause little change in cardiac substrate exchange.

It should be noted that in dogs receiving intravenous insulin (groups I and II), no change in coronary blood flow occurred despite an increment in plasma insulin of ~60 \( \mu \text{U/ml} \). These data stand in contrast to the work of Liang et al. (14). They recently reported that insulin, given in doses four to eight times higher than those employed in our studies, caused myocardial blood flow, measured by the radioactive microsphere technique, to increase by ~40% if blood glucose was allowed to fall. Maintenance of euglycemia resulted in a lesser (~30%) but still significant increase in myocardial blood flow. Beta adrenergic blockade prevented the insulin-induced rise in coronary flow. The higher insulin infusion rates employed in that study likely account for the increased flow observed, since insulin stimulation of sympathetic discharge appears to be dose-dependent (32). Liang et al. (14) also noted increases in heart rate, cardiac output, and left ventricular dP/dt in response to insulin. Propranolol effectively blocked each of the changes in cardiac function, which accompanied insulin infusion in both euglycemic and hypoglycemic animals. These observations again suggest that insulin’s effect was indirect and mediated via beta adrenergic stimulation. We observed no change in heart rate, arterial blood pressure, or pulse pressure during protocols 1 and 2 when insulin is infused at a rate of the 1 \( \mu \text{U/min per kilogram} \).

The current study also provides physiologic data which have implications concerning the use of radionuclide imaging to assess myocardial metabolic events. The advent of positron and single photon-computed tomography and the production of radiolabeled carbon-11 and fluorine-18 metabolites currently allows direct assessment of regional metabolic function. Studies have involved qualitative as well as quantitative measures of regional myocardial uptake and distribution of fatty acid and glucose analogs, and regional perfusion in experimental animals and man (33, 34). Increased regional accumulation of 2-fluoro-2-deoxyglucose has been used as a marker of myocardial viability in ischemic portions of the left ventricle where anerobic glucose metabolism has been operative (34). Awareness of the effect of a varying metabolic milieu upon myocardial utilization of these substrates is critical for understanding measurements of altered metabolism under pathologic circumstances.

In summary, findings in the current study support the hypothesis that at physiologic concentrations, plasma insulin is a major regulator of myocardial substrate exchange in the conscious dog. Insulin promotes myocardial carbohydrate metabolism directly by stimulating carbohydrate uptake and indirectly through inhibition of lipolysis. The lowered plasma concentration of FFA mediates the indirect action of insulin, and infusion of FFA significantly blunts insulin stimulation of myocardial carbohydrate removal. Hyperglycemia, in the absence of elevated insulin levels, has little effect on heart glucose uptake. Finally, our results do not support a significant role for insulin at physiological concentrations in the modulation of coronary blood flow.

Acknowledgments

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