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Role of Cortisol in Cardiac Glucose Metabolism In Vivo

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ABSTRACT

Little is known about how physiological concentrations of glucocorticoid relate to cardiac metabolism in vivo. Healthy conscious dogs with catheters implanted for blood sampling and glucose infusion were studied. The range of blood glucose values produced by glucose infusion was 3700 to 74,400 μmol/liter. Arterial glucose concentration (C_a) was not significantly correlated with the arterial–coronary sinus difference in concentration of glucose, C_{a-cs} glucose ($N = 50$, $r = 0.23$). However, at or above glucose infusion rates of 2120 μmol/min, significant increases in cardiac glucose extractions were seen. The range of plasma cortisol values was 13 to 438 nmol/liter. Cortisol immunoreactivity in arterial plasma (I_a cortisol) was significantly and negatively correlated with C_{a-cs} glucose ($N = 50$, $r = -0.37$, $P < 0.01$). Nonparametric analysis confirmed this association (Spearman rank correlation coefficient, $r_s = -0.44$, $P < 0.01$). The heart took up and released cortisol in relation to I_a cortisol ($N = 50$, $r = 0.75$, $P < 0.001$; $r_s = 0.58$, $P < 0.001$). The range of I_{a-cs} cortisol was −31 to 138 nmol/liter (mean ± SEM, 12 ± 4, $P < 0.01$). The C_{a-cs} glucose was negatively correlated with I_{a-cs} cortisol ($N = 50$, $r = -0.43$, $P < 0.01$; $r_s = -0.50$, $P < 0.001$). Thus, higher plasma cortisol immunoreactivity may lead to greater myocardial cortisol extraction and suppression of myocardial glucose extraction in vivo. At the same time arterial insulin immunoreactivity had a significant positive relationship to myocardial glucose extraction ($N = 50$, $r = 0.37$, $P < 0.05$; $r_s = 0.38$, $P < 0.01$) and arterial plasma free fatty acids a negative relationship ($N = 50$, $r_s = -0.30$, $P < 0.05$).

The hemodynamic effects of pharmacological doses of glucocorticoid on the heart have been studied in some detail (16). However, the role of physiological concentrations of cortisol in the metabolism of the heart in vivo requires clarification. In the isolated perfused heart glucocorticoid decreases glucose uptake by inhibition of phosphofructokinase (6, 7, 12). There is some evidence that glucocorticoid decreases myocardial glucose extraction in man (19). Wahlqvist et al. (18) studied arterial and coronary sinus glucocorticoid concentrations in fasting healthy men and demonstrated uptake and release of glucocorticoid by the myocardium. This phenomenon, however, was not related to cardiac glucose metabolism.

In the present investigation, conscious dogs were studied to define the role of cortisol in cardiac glucose metabolism over a wide range of blood glucose levels. A specific radioimmunoassay for cortisol was employed.

METHODS

Vinyl catheters were implanted into the aorta, inferior vena cava, and coronary sinus of healthy mongrel dogs (18–25 kg body weight) under general anesthesia. The aortic and coronary sinus lines were used for the simultaneous measurement of arterial and coronary sinus concentrations of glucose, free fatty acids (FFA), cortisol, and insulin. The venous line was used for infusions of glucose.

Nine studies were performed on five dogs 1–30 days postoperatively. After an overnight fast, the dogs were studied in the conscious state with the first blood samples taken at 9 A.M. and the last at 1 P.M. Blood samples were obtained before infusion of glucose and after 40-min periods of infusion of glucose at 420, 850, 1060, 2120, 5300, and 10,600 μmol/min.

Whole blood glucose was determined with a Yellow Springs Instruments glucose oxidase electrode system (1). Plasma FFA were extracted according to the method of Trout, Estes, and Freidberg (15) and determined by the colorimetric method of Duncombe (3). Plasma insulin was measured by a modification of a double antibody technique (5).

Plasma cortisol was estimated by an iodinated double antibody technique. In this assay, 100-μl plasma aliquots were diluted with 1 ml of H_2O, then extracted with 4 ml of dichloromethane. After centrifugation, the aqueous layer was aspirated. One-ml aliquots of the dichloromethane extract were transferred to clean glass tubes and evaporated to dryness. The residue was dissolved in a 0.5-ml phosphosaline buffer, which provided two 100-μl aliquots. To each aliquot, 400 μl of "assay binding solution" was added. Assay tubes were vortexed before incubation at 37°C for 1 hr and at 4°C for 30 min. The assay binding solution contained four reagents mixed in a ratio of 1:1:1:1: 1) cortisol antibody raised in rabbits to cortisol conjugated to bovine serum albumin (obtained lyophilized from the Diagnostic Products Corporation), 2) ^{125}I-cortisol (Diagnostic Products Corporation), 3) normal rabbit serum as a "carrier" to aid precipitation of the second antibody–cortisol antibody complex, and 4) second antibody, a goat antibody to rabbit serum (Calbiochem). After incubation, 0.2 ml of cold 20% polyethylene glycol in phosphosaline buffer was added to all tubes, which were then vortexed and centrifuged at 4°C. The supernatant was aspirated and the precipitate counted. The calibration curve was computed by a least squares fit to a logarithmic second-degree polynomial. The inter-assay coefficient of variation for plasma cortisol was 15% and the intra-assay coefficient of variation was less than 10%. Cross-reactivity with corticosterone was less than 1%.

Paired student's t tests and linear regression analyses were applied where appropriate, as found in Snedecor and Cochran (14). The data were

also assessed nonparametrically with the Spearman rank correlation coefficient (r_s) (13). A correction factor for tied observations was incorporated in the computation of r_s. A two-tailed table for critical values of r_s was used to determine levels of significance (2).

RESULTS

Glucose

Arterial concentration (C_a) of glucose rose progressively as glucose infusion rate increased (Figure 1, lower panel). The range of blood glucose concentrations produced was 3700 to 74,400 μmol/liter. C_a glucose was significantly different at each infusion rate by comparison with control. Below

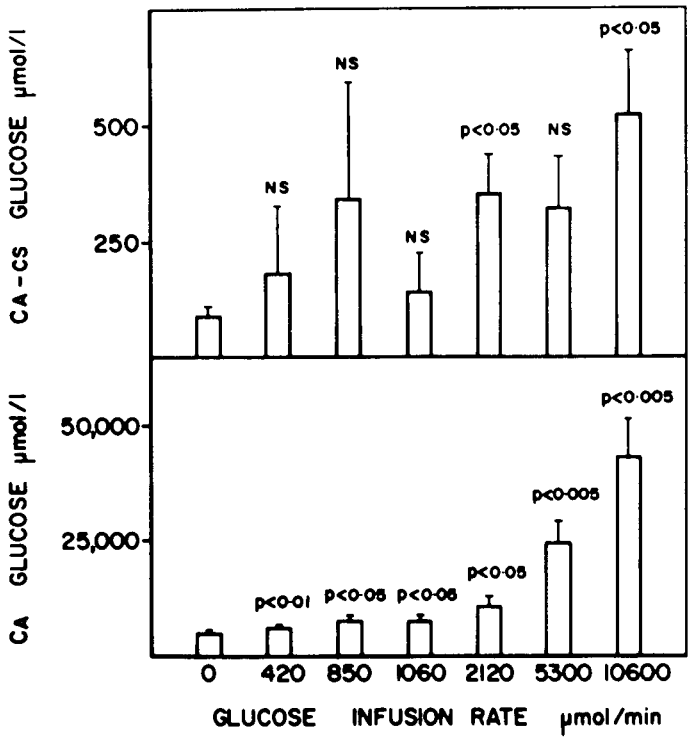

Figure 1. Arterial blood glucose concentrations (C_a glucose) and myocardial glucose extractions (C_{a-cs} glucose) during different rates of intravenous infusion of glucose. Mean ± SEM for nine studies are shown. P values indicate significance of difference from observations where no glucose was infused, assessed by a paired t test.

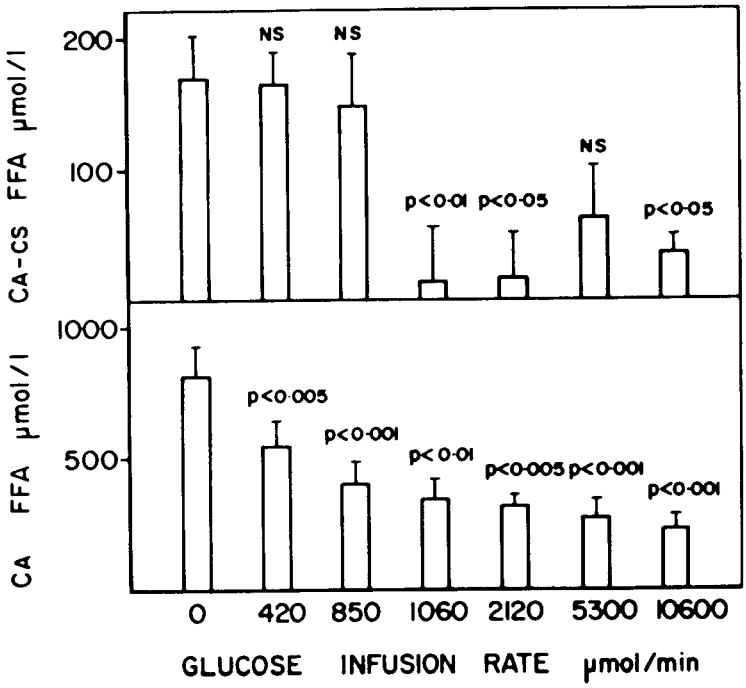

Figure 2. Arterial plasma free fatty acid concentrations (C_a FFA) and myocardial FFA extractions (C_{a-cs} FFA) during different rates of intravenous infusion of glucose (see legend to Figure 1).

the infusion rate of 2120 μmol/min, C_{a-cs} (arterial-coronary sinus concentration difference or myocardial extraction) for glucose was not significantly different from control (Figure 1, upper panel). C_{a-cs} glucose was not significantly correlated with C_a glucose ($N = 50$, $r = 0.23$, $P > 0.05$; $r_s = 0.28$, $P > 0.05$).

Free Fatty Acids (FFA)

As glucose infusion rate increased, C_a FFA fell from 820 ± 110 μmol/liter (mean ± SEM) in the control period to 230 ± 60 μmol/liter (mean ± SEM) at a glucose infusion rate of 10,600 μmol/min (Figure 2, lower panel). Myocardial FFA extraction was not significantly different from control until the glucose infusion rate reached 1060 μmol/min (Figure 3, upper panel). C_{a-cs} FFA was significantly related to C_a FFA ($N = 50$, $r = 0.62$, $P < 0.001$).

Glucose and FFA Interrelationships

By conventional correlation analysis C_{a-cs} glucose was not significantly related to C_a FFA ($N = 50$, $r = -0.26$, $P > 0.05$) or to myocardial FFA extraction ($N = 50$, $r = -0.26$, $P > 0.05$). However, nonparametric assessment with the Spearman rank correlation coefficient, r_s, gave significant values of -0.30 ($P < 0.05$) and -0.39 ($P < 0.02$), respectively, for these relationships.

Cortisol

I_a cortisol during infusions of glucose did not differ significantly from control (Figure 3, lower panel). Myocardial extraction of cortisol (I_{a-cs} cortisol) ranged from -31 to 138 nmol/liter (mean ± SEM, 12 ± 4, $P < 0.01$).

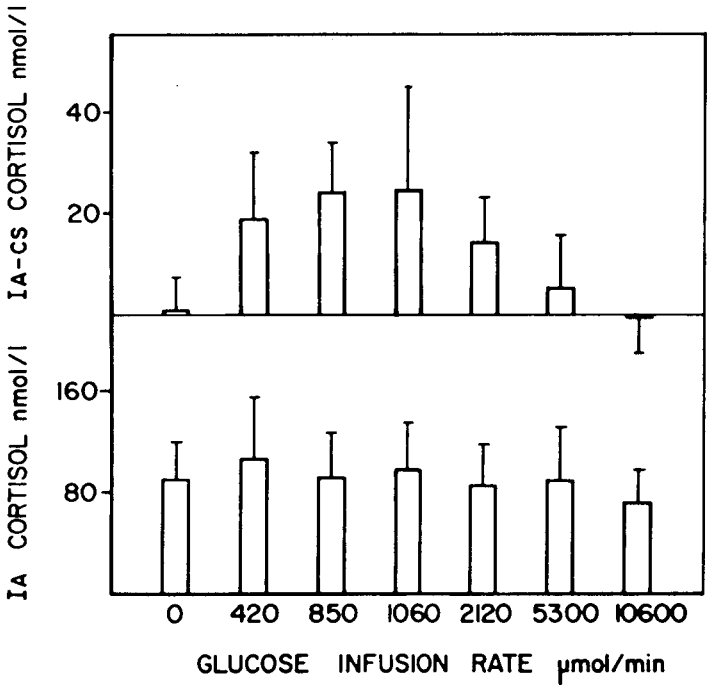

Figure 3. Arterial plasma cortisol immunoreactivity (I_a cortisol) and arterial–coronary sinus differences in cortisol immunoreactivity (I_{a-cs} cortisol) during different rates of intravenous infusion of glucose (see legend to Figure 1).

I_a cortisol was significantly and negatively correlated with C_{a-cs} glucose ($N = 50$, $r = -0.37$, $P < 0.01$; $r_s = -0.44$, $P < 0.01$) (Figure 4). Likewise, I_{a-cs} cortisol was significantly and negatively correlated with C_{a-cs} glucose ($N = 50$, $r = -0.43$, $P < 0.01$; $r_s = -0.50$, $P < 0.001$) (Figure 5).

There was a significant and positive relationship between cortisol and I_a cortisol whether assessed parametrically ($N = 50$, $r = 0.75$, $P < 0.001$) or nonparametrically ($N = 50$, $r_s = 0.58$, $P < 0.001$) (Figure 6).

Insulin

Arterial insulin concentrations (I_a insulin) increased significantly and progressively as glucose infusion rates increased. I_a insulin was 17.3 ± 2.5, 39.4 ± 8.0, 66.1 ± 15.9, 66.7 ± 15.3, 103.0 ± 24.5, 212.9 ± 42.8, and 617.4 ± 141.2 μU/ml (mean ± SEM) at glucose infusion rates of 0, 420, 850, 1060, 2120, 5300, and 10,600 μmol/min, respectively. There was a significant relationship between C_{a-cs} glucose and I_a insulin by both parametric analysis ($N = 50$, $r = 0.31$, $P < 0.05$) and nonparametric analysis ($N = 50$, $r_s = 0.38$, $P < 0.01$) (Figure 7).

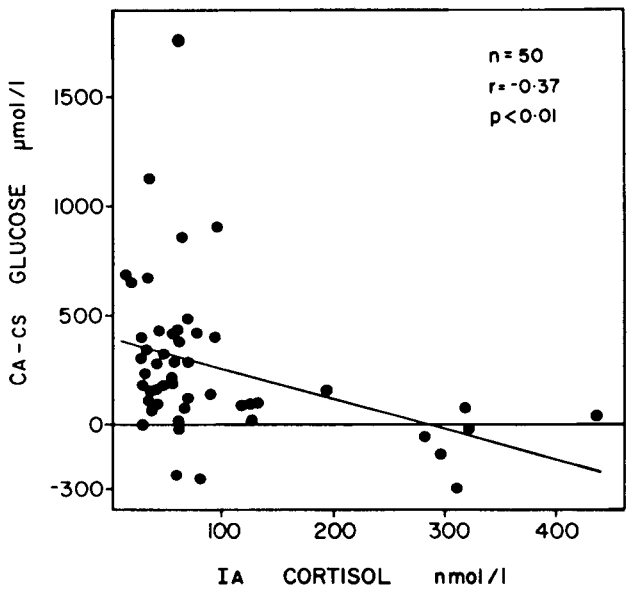

Figure 4. Relationship between myocardial glucose extraction and arterial plasma cortisol immunoreactivity. An equally significant relationship to that obtained on linear regression analysis is the Spearman rank correlation coefficient, $r_s = -0.44$ ($P < 0.01$).

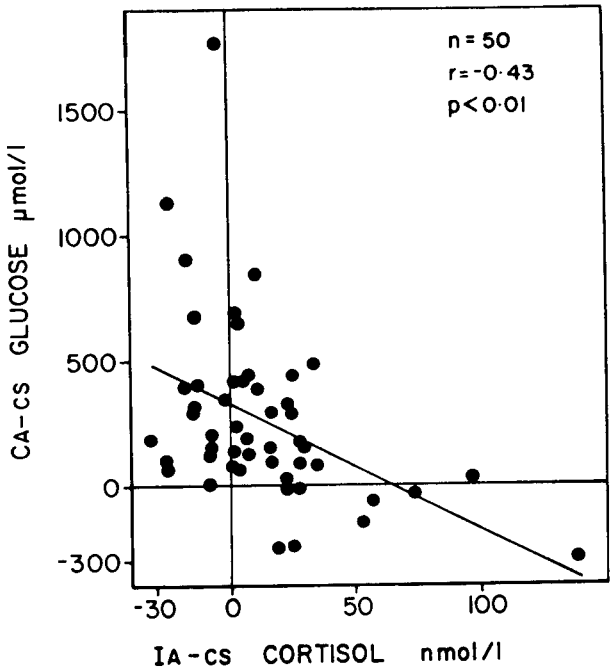

Figure 5. Relationship between myocardial glucose extraction and arterial-coronary sinus difference in cortisol immunoreactivity. An equally significant relationship to that obtained on linear regression analysis is the Spearman rank correlation coefficient, $r_s = -0.50$ ($P < 0.001$).

DISCUSSION

Role of Glucose

Does an increased supply of glucose to the myocardium improve cardiac glucose utilization (17)? In the present investigation, conscious dogs infused with glucose at a rate of 2120 μmol/min had a significant increase in myocardial glucose extraction. On a body weight basis, this would correspond to a glucose infusion rate about three times that which might be used in parenteral nutrition. For this increase in cardiac glucose extraction to have occurred, the mean arterial blood glucose concentration had more than doubled from 4600 μmol/liter to 10,600 μmol/liter. Even so, the increment in arterial glucose concentration was not responsible for the increase in myocardial glucose extraction. Several metabolic events accompany the intravenous infusion of glucose, including release of insulin from the pancreas, possible alterations in insulin-antagonistic hormones, and a decrease in plasma FFA concentrations. Any one of these changes, in its

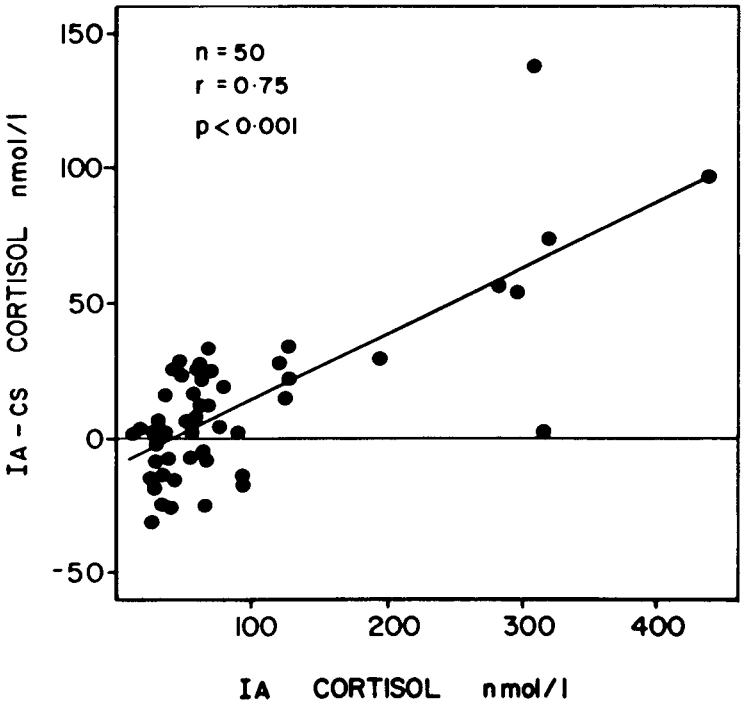

Figure 6. Relationship between arterial–coronary sinus differences in cortisol immunoreactivity and plasma immunoreactivity. The Spearman rank correlation coefficient, r_s, is 0.50 ($P < 0.001$).

own right, could modify myocardial glucose metabolism. In fact, there was no significant correlation between myocardial glucose extraction and arterial glucose concentration, over a wide range.

Role of Free Fatty Acids

Previous work (17) has suggested that there is competition between FFA and glucose for extraction by the human myocardium which would support the hypothesis of Randle and co-workers developed in vitro (11). In the dogs studied, glucose infusion at 1060 μmol/liter or above led to a marked decrease in myocardial FFA extraction, although arterial plasma FFA concentrations fell significantly at glucose infusion rates of 420 μmol/min. The application of nonparametric statistical analysis revealed that there was a negative relationship between myocardial glucose extraction and either arterial plasma FFA concentration or myocardial FFA extraction. These negative relationships were not evident by conventional correlation analysis,

however. It would appear that in the present model, FFA are not a strong negative determinant of myocardial glucose extraction.

Role of Cortisol

Despite the rapid intravenous infusion of glucose, no significant change in arterial plasma cortisol immunoreactivity occurred. To some extent, variations in plasma cortisol immunoreactivities may be attributed to the proximity of a given study to thoracic surgery. Dogs were studied between 1 and 30 days postoperatively. In any case, there was a range of plasma cortisol at which myocardial glucose extraction could be examined.

There was a significant negative relationship between myocardial glucose extraction and arterial plasma cortisol immunoreactivity. This was so whether parametric or nonparametric statistical analyses were applied. In view of the distribution of data in the present investigation, the nonparametric mode of analysis is probably preferable. The data are consistent

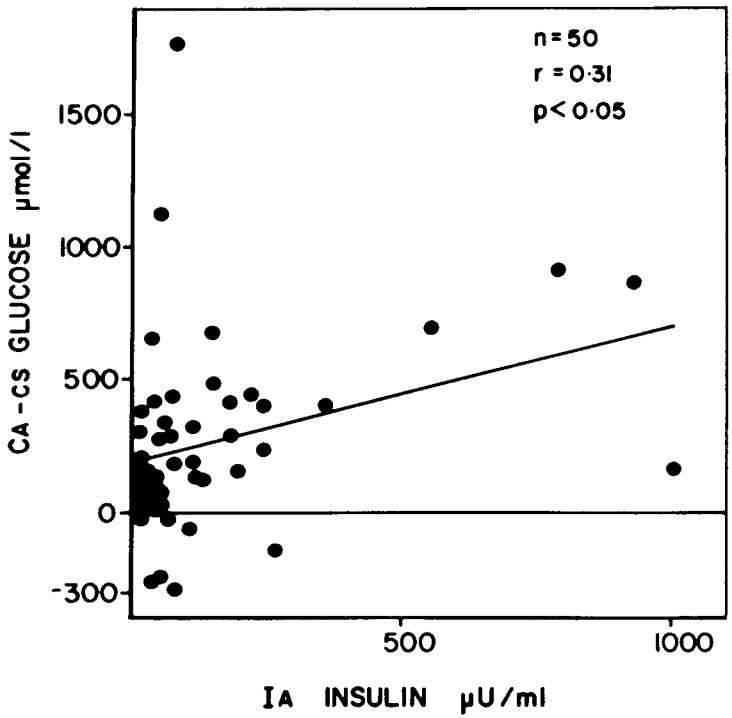

Figure 7. Relationship between myocardial glucose extraction and arterial plasma insulin immunoreactivity. The Spearman rank correlation coefficient, r_s, is 0.38 ($P < 0.01$).

with observations in the isolated perfused heart (6, 7, 12) showing that glucocorticoid decreases myocardial glucose utilization probably through inhibition of phosphofructokinase. In vivo, where a number of other variables might operate to influence cardiac glucose metabolism, cortisol emerges with an effect comparable to that found in vitro.

Previous work has shown that glucocorticoid measured fluorimetrically can be taken up and released in the human coronary circulation (18). However, these events have not been linked to myocardial glucose metabolism. An assay more specific for cortisol and the dog model have allowed a significant negative relationship between myocardial glucose extraction and arteriovenous differences of cortisol across the coronary circulation to be recognized. Glucocorticoid associated with cytosol receptors in dog heart probably amounts to about 30 pmol/g wet wt (4). For a 100-g heart this would total 3 nmol of glucocorticoid. With a mean cortisol extraction of 12 nmol/ml in the present investigation and a presumed coronary plasma flow of about 50 ml/min, cortisol would be taken up by the heart at a rate of 600 nmol/min, greatly in excess of receptor binding potential. Corticosterone, a less important glucocorticoid in the dog than cortisol (9), has not been considered in the present investigation and may further contribute to cardiac glucocorticoid turnover. Although the mass changes of cortisol across the coronary circulation apparently exceed those predicted from considerations of steroid/receptor interaction, there seems to be some way in which these arteriovenous differences in cortisol are linked to glucose metabolism. There could, of course, be a diminishing concentration gradient of cortisol from coronary circulation to receptor (10). Cortisol movement through the extracellular space to lymphatics or degradation in the heart may account for the difference between the flux of cortisol from the vascular compartment and the mass present at the cytosolic receptor.

Another possibility is that glucose extraction decreases cortisol extraction. However, glucocorticoid-receptor interaction appears energy dependent, and therefore an additional energy supply such as glucose should actually increase cortisol extraction (8). The negative relationship seen in the present study would indicate that changes in glucose extraction are unlikely to have led to the arteriovenous differences of cortisol in the coronary circulation.

The present study confirmed previous work in man postulating that, in certain circumstances, the higher the plasma cortisol, the greater the arterial–coronary sinus difference of cortisol (18).

Role of Insulin

Although there is little doubt that insulin is a determinant of myocardial glucose utilization in vitro, evidence for a direct role of insulin in vivo has

been scanty. By multivariate analysis, Wahlqvist et al. (19) provided evidence that insulin acted independent of FFA and glucocorticoid in man to determine glucose extraction. This work in conscious dogs indicates a direct relationship between myocardial glucose extraction and arterial insulin immunoreactivity. Nonparametric and parametric analyses have been applied because of the non-normal distribution of the observations. Insulin appears to be a determinant of glucose extraction using either method. Further work is required to determine whether in the present model insulin operates independent of FFA, cortisol, and arterial glucose concentrations to determine myocardial glucose extraction.

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