Morning Cortisol Levels and Glucose Effectiveness

J.M. Fernández-Real, R. Casamitjana, and W. Ricart

Anabolic corticosteroids have been reported to enhance glucose effectiveness (S_G). In experimental models of long-term cortisol infusion in diabetic dogs, the maintenance of normal S_G during chronic hypercortisolemia prevented a significant deterioration of glucose tolerance. We hypothesized that in analogy with exogenous corticosteroids, endogenous cortisol might influence S_G. We aimed to study the influence of serum cortisol on S_G prior to a frequently sampled intravenous glucose tolerance test (FSIVGTT) in 18 otherwise healthy men. The serum cortisol level or free cortisol index (ratio of cortisol to cortisol-binding globulin [CBG]) were not associated with the body mass index (BMI), waist to hip ratio (WHR), fasting insulin, or insulin sensitivity ([S_I] all $r < .20$, P = NS). Conversely, S_G correlated with serum cortisol levels measured prior to the FSIVGTT ($r = .60$, $P = .008$) and with the free cortisol index ($r = .48$, $P = .03$). The association was stronger in lean subjects (BMI < 25 kg/m^2, $r = .90$, $P = .002$, n = 8). Men with a pre-FSIVGTT serum cortisol level above the median (431 nmol/L) were similar by age, BMI, WHR, and S_I to the subjects with cortisol levels below the median, but the latter presented a significantly decreased S_G (0.0014 ± 0.006 *v* 0.022 ± 0.007 min^{-1}, $P = .03$). In multiple linear regression analysis, fasting glucose ($P = .02$) and serum cortisol ($P = .027$) independently predicted S_G, contributing to 26% of its variance. In summary, our findings suggest that the prevailing cortisol level appears to be associated with S_G. The lower cortisol levels usually found in abdominally obese men could contribute to their altered glucose tolerance, perhaps via decreased S_G.

GLUCOSE DISPOSAL in humans occurs as a result of both insulin-mediated and non–insulin-mediated glucose uptake. The latter contributes to approximately 75% of glucose disposal under euglycemic conditions. Glucose effectiveness (S_G) is a measure of the action of glucose independent of insulin. S_G is defined as the effect of glucose itself, at basal insulin, to promote its own disposal through uptake by mass action into the tissues and through suppression of endogenous glucose production.[1] The mass-action effect of glucose to stimulate its own uptake is well described.[2] Hyperglycemia recruits insulin-independent glucose transporters (GLUT1 and GLUT2) to the cell surface and stimulates calcium-mediated intracellular enzymes that increase glucose uptake.[2]

Different factors that influence S_G have been described.[2] Among them, exercise conditioning, certain oral hypoglycemic agents, glucagon-like peptide, and a reduction of free fatty acid levels all enhance S_G.[2-6] Anabolic corticosteroids also have been reported to enhance S_G.[7] Corticoid-like effects of some of these anabolic steroids have been observed in humans.[8] In experimental models of long-term cortisol infusions in diabetic dogs, the maintenance of normal S_G during chronic hypercortisolemia prevented a significant deterioration of glucose tolerance despite marked insulin resistance.[9]

We hypothesized that, in analogy with exogenous corticosteroids, endogenous cortisol may influence S_G.

SUBJECTS AND METHODS

Subjects

Eighteen healthy men with a body mass index ([BMI] weight in kilograms divided by the square of the height in meters) less than 35 kg/m^2 were studied. The waist circumference was measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteal region. None of the subjects used any medication (including steroids) or had any evidence of metabolic disease other than obesity. Liver disease and thyroid dysfunction were specifically excluded by a biochemical workup. All subjects reported that their body weight was stable for at least 3 months before the study, and all were normotensive and had normal blood lipid levels (data not shown). The subjects consumed a weight-maintaining diet containing at least 300 g carbohydrate per day, refrained from exertion, and abstained from caffeine and alcohol use for 72 hours before the tests. The protocol was approved by the Hospital Ethics Committee, and informed consent was obtained from each subject.

Study Protocol

A standard oral glucose tolerance test was performed in all subjects. Insulin sensitivity was analyzed using the frequently sampled intravenous glucose tolerance test (FSIVGTT) with minimal-model analysis as described elsewhere.[10] In brief, the experimental protocol started between 8:00 and 8:30 AM after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at −30, −10, and −5 minutes, after which glucose (300 mg/kg body weight) was injected over 1 minute starting at time 0. Additional samples were obtained from a contralateral antecubital vein until 180 minutes.

The serum glucose level during the FSIVGTT was measured in duplicate by the glucose oxidase method with a Glucose Analyzer 2 (Beckman, Brea, CA). The coefficient of variation was 1.9%. The serum insulin level during the FSIVGTT was measured in duplicate by monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). The lowest limit of detection was 4.0 mU/L. The intrassay coefficient of variation was 5.2% at a concentration of 10 mU/L and 3.4% at 130 mU/L. The interassay coefficient of variation was 6.9% and 4.5% at 14 and 89 mU/L, respectively.

Serum cortisol was evaluated prior to the FSIVGTT in basal samples obtained from the indwelling catheter at −10 and 0 minutes, 20 and 30 minutes after venipuncture. The mean arithmetic value of the 2 measurements was considered as basal cortisol. Its concentration was determined by a microparticle enzyme immunoassay, (IMX System; Abbott Laboratories, Abbott Park, IL) with an intraassay and interassay coefficient of variation less than 8%. Plasma cortisol-binding globulin (CBG) was determined by radioimmunoassay (Radim KP31; Angleur, Liège, Belgium). The intraassay and interassay coefficient of variation

From the Diabetes Unit, Department of Endocrinology and Nutrition, University Hospital of Girona "Dr Josep Trueta," Girona; and the Hormone Laboratory, University Hospital Clinic, Barcelona, Spain.

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Address reprint requests to J.M. Fernández-Real, MD, PhD, Department de Endocrinología, Hospital de Girona, Ctra. França s/n, 17007 Girona, Spain.

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was 3.6% and 7.5%, respectively. To estimate the level of free circulating cortisol (free cortisol), the ratio of cortisol to plasma CBG was used.[11]

Data from the FSIVGTT were entered into computer programs that calculate the characteristic metabolic parameters by fitting glucose and insulin to the minimal model that describes the time course of glucose and insulin concentrations. The glucose disappearance model, by accounting for the effect of insulin and glucose on glucose disappearance, provides the parameters S_I (10^{-4} per minute per milliunit per liter), a measure of the effect of insulin above the basal level to enhance glucose disappearance, and S_G (per minute), or glucose effectiveness, defined as the effect of glucose itself, at basal insulin, to promote its own disposal through uptake by mass action into the tissues and via suppression of endogenous glucose production. The estimation of model parameters was performed according to the MINMOD computer program.[1]

Statistical Analysis

Descriptive results of continuous variables are expressed as the mean and range. Before the statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these criteria (CBG, free cortisol, and S_G) were logarithmically transformed. We used unpaired *t* tests for comparisons of quantitative variables. Relationships between variables were tested by Pearson's correlation coefficient and stepwise multivariate linear regression analysis with forward selection. The regression coefficient generated by this analysis indicates the slope of the association between the dependent variable and the specified independent variable after adjustment for other independent variables in the model. The level of statistical significance was set at a value *P* value less than .05. Statistical analyses were performed with the BMDP statistical package (BMDP Statistical Software, Cork, Ireland).

RESULTS

Anthropometric and biochemical characteristics of the subjects are shown in Table 1. Four obese subjects showed impaired glucose tolerance on a standard oral glucose tolerance test. Exclusion of these subjects resulted in little change in the findings, so they were retained in the analysis to increase statistical power. Serum cortisol or free cortisol levels were not associated with the BMI, waist to hip ratio (WHR), fasting insulin, or insulin sensitivity (all $r < .20$, P = nonsignificant [NS]).

S_G correlated weakly with serum glucose ($r = -.47$, $P = .054$). S_G was positively associated with the morning serum cortisol level ($r = .60$, $P = .008$) and free cortisol level ($r = .48$, $P = .03$) (Fig 1). When only lean subjects (BMI < 25 kg/m^2) were considered, the association between S_G and serum cortisol was even stronger ($r = .90$, $P = .002$, n = 8), although only a trend between S_G and free cortisol ($r = .55$, $P = .1$) was observed.

Table 1. Anthropometric and Biochemical Variables in the Study Subjects (N = 18)

Variable	Mean	Range
Age (yr)	37.3	20-46
BMI (kg/m^2)	29.4	22-35
WHR	1.00	0.9-1.1
Fasting glucose (mmol/L)	5.4	4.5-6.5
Fasting insulin (mU/L)	12.9	4.5-32
Insulin sensitivity (min^{-1}/mU/L)	1.8	0.01-4.2
S_G (min^{-1})	0.018	0.006-0.034
Plasma cortisol (nmol/L)	428	187-595
CBG (mg/L)	34	25-49
Free cortisol index	12.8	5.8-17.4

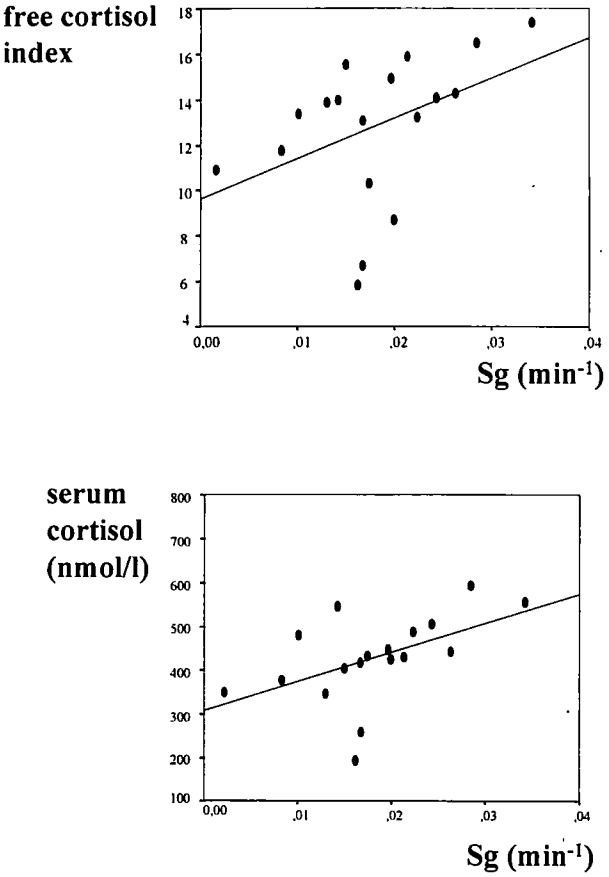

Fig 1. Linear correlation between S_G and the free cortisol index ($r = .48$, $P = .03$) and serum cortisol ($r = .60$, $P = .008$).

The men with a pre-FSIVGTT serum cortisol level below the median (431 nmol/L) presented a significantly decreased S_G (0.0014 ± 0.006 *v* 0.022 ± 0.007 min^{-1}, $P = .03$). Men with a serum cortisol level below and above the median were similar in age, BMI, WHR, and insulin sensitivity (Table 2). S_G correlated significantly with serum glucose ($r = -.76$, $P = .016$) in men with serum cortisol above the median level.

A multiple linear regression analysis to predict S_G was constructed with fasting glucose, WHR, and serum cortisol as independent variables. In this model, both fasting glucose ($P = .02$) and serum cortisol ($P = .027$) independently predicted S_G, contributing to 26% of its variance.

DISCUSSION

In this study, we have found that serum cortisol levels measured prior to the FSIVGTT seem to influence S_G. This association was maintained even after controlling for CBG levels. However, the design of the study does not allow an isolation of the direct effects of cortisol on S_G. Different cortisol

Table 2. Comparison of Men with Serum Cortisol Levels Below and Above the Median

Variable	Cortisol ≤431 nmol/L	Cortisol >431 nmol/L	P
No. of subjects	9	9	–
Age (yr)	39.2 ± 7.3	35.4 ± 9	NS
BMI (kg/m²)	29.4 ± 3.6	29.4 ± 5.4	NS
WHR	1.02 ± 0.05	0.99 ± 0.03	NS
Fasting glucose (mmol/L)	5.5 ± 0.5	5.4 ± 0.6	NS
Fasting insulin (mU/L)	13.3 ± 7.5	12.4 ± 7.8	NS
Insulin sensitivity (min^{-1}/mU/L)	1.54 ± 1.4	2.07 ± 1.4	NS
S_G (min^{-1})	0.014 ± 0.006	0.022 ± 0.007	.03
Plasma cortisol (nmol/L)	355.9 ± 82.9	500.3 ± 56	.001
CBG (mg/L)	32.6 ± 7.4	35.4 ± 3.8	NS
Free cortisol index	11.4 ± 3.6	15.2 ± 2	.04

clamps, at low and high cortisol levels, will be required to better characterize this hypothesis. Acute exercise has been described to augment S_G,[12] and is a situation in which increased cortisol levels are usually found.[13] In the opposite way, anorexia nervosa, a state of relative cortisol resistance,[14] is accompanied by lower S_G.[15] As described earlier, anabolic corticosteroids with corticoid-like effects have been reported to enhance S_G,[7] and in experimental models of long-term cortisol infusions, maintenance of normal S_G during chronic hypercortisolemia prevented a significant deterioration of glucose tolerance.[9] We have found that endogenous cortisol is associated with enhanced S_G similarly to exogenous corticosteroids.

The relationship between cortisol levels and S_G was stronger in men with a BMI less than 25 kg/m², suggesting that the usually higher morning cortisol levels of lean subjects[16-18] are associated with enhanced S_G. On the contrary, men with abdominal obesity are characterized by lower morning cortisol levels.[16-18] We describe here a decrease in S_G in subjects with cortisol levels below the median, even after accounting for the BMI and WHR. However, the WHR is an imperfect marker of central obesity. We cannot exclude the possibility that subjects with cortisol levels below the median may in fact have shown more evidence of central obesity with the use of more sophisticated methodology. Together with insulin resistance, the lower cortisol levels could contribute, perhaps via impaired S_G, to the altered glucose tolerance usually found in abdominal obesity. Furthermore, a decrease in S_G, usually found in patients with type 2 diabetes[19] and partially confirmed by the negative correlation between S_G and fasting glucose in our study subjects, would worsen intracellular glucopenia.

It will be necessary to study the molecular events involved in the association between S_G and cortisol clearance to properly characterize the mechanisms. In fact, it is formally possible that both S_G and cortisol levels are associated with a third, unknown factor that influences both parameters. In summary, the results of the present study suggest that prevailing cortisol levels appear to be associated with S_G in men.

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