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High anticipatory stress plasma cortisol levels and sensitivity to glucocorticoids predict severity of coronary artery disease in subjects undergoing coronary angiography

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Abstract

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and/or increased sensitivity of peripheral tissues to glucocorticoids may be associated with the dysmetabolic syndrome and its cardiovascular sequelae. In this prospective pilot clinical study, we examined possible associations between HPA axis activity and severity of cardiovascular disease. We measured morning serum cortisol and intima media thickness (IMT) of carotid and femoral arteries in 105 subjects before undergoing coronary angiography for suspected coronary artery disease (CAD). In a randomly selected 46 of these subjects, we obtained late afternoon and morning cortisol levels (after ultralow-dose dexamethasone [0.25 mg] treatment) and determined their genotype for the *Bcl1* polymorphism of the glucocorticoid receptor gene, which has been associated with increased sensitivity to glucocorticoids. There was significant association between morning preangiography cortisol levels and the number of vessels with severe stenosis in the angiography, independently of age or sex ($P = .002$), and a trend for a positive correlation between morning cortisol and the IMT of the femoral artery ($P = .057$). *Bcl1* G allele homozygotes had a significantly higher carotid IMT ($P = .005$) and a nonsignificant tendency for higher waist-hip ratio ($P = .059$). Hyperactivity of the HPA axis in anticipation of a stressful procedure, such as angiography, may be an index of CAD severity. Chronic HPA axis hyperreactivity combined with tissue hypersensitivity to glucocorticoids may contribute to more severe atherosclerosis and CAD.

1. Introduction

Strong associations between increased hypothalamic-pituitary-adrenal (HPA) axis activity and the dysmetabolic syndrome and, hence, the resultant atherosclerotic cardiovascular disease, have been reported or suggested, respectively [1-4]. In about 35% of normal volunteers, the corticotropin and cortisol response to exercise is not suppressed after pretreatment with a high dose (4 mg) of dexamethasone [5]. These individuals, designated *high responders*, also had high cortisol levels before the exercise [6], increased cortisol responses to a compound psychological stress, and a high Spielberger anxiety score compared with *low responders* [7]. It is not known whether high-responder types of individuals have any long-term health consequences from the hyperactivity of their HPA axis.

Individuals carrying the *Bcl1* polymorphism of the glucocorticoid receptor (GR) gene have increased sensitivity to an ultralow dose (0.25 mg) of dexamethasone and features of the dysmetabolic syndrome [8-10]. Associations with increased cardiovascular risk have also been suggested [11]. This intronic polymorphism may indirectly affect promoter activity [8].

We hypothesized that patients with atherosclerotic cardiovascular disease may represent a population of persons with increased activity or reactivity of the HPA axis and/or increased target tissue sensitivity to glucocorticoids. In this pilot study, we assessed basal serum cortisol

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concentrations in a group of highly selected patients undergoing coronary angiography. Cortisol was measured at a moment of severe anticipatory stress at the morning of the angiography. In a subgroup of these subjects an ultralow-dose overnight dexamethasone-suppression test was performed. This has been proposed as an index of pituitary cell sensitivity to glucocorticoids; we aimed to use the responsiveness of pituitary cells as an indirect index of general tissue sensitivity to glucocorticoids. In the same group of subjects, late afternoon serum cortisol was also measured to examine diurnal variation of the axis. Possible associations of these indices with the severity of coronary artery disease (CAD) and peripheral atherosclerosis were evaluated.

2. Subjects and methods

A group of 105 patients (age range, 36-78 years; 81 men, 24 women; Group A) referred for coronary angiography were examined. The extent of CAD was coded as 0, 1, 2, or 3 according to the number of coronary vessels with a 50% or greater narrowing in the coronary angiography; thus, an individual who had 2 coronary vessels with stenosis of the luminal diameter exceeding 50% was coded as 2, and an individual with 0 coronary arteries with 50% stenosis was coded as 0.

In the morning of the angiography procedure, before the catheterization, a fasting serum sample was taken for determination of biochemical parameters and cortisol and insulin. Testosterone levels were also measured as an indirect index of stress because lower levels have been reported in stressed male individuals; on the other hand, higher levels have been reported to be harmful for vascular health in women. Serum cortisol was determined by a two-site chemiluminescence immunoassay, using the Nichols "Advantage" (Nichols Diagnostics, Los Angeles, CA), with intra-assay and interassay coefficients of variation of 3.67% and 4.5%, respectively, and a reference range of 2.9 to 18.8 μg/dL (80-520 nmol/L). Serum insulin was measured by enzyme-linked immunosorbent assay (Boehringer Mannheim, Indianapolis, IN); serum total testosterone was measured by chemiluminescence (ACS-180, Bayer, Hollistone, MA). Basal insulin resistance was estimated by using the homeostasis model assessment, applying the formula: Insulin resistance = FI × G/22.5, where FI is fasting insulin (μU/mL) and G is fasting glucose (mmol/L).

In all subjects, the intima media thickness (IMT) of the left carotid artery, carotid bulb and left femoral artery were measured by ultrasonography as an index of peripheral atherosclerosis as previously described [12]. In a random subgroup of 46 subjects (age, 36-76 years; 37 men, 9 women; Group B), an afternoon sample for cortisol determination was also taken between 5:30 and 6:00 PM. A low dose of dexamethasone (0.25 mg) was administered orally in the form of a tablet to these patients between 10:30 and 11:00 PM on the same day and an additional blood sample was taken for cortisol determination (after dexamethasone treatment) the following morning (8:30-9:00 AM) (blood sampling was not done in 3 subjects). To ensure compliance, we asked the physician on call to administer the dexamethasone and to ascertain that it had been taken in his presence.

DNA was extracted from peripheral blood lymphocytes by using standard methods (n = 36 from group B because cells for DNA extraction were not available in the first 10 individuals). The method used involves collection of 3 mL of peripheral blood in EDTA, separation of the buffy coat containing the lymphocytes after brief centrifugation, incubation with proteinase-K and sodium dodecyl sulfate, followed by phenol/chloroform extraction. The first intron of the GlucR gene was amplified using the conditions described by Fleury et al [13]. The polymerase chain reaction fragments were digested with the enzyme *Bcl1*, which recognizes the polymorphic sequence (C to G) in intron 2 of the GR gene. The fragment analysis was performed by electrophoresis in polyacrylamide gel with appropriate molecular size markers and ethidium bromide staining. The most common genotype (wild type) is designated CC; the heterozygotes, CG; and the homozygotes, GG.

2.1. Statistical analyses

All parametric data are expressed as mean ± SEM. Univariate analysis of the association between cortisol levels and the number of arteries with significant stenosis, as well as with arterial IMT, was performed with standard regression techniques for continuous and categorical variables. The interaction between hormonal and vascular parameters was evaluated by multiple stepwise regression analysis. When appropriate, χ^2 test with Yates continuity correction was used. Student t test was used to compare mean values between groups of the 3 *Bcl1* genotypes, when

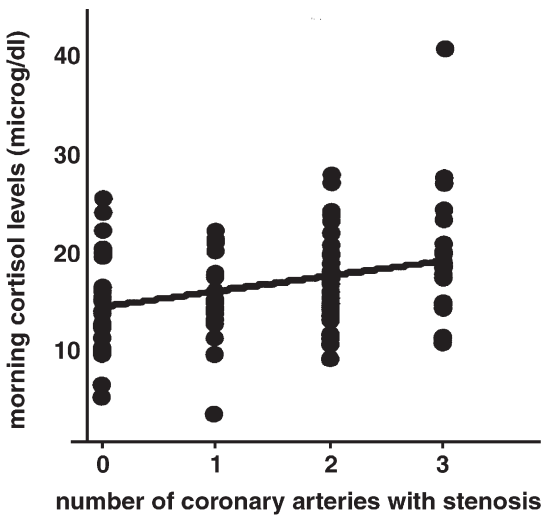

Fig. 1. Association between early morning anticipatory stress plasma cortisol levels and severity of CAD in subjects undergoing coronary angiography (r = 0.384, P < .001).

Table 1
Stepwise multiple regression model for predicting extent of CAD (0, 1, 2, or 3 arteries with stenosis): models 1 and 2

Variable	Predictor	β Coefficient	T	P	Overall r^2
Model 1					
Extent of CAD	Cortisol	0.412	2.857	.0071	0.334,
	Age	0.365	2.55	.0152	P = .002
	Sex	−0.325	−1.28	.0249	
Model 2					
Extent of CAD	Cortisol	0.333	3.75	<.001	0.220,
	Hypertension		0.48	NS	P = .000
	Smoking		0.58	NS	
	Diabetes mellitus	0.340	3.83	<.001	
	Cholesterol		0.39	NS	

NS indicates not significant.

the distribution was normal; the Mann-Whitney rank test was used otherwise.

3. Results

The range of morning cortisol levels was 2.6 to 36.7 μg/dL. There was a highly significant positive linear correlation between morning anticipatory cortisol levels and the number of arteries (0, 1, 2, 3) with significant stenosis (>50%) in the arteriography (r = 0.384, P < .001; Fig. 1). Stepwise multiple regression analysis showed that this association was independent of age or sex (Table 1, model 1). In further analysis, other predisposing factors such as hypertension, smoking, diabetes mellitus, and cholesterol were included in the model, and the results showed that the association with cortisol remained significant (Table 1, model 2). There was a trend for a positive correlation between morning cortisol levels and the IMT of the left femoral artery (r = 0.184, P = .057). In the univariate analysis, there was no significant association between the extent of CAD and insulin levels (r = 0.046, P = .508), testosterone levels (men: r = 0.044, P = .639), or the HOMA basal insulin resistance index (r = 0.117, P = .092). No association of testosterone with CAD severity was found in women either; however, the number of female subjects examined was small.

The cortisol responses to the ultralow-dose dexamethasone suppression appeared to have a bimodal distribution: because there is no standard to define the response, a cutoff value of 3.6 μg/dL (100 nmol/L) for dexamethasone suppression was chosen because this was the point separating the 2 populations. Subjects with suppression of cortisol (n = 34) after the low-dose dexamethasone to values less than 3.6 μg/dL (100 nmol/L) showed a tendency to lower anticipatory morning cortisol levels (12.9 ± 0.44 μg/dL, mean ± SEM) than those who had no such suppression (n = 9) (14.8 ± 0.88 μg/dL, P = .07). The group that did not show suppression after dexamethasone had severe CAD (3-vessel disease) more frequently (4/10, 40%) than those who demonstrated suppression (5/32, 16%); however, this difference was not significant.

The distribution of the *Bcl1* polymorphism in the 36 patients where DNA was available was as follows: CC = 15 (41.6%), CG = 17 (47.2%), GG = 4 (11.1%) subjects. Allele frequency was as follows: C = 65.3%, G = 34.7%. This distribution did not differ from that expected according to the Hardy-Weinberg equilibrium and is similar to that reported in other Caucasian populations [8].

The degree of decline in late afternoon cortisol levels (delta PM cortisol, ie, the difference between the morning and afternoon values) was significantly greater in homozygous carriers of the G allele; accordingly, the afternoon cortisol levels tended to be lower in GG homozygotes (Table 2). Furthermore, the waist-hip ratio tended to be higher in the GG homozygotes (Table 2). The degree of cortisol suppression by the low-dose dexamethasone was also greater in homozygotes; however, this difference was not significant (Table 2). Finally, the IMT of the left common carotid artery was significantly greater in GG homozygotes (Table 2). The severity of CAD did not differ according to *Bcl1* genotype, CC, CG, and GG, respectively: 0 vessel: 11.8%, 11.8%, and 2.9%; 1 vessel: 8.8%, 2.9%, and 2.9%; 2 vessels: 8.8%, 20.6%, and 0%; 3 vessels with stenosis: 14.7%, 8.8%, and 5.9%; Pearson χ^2 = 1.461, not significant.

4. Discussion

Preangiography cortisol concentrations correlated significantly and independently with the severity of CAD, possibly

Table 2
Associations of *Bcl1* genotype of the glucocorticoid receptor with biochemical and clinical indices in subjects that underwent coronary angiography

	CC (n = 15)	GC (n = 17)	GG (n = 4)	P
Basal cortisol (μg/dL)	12.7 ± 4.1 (2.6-17.5)	13.3 ± 2.7 (9.5-17.5)	14.1 ± 2.5 (11.8-17.7)	.39
6 PM cortisol	6.3 ± 3 (1.7-11)	5.1 ± 3.2 (2.1-14.4)	3.5 ± 1.6 (1.5-5.3)	.11
Postdexamethasone cortisol (μg/dL)	4.2 ± 4.3 (0.7-12.9)	2.7 ± 3.5 (0.8-9.1)	2.5 ± 2.7 (0.7-6.4)	.27
Delta PM cortisol	7.1 ± 2.4 (3-9.5)	8.5 ± 4.1 (2.9-13.5)	10.6 ± 1.5 (8.8-12.4)	.009[a]
Delta postdexamethasone cortisol	9.1 ± 4.1 (2-16.4)	10.1 ± 3.4 (3.1-15.8)	11.6 ± 1.1 (10.9-13.3)	.1
Waist-hip ratio	0.91 ± 0.13	0.91 ± 0.08	1.0 ± 0	.059[a]
Waist	90.7 ± 29	94.7 ± 11	106 ± 4	.09[a]
BMI	26.9 ± 3.5	26.5 ± 3.9	27.4 ± 1.8	.8
LICA (IMT)	0.7 ± 0.23	0.8 ± 0.7	1.3 ± 0.22	.005[a]

LICA = left internal carotid artery.
[a] For the difference between CC and GG homozygotes, Mann-Whitney test.

showing that hyperactivity of the axis, obviously over many years, might have been detrimental to the cardiovascular health of these individuals. Several studies have examined the potential association of the activity of HPA axis with coronary atherosclerosis. Some studies reported that higher cortisol levels were associated with more severe CAD, whereas others did not [14-16]. A very recent study performed in middle-aged men showed a significant predictive value of elevated cortisol–testosterone ratio for the future development of ischemic heart disease. Cortisol levels alone were not predictive of future events in that study, however; these were samples taken under unstressed routine examination [17]. It is possible that we found this significant correlation because cortisol samples were obtained under conditions of significant anticipatory stress. In our study, increased cortisol levels were also marginally associated with the severity of peripheral atherosclerosis. A similar association was previously reported for urinary free cortisol in young diabetic subjects [18].

Person-to-person variability of the HPA axis activity in healthy individuals has been previously described [4-6]. In a series of earlier studies, the exercise-induced stimulation of the pituitary adrenal axis was examined. The degree of suppression of this activation by dexamethasone pretreatment helped identify 2 groups showing different patterns of HPA axis activity: one group had full suppression of the axis and low basal preexercise cortisol levels (*low responders* to stress) and one group escaped dexamethasone suppression and had high basal preexercise cortisol levels and thus a hyperactive axis (*high responders* to stress). The latter also had indices of sympathetic activation, such as increased heart rate and postexercise glucose increase, as well as high arginine-vasopressin response to exercise. It was speculated then that these individuals might have long-term adverse effects of stress system hyperactivity on health. On the basis of the results of the present study, one could speculate that in this highly selected group of individuals with CAD who underwent coronary angiography, those with a hyperactive axis, as reflected by higher basal preangiography cortisol levels, possibly resemble the model of high responders of the earlier studies, although a totally different experimental setting was used then. This suggestion is strengthened by our findings that the individuals who did not show suppression of their cortisol by ultralow dexamethasone dose to less than 100 nmol/L (3.6 μg/dL) tended to have more severe coronary disease and higher basal cortisol levels.

In addition and in parallel to the possible detrimental effect of cortisol on the vasculature due to chronic hyperactivity of the HPA axis, increased tissue sensitivity to glucocorticoids resulting from a more sensitive glucocorticoid signaling cascade may also contribute to atherosclerosis in a non–mutually exclusive fashion [19]. Association of sensitivity or exposure to glucocorticoids and increased risk for cardiovascular disease has been suggested by different studies [1,20,21]. To assess the sensitivity of the HPA axis to glucocorticoids, we performed the ultralow-dose overnight dexamethasone suppression test [8,22]. Although the response to this test examines specifically the sensitivity of pituitary cells to the negative feedback action and does not in any way preclude the level of glucocorticoid action in other tissues, especially because of the complexity of the glucocorticoid signaling system, we reasoned that it could be viewed as an indirect index of sensitivity to glucocorticoids in at least one cell type. However, we did not find any significant findings concerning associations between sensitivity assessed in this indirect manner and severity of CAD.

Several studies have shown that the *Bcl1* polymorphism is associated with increased sensitivity to glucocorticoids. This was confirmed in our study despite the limited number of subjects studied, as homozygous carriers of the most sensitive *Bcl1* allele had a tendency for higher post–dexamethasone cortisol suppression, possibly reflecting pituitary cell sensitivity [8,22]. It is interesting that the G carriers had normal diurnal variation of their serum cortisol; in fact, their afternoon levels were even lower than those of CC carriers, thus showing that in this group glucocorticoid sensitivity is independent of hyperactivity of the axis. It is likely that the higher delta PM cortisol difference in these subjects is due to the fact that they had the highest morning levels. It should be noted that the functional effect of polymorphisms of the glucocorticoid receptor on the sensitivity to glucocorticoids may vary from tissue to tissue and that it is possible that the sensitivity of peripheral tissues may be higher than at the feedback level, as already suggested [23,24].

Interestingly, in our patients carrying the sensitive GR signaling system (GG homozygotes), the IMT of the left carotid artery was significantly increased, showing indirectly that increased glucocorticoid action over the years might also be an important factor for peripheral atherosclerosis. Accordingly, an increased waist-hip ratio, a possible clinical index of excess chronic cortisol action, was also present in the GG homozygotes, although this just missed statistical significance. An analogous observation in GG homozygotes was previously reported [9,10].

A few earlier studies explored the possible associations of other genetic variants of the GR with CAD. One such study found an increased frequency of the N363S polymorphism in subjects with angina compared with those without [25], whereas another correlated this polymorphism with visceral obesity and markers of cardiovascular disease [26]. Thus, although this was a pilot study and the number of individuals positive for the GG polymorphism was obviously small, it is interesting that the results observed point to the same direction as the relevant literature data. The lack of association with coronary artery stenosis may be due to the small number of subjects that were genotyped.

The results concerning sensitivity of the glucocorticoid signaling system may be seen as contradicting the results of the dexamethasone suppression, which was also performed as an index of sensitivity, because those who showed the

least suppression after the dexamethasone treatment also tended to have more severe CAD. This association is obviously related to the hyperactivity of the axis, as we have found that those who did not suppress had higher preangiography cortisol levels. Although this fact may at first seem to be a paradox, it probably shows that hyperactivity as well as sensitivity may both independently contribute to more severe CAD, and does not necessarily mean that one individual carrying GG genotype cannot belong to the chronically stressed who have hyperactivity of the axis. The interpretation of the results concerning the indirect indices of sensitivity is obviously difficult because one cannot separate these 2 independent effects.

In conclusion, basal preangiography cortisol levels may be an independent index of the severity of CAD in subjects undergoing coronary arteriography showing hyperactivity of the HPA axis in anticipation of a stressful event. It is possible that the high cortisol levels in these individuals reflect chronic hyperreactivity of the HPA axis to stress. Associations of peripheral atherosclerosis with the homozygous presence of the *Bcl1* polymorphism in the glucocorticoid receptor gene may suggest that the sensitivity to cortisol may also be one of the factors involved in the development of more severe atherosclerotic disease, although this has to be confirmed in a larger series. On the basis of these preliminary observations we may suggest that both hyperactivity of the HPA axis and hypersensitivity of tissues to glucocorticoids may independently contribute to the severity of coronary and peripheral atherosclerosis. We speculate that when these 2 predisposing factors are present in the same individual, more severe consequences for vascular health are possible.

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