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Cardiovascular Risk Factors and Common Carotid Artery Caliber and Stiffness in Patients with Cushing's Disease during Active Disease and 1 Year after Disease Remission

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Cardiovascular accidents represent the most important cause of death in patients with Cushing's syndrome. This prospective study aims at evaluating carotid arteries by echo-Doppler ultrasonography and clinical and metabolic markers of atherosclerosis in 25 patients with Cushing's disease (CD) before and after 1 yr of remission. Thirty-two sex- and age-matched subjects (control-1) and 32 body mass index-matched subjects (control-2) served as controls. At diagnosis, CD patients had higher body mass index, waist to hip ratio (WHR), total, low-density lipoprotein-cholesterol and total/high-density lipoprotein (HDL) ratio, glucose and insulin, as well as lower HDL-cholesterol than control-1; they had higher WHR and total/HDL ratio and lower HDL-cholesterol than control-2. They also had higher intima-media thickness (IMT), and lower systolic lumen diameter and distensibility coefficient (DC) than either control group. Atherosclerotic plaques were detected in 31.2% of patients, 0 control-1, and 6.2% of control-2 subjects. One year after remission, WHR, LDL-cholesterol, and IMT significantly decreased, whereas systolic lumen diameter and DC significantly increased. However, all of the above parameters were still abnormal compared with control-1, but not control-2. A significant correlation was found between WHR, glucose and insulin levels, and right and left carotid IMT. WHR was the best predictor of left IMT and left DC in active, but not in cured, patients. The duration of hypercortisolism was the best predictor of right DC in active but not in cured patients. In conclusion, patients with CD have severe atherosclerotic damage. The persistence of a metabolic syndrome, vascular damage, and atherosclerotic plaques after cortisol level normalization makes these subjects still at high cardiovascular risk despite disease remission. (*J Clin Endocrinol Metab* 88: 2527–2533, 2003)

AMONG ALL SYSTEMIC consequences of hypercortisolism, cardiovascular complications are the most severe, causing a mortality rate 4-fold higher than that expected in the normal population (1, 2). High prevalence of atherosclerosis due to visceral obesity, systemic arterial hypertension, impairment of glucose tolerance, hyperlipidemia, and thrombotic diathesis is known to occur in hypercortisolism (3, 4). In a recent retrospective study, we investigated the consequences of a previous hypercortisolism on glucose and lipid metabolism and vascular system as well (5). Patients cured from Cushing's disease (CD) for 5 yr have a higher prevalence of atherosclerosis compared with age-, sex-, and body mass index (BMI)-matched controls so maintaining a high cardiovascular risk (5). The starting point of the accelerated atherosclerosis of these patients is likely the persistent visceral obesity and/or the insulin resistance syndrome, with their functional and structural negative consequences on the cardiovascular system.

Visceral obesity, glucose intolerance, insulin resistance, and hyperlipidemia are known features of hypercortisolism (1–4, 6); conversely, the occurrence of vascular atherosclerotic damage during the active disease and its changes after the disease remission have never been clearly investigated.

This longitudinal study aims at investigating the atherosclerotic vascular damage and its metabolic origin in CD patients, focusing on potential changes occurring between active phase and remission.

Subjects and Methods

Twenty-five patients with CD (8 men and 17 women; age range, 20–50 yr; mean, 34.2 ± 1.9 yr; median, 33 yr) were enrolled in this open longitudinal study after their informed consent had been obtained. The diagnosis of CD was based on the presence of the following criteria: 1) increase of daily urinary cortisol excretion with inappropriately high plasma ACTH concentrations; 2) increase of basal serum cortisol concentrations with lack of the physiological circadian rhythm; and 3) failure of urinary and serum cortisol suppression after low-dose dexamethasone test but greater than 50% decrease after high-dose dexamethasone test. The diagnosis of remission of CD was based on the presence of the following criteria: 1) urinary daily cortisol excretion and plasma ACTH concentrations below or within the normal range; 2) serum cortisol concentrations below or within the normal range with restoration of physiological circadian rhythm; and 3) suppression of urinary and serum cortisol concentrations after low-dose dexamethasone test (5). All patients had undergone selective surgical resection of

Abbreviations: BMI, Body mass index; CD, Cushing's disease; DBP, diastolic blood pressure; DC, distensibility coefficient; DLD, diastolic LD; HR, heart rate; IMT, intima-media thickness; LD, lumen diameter; MM, media-media distance; NS, not significant; PV, peak velocity; SBP, systolic blood pressure; SLD, systolic LD; US, ultrasonography; WHR, waist to hip ratio.

an ACTH-secreting pituitary adenoma by transsphenoidal approach: the results of immunohistochemistry on surgically removed pituitary adenomas confirmed the diagnosis in all patients. In 16 of 25 patients, stable normalization of cortisol levels was achieved after surgery, and it was preceded in 12 of them by a transient hypocortisolism; only 5 patients with persistent hypocortisolism received cortisone acetate at standard doses (25–37.5 mg/d). These latter patients were under treatment with cortisone acetate for the duration of the study. The remaining nine patients had unsuccessful transsphenoidal surgery; six of them had undergone pituitary irradiation, and three had been operated on twice, achieving two remissions and one persistence of disease. This latter patient and the six irradiated ones had been treated with adrenocortical blocking drugs, which induced stable suppression of serum and urinary cortisol levels, while awaiting the effects of radiotherapy. After surgery, the evaluation of residual pituitary function revealed secondary hypogonadism, hypothyroidism, and GH deficiency in four, two, and four patients, respectively; diabetes insipidus occurred in three patients. These patients received hormone replacement therapy with estroprogestinic preparation, testosterone enanthate at the monthly dose of 250 mg im, L-thyroxin at the dose 75–125 μg/d, recombinant human GH at the dose of 0.0125 U/kg·d, and 1-deamino-8-D-arginin-vasopressin at the dose of 10–20 μg/d. Three to 6 months after CD remission, primary hypothyroidism, following chronic lymphocytic thyroiditis, occurred in five patients; L-thyroxin replacement was given at the dose 75–125 μg/d. The adequacy of replacement therapy was periodically monitored during the follow-up by measuring free thyroid and sex steroid hormones and IGF-I levels, daily water balance, blood pressure, serum electrolyte levels, and regularity of menses. At study entry, 18 patients (72.0%) had arterial hypertension, 5 (20%) had overt diabetes mellitus, and 16 (64.0%) had reduced glucose tolerance. In the six patients with moderate/severe hypertension, treatment with calcium-antagonists (in three) and/or angiotensin-converting enzyme inhibitors (in six) controlled blood pressure. In three patients with diabetes mellitus, oral glucose-lowering drugs were given, whereas the remaining two underwent a low-lipid/carbohydrate diet regimen only. The other 20 patients and the controls were kept at balanced normocaloric regimen since at least 4 wk before entering the study. In patients treated with antihypertensive and/or glucose-lowering drugs at entry study, biochemical pretreatment values were considered for analysis. All patients were nonsmokers, whereas six of them reported a cardiovascular accident in one or more members of their families. A silent myocardial infarction, documented by electrocardiogram, was observed in three patients during active (patient 23) disease or after 3–6 months of disease remission (patients 8 and 22), whereas previous transient ischemic attack occurred in four patients during active disease (patient 25) or 5–10 months after disease remission (patients 8, 19, and 22). After remission, all patients had been followed at least twice yearly to verify the persistent control of cortisol secretion and the possible onset of other pituitary insufficiencies. The presumed disease duration was estimated by the time of appearance of symptoms that were likely related to the presence of hypercortisolism, such as weight gain, purple striae, hirsutism, irregular menses, gonadal dysfunction, hypertension, hyperglycemia, and dyslipidemia. In this series, disease duration ranged from 1–10 yr (mean, 3.6 $\pm$ 0.5 yr; median, 3 yr), and age of disease onset ranged from 18–47 yr (mean, 30.5 $\pm$ 1.9 yr; median, 29.0 yr). Patient profiles at study entry in individual patients are shown in Table 1. After disease remission, clinical and biochemical parameters were reevaluated in all patients after withdrawal of antihypertensive and antidiabetic treatments.

Controls

Two different control groups were enrolled in the study: 32 sex- and age-matched healthy subjects (control-1) and 32 BMI-matched subjects (control-2). All control subjects agreed to participate in the study and were recruited among the medical and paramedical personnel of the Department of Molecular and Clinical Endocrinology and Oncology of the "Federico II" University of Naples, Italy. None of these subjects had ever received chronic treatment with glucocorticoids or drugs known to interfere with glucose or lipid metabolism or to influence blood pressure. All were nonsmokers, and none had familial or personal history of cardiovascular diseases. The comparison between patients and the two different control groups was performed separately to estimate the role of BMI in the pathogenesis of clinical, metabolic, and vascular features of the patients of the study.

Study protocol

In accordance with a previous study (5), a clinical, biochemical, and vascular study was performed in patients with CD during the active phase of the disease and 1 yr after disease remission, whereas it was performed in all controls at study entry.

Clinical study. Height, weight, BMI, waist to hip ratio (WHR), and measurements of heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were evaluated by standard methods. BMI was measured as the ratio between the weight and the square of the height. A BMI between 25 and 30 kg/m^2 was considered as the index of overweight, whereas BMI greater than 30 kg/m^2 was considered the index of obesity (7). WHR was measured as the ratio between the waist, considered as the smallest torso circumference between the 12th rib and the iliac crest, and the circumference of the hip, considered as the maximal extension of the buttocks. The measurements were performed with the patients in standing position with relaxed abdomen, arms at sides, and joined feet (8). Blood pressure was measured in the right arm, with the subjects in a relaxed sitting position. The average of six measurements (three taken by each of two examiners) with a mercury sphygmomanometer was used. Hypertension was diagnosed when DBP values were greater than 90 mm Hg and was graded as mild between 91 and 104 mm Hg, moderate between 105 and 114 mm Hg, and severe when 115 mm Hg or greater, in line with World Health Organization criteria (9). In patients treated with antihypertensive drugs, blood pressure values before starting antihypertensive therapy were considered for the diagnosis and evaluation of the severity of hypertension.

Biochemical study. Fasting glucose and insulin, triglycerides, and total, LDL, and HDL cholesterol were measured by standard procedures. The total/HDL-cholesterol ratio, considered to be the index of severe cardiovascular risk (10), was also calculated. Hypertriglyceridemia was diagnosed when triglyceride levels were above 2.8 mmol/liter (11), whereas hypercholesterolemia was diagnosed when total cholesterol levels were above 6.2 mmol/liter (12). Glucose tolerance and insulin resistance were evaluated on the basis of fasting blood glucose and insulin levels or the response of blood glucose levels to a standard oral glucose tolerance test (75 g glucose diluted in 250 ml saline solution, measuring blood glucose every 30 min for 2 h). Diabetes mellitus was diagnosed when fasting blood glucose levels were above 7 mmol/liter in two consecutive determinations or at least 11.1 mmol/liter 2 h after oral glucose, whereas an impairment of glucose tolerance was diagnosed when blood glucose levels were between 7 and 11.1 mmol/liter 2 h after oral glucose with an additional measurement of 11.1 mmol/liter or more between 0 and 2 h after glucose load (13). Plasma ACTH and serum and urinary cortisol, assayed by RIA using commercially available kits, were measured to assess the hypothalamus-pituitary-adrenal axis.

Vascular study. Carotid artery ultrasound imaging was performed by echo-Doppler ultrasonography (US), carried out with a Vingmed Sound CMF 725 (Vingmed Sound, Horten, Norway) using a 7.5-MHz annular phased array transducer. Right and left carotid arteries were scanned longitudinally, 2.5 cm proximal to the bifurcation. When satisfactory B-mode imaging was achieved, the volume sample was placed in the middle of the vessel lumen, and consequently M-mode images were taken for several cardiac cycles. The pictures were stored on magnetic media and analyzed later. US imaging studies were performed by one operator (S.S.) who was blind in respect to patient or control study. Each measurement was repeated three times, and the mean was taken into consideration. Wall thickness, lumen, and distensibility of both carotids were investigated by measuring the intima-media thickness (IMT), systolic and diastolic media-media distance (MM), systolic lumen diameter (SLD) and diastolic lumen diameter (DLD), blood systolic and diastolic peak velocity (PV), and distensibility coefficient (DC). The lumen diameter (LD) was calculated by the following equation: LD = MM $-$ (2 $\times$ IMT). The DC was calculated using the following equation: (2δ/SLD)/P, where δ is the change in LD (peak systole to peak diastole) and P is the pulse pressure (in kilopascals; Ref. 14). In all subjects, presence, location, and size of plaques were also evaluated at the level of common, internal, and external carotid arteries.

TABLE 1. Patient profiles during active disease and 1 yr after disease remission

Patients		Disease duration (yr)	Therapy	Plasma ACTH (pmol/liter)		0800 h Serum cortisol (nmol/liter)		2400 h Serum cortisol (nmol/liter)		Urinary cortisol (nmol/d)	
Sex	Age (yr)			Activity	Remission	Activity	Remission	Activity	Remission	Activity	Remission
1. F	20	1	TS	42.9	10.2	1243	419	1016	126	2162	280
2. F	20	2	TS	43.9	11.2	1143	317	1219	126	2654	254
3. F	24	2	TS	37.5	17.5	1520	316	1200	110	1980	194
4. F	24	6	TS RT AA	23.1	7.3	979	367	987	123	2971	255
5. F	25	3	TS	36.5	18.5	1421	290	1098	108	1887	178
6. F	25	7	TS RT AA	22.1	6.3	749	467	707	138	2840	245
7. M	27	3	TS	30.0	10.6	818	318	914	97	1350	238
8. M	28	4	TS	31.0	11.6	998	400	888	123	1976	243
9. F	28	2	TS	32.5	8.9	915	374	540	105	1460	179
10. F	29	5	TS^{α}	57.9	14.6	1016	420	786	110	914	180
11. M	30	5	TS RT AA	40.3	12.0	1720	510	1540	120	2100	215
12. F	30	1	TS	36.4	16.4	1314	254	1050	118	1500	220
13. F	33	7	TS^{α}	20.6	2.2	870	254	586	133	764	270
14. F	35	1	TS	25.8	7.8	750	189	340	114	980	210
15. M	36	3	TS	55.9	10.1	844	366	929	135	1267	240
16. M	37	4	TS	28.8	8.5	2150	298	1750	110	3380	290
17. F	42	3	TS	37.0	11.6	1990	431	1323	99	2765	188
18. F	43	1	TS	25.5	7.0	1420	440	1049	95	1948	265
19. F	43	4	TS	36.0	12.6	1890	410	1300	90	2689	194
20. F	44	2	TS	24.5	6.0	1621	345	1654	103	1976	276
21. M	45	1	TS	22.0	8.8	1279	435	583	118	1015	234
22. F	45	9	TS^{α} AA	28.5	6.6	1281	431	765	123	1003	271
23. F	46	10	TS^{α} AA	29.5	5.6	688	380	586	116	830	251
24. M	46	2	TS	21.0	9.8	889	339	999	105	1546	221
25. M	50	3	TS	29.8	9.5	2450	287	1545	88	3234	299
Mean ± SEM	34.2 ± 1.9	3.6 ± 0.5		32.7 ± 2.0	10.0 ± 0.8	1278 ± 95.2	362.3 ± 15.3	1014.2 ± 73.6	113.3 ± 2.7	1887.6 ± 158.6	235.6 ± 7.3
Range	20–50	1–10		20.6–57.9	2.2–17.5	688–2150	189–510	340–1750	90–138	764–3380	179–290

TS, Transsphenoidal surgery; TS^{α}, surgery performed twice before the study; RT, radiotherapy; AA, adrenolitic agents. Normal hormones ranges: plasma ACTH, 4–22 pmol/liter at 0800 h; serum cortisol, 110–520 nmol/liter at 0800 h and 50–140 nmol/liter at 2400 h; free urinary cortisol, 100–370 nmol/d.

Statistical analysis

The statistical analysis was performed by SPSS for Windows version 9.0 (SPSS, Inc., Chicago, IL). The comparison between the numerical data was performed by ANOVA, followed by Newman-Keuls' test, or Student's *t* test for unpaired or paired data where appropriate. The comparisons between the categorical data were performed by χ^2 test with Yates correction and Fisher exact test where appropriate. The correlation study was performed by the linear regression analysis calculating the Pearson's coefficient. The multiple regression analysis was performed among the variables correlated at the linear correlation. Data were reported as mean ± SEM. The significance was set at 5%.

Results

Active disease

Overweight was present in 12 patients (48.0%), 5 control-1 subjects (15.6%), and 14 control-2 subjects (43.7%); obesity was present in 8 patients (32.0%), 0 control-1 and 10 control-2 subjects (31.2%). Overweight or obesity was significantly more prevalent in patients than in control-1 ($\chi^2 = 21.1$; $P < 0.001$) but not control-2 [$\chi^2 = 0.02$; P = not significant (NS)]. Hypertension was found in 18 patients [72.0% (mild in 9, moderate in 6, severe in 3)], 3 control-1 subjects [9.4% (mild in 2, moderate in 1); $\chi^2 = 21.0$; $P < 0.001$], and 8 control-2 subjects [25% (mild in 4, moderate in 4); χ^2=4.6; $P < 0.05$]. BMI, WHR, and DBP were higher in the patients with active CD than control-1 and control-2 subjects. HR was similar among groups. Mean values of any parameter are shown in Table 2.

Diabetes mellitus was diagnosed in 5 patients (20.0%) and 2 control-2 subjects (6.2%); reduced glucose tolerance was diagnosed in 16 patients (64.0%), 4 control-1 (12.5%), and 9 control-2 (28.1%) subjects. Diabetes mellitus or reduced glucose tolerance was more prevalent in patients than in control-1 ($\chi^2 = 26.3$; $P < 0.001$) and control-2 ($\chi^2 = 6.1$; $P < 0.05$). Hypercholesterolemia was found in 13 patients (52%), 0 control-1 ($\chi^2 = 18.7$; $P < 0.001$), and 10 control-2 subjects (31.2%; $\chi^2 = 0.9$; P = NS). HDL-cholesterol levels lower than normal were found in 9 patients (36.0%), 0 control-1 ($\chi^2 = 11.1$; $P < 0.001$), and 7 control-2 subjects (21.9%; $\chi^2 = 0.78$; P = NS). Hypertriglyceridemia was found in 5 patients (20.0%), 0 control-1 ($\chi^2 = 4.7$; $P < 0.05$), and 4 control-2 subjects (12.5%; ($\chi^2 = 0.16$; P = NS). A total/HDL cholesterol ratio greater than 5 was found in 14 patients (56.0%), 0 control-1 ($\chi^2 = 23.0$; $P < 0.001$), and 5 control-2 subjects (15.6%; $\chi^2 = 10.2$; $P = 0.001$). Mean values of any parameter are shown in Table 2.

In CD patients, right and left IMT were higher, SLD and DC were lower than either control group and DLD was lower than control-1 (Table 3). Well defined carotid wall plaques were detected in eight patients (32.0%), no control-1 ($\chi^2 = 9.4$; $P < 0.01$), and two control-2 subjects (6.2%; $\chi^2 = 4.8$; $P < 0.05$). Four patients had bilaterally localized carotid plaques (Table 4).

Disease remission

BMI did not change, whereas WHR, SBP, and DBP decreased, although WHR and DBP were still significantly higher than control-1 (Table 2). Obesity recovered in three patients (37.5%); hypertension in eight (44.4%); diabetes mellitus in two (40%); hypercholesterolemia in three (23%); and hypertriglyceridemia in two (40%). The total/HDL cholesterol ratio normalized in five patients (35.7%). Among the different biochemical parameters, only LDL-cholesterol levels significantly reduced compared with baseline, although they were still significantly higher than control-1 (Table 2).

Common carotid artery IMT decreased; SLD and DC increased compared with baseline, but they remained abnormal compared with control-1 (Table 3). Well defined carotid wall plaques were still detected in eight patients (32.0%), without any change compared with baseline (Table 4). Individual IMT data, before and 1 yr after CD remission, are shown in Fig. 1.

TABLE 2. Clinical, metabolic, and hormonal parameters in patients with CD before and after remission compared with controls

	Patients		Controls	
	Active disease	1-yr remission	1 (Sex- and age-matched)	2 (BMI-matched)
No.	25	25	32	32
BMI (kg/m^2)	29.2 ± 1.80[a]	26.8 ± 1.50[a]	22.8 ± 1.60	28.4 ± 1.80
WHR	0.99 ± 0.03[b,c,e]	0.88 ± 0.04[a]	0.76 ± 0.04	0.82 ± 0.04
HR (bpm)	68.6 ± 2.85	68.0 ± 2.00	70.5 ± 3.50	73.0 ± 4.70
SBP (mm Hg)	141 ± 7.45[c,e]	125 ± 3.90	116 ± 3.20	128 ± 5.50
DBP (mm Hg)	99.0 ± 2.65[b,c,e]	86.5 ± 2.45[c]	76.4 ± 2.50	84.5 ± 4.10
Fasting blood glucose levels (mmol/liter)	5.60 ± 0.40[a]	5.00 ± 0.40	4.00 ± 0.40	5.10 ± 0.05
Fasting serum insulin levels (μU/ml)	19.8 ± 3.80[a]	13.5 ± 2.50	9.10 ± 1.80	15.0 ± 3.20
2 h after OGTT blood glucose levels (mmol/liter)	9.40 ± 0.60[b,c]	8.50 ± 0.70	6.98 ± 0.50	7.62 ± 0.40
2 h after OGTT serum insulin levels (μU/ml)	125 ± 22.5[b,c]	120 ± 15.8[c,d]	35.4 ± 6.30	86.2 ± 5.10
Serum triglyceride levels (mmol/liter)	1.95 ± 0.40	1.80 ± 0.30	1.51 ± 0.30	2.06 ± 0.40
Total blood cholesterol levels (mmol/liter)	6.20 ± 0.65[a]	5.50 ± 0.55	4.50 ± 0.40	5.66 ± 0.50
LDL-cholesterol levels (mmol/liter)	4.35 ± 0.60[c,e]	3.75 ± 0.50[a]	2.67 ± 0.30	3.85 ± 0.40
HDL-cholesterol levels (mmol/liter)	1.00 ± 0.08[b,c]	1.12 ± 0.09[c]	1.44 ± 0.06	1.31 ± 0.07
Total/HDL cholesterol ratio	6.10 ± 0.60[b,c]	5.10 ± 0.55[c]	3.10 ± 0.30	4.30 ± 0.40
Plasma ACTH levels (pmol/liter)	32.7 ± 2.0[c,d,e]	10.0 ± 0.8	14.1 ± 1.50	16.2 ± 2.20
Serum cortisol levels (nmol/liter)	1278 ± 95.2[c,d,e]	362.3 ± 15.3	298 ± 24.2	395 ± 30.2
Urinary cortisol levels (nmol/24 h)	1887.6 ± 158.6[c,d,f]	235.6 ± 7.3	214 ± 14.5	245 ± 19.4
Serum IGF-I levels (ng/ml)	219.5 ± 13.5[e]	239.6 ± 12.1	248.3 ± 13.6	258.1 ± 16.1

OGTT, Oral glucose tolerance test.
[a] $P < 0.05$ *vs.* control-1; [b] $P < 0.05$ *vs.* control-2; [c] $P < 0.01$ *vs.* control-1; [d] $P < 0.01$ *vs.* control-2; [e] $P < 0.05$ *vs.* remission; [f] $P < 0.01$ *vs.* remission.

TABLE 3. Hemodynamic parameters in patients with CD before and after remission compared with controls

	Patients		Controls	
	Active disease (n = 25)	1-yr remission (n = 25)	1 (Sex- and age-matched) (n = 32)	2 (BMI-matched) (n = 32)
IMT (mm)				
Right	1.25 ± 0.05[c,d,e]	1.10 ± 0.04[a]	0.89 ± 0.06	1.00 ± 0.04
Left	1.28 ± 0.04[c,d,e]	1.15 ± 0.03[c]	0.88 ± 0.05	1.05 ± 0.05
Systolic MM (mm)				
Right	8.40 ± 0.25	8.40 ± 0.20	8.63 ± 0.25	8.64 ± 0.24
Left	8.41 ± 0.27	8.45 ± 0.25	8.56 ± 0.22	8.82 ± 0.30
Diastolic MM (mm)				
Right	7.95 ± 0.20	7.90 ± 0.25	8.03 ± 0.25	8.15 ± 0.26
Left	8.06 ± 0.28	8.05 ± 0.30	8.10 ± 0.25	8.22 ± 0.28
SLD (mm)				
Right	5.90 ± 0.18[b,c,f]	6.20 ± 0.16[a]	6.85 ± 0.20	6.64 ± 0.18
Left	5.85 ± 0.15[a,b,f]	6.15 ± 0.16[a]	6.80 ± 0.24	6.72 ± 0.22
DLD (mm)				
Right	5.45 ± 0.16[a]	5.70 ± 0.19	6.25 ± 0.21	6.15 ± 0.20
Left	5.50 ± 0.18[a]	5.75 ± 0.18	6.34 ± 0.20	6.12 ± 0.26
Systolic PV (cm/sec)				
Right	59.5 ± 2.85	62.5 ± 2.80	65.0 ± 3.60	68.4 ± 4.70
Left	61.5 ± 2.50	63.8 ± 2.65	64.1 ± 4.20	69.5 ± 5.10
Diastolic PV (cm/sec)				
Right	17.5 ± 1.50	17.5 ± 1.24	18.5 ± 2.40	19.3 ± 1.20
Left	17.8 ± 1.10	17.5 ± 0.12	18.9 ± 2.10	20.2 ± 1.60
DC (10^{-3} kPa^{-1})				
Right	23.5 ± 1.54[c,d,f]	29.4 ± 2.20[c]	37.7 ± 3.00	34.3 ± 2.2
Left	24.7 ± 1.76[c,d,f]	30.0 ± 1.90[c]	38.1 ± 2.90	34.9 ± 1.9

[a] $P < 0.05$ *vs.* control-1; [b] $P < 0.05$ *vs.* control-2; [c] $P < 0.01$ *vs.* control-1; [d] $P < 0.01$ *vs.* control-2; [e] $P < 0.05$ *vs.* remission; [f] $P < 0.01$ *vs.* remission.

TABLE 4. Size and localization of the plaques detected by US in patients with CD before and after remission and in controls

	Site	Study entry		1-yr study	
		Maximum diameter (mm)	IMT (mm)	Maximum diameter (mm)	IMT (mm)
Patient no.					
8	Right internal carotid	13	1.9	14	1.7
9	Right internal carotid	12	1.7	13	1.7
13	Right carotid bifurcation	29	2.1	28	2.0
	Left internal carotid	13	1.8	14	1.8
16	Left internal carotid	21	2.0	21	1.9
19	Left carotid bifurcation	17	1.8	18	1.7
22	Right carotid bifurcation	27	2.6	26	2.4
	Left carotid bifurcation	20	1.4	20	1.4
23	Right carotid bifurcation	31	3.3	33	3.4
	Left carotid bifurcation	22	1.6	20	1.5
25	Right carotid bifurcation	27	2.0	26	1.8
	Left internal carotid	13	1.9	15	1.7
Control no.					
14	Left carotid bifurcation	15	1.6	15	1.6
16	Right internal carotid	21	2.2	20	2.3

Clinical, metabolic, and US parameters were not significantly different in patients receiving cortisone acetate replacement therapy and those who did not.

Correlation analysis

In active CD patients, no significant correlation was found between the BMI and any clinical, biochemical, or vascular parameter, whereas WHR was significantly correlated to SBP ($r = 0.61$; $P < 0.05$), DBP ($r = 0.68$; $P < 0.05$), fasting and post-glucose load glucose ($r = 0.78$; $P < 0.01$; and $r = 0.81$; $P < 0.01$, respectively), and insulin levels ($r = 0.82$; $P < 0.01$; and $r = 0.81$; $P < 0.01$); right ($r = 0.65$; $P < 0.05$) and left IMT ($r = 0.86$; $P < 0.01$); and left DC ($r = -0.74$; $P < 0.05$). After remission, WHR was significantly correlated to SBP ($r = 0.69$; $P < 0.05$), DBP ($r = 0.72$; $P < 0.05$), fasting and post-glucose load glucose ($r = 0.71$, $P < 0.05$; and $r = 0.64$, $P < 0.05$) and insulin levels ($r = 0.74$, $P < 0.05$; and $r = 0.75$, $P < 0.05$); right ($r = 0.62$; $P < 0.05$) and left carotid IMT ($r = 0.73$; $P < 0.01$). In active patients, the duration of hypercortisolism was significantly correlated to right carotid IMT ($r = 0.71$; $P < 0.05$) and right ($r = -0.77$; $P < 0.01$) and left ($r = -0.68$; $P < 0.05$) carotid DC.

At the multiple regression analysis, WHR was the best predictor of post-glucose load insulin concentration both before ($\beta = 0.88$; $P < 0.01$) and after ($\beta = 0.79$; $P < 0.05$) CD remission, and of left carotid IMT ($\beta = 0.86$; $P < 0.01$) and left carotid DC ($\beta = -0.73$; $P < 0.05$) in active patients. The duration of hypercortisolism was the best predictor of right

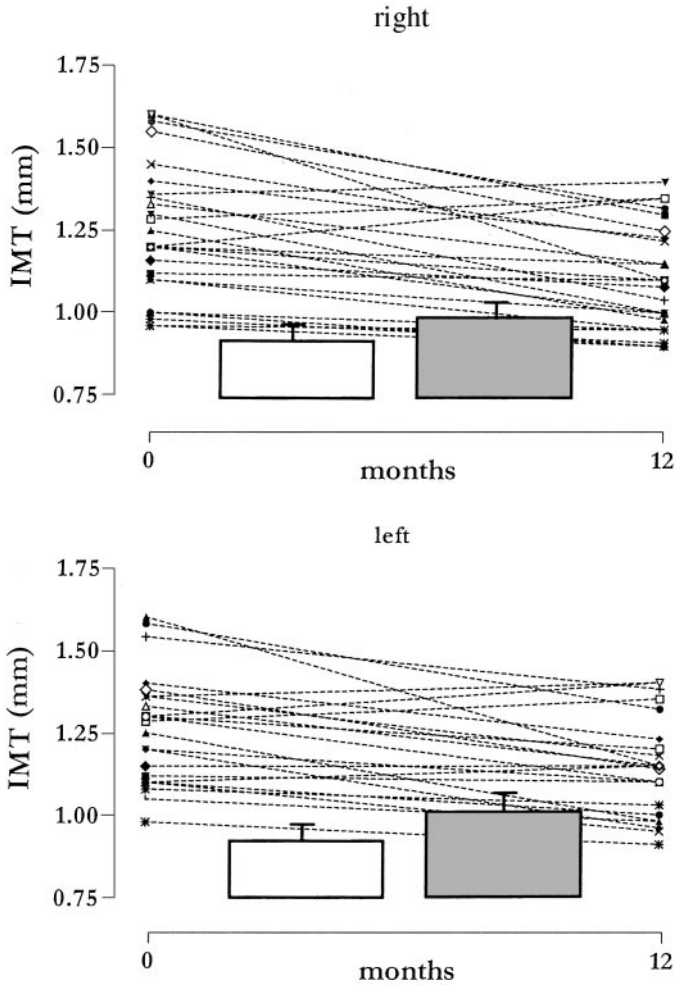

FIG. 1. Individual data of the right and left IMT before and 1 yr after remission from CD, measured by ultrasonography. The *bar* indicates the IMT of control-1 (□) and control-2 (▨) subjects expressed as mean ± SEM.

carotid DC in active ($\beta = -0.77$; $P < 0.01$) but not in remitted patients.

Discussion

The most relevant finding of the current study is a higher prevalence of atherosclerotic damage in patients with active CD. The abnormalities of carotid artery wall were accompanied by obesity, hypertension, impairment of glucose tolerance, and hyperlipidemia. This result supports the evidence of a 4-fold higher than that expected mortality rate for cardiovascular accidents in patients with Cushing's syndrome (2–4). One year after remission from hypercortisolism, despite a moderate improvement of clinical, biochemical, and vascular parameters, reduced caliber, and increased stiffness of carotid arteries wall, and atherosclerotic plaques as well, persisted. Therefore, disease remission, defined by endocrine and radiological parameters, is not followed by decreased parameters and clinical conditions associated with cardiovascular risk at 1 yr.

The metabolic syndrome associated with chronic glucocorticoid excess is well known; obesity, insulin resistance, hyperglycemia, hypercholesterolemia, and hypertriglyceridemia gradually develop in patients with Cushing's syndrome, as well as hypertension and thrombotic diathesis (1–4, 6, 15). The development of multiple atherogenic factors, as a consequence of supraphysiological levels of cortisol, is the trigger mechanism of endothelial damage and artery plaque formation. Similar pathogenesis is claimed in non-insulin-dependent diabetes mellitus to justify the earlier onset and the accelerated course of atherosclerosis (16). Although in patients with endogenous hypercortisolism the occurrence of arterial atherosclerotic or preatherosclerotic lesions has never been evaluated, accelerated atherosclerosis after prolonged corticosteroid administration has been shown in both animals (17, 18) and humans (19).

Among the multiple factors featuring the metabolic syndrome in CD, abdominal obesity and insulin resistance play a central role in initiating and maintaining atherosclerosis. Excessive accumulation of central adiposity has been demonstrated to relate to increased mortality and cardiovascular risk for disorders such as diabetes, hyperlipidemia, hypertension, and atherosclerosis (20). In the current study, abdominal obesity, measured by WHR, and most clinical, biochemical, and vascular parameters were abnormal in CD patients compared with both sex- and age-matched and BMI-matched control populations. Furthermore, WHR was correlated to clinical, metabolic, and vascular parameters and was independently related to the most important parameters of insulin resistance and atherosclerotic damage during active disease. Therefore, abdominal obesity is the most likely candidate to explain the increased vascular risk of patients with chronic hypercortisolism. Insulin resistance is recognized as a basic prerequisite to generate the metabolic syndrome (21), and when associated with abdominal obesity, as in patients with hypercortisolism, it increases the cardiovascular risk (22); in our patients, fasting glucose and insulin levels were undoubtedly increased.

The vascular damage of patients with the metabolic syndrome starts with endothelial dysfunction (23). In fact, impaired vasodilation after acetylcholine or hyperemia (24), enhanced large artery stiffness, (25) and increased prothrombotic and procoagulant activity (26) have been shown in all states associated with metabolic syndrome/insulin resistance development, like diabetes mellitus (24, 27), obesity (28), impairment of glucose tolerance (29), and gestational diabetes (30). Summarizing, the sequence of events bringing to the atherosclerotic plaque formation in patients with CD seems to begin with visceral adiposity excess and reduced insulin sensitivity, then undergoing gradual development of an overt metabolic syndrome with endothelial damage and atherosclerotic plaque formation.

One year after stable remission from hypercortisolism, the prevalence of the above-mentioned clinical and metabolic disorders, although reduced compared with the active phase of the disease, was still significantly higher than that observed in the control population. These results were similar to those found in another cohort of patients studied 5 yr after disease remission (5). These findings indicate that long-term normalization of circulating cortisol levels is not followed by the disappearance of clinical and metabolic features of active hypercortisolism and further explains the persistence of vas-

cular damage and atherosclerotic plaques in patients with previous CD. These results are in line with previous studies demonstrating persistence of moderate hypertension after removal of adrenal cortisol-secreting tumors (31, 32) and, interestingly, postoperative persistence of hypertension was correlated with entity and duration of hypertension during the active phase of hypercortisolism (32). It is likely that patients with longer disease duration and higher cortisol levels maintain a higher cardiovascular risk also after disease remission. In line with this hypothesis, the results of the current study demonstrate that carotid artery compliance was correlated with disease duration both in active disease and after its remission. The persistence of metabolic syndrome in patients cured from CD further confirms its pathogenetic role in developing vascular atherosclerotic damage in these patients. Interestingly, worsening of atherosclerosis and cardiovascular damage seems to characterize the long-term cured CD patients (5) compared with those studied 1 yr after CD remission. However, a long-term prospective study is necessary to confirm this observation.

In conclusion, patients with CD have severe atherosclerotic damage, as indicated by reduced caliber, increased stiffness of carotid artery wall, and increased prevalence of atherosclerotic plaques. Vascular damage developed in parallel to an acquired metabolic syndrome. Both metabolic and vascular alterations resulted markedly correlated to visceral obesity and insulin resistance, appearing strictly interacting each other. Remission from hypercortisolism is followed by improvement, but not normalization, of biochemical and vascular parameters. Therefore, present or past exposure to glucocorticoid excess has to be considered a condition associated with a high cardiovascular risk; these patients should be included in a lifelong follow-up.

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