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Original Article

Serum cortisol as a novel biomarker of atherosclerosis under chronic stress

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Abstract: Background: Adrenal necrosis score (ANS) and serum cortisol concentration (SCC) have been associated with atherosclerosis (AS), characterized by increased intimal and medial thickness. However, under chronic stress (CS), detailed relationships between SCC, ANS, increased intimal/medial thickness are indistinct. Aims of research, under CS, were to: 1) determine separated relationships between them; 2) investigate whether ANS is independent risk factor; 3) further explore whether SCC could be used to reflect ANS and predict intimal/medial thickness. Methods: Thirty-seven rabbits under CS underwent measures of ANS by pathological observation, serum cortisol level, lipid content of abdominal artery wall by Oil red O assay, macrophagocyte by immunohistochemistry. Results: Intimal thickness was related to ANS (P<0.05, β=0.375, and the $b_{constant\ quantity}$ =0.069). ANS (odd ratio [OR]: 6.525, P=0.043) independently affected intimal thickness. There is a linear correlation between ANS and SCC (P=0.009, β=-0.422, and the $b_{constant\ quantity}$ =7.307). Under CS, there are more macrophagocyte and lipids in the artery wall. Conclusion: SCC can influence and predict ANS and intimal thickness. Finally, intimal thickness =-0.158×SCC+2.809.

Keywords: Adrenal necrosis score, serum cortisol concentration, atherosclerosis, intimal thickness, medial thickness

Introduction

AS, featured with an increased intimal and medial thickness of the large arteries, is a potential inducement of numerous cardio-cerebrovascular diseases (CCVDs) [1]. Research suggested that CS could affect the balance of the hypothalamic-pituitary-adrenal (HPA) axis, leading to adrenal dysfunction and necrosis, which reduces cortisol secretion, and augments the hazard of atherosclerotic diseases [2]. Moreover, cortisol also can help the body recover from stress and regain a status of homeostasis [3]. The detailed relationships between adrenal status, serum cortisol level, and increased atherosclerotic intimal/medial thickness is unclear.

Carotid intima-media thickness (CIMT) has been used as a surrogate measurement of subclinical AS, which has relation to risk for cardiovascular events [4]. Among the literature, intimal and medial thickness are analyzed together in plentiful research [5]. The changes of the endarterium contain accumulation of intracellular lipids, local thickening of intima, formation of foam cells. However, increasing smooth muscle cells is the most relevant factor causing thickened vascular tunica, and morphological change is obvious [6]. Under CS, it is ill-defined what the most critical factor (adrenal-cortisol versus inflammatory factor) for AS is.

Therefore, the research aims to explore whether the serum cortisol level could be regarded as an indicator to reflect adrenal necrosis and predict the intimal/medial thickness.

Methods

Animals and diet [7]

Thirty-seven 3-month-old New Zealand rabbits, weighing 3.0±0.2 kg were used in this study. The rabbits were obtained from the Beijing

Fulong Tengfei Laboratory Animal Research Institute Co. LTD. Each rabbit was placed in one of in 3 randomly assigned groups in separate boxes (60×60×60 cm) for 1 week to adapt to it's environment. The animals could freely acquire food and water in an agreeable condition (relative humidity, 60%±2%; temperature, 25°C±2°C; light on at 08:00, 12-hour dark/light cycle). The Animal Care and Use Committee authorized the experiments and they were perfomed under the experimental ethics agreement. During the experiments, all procedures were designed to reduce any pain caused to the rabbits.

The fodder was provided by Beijing Keao third-Feed Co. Rabbits in the control group (n=11) were fed normal chow, totaling 11.4% of kcal from fat, composed of 4.0% saturated fat, 45% carbohydrates, 2.0% polyunsaturated fat, 4.2% monounsaturated fat. Rabbits in the high-fat (HF) diet group (HF group, n=13) were fed with 91.23% basic feed, 2.5% sugar, 5.2% lard, 2.2% cholesterol, 0.36% cholate, 0.3% propylthiouracil. Rabbits in the HF diet plus CS group (HF+CS group, n=13) were fed with 91.23% basic feed, 2.5% sugar, 5.2% lard, 2.2% cholesterol, 0.36% cholate, 0.3% propylthiouracil under conditions of CS for 8 weeks.

CS assay [7]

Firstly, noise stimulation was performed with 5 seconds of an alarm at about 110 decibels (dB), followed by 5 minutes of silence for 3 hours. Secondly, flash + continuous alarm (about 85 dB) stimulation was carried out in room within 2 hours after lights going out. Thirdly, rabbits were restrained with cages for 3 hours. Fourthly, continuous lighting stimulation after 19:00 was used. THERE IS SOMETHING MISSING HERE... hang rabbit's box by heip of cleek, which tilts when the rabbit moves. This step took 2 hours. Sixthly, no water was given for 24 hours, then no food for 24 hours. The CS period was 7 days, one cycle was once for 30 days. The process took 60 days.

Animal experiments

Researchers monitored animal health and behavior in the morning everyday. Thirty-nine rabbits were used in experiment, of which 37 were euthanized and 2 died due to diarrhea. Experimental animals were euthanized at the end of the study, 3 months after modeling. Following euthanasia of rabbits with 3% pentobarbital solution (300 mg/kg of animal body weight). The indicators of determening death included breathing and cardiac arrest, pupil dilation, and nerve reflex disappeared. We ensured that all aspects of animal experimental research protocols reduce the suffering and distress caused, and we considered alternative measures of suffering and distress. Anesthetics and painkillers were used to minimize or eliminate suffering and distress without interfering with the objectives of the study. We established humane endpoints for all situations of suffering and distress that could not be avoided or eliminated. Death or severe suffering and distress were avoided as experimental end points if possible. Important factors during the study were: enjoyment of freedom from hunger and thirst (physiological welfare); freedom to live comfortably (environmental well-being); the right to freedom from pain, injury and illness (health benefits); treedom to express nature (behavioral welfare).

Pathological observation and necrosis score of the adrenal gland

Blunt dissection of bilateral adrenal glands was implemented, adrenal tissue was fixed in 10% forma forest for 24 h. Then, we removed excess connective tissue with forceps, the researcher weighed the clean adrenal glands bilaterally with an electronic scale. We recorded the weight of right adrenal (WRA) and weight of left adrenal (WLA). After immobilization, tissue was dehydrated with an automatic dewatering machine. Paraffin sections were made with 6 μm thickness. H&E staining was performed on the tissue sections, ANS was graded with a total of four grades: 0 for no necrosis, 1 for point necrosis, 2 for focal necrosis, 3 for sheet necrosis.

Detection of serum cortisol and inflammatory factors

Collection of blood samples was under chloral hydrate anesthesia. A small amount of blood was gently drawn from the marginal vein of the rabbit ear. We gently rubbed or warmed the ears to increase blood flow. An EDTA anticoagulant tube was used to store a total of 0.4 mL blood. Enhanced immunoturbidimetric assay was employed to measure high-sensitivity

c-reactive protein (hs-CRP). Interleukin-6 (IL-6), matrix metallopeptidase-9 (MMP-9), SCC were detected by commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) [7]. The threshold for SCC: the usual range of serum cortisol was divided into three periods. At eight o'clock, normal range was 4.2-24.8 ug/dl; at 16 o'clock, normal range was 2.9-17.3 ug/dl. At 0 o'clock normal range was 0-6.7 ug/dl.

Measurement of enzyme linked immunosorbent assay (ELISA)

ELISA is an immunoassay technique widely used in the determination of proteins, antibodies, or hormones in liquid samples. It has high sensitivity, specificity, safety, and reliability. The basis of ELISA is the solid phase of antigen or antibody and the enzymatic labeling of antigen or antibody. ELISA binds soluble antigens or antibodies to solid phase carriers such as polystyrene, and uses the specific binding of antigens and antibodies to qualitatively and quantitatively detect immune responses. Operations were performed according to kit instructions. Basic steps are as follows: 1. Coating; 2. Adding sample; 3. Adding enzyme-labeled antibody; 4. Adding substrate solution to develop color; 5. Terminating reaction; 6. Determination of results.

Measurements of intimal thickness and medial thickness of abdominal artery

The celiac artery was isolated and dissected after blood collection. Part of the artery was fixed in 4% poly-formaldehyde solution, embedded in paraffin, and sectioned. The carotid artery was cut into 6-μm-thick transverse sections, stained with hematoxylin-eosin (H&E) after fixation in 4% poly-formaldehyde solution embedded in paraffin wax. After slicing, remaining embedded vessels (about 3 mm) were placed in normal saline and gross observation was performed under a stereoscopic microscope (Axio Zoom V16, ZEISS, Germany). Intima and media thickness of atherosclerotic plaques were measured in H&E-stained carotid artery sections under a microscope (Axio Zoom.V16; ZEISS, Oberkochen, Germany) using Image-Pro Plus 5.0 software (Media Cybernetics, Rockville, MD, USA). Intimal thickness is equal to radius of elastic intima minus the radius of the cavity. Media thickness equals radius bounded by external elastic membrane minus radius bounded by the inner elastic membrane. The threshold for intimal thickness is 2-10 mm.

Oil red O assay for lipid content of abdominal artery wall

Abdominal artery was placed into a Tissue Tek container which was then filled with Tissue Tek OCT compound gel (Sakura, Tokyo, Japan). Samples were frozen in liquid nitrogen, cut into 6-μm-thick sections for lipid staining using standard Oil red O protocol. Microscope and software for observation and measurement were the same as H&E assay.

Immunohistochemistry to detect macrophagocyte

Celiac artery tissue was used to make paraffin sections. Paraffin sections were deparaffinized with water, sealed with hydrogen peroxide, washed with double distilled water. Macrophagocytes were detected by immunohistochemistryand anti-CD68 monoclonal antibody after antigen repair. Assay steps were performed as required by VECTASTAIN Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA). First, antigen-fixed paraffin sections were washed 2 to 3 times (5 min/times) with phosphate-buffered saline (PBS), blocked with 10% goat serum (TransGen Biotech, Beijing, China) for 20 min at 37°C. Second, remove serum using filter paper, add CD68 antibody dropwise, then incubated overnight at 4°C. Third, sections were washed with PBS 3 times (5 min/time), incubated with goat anti-rabbit monoclonal antibody at 37°C for 1 hour. Fourth, color development was performed with diaminobenzidine. Each paraffin section was photographed and counted in six areas.

Statistical analysis

Data were expressed as percentage of total and mean ± SD. Used Student's was used t test to compare the 2 groups, or a one-way ANOVA test was performed when 3 groups were compared. When variance met homogeneity test, LSD post hoc test was used. When the variance did not meet the homogeneity test, Tamhane was used for post hoc test.

We used Pearson's chi-squared test for associations between underlying risk factors, intimal

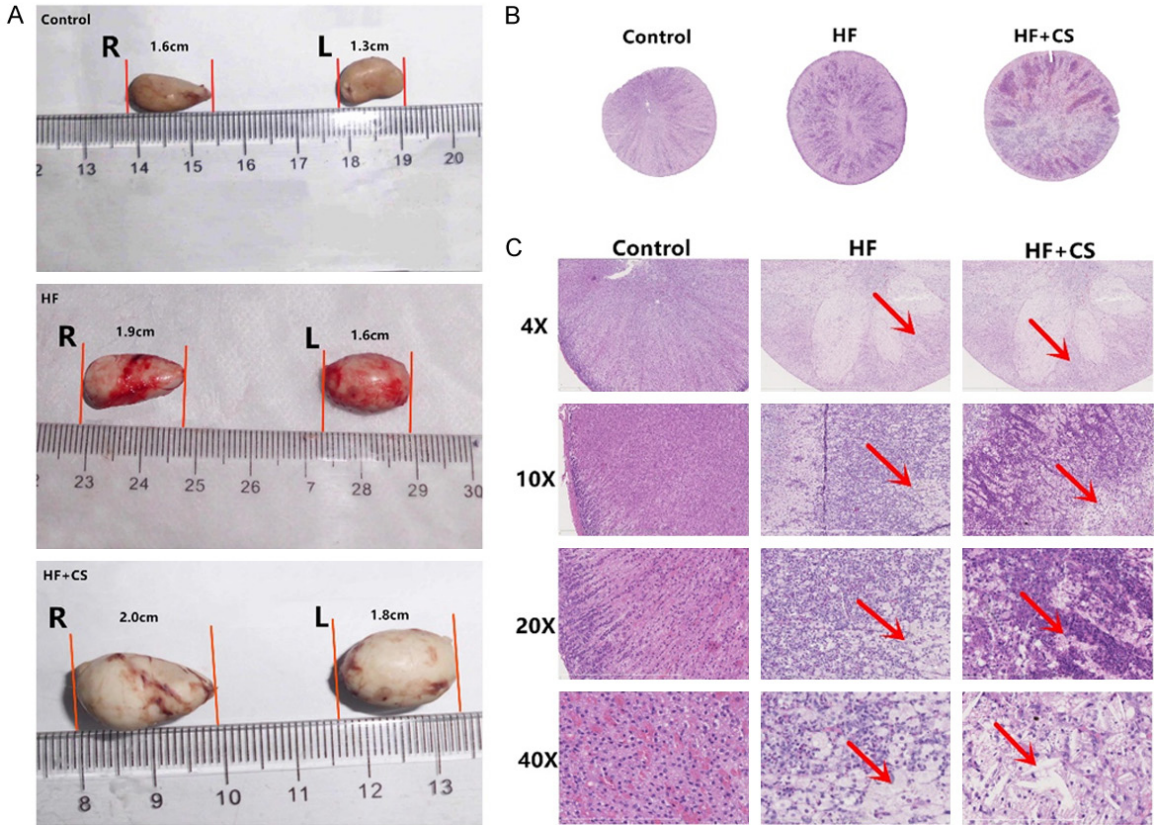

Figure 1. The distinction of adrenal size and pathogeny structure between the three groups. A. Gross appearance; B. The overall view in the microscope; C. Implementing H&E staining (4×, 10×, 20×, 40×). H&E: Hematoxylin-eosin; HF: High-fat diet; HF+CS: High-fat diet plus chronic stress.

and medial thickness. Spearman-rho test was used to compare adrenal characteristics, SCC, inflammatory factors for correlation analysis. When analytic results reached a liberal statistical threshold of $P<0.2$ for each comparison, key factors were forced into multivariable linear regression model to confirm key factors of abdominal intimal and medial thickness. In order to recognize residual distribution, histogram and Shapiro-Wilk test were performed. Wilcoxon signed-rank test was performed to compare intimal and medial thickness. Univariate and multivariate logistic regression analysis was used to get each variable's odds ratios (ORs) for intimal and medial thickness. Finally, we used linear regression analysis to explore linear correlations between ANS, SCC, intimal thickness. Receiver operating characteristic (ROC) curve analysis was used to analyze predictive ability of ANS and SCC for atherosclerotic intimal thickness.

All statistical analyses were conducted using SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA). A $P<0.05$ was considered statistically significant.

Results

Adrenal pathological changes of different groups

Though gross appearance, in the HF group, adrenal volume becomes was bigger than in the control samples but was smaller than in HF+CS group (**Figure 1A** and **1B**). H&E staining presented that in order of control, HF, HF+CS groups, adrenal inflammatory reaction was more and more enhanced. Under condition of CS, adrenal tissue has obvious cholesterol crystals (**Figure 1C**).

Effects of CS on the abdominal aorta in aspects of morphology, lipid deposition, macrophagocytes

Figure 2 presents status of the abdominal aorta in the 3 groups (control group, HF group, HF+CS group) both macroscopically and micro-

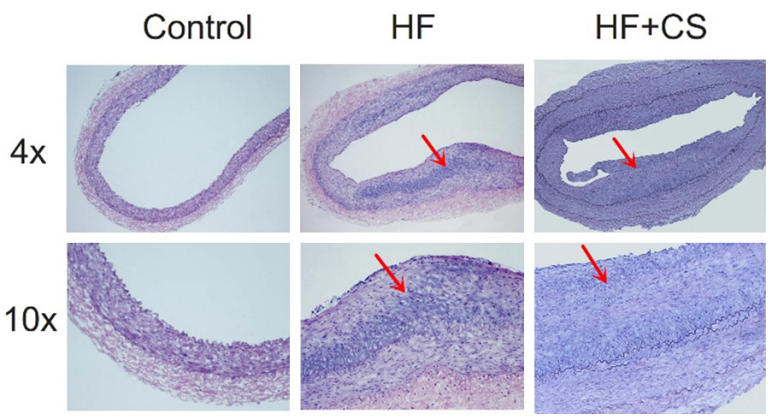

Figure 2. The pathological observation of the abdominal aorta of the control, HF, and HF+CS groups through the H&E staining. (Gross appearance, 4×, 10×). H&E: Hematoxylin-eosin; HF: High-fat diet; HF+CS: High-fat diet plus chronic stress.

scopically, which showed that the intimal and medial thickness was incrassated. Compared with HF group, there was more lipids in the abdominal aorta wall in the HF+CS group (**Figure 3**). Through immunohistochemical staining, the intima of the HF group was more abundant in macrophagocytes than the control group. However, compared with HF+CS group, there were less macrophagocytes than in the HF group (**Figure 4**).

SCC can sensitively and precisely predict ANS, which can predict intimal thickness through ROC curve

We created ROC curves to recognize accurate threshold for SCC to predict ANS and ANS to predict intimal thickness. ANS was most closely related to incresead intimal thickness (area under the curve for intimal thickness, 0.729; 95% CI, 0.552-0.905; P=0.020) (**Figure 5A**). SCC can predict ANS (area under the curve for medial thickness, 0.778; 95% CI, 0.624-0.933; *P*=0.007) (**Figure 5B**).

Associations between characteristics and intimal and medial thickness based on χ^2 test

Table 1 showed the relationship between potential risk factors and intimal and medial thickness by Pearson's chi-squared test. In individuals, weight of right adrenal (WRA) (*P*=0.018), importance of left adrenal (WLA) (*P*=0.036), ANS (*P*=0.037) all correlate with intimal thickness. No clear correlation was found between all potentially related characteristics and medial thickness.

Further associations between potential characteristics and intimal and medial thickness by Spearman's correlation test and multiple linear regression

To explore whether potential characteristics about adrenal and inflammatory factors influence intimal and medial thickness, correlation analysis was continued. Spearman's correlation coefficient showed, intimal thickness was related to WRA (ρ=0.428, *P*=0.008), WLA (ρ=0.487, *P*=0.002), ANS (ρ=0.380, *P*=0.020), IL-6 (ρ=-0.374, *P*=0.023) (**Table 2**). Natural logarithmic intimal thickness was related to ANS (β=0.372, *P*=0.040) after holding all other variables at a fixed value in multivariate linear regression model. However, correlation analysis confirmed, medial thickness was related to WRA (ρ=0.332, *P*=0.045) (**Table 2**).

Univariate logistic regression for proportional hazards analysis of correlative factors for intimal and medial thickness

Table 3 presents univariate odd ratios (ORs) and 95% confidence intervals (95% CI) for individuals' intimal and medial thickness. OR for intimal thickness was 5.778 (95% CI, 1.258-26.526, *P*=0.024) in group with high WRA compared with low WRA. On intimal thickness, high WLA subjects had a higher OR of 4.800 (95% CI, 1.052-21.907, *P*=0.043) than subjects with low WLA. Subjects with high ANS had higher intimal thickness than subjects with low ANS, OR is 4.286 (95% CI, 1.058-17.363, *P*=0.041). However, no characteristic is disadvantageous for medial thickness significantly.

Independent risk factor for intimal thickness based on multivariate logistic regression: ANS

Multivariate logistic regression analysis confirmed. Higher ANS, higher risk of disease (OR: 6.525, 95% CI, 1.065-39.991; *P*=0.043) (**Table 4**).

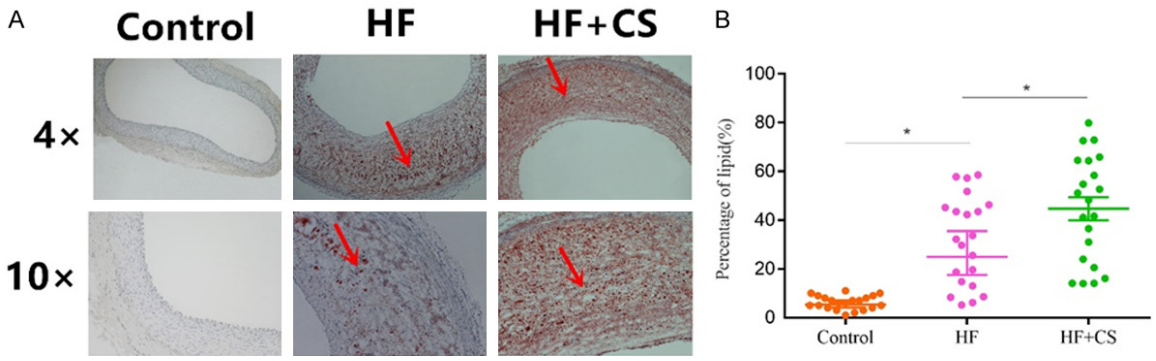

Figure 3. The lipid content of the abdominal artery wall is detected by Oil red O assay. A. Microscopic view (4×, 10×); B. Quantitative comparison between the three groups. HF: High-fat diet; HF+CS: High-fat diet plus chronic stress. *P<0.05.

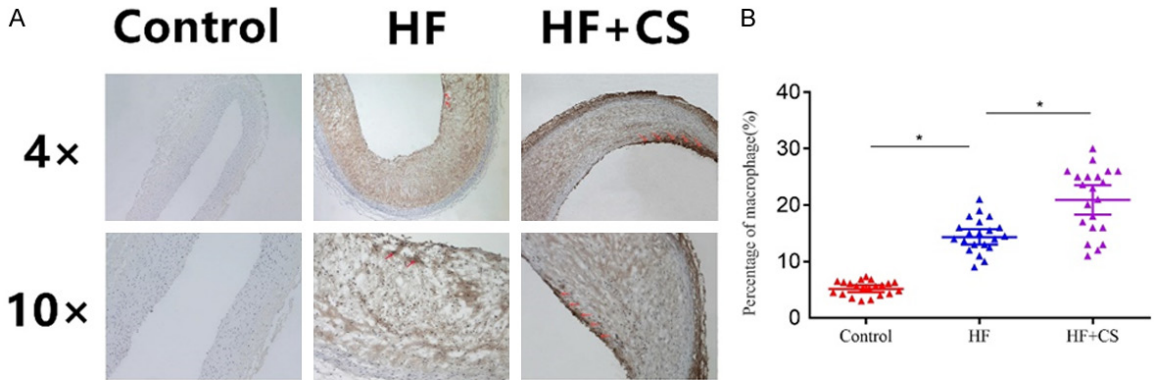

Figure 4. The detection of macrophage of the abdominal artery wall by immunohistochemical staining with anti-CD68 monoclonal antibody. A. Microscopic view (4×, 10×); B. Quantitative comparison between the three groups. HF: High-fat diet; HF+CS: High-fat diet plus chronic stress. *P<0.05.

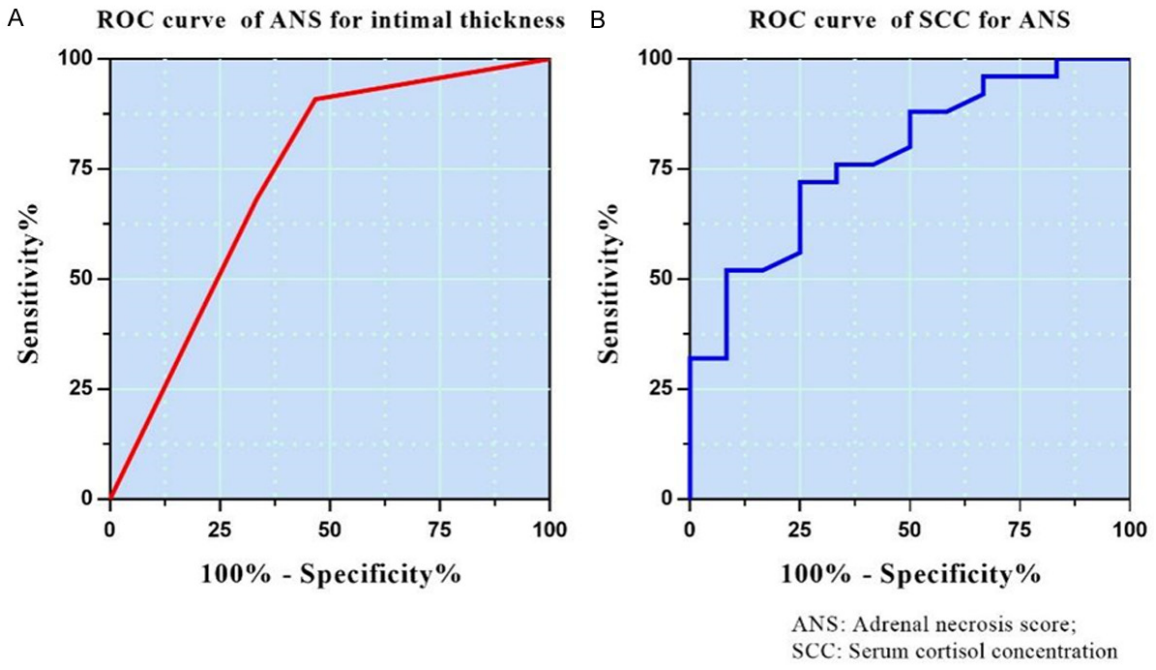

Figure 5. ROC curve of the predictive value between ANS, SCC, and intimal thickness. A. The ROC curve of ANS for intimal thickness. Increasing the ANS was predictive of the intimal thickness (area under the curve for intimal thickness, 0.729; 95% CI, 0.552-0.905; *P*=0.020). B. The ROC curve of SCC for ANS (area under the curve for medial thickness, 0.778; 95% CI, 0.624-0.933; *P*=0.007). ANS: Adrenal necrosis score; SCC: Serum cortisol concentration.

Table 1. Physical and metabolic characteristics and the status of intimal and media thickness

Characteristics			Intimal thickness		*P*	Media thickness		*P*
			Low (%) <0.096 cm	High (%) ≥0.096 cm		Low (%) <0.280 cm	High (%) ≥0.280 cm	
WRA	Low	<0.356 g	12 (32.4%)	9 (24.3%)	0.018*	16 (43.2%)	5 (13.5%)	0.098
	High	≥0.356 g	3 (8.1%)	13 (35.1%)		8 (21.6%)	8 (21.6%)	
WLA	Low	<0.388 g	12 (32.4%)	10 (27.0%)	0.036*	16 (43.2%)	6 (16.2%)	0.225
	High	≥0.388 g	3 (8.1%)	12 (32.4%)		8 (21.6%)	7 (18.9%)	
ANS	Low	<3	10 (27.0%)	7 (18.9%)	0.037*	12 (32.4%)	5 (13.5%)	0.501
	High	≥3	5 (13.5%)	15 (40.5%)		12 (32.4%)	8 (21.6%)	
SCC	Low	<159.766 ng/ml	7 (18.9%)	13 (35.1%)	0.457	13 (35.1%)	7 (18.9%)	0.985
	High	≥159.766 ng/ml	8 (21.6%)	9 (24.3%)		11 (29.7%)	6 (16.2%)	
hs-CRP	Low	<0.931 pg/ml	8 (21.6%)	9 (24.3%)	0.457	12 (32.4%)	5 (13.5%)	0.501
	High	≥0.931 pg/ml	7 (18.9%)	13 (35.1%)		12 (32.4%)	8 (21.6%)	
IL-6	Low	<67.412 pg/ml	6 (16.2%)	15 (40.5%)	0.089	14 (37.8%)	7 (18.9%)	0.793
	High	≥67.412 pg/ml	9 (24.3%)	7 (18.9%)		10 (27.0%)	6 (16.2%)	
MMP-9	Low	<47.920 pg/ml	5 (13.5%)	13 (35.1%)	0.124	10 (27.0%)	8 (21.6%)	0.248
	High	≥47.920 pg/ml	10 (27.0%)	9 (24.3%)		14 (37.8%)	5 (13.5%)	

Pearson's chi-squared test was used. *$P<0.05$. WRA: Weight of right adrenal; WLA: Weight of left adrenal; ANS: Adrenal necrosis score; SCC: Serum cortisol concentration; hs-CRP: high-sensitivity C-reactive protein; IL-6: Interleukin-6; MMP-9: Matrix metallopeptidase-9.

Table 2. Associations between intimal (and media) thickness and relevant metabolic characteristics

Characteristics	Intimal thickness					Media thickness				
	Spearman's rank correlation coefficient		Multiple linear regression			Spearman's rank correlation coefficient		Multiple linear regression		
	ρ[a]	*P*	β[b]	*P*	VIF	ρ[a]	*P*	β[b]	*P*	VIF
WRA	0.428	0.008*	-0.091	0.815	6.924	0.332	0.045*	0.463	0.274	6.924
WLA	0.487	0.002*	0.443	0.279	7.553	0.274	0.101	-0.085	0.847	7.553
ANS	0.380	0.020*	0.372	0.040*	1.409	0.175	0.300	0.089	0.639	1.409
SCC	-0.028	0.869	0.136	0.439	1.401	-0.043	0.801	0.109	0.564	1.401
hs-CRP	0.198	0.240	0.018	0.918	1.350	0.280	0.093	0.286	0.130	1.350
IL-6	-0.374	0.023*	-0.082	0.619	1.255	-0.007	0.965	0.141	0.432	1.255
MMP-9	-0.102	0.548	-0.202	0.213	1.179	-0.104	0.539	0.013	0.941	1.179

[a]Spearman's rank correlation coefficient between intimal (and media) thickness and relevant characteristics; ρ: Spearman's correlation coefficient. [b]Multiple linear regression analysis, β: parameter estimate; *Significant variables: $P<0.05$. WRA: Weight of right adrenal; WLA: Weight of left adrenal; ANS: Adrenal necrosis score; SCC: Serum cortisol concentration; hs-CRP: high-sensitivity C-reactive protein; IL-6: Interleukin-6; MMP-9: Matrix metallopeptidase-9.

SCC and ANS present a close linear correlation

Through linear regression analysis, ANS has correlation with intimal thickness ($P<0.05$, β=0.375, $b_{constant\ quantity}$ =0.069). Furthermore, among all risk factors, there is only one significant linear correlation: between ANS and SCC ($P=0.009$, β=-0.422, $b_{constant\ quantity}$ =7.307) (**Table 5**). According to two equality relationships: Intimal thickness =0.375×ANS+0.069, ANS=-0.422×SCC+7.307, thus we can get that: Intimal thickness =-0.158×SCC+2.809.

Table 3. Correlative characteristics' effect on intimal and media thickness based on univariate Logistic proportional regression analysis analysis

Characteristics		Intimal thickness			Media thickness		
		OR	95% CI	*P*	OR	95% CI	*P*
WRA	Low	1			1		
	High	5.778	1.258-26.526	0.024*	3.200	0.787-13.017	0.104
WLA	Low	1			1		
	High	4.800	1.052-21.907	0.043*	2.333	0.586-9.291	0.229
ANS	Low	1			1		
	High	4.286	1.058-17.363	0.041*	1.600	0.405-6.324	0.503
SCC	Low	1			1		
	High	0.606	0.161-2.275	0.458	1.013	0.262-3.924	0.985
hs-CRP	Low	1			1		
	High	1.651	0.440-6.200	0.458	1.600	0.405-6.324	0.503
IL-6	Low	1			1		
	High	0.311	0.079-1.222	0.094	1.200	0.308-4.672	0.793
MMP-9	Low	1			1		
	High	0.346	0.088-1.361	0.129	0.446	0.112-1.776	0.252

OR, odds ratio; 95% CI, 95% confidence interval. *$P<0.05$. WRA: Weight of right adrenal; WLA: Weight of left adrenal; ANS: Adrenal necrosis score; SCC: Serum cortisol concentration; hs-CRP: high-sensitivity C-reactive protein; IL-6: Interleukin-6; MMP-9: Matrix metallopeptidase-9.

Table 4. The characteristics and their effect on intimal and media thickness based on multivariate Logistic proportional regression analysis

Characteristics	Intimal thickness			Media thickness		
	OR	95% CI	*P*	OR	95% CI	*P*
WRA	2.605	0.115-59.203	0.548	2.398	0.165-34.901	0.522
WLA	2.571	0.104-63.691	0.564	1.454	0.081-26.015	0.799
ANS	6.525	1.065-39.991	0.043*	1.854	0.398-8.639	0.432
SCC	0.316	0.046-2.169	0.241	0.957	0.214-4.277	0.955
hs-CRP	0.731	0.101-5.270	0.756	1.374	0.274-6.895	0.700
IL-6	0.286	0.042-1.948	0.201	2.065	0.398-10.730	0.388
MMP-9	0.206	0.029-1.482	0.117	0.382	0.074-1.967	0.250

OR, odds ratio; 95% CI, 95% confidence interval. *$P<0.05$. WRA: Weight of right adrenal; WLA: Weight of left adrenal; ANS: Adrenal necrosis score; SCC: Serum cortisol concentration; hs-CRP: high-sensitivity C-reactive protein; IL-6: Interleukin-6; MMP-9: Matrix metallopeptidase-9.

Discussion

First, this study concludes that adrenal necrosis has an independently strong correlation and precisely sensitive prediction for changes in intimal thickness of AS. In addition, SCC is strongly correlated with adrenal necrosis, which could predict ANS precisely and sensitively.

Cortisol, a kind of adrenocortical hormone, affects carbohydrate metabolism [8]. Sometimes, cortisol is refered to as the basic "stress hormone". Cortisol can be converted into corticosteroids through the action of 11β-hydroxyl steroid dehydrogenase. Under stress, cortisol generally controls excessive inflammation, and plays the role of anti-allergy and inhibiting immune response [9]. Cortisol metabolism helps with physical activity. Under normal circumstances, the body is in an good position to control production and regulation of cortisol in the blood, but not always [10]. However, many people suffer from CS [11]. In CS state, when SCC is high for a long time, it will feedback to inhibit the hypothalamus-pituitary-adrenal cortical axis, so that pituitary secretion of adreno-

Table 5. The linear regression analysis of other characteristics for adrenal necrosis score

Characteristics	Adrenal necrosis score		
	β	*P*	constant quantity
WRA	0.223	0.185	1.112
WLA	0.199	0.237	1.170
SCC	-0.422	0.009*	7.307
hs-CRP	0.019	0.912	1.542
IL-6	-0.111	0.515	2.172
MMP-9	0.015	0.621	0.886

β: parameter estimate; *Significant variables: $P<0.05$. WRA: Weight of right adrenal; WLA: Weight of left adrenal; SCC: Serum cortisol concentration; hs-CRP: high-sensitivity C-reactive protein; IL-6: Interleukin-6; MMP-9: Matrix metallopeptidase-9.

corticotrophic hormone decreases, which leads to atrophy or even necrosis of adrenal cortex, and then secretion of cortisol decreased [12].

Cortisol has a powerful anti-inflammatory effect [13]. Its main mechanism is to inhibit inflammatory factors, inhibit antigen-antibody reaction, reduce capillary permeability, reduce toxin damage to body. In addition, cortisol can promote adherent granulocytes on the wall of small blood vessels into the blood circulation, the blood neutrophils increase, thereby symptoms of inflammation could be reduced [14, 15]. In the initial stage of inflammation, it can alleviate exudation, phagocytic response, so as to improve the symptoms of pain. At a later stage, it could inhibit the proliferation of capillaries, fibroblasts, reduce sequelae [16]. However, when CS occurs for a long time and cortisol levels decrease, the body's anti-inflammatory effect is weakened. Inflammatory factors may rampantly destroy the arterial endothelium, breaking the dynamic balance of endothelial injury and repair, thereby exacerbating AS.

Moreover, this research found that, according to immunohistochemical staining, the abdominal aortic endothelium showed more macrophage expression under CS than HF and control groups. Previous studies have demonstrated that cortisol impacts the immune process's suppression, including inhibition of phagocytosis, antigen processing of macrophages, and induction of lymphocyte depletion [17, 18]. It inhibits many links in the immune process. However, being under CS for a long time, the serum cortisol levels decrease, so that macrophages become more active, aggregate in the arterial endothelium where the inflammation reaction is stronger, and phagocytose necrotic or inflammatory substances, resulting in more aggregation of macrophages in the arterial endothelium under CS [19].

Furthermore, according to result of Oil red O assay, compared with HF group, there are more lipids in the abdominal aorta wall in HF+CS group. Lipids are an indispensable killer in atherosclerotic formation and intimal thickening [20]. Several studies have proved that cortisol promotes glyconeogenesis, accelerates fat decomposition, and thus reduces fat concentration in blood [21]. However, when CS occurs over a long period of time and serum cortisol level decreases, glyconeogenesis decreases, fat decomposition becomes slow, blood fat concentrations increase, augmenting risk of AS [22].

Finally, in terms of arterial blood pressure and blood flow velocity, cortisol is necessary to maintain normal blood pressure and flow [23, 24]. The reason is that cortisol could increase the number of catecholamine receptors on vascular smooth muscle cells. It also could regulate receptor-mediated information transfer process to enhance vascular smooth muscle cells' sensitivity to catecholamine and vascular smooth muscle tension [25]. It could inhibit the synthesis of prostaglandins from promoting vasodilation and reducing blood capillary permeability, which is beneficial to maintaining blood volume [26]. However, when CS exists for a long time, SCC decreases, blood vessels dilate, permeability increases, and blood flow slows down, which facilitates deposition of lipids on blood vessel wall, formation of atherosclerotic plaques [27].

Limitations

There are some areas which can be improved in this research. First of all, in the spearman's rank correlation coefficients, a direct correlation between SCC and intimal thickness of AS couldn't be found, and only through the intermediary role of ANS, can we speculate that there is indirect correlation and predictability between SCC and intimal thickness of AS. This may be due to limited number of samples. Small sample size may have led to bias and

affected ROC analyses and linear correlation results. Therefore, it is necessary to increase sample size. Second, this study did not involve genetic and molecular mechanisms of adrenal necrosis and serum cortisol in AS. In conclusion, we will continue to explore the relationship between adrenal necrosis, serum cortisol on AS.

Conclusion

In summary, this research demonstrated, adrenal necrosis is closely related to intimal thickness of abdominal aorta under CS with precise predictability. Serum cortisol level affects development of adrenal necrosis and CCVDs.

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Disclosure of conflict of interest

None.

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