The discrepancy of aromatase expression in epicardial adipose tissue between CHD and non-CHD patients

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Abstract

Objectives: Epicardial adipose tissue (EAT) aromatase converts androstenedione and other adrenal androgens into oestrogens. The locally produced oestradiol (E_2) may have cardiovascular protective effects. Little is known about the relationship between EAT aromatase level and coronary heart disease (CHD). Here, we compared EAT aromatase levels in CHD versus non-CHD patients and assessed the relationship between EAT aromatase levels and lesion degree in the coronary arteries.

Methods: EAT and blood specimens were obtained from patients undergoing thoracotomy prior to cardiopulmonary bypass. Serum E_2 levels were obtained from our hospital laboratory. EAT aromatase expression was determined by RT-qPCR and ELISA assays. All patients underwent coronary angiography and the level of coronary lesions was evaluated with the SYNTAX score.

Results: Compared with non-CHD patients, CHD patients had lower EAT aromatase mRNA and protein levels. In the CHD patients, EAT aromatase and oestrogen levels negatively correlated with the severity of coronary artery disease.

Conclusion: Our data revealed that reduced EAT aromatase levels correlated with coronary atherosclerotic lesions. Reduced EAT aromatase protein levels may aggravate the severity of atherosclerosis. Future studies should investigate the mechanisms regulating aromatase expression in epicardial fat.

Keywords: coronary heart disease, epicardial adipose tissue, aromatase, oestrogen

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Due to the close association between obesity and cardiovascular diseases such as coronary heart disease (CHD), heart failure, hypertension, stroke, atrial fibrillation and sudden cardiac death, the roles of adipose tissue have been widely studied. In the past 20 years, adipose tissue, which is regarded as the largest endocrine organ, has been shown to have complex secretory functions with local and systemic effects.[1] As a consequence of maladaptive adipose tissue expansion, adipose tissue cells undergo phenotypic modifications that alter their secretory output.[2]

Adipose tissue can transform steroid precursors into steroid hormones to influence fat distribution and lipid metabolism.[3] This function of adipose tissue depends on aromatase, an enzyme encoded by the cytochrome P450 family 19 subfamily A member 1 (CYP19A1), which catalyses the production of oestrone and oestradiol (oestrogens) from androstenedione and testosterone (androgens), respectively.[4] Even with low levels of aromatase, the abundance of adipose tissue makes it a major source of oestrogen in postmenopausal women and aging men.[5] The oestrogens generated in this way bind to specific receptors to exert cardiovascular protection.[6]

Both epicardial and visceral adipose tissue (EAT and VAT) derive from the splanchnopleuritic mesoderm.[7] EAT is wrapped by the visceral pericardium and directly adheres to the myocardial surface and coronary arteries.[7] In physiological settings, EAT accounts for about 20% of the heart's weight and is mainly distributed along the coronary artery in the atrioventricular sulcus, interventricular sulcus, right ventricular free wall and left ventricular apex, with small amounts around the left and right atria and auricle. There is no fascial structure between EAT and the adjacent myocardial and vascular walls.[8]

EAT releases factors such as adiponectin, interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF)-α and nitric oxide, which directly infiltrate into the myocardium (paracrine) or go through the coronary vasa vasorum (vasocrine) to influence the coronary arteries.[9-11] Currently there is no evidence confirming the relationship between aromatase levels in EAT and CHD. Here, we examined the relationship between EAT aromatase levels and CHD.

Methods

The case group (n = 30) (CHD group) comprised male patients, aged 50 years and older, who underwent coronary artery bypass grafting due to coronary atherosclerotic heart disease, with at least one coronary artery stenosis > 90% as revealed by coronary angiography. The control group (n = 30) comprised non-CHD patients, aged 50 years and older, undergoing thoracotomy due to other cardiac implications, with no significant stenosis on coronary angiography. Patients with severe hepatorenal dysfunction or under treatment with hormones, immunosuppressants, chemotherapy or other special drugs were excluded from the study.

After coronary angiography in our hospital, three researchers with senior attending physicians examined the angiography images and entered the values into the SYNTAX score calculator.

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The patients' data were obtained and the average for each person was calculated.

Ethical approval for the study was granted by the ethics committee of ShanXi Cardiovascular Hospital. All participants gave written informed consent and the study adhered to the Declaration of Helsinki.

Fasting venous blood samples were collected in the ward between 06:00 and 07:00 on the day after hospitalisation. Blood samples were put into the centrifuge tube without anticoagulant and centrifuged at 3 000 rpm for 15 minutes. The serum was separated and the serum oestradiol level was determined with chemiluminescence on a Beckman Coulter unicell DXL 800 immunoassay system.

During thoracotomy, five to 10 g EAT was taken from the initial segment of the right atrioventricular sulcus, close to the right coronary artery, before cardiopulmonary bypass (CPB). The samples were stored in liquid nitrogen for future analysis.

Total RNA was extracted using an RNA extraction kit (Qiagen, 205111) following the manufacturer's instructions. RT-qPCR analysis was done using the SYBR GreenER qPCR kit (Takara, RR820A) following the manufacturer's instructions. Relative gene expression was determined using the $2^{-\Delta\Delta}$Ct method. The results for each gene came from 30 independent repeated measurements (n = 30/group). Primer sequences are shown in Table 1.

To determine the presence of aromatase in EAT and compare its levels in CHD and non-CHD patients, samples were analysed using a human CYP19A1 ELISA kit (Cusabio Biotech, Life Sciences Advanced Technologies). The analysis was done in three independent replicates (n = 30/group).

Statistical analysis

Measurements are presented as mean ± SD and patient proportions as percentages. The Spearman correlation test was used for correlation analysis. The t-test was used for intergroup comparison of measured data and the chi-squared test was used for intergroup comparison of counted data. A p-value ≤ 0.05 indicated statistical significance. Data were analysed on SPSS version 26.

Results

A total of 60 patients were included in this study, with a median age of 59.17 ± 11.66 years in the CHD group and 57 years (52–66) in the control group. Hypertension was more prevalent in the CHD group than in the control group. Low-density lipoprotein cholesterol level and left ventricular ejection fraction were lower in the CHD group relative to the control group ($p \leq 0.05$). Other indicators did not vary significantly between the two groups (Table 2).

Table 1. The primers of related genes for aromatase RT-qPCR

Name	*Sequences (5′→3′)*
Aromatase forward	TGGAAATGCTGAACCCGATAC
Aromatase reverse	AATTCCCATGCAGTAGCCAGG
Internal reference GAPDH forward	CCACCCATGGCAAATTCCAATGGCA
Internal reference GAPDH reverse	TCTAGACGGCAGGTCAGGTCCACC

PCR, polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

Table 2. Basic parameters of the patients

Parameters	*Non-CHD group* (n = *30*)	*CHD group* (n = *30*)	χ^2 / t	p-*value*
Hypertension, *n* (%)	11 (34.4)	21 (65.6)	6.696	0.010
Hyperlipidaemia, *n* (%)	16 (47.1)	18 (52.9)	0.271	0.602
Diabetes, *n* (%)	5 (35.7)	9 (64.3)	1.491	0.222
Smoking, *n* (%)	18 (58.1)	13 (41.9)	1.669	0.196
Age, years	59.03 ± 9.90	59.30 ± 13.36	0.088	0.930
BMI, kg/m^2	26.39 ± 2.62	27.19 ± 2.71	1.163	0.250
SBP, mmHg	126.80 ± 18.09	130.43 ± 18.48	0.770	0.445
LDL-C, mmol/l	3.11 ± 0.85	2.67 ± 0.66	2.202	0.032
HDL-C, mmol/l	1.15 ± 0.31	1.16 ± 0.30	0.042	0.967
Scr, µmol/l	75.22 ± 13.48	78.78 ± 13.97	1.004	0.319
LVEF, %	64.77 ± 5.81	44.10 ± 9.22	10.384	< 0.001
LVEDD, mm	46.47 ± 6.44	47.87 ± 11.92	0.566	0.574

Values are presented as mean ± SD or number (%).
BMI, body mass index; SBP, systolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Scr, serum creatinine; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter.

To exclude the effects of serum oestrogen differences on coronary artery lesions, oestrogen levels were measured by chemiluminescence in the blood samples collected before CPB. This analysis did not reveal significant differences in serum oestrogen levels in the two groups (p = 0.7011, Fig. 1A).

RT-qPCR analysis indicated that relative to the non-CHD group, EAT aromatase levels were significantly lower in the CHD group (p < 0.0001, Fig. 1B). ELISA showed that EAT aromatase protein levels were also significantly lower in the CHD group (p < 0.0001, Fig. 1C).

Correlative analysis revealed no correlation between aromatase mRNA and protein levels in the control versus CHD groups ($r_{control}$ = –0.069; $p_{control}$ = 0.717; $r_{CHD\ group}$ = –0.057; $p_{CHD\ group}$ = 0.764) (Fig. 2A, B). There was a negative correlation between aromatase protein content and SYNTAX score in the CHD patients, hence, the higher the SYNTAX score, the lower the aromatase protein content (correlation coefficient = –0.430, p = 0.018, Fig. 2C).

Discussion

Based on clinical and experimental investigation, we report for the first time that aromatase level negatively correlated with CHD severity. Since EAT and VAT have the same embryological origin, EAT can be considered the visceral adipose depot in the heart.[12] There is no fascial structure between EAT, the adjacent myocardium and the vascular walls, and high-density adipose tissue can directly infiltrate into the cardiomyocytes, making contact with the adventitia of the coronary arteries.[13] This offers an important structural basis for the endocrine role of adipose tissue.

Coronary atherosclerotic plaques are reported to mainly occur in arterial segments surrounded by EAT,[14] while intramyocardial coronary artery segments are largely unaffected by atherosclerosis.[15] EAT volume is proposed as a biomarker for subclinical atherosclerosis, independent of other coronary artery disease risk factors.[12] Based on these facts, we hypothesised that EAT plays a critical role in the pathogenesis of coronary artery disease.

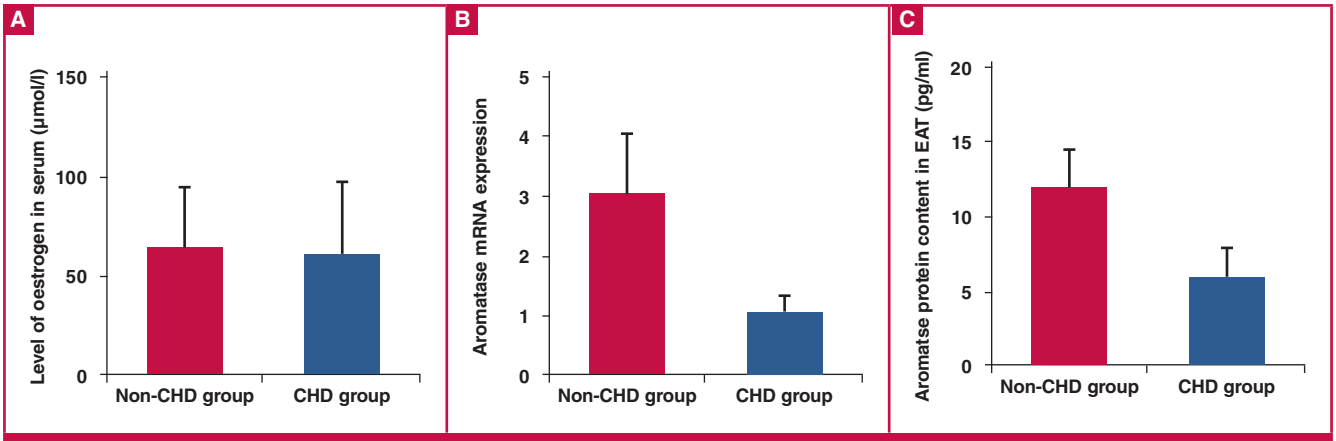

Fig. 1. A. Comparison of oestrogen levels in the serum between the two groups. CHD group: 63.20 ± 32.12 (µmol/l); non-CHD group: 59.79 ± 36.23 (µmol/l). There was no significant difference between the two groups ($p > 0.05$). B. Comparison of the expression of aromatase mRNA in EAT between the two groups. CHD group: 1.0 ± 0.37; non-CHD group: 3.05 ± 0.99. The mRNA expression of aromatase was significantly reduced ($p < 0.05$) in the CHD group. C. Comparison of aromatase protein content in EAT using the *t*-test. CHD group: 5.74 ± 1.97 (pg/ml); non-CHD group: 11.79 ± 2.60 (pg/ml) ($p < 0.0001$). The difference was statistically significant ($p < 0.05$).

CHD incidence in postmenopausal women is almost four times higher than in men.[16] Although adipose tissues has relatively low levels of aromatase and androgens (which are often < 1% in any tissue), their influence on hormone function may be high.[17] For this locally produced oestrogen, especially with gonadal failure, there may be increased cause to exert a cardiovascular protective effect.

Aromatase is involved in sex hormone transformation. Differences in EAT aromatase expression may directly affect local oestrogen levels, the oestrogen/androgen ratio and their biological functions.[18] CYP19 polymorphisms are associated with oestrogen inactivation and CYP19 mutations may alter aromatase protein structure, affecting its activity.[19]

It should be noted that adipose tissue is not homogeneous and control of aromatase expression is tissue specific. For example, while in the ovaries, aromatase expression is regulated by cAMP, and in the breasts it is controlled by prostaglandins.[17] Some studies show that in breast adipose tissue, obesity and low-grade inflammation upregulate aromatase gene expression and oestrogen production.[20-22]

In this study we did not find a correlation between aromatase mRNA and protein levels in the control versus CHD groups. This indicates that there may be other regulatory mechanisms affecting aromatase protein synthesis. The regulatory mechanisms of aromatase expression in EAT have not been studied as yet.

Numerous studies have examined the effects of oestrogen on cardiovascular diseases and found that its protective effects include reduced fibrosis, stimulation of angiogenesis and vasodilation, improved mitochondrial function and reduced oxidative stress.[6] Many of these oestrogen effects have been associated with local EAT aromatase and atherosclerosis, arrhythmia and ischaemia–reperfusion injury.

Some patients with oestrogen-associated breast cancer may require aromatase inhibitor chemotherapy. Cardiovascular events are suggested as primary causes of the low quality of life in breast cancer patients undergoing treatment with aromatase

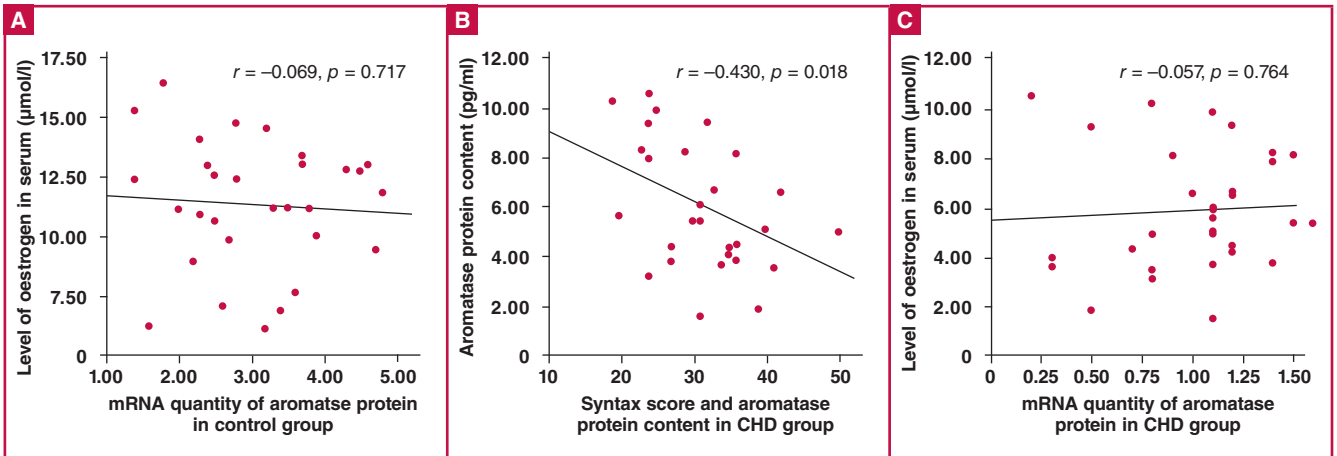

Fig. 2. A. Scatter diagram of aromatase mRNA quantity and aromatase protein content in the control group. B. Scatter diagram of aromatase mRNA quantity and aromatase protein content in the CHD group. C. Scatter diagram of SYNTAX score and aromatase protein content in the CHD group.

inhibitors.[23] This fact is illustrated by findings that elevated EAT aromatase levels may delay or prevent the occurrence of various cardiovascular diseases.

In this study we found a significant negative correlation between severity of coronary artery lesions and level of aromatase protein in the CHD group. However, aromatase activity was not evaluated in this study and the relationship between aromatase protein level, aromatase activity and coronary artery disease needs further study.

Conclusion

Our data show that reduced aromatase expression in EAT correlated with coronary atherosclerotic lesions. Decreased EAT aromatase protein levels may aggravate the severity of atherosclerosis. Future studies should evaluate the mechanisms regulating aromatase transcription and translation in EAT.

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