J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-010-0547-9 30(5):589-593,2010

Roles of Human Epicardial Adipose Tissue in Coronary Artery Atherosclerosis*

Xinzhong CHEN (陈新忠)[1#], Zhouyang JIAO (焦周阳)[1], Lei WANG (汪 雷)[2], Zongquan SUN (孙宗全)[1], Yutao WEI (魏玉涛)[1], Xianguo WANG (王现国)[1], Dongsheng XIA (夏东升)[1]
[1]*Department of Cardiovascular Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*
[2]*The First College of Clinical Medical Science, China Three Gorges University, Yichang 443003, China*

Summary: This study examined the adipocytokine-vascular interactions and link between epicardial adipose tissue and coronary artery atherosclerosis. Thirty-four patients undergoing open heart surgery were chosen randomly, and divided into group Ⅰ (non-coronary artery disease group) and group Ⅱ (coronary artery disease group). Blood samples were taken through peripheral vein prior to surgery. Plasma levels of a panel of proteins (adiponectin, IL-10, TNF-α) were detected by using ELISA. Epicardial adipose tissue was taken near the proximal tract of the right coronary artery and subcutaneous adipose was taken from the leg before cardiopulmonary bypassing, adiponectin and CD68 + were detected by using RT-PCR and immunohistochemistry. Our results showed that plasma adiponectin level was significantly lower in the group Ⅱ as compared with group Ⅰ (P<0.05). There were no differences in plasma concentration (IL-10, TNF-α, tatal-chol, HDL-chol, LDL-chol) between group Ⅰ and group Ⅱ. The number of CD68＋ cells in epicardial adipose tissue of group Ⅱ was significantly higher than that in subcutaneous adipose tissue. Adiponectin mRNA expression was 6 fold higher in subcutaneous adipose tissue than in epicardial adipose tissue of group Ⅱ (P<0.01). Furthermore, the level of adiponectin mRNA in the epicardial adipose tissue in group Ⅱ was also significantly lower than in group Ⅰ (P<0.05). We are led to conclude that inflammation that occurs locally in epicardial adipose tissue of CAD contributes to the pathogenesis of coronary artery disease.
Key words: adiponectin; epicardial adipose tissue; inflammation; adipo-vascular axis; atherosclerosis

Obesity is a great risk for vascular diseases, including atherosclerosis and restenotic change after coronary arterial bypass graft[1]. Obesity is also linked to a state of chronic inflammation that is associated with the production of pro- and anti-inflammatory cytokines secreted by white adipose tissue (WAT)[2], such as interleukin (IL)-6, tumor necrosis factor (TNF-α), IL-1 and IL-1 receptor antagonist. Adiponectin is a fat-derived hormone directly bridging the adipose-vascular axis[3].

Adiponectin is an adipose-derived factor identified in the human adipose tissue. A recent study found that adiponectin has the potential of anti-atherosclerosis, anti-inflammation and anti-injury-induced intimal hyperplasia[4]. In this study, we examined the effect of adiponectin protein expression on coronary artery.

Xinzhong CHEN, E-mail: xinzhongchen@yahoo.com.cn
#Corresponding author
*This project was supported by a grant from the National Natural Science Foundation of China (No. 30872541 C160502), and fund for Doctoral Program of Ministry of Education of China (No. 200804871116).

1 SUBJECTS AND METHODS

1.1 Patient Data

Thirty-four consecutive subjects (including 13 women and 21 men, age range: 39–72 y) were included and divided into 2 groups: non-coronary artery disease group (group Ⅰ) and coronary heart disease group (group Ⅱ). In the group Ⅰ, seven subjects had undergone surgery for valve replacement and four for correction of congenital heart disease. In group Ⅱ, 23 patients who had undergone elective coronary artery bypass transplant for critical CAD. The subjects had no signs, symptoms and history of chronic pulmonary hypertension, diabetes, dyslipidemia, and other metabolic diseases. None of them was taking drugs. Echocardiography was performed in all subjects during routine examinations. This study was approved by the review committee of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Informed consent was obtained from all the subjects before the study began.

1.2 Methods

1.2.1 Blood and Pericardial Cavity Fluid Measurements For blood collection and treatment, before the

surgery, peripheral venous blood was drawn into pyrogen-free tubes with EDTA as an anticoagulant and stored at –80°C. Adiponectin, IL-10 and TNF-α were quantitatively determined by double antibody sandwich ELISA. IL-10, TNF-α, EK-ADI-01 ELISA kits were purchased from Jingmei Co., China.

Pericardial cavity fluid was obtained during the cardiac thoracic surgery and centrifuged at 2500 r/min for 10 min. Then the supernatant was harvested and preserved at –80°C. Adiponectin, IL-10 and TNF-α concentration was assayed by ELISA.

Concentrations of tatal-chol, HDL-chol and LDL-chol were measured by using automatic biochemistry analyzer.

1.2.2 Immunohistochemistry For adipose tissue collection and detection, epicardial adipose tissue samples (group Ⅰ) and subcutaneous fat surrounding great saphenous vein (group Ⅱ) was harvested before the cardiopulmonary bypassing. Then the adipose tissue was divided into three groups: group A (epicardial adipose tissue in group Ⅰ), group B (epicardial adipose tissue in group Ⅱ) and group C (subcutaneous adipose tissue in group Ⅱ). CD68＋was detected by immunohistochemical staining with a polyclonal goat antibody (Santa Cruz Biotechnology, USA) and a secondary fluorescein isothiocyanate-coupled donkey anti-goat IgG antibody (Santa Cruz, USA). For cell counting, 4 different fields of view on each slide were examined at 400 magnifications. The total number of cells expressing CD68＋ in each field was calculated by using the Leica Qwin software pacakage (Leica, USA).

1.2.3 Total RNA Isolation and RT-PCR

For adiponectin mRNA detection, total RNA was prepared with an RNASTAT-60 kit (Tel-Test, Friendswood, TX, USA). cDNA was produced by using Taqman reverse transcription kits (PerkinElmer Life Sciences, USA). RT-PCR was performed according to the manufacturer's instructions. Primers for adiponectin were: 5'-GTCCTAAGGGAGACATCG and 5'-GAGGCTGAC CTTCACCTA, and primers for β-actin 5'-CCAACC GCFAFAAFATGACC and 5'-GATCTTCATGAGGTA GTCAGT. PCR amplification of adiponectin was performed under the following conditions: 35 cycles of denaturation for 55 s at 95°C, annealing for 55 s at 49°C, and extension for 55 s at 72°C. Perilipin primer sequence was based on that by Faber *et al*.

1.3 Statistical Analysis

The experimental data were expressed as $\bar{x}\pm s$ and processed by Student's *t* test by using SPSS software package.

2 RESULTS

2.1 Adiponectin, IL-10, TNF-α, Tatal-chol, HDL-chol, LDL-chol Levels in Peripheral Venous Blood

Plasma adiponectin level was significantly lower in group Ⅱ as compared with group Ⅰ (P<0.05). There were no differences in plasma concentration (IL-10, TNF-α, tatal-chol, HDL-chol, LDL-chol) between group Ⅱ and group Ⅰ (table 1).

Table 1 Adiponectin, IL-10, TNF-α, tatal-chol, HDL-chol, LDL-chol levels in peripheral venous blood in the two groups

Groups	Adiponectin (μg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)	Tatal-chol (mmol/L)	HDL-chol (mmol/L)	LDL-chol (mmol/L)
Ⅰ	14.3±6.9	4.7±0.51	2.63±0.77	4.8±0.49	1.24±0.21	2.93±0.49
Ⅱ	9.7±6.6	4.1±0.26	2.58±0.61	5.1±0.47	1.09±0.38	3.08±0.51

2.2 Adiponectin, IL-10, TNF-α in Pericardial Cavity

Adiponectin concentration in pericardial cavity was significantly lower in group Ⅱ group as compared with group Ⅰ (P<0.05), but TNF-α concentration was significantly higher in group Ⅱ (P<0.05). There was no differences in IL-10 concentration between group Ⅱ and group Ⅰ (table 2).

Table 2 Adiponectin, IL-10, TNF-α in Pericardial Cavity

Groups	Adiponectin (μg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)
Ⅰ	18.1±8.9	4.3±0.53	3.71±0.87
Ⅱ	13.4±7.4	4.1±0.44	5.89±0.74

2.3 The CD68+ Expression

The quantity of CD68＋ cells in epicardial adipose tissue of group Ⅱ was significantly higher than that in group Ⅰ (fig. 1–3).

2.4 Adiponectin mRNA Expression

Adiponectin mRNA expression was higher in subcutaneous adipose tissue than in epicardial adipose tissue of group Ⅱ (P<0.01). Furthermore, the level of adiponectin mRNA in the epicardial adipose tissue in group Ⅱ was also significantly lower than in group Ⅰ (P<0.05) (fig. 4).

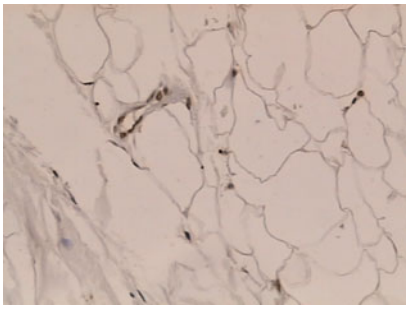

Fig. 1 The CD68＋ expression in epicardial adipose tissue in group Ⅰ

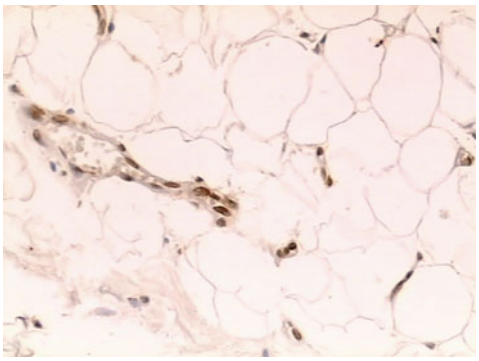

Fig. 2 The CD68＋expression in epicardial adipose tissue in group Ⅱ

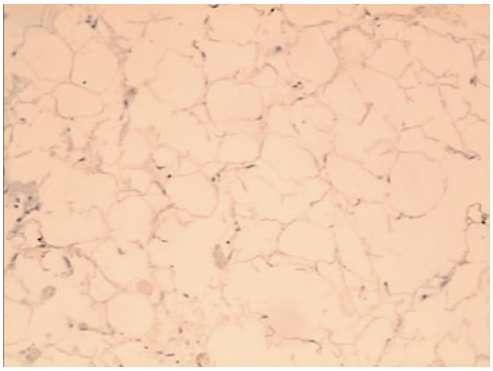

Fig. 3 The CD68＋expression in subcutaneous adipose tissue in group Ⅱ

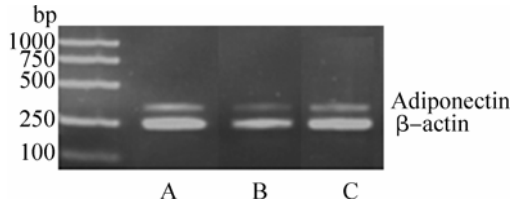

Fig. 4 Adiponectin mRNA expression (CNT/mm^2) in three groups
A: Epicardial adipose tissue in group Ⅰ; B: Epicardial adipose tissue in group Ⅱ; C: Subcutaneous adipose tissue in group Ⅱ

3 DISCUSSION

Adiponectin, an adipocyte-derived plasma protein that contains 244 amino acids is a serum protein of 30 kD. Serum adiponectin concentration is at 2–30 mg/L. Adiponectin, a member of the complement factor C1q family, produced and secreted exclusively by adipose tissues, has been reported to be linked with visceral adiposity, insulin resistance and cardiovascular risk[5–7]. Recently, immunohistochemical studies found adiponectin accumulates in the subendothelial space of the injured human artery and repairs it[8, 9]. In tissue cultures, adiponectin attenuates monocyte attachment to endothelial cells by reducing the expression of adhesion molecules on endothelial cells[10, 11]. Adiponectin also suppresses lipid accumulation in monocyte-derived macrophages by suppressing the expression of macrophage scavenger receptor and its deficiency aggravates neointimal thickening[12, 13]. Moreover, adiponectin supplement attenuates neointimal thickening in mechanically injured arteries through the suppressive effect of adiponectin on the proliferation and migration of vascular smooth muscle cells. Adiponectin serves as a protector for human coronary artery[14, 15].

Epicardial adipose tissue is a true visceral fat tissue that deposits not only on the free wall of right ventricle and on the left ventricular apex, but also around the atria[16, 17]. The epicardial adipose tissue is significantly associated with the development of heart diseases[18–20]. In fact, both epicardial fat and intra-abdominal fat originate from the brown adipose tissue in infancy. The biochemical properties of epicardial adipose tissue suggest it might serve as cardiovascular and metabolic risk indicator[21]. Epicardial adipose tissue is a source of several inflammatory mediators. It was reported that in young adult guinea pigs, the rate of free fatty acids released by epicardial adipose tissue was twice that of the perirenal fat deposits, indicating an increased lipolytic activity. So far, there is little research concerning the role of epicardial adipose for ethical considerations prevented obtaining such tissues from patients undergoing heart surgery.

Human IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of the major proinflammatory cytokines, chemokines and antigen-specific T cell response[22, 23]. In our study, we found that plasma adiponectin level was significantly higher in group Ⅰ as compared with group Ⅱ (P<0.05), which suggests it is, to some extent, linked to coronary heart disease. There were no significant differences in plasma concentration (IL-10, TNF-α, tatal-chol, HDL-chol, LDL-chol) between the two groups, suggesting that plasma inflammatory biomarkers can't reflect the epicardial tissue inflammation. In fact, we found that IL-10 and TNF-α in epicardial adipose tissue is significantly higher in group Ⅰ than in group Ⅱ. However, adiponectin expression in epicardial adipose tissue is significantly lower in group Ⅰ than in group Ⅱ. In this study, we found that the presence of more epicardial adipose is associated with elevated plasma inflammatory factor concentrations and lower plasma adiponectin levels, indicating that inflammation in epicardial adipose tissue contributes to the development of coronary artery disease[24, 25].

A growing body of evidence shows that macrophages, as well as T lymphocytes, preferentially accumulate and activate at the epicardial adipose[26]. Histological studies of the human coronary artery revealed adipocytes producing IL-8 and MCP-1 to the smooth muscle cells of the media and the endothelial cells of the *vasa vasorum*[27]. Moreover, it has been shown that smooth muscle cells and endothelial cells possess functional chemokine receptors, including receptors for MCP-1 and IL-8. Macrophages, as well as T lymphocytes, preferentially accumulate at the interface between WAT and the adventitia of atherosclerotic aorta.

The epicardial adipose tissue themselves are the major source of chemokines[28]. WAT is an active endocrine and paracrine organ secreting various pro- and anti-inflammatory cytokines and chemokines[29]. The paracrine role of adipose depots in local coronary atherosclerosis was recently illustrated in epicardial WAT, which has been shown to produce significantly higher levels of inflammatory factors than subcutaneous

adipose tissue (scWAT), including IL-1, IL-6, MCP-1, and TNF-α. The local infiltration by leukocytes was associated with the production of these cytokines by the epicardial adipose depots. Similarly, others have speculated that the presence of WAT surrounding epicardial arteries leads to an amplification of vascular inflammation.

Epicardial fat cells play a key role in the pathogenesis of cardiovascular diseases. Under certain conditions, epicardial fat cells produce a great deal of bioactive substances and induce the inflammatory response. So far, the treatment of coronary heart disease has failed to eliminate the inflammation produced by epicardial adipose tissue. The investigation of inflammation and ischemia caused by epicardial fat cells will contribute to the development of new and more effective treatment of cardiovascular diseases.

REFERENCES

1 Matsuda M, Shimomura I, Sata M, *et al*. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J Biol Chem, 2002, 277(40):37487-37491

2 Moro C, Klimcakova E, Lolmède K, *et al.* Atrial natriuretic peptide inhibits the production of adipokines and cytokines linked to inflammation and insulin resistance in human subcutaneous adipose tissue. J Diabetologia, 2007,50(5):1038-1047

3 Tsuchida A, Yamauchi T, Takekawa S, *et al.* Peroxisome proliferator-activated receptor (PPAR) activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPAR, PPAR, and their combination. J Diabetes, 2005, 54(12):3358-3370

4 Takaoka M, Suzuki H, Shioda S, *et al.* Endovascular injury induces rapid phenotypic changes in perivascular adipose tissue. J Arterioscler Thromb Vasc Biol, 2010,30(8):1576-1582

5 Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, *et al.* Identification and characterization of CTRP9, a novel secreted glycoprotein, from adipose tissue that reduces serum glucose in mice and forms heterotrimers with adiponectin. FASEB J, 2009,23(1):241-258

6 Li S, Shin HJ, Ding EL, *et al*. Adiponectin levels and risk of type 2 diabetes: A systematic review and meta-analysis. JAMA, 2009,302(2): 179-188

7 Chen XZ, Sun ZQ, Zheng XY, *et al.* Adiponectin inhibits free fatty acids -activated Toll-like receptor 4 signaling pathway in monocytes. Chin J Pathophysiol, 2008, 24(11):2117-2120

8 Nakamura Y, Shimada K, Fukuda D, *et al.* Implications of plasma concentrations of adiponectin in patients with coronary artery disease. J Heart, 2004,90(5):528-533

9 Arita Y, Kihara S, Ouchi N, *et al*. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB–binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. J Circulation. 2002,105(24):2893-2898

10 Ouchi N, Kihara S, Arita Y, *et al*. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation, 2001,103(8):1057-1063

11 Chen XZ, Sun ZQ, Du XL, *et al*. Study on the relationship between heat shock protein 70 and toll-like receptor-4 of monocytes. J Huazhong Univ Sci Technolog [Med Sci], 2004,24(6):560-562

12 Waki H, Yamauchi T, Kamon J, *et al*. Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1. J Endocrinology, 2005,146(2):790-796

13 Yin X, Tu L, Yang H. Effect of simvastatin on IL-6 and adiponectin secretion and mRNA expression in 3T3-L1 adipocytes. J Huazhong Univ Sci Technolog [Med Sci], 2007,27(3):248-251

14 Wang TD, Lee WJ, Shih FY, *et al*. Relations of epicardial adipose tissue measured by multidetector computed tomography to components of the metabolic syndrome are region-specific and independent of anthropometric indexes and intraabdominal visceral fat. J Clin Endocrinol Metab, 2009,94(2):662-669

15 Wolk R, Berger P, Lennon RJ, *et al*. Association between plasma adiponectin levels and unstable coronary syndromes. J Eur Heart J, 2007,28(3):292-298

16 Langheim S, Dreas L, Veschini L, *et al*. Increased expression and secretion of resistin in epicardial adipose tissue of patients with acute coronary syndrome. J Am J Physiol Heart Circ Physiol, 2010,298(3):H746-H753

17 Iacobellis G, Ribaudo MC, Assael F, *et al*. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. J Clin Endocrinol Metab, 2003,88(11):5163-5168

18 Roubícek T, Dolinková M, Bláha J, *et al*. Increased angiotensinogen production in epicardial adipose tissue during cardiac surgery: possible role in a postoperative insulin resistance. J Physiol Res, 2008,57(6):911-917

19 Dutour A, Achard V, Sell H, *et al*. Secretory type II phospholipase A2 is produced and secreted by epicardial adipose tissue and overexpressed in patients with coronary artery disease. J Clin Endocrinol Metab, 2010,95(2): 963-967

20 Kremen J, Dolinkova M, Krajickova J, *et al*. Increased subcutaneous and epicardial adipose tissue production of proinflammatory cytokines in cardiac surgery patients: possible role in postoperative insulin resistance.J Clin Endocrinol Metab, 2006,91(11):4620-4607

21 Zhou Y, Zhang M, Guo W, *et al*. Expression of resistin protein in normal human subcutaneous adipose tissue and pregnant women subcutaneous adipose tissue and placenta. J Huazhong Univ Sci Technolog [Med Sci], 2006, 26(3):288-291

22 Wei G, Zhang M, Mei Y, Dong J. Expression of cytokines IL-2, IL-10 and TNF-alpha in mice with herpes simplex viral encephalitis. J Huazhong Univ Sci Technolog [Med Sci], 2006,26(3):308-310

23 Salvati VM, Mazzarella G, Gianfrani C, *et al*. Recombinant human interleukin 10 suppresses gliadin dependent T cell activation in ex vivo cultured coeliac intestinal mucosa. J Gut, 2005,54(1):46-53

24 Baker AR, Harte AL, Howell N, *et al*. Epicardial adipose tissue as a source of nuclear factor-kappaB and c-Jun N-terminal kinase mediated inflammation in patients with coronary artery disease. J Clin Endocrinol Metab, 2009, 94(1):261-267

25 Silaghi A, Achard V, Paulmyer-Lacroix O, *et al*. Expression of adrenomedullin in human epicardial adipose tissue: role of coronary status. J Am J Physiol Endocrinol Metab, 2007,293(5):E1443-E1450

26 Gruen ML, Hao M, Piston DW, *et al*. Leptin requires canonical migratory signaling pathways for induction of

monocyte and macrophage chemotaxis.Am J Physiol Cell Physiol, 2007,293(5):C1481-8148

27 Bumrungpert A, Kalpravidh RW, Chuang CC, *et al*. Xanthones from mangosteen inhibit inflammation in human macrophages and in human adipocytes exposed to macrophage-conditioned media. J Nutr, 2010,140(4): 842-847

28 Henrichot E, Juge-Aubry CE, Pernin A, *et al*. Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis? J Arterioscler Thromb Vasc Biol. 2005,25(12):2594-2599

29 Herder C, Baumert J, Thorand B, *et al*. Chemokines and incident coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. J Arterioscler Thromb Vasc Biol, 2006,26(9):2147-2152

(Received March 5, 2010)