INCREASED ANGIOTENSINOGEN PRODUCTION IN EPICARDIAL ADIPOSE TISSUE DURING CARDIAC SURGERY: POSSIBLE ROLE IN A POSTOPERATIVE INSULIN RESISTANCE

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Short title:
Fat angiotensinogen expression in critical illness

SUMMARY

Critical illness induces among other events production of proinflammatory cytokines that in turn interfere with insulin signalling cascade and induce insulin resistance on a postreceptor level. Recently, local renin-angiotensin system of adipose tissue has been suggested as a possible contributor to the developement of insulin resistance in patients with obesity. The aim of our study was to determine local changes of the renin-angiotensin system of subcutaneous and epicardial adipose tissue during a major cardiac surgery, which may serve as a model of an acute stress potenatially afecting endocrine function of adipose tissue. 10 patients undergoing elective cardiac surgery were included into the study. Blood samples and samples of subcutaneous and epicardial adipose tissue were collected at the beginning and at the end of the surgery. Blood glucose, serum insulin and adiponectin levels were measured and mRNA for angiotensinogen, angiotensin-converting enzyme and angiotensin II type 1 receptor were determined in adipose tissue samples using RT PCR. Cardiac surgery significantly increased both insulin and blood glucose levels suggesting the development of insulin resistance while serum adiponectin levels did not change. Expression of angiotensinogen mRNA significantly increased in epicardial adipose tissue at the end of surgery relative to baseline but remained unchanged in subcutaneous adipose tissue. Fat expression of angiotensin-converting enzyme and type 1 receptor for angiotensin II were not affected by surgery. Our study suggests that increased angiotensinogen production in epicardial adipose tissue may contribute to the developement of postoperative insulin resistance.

Key words: adiponectin, adipose tissue, angiotensinogen, critical illness, insulin resistance, renin angiotensin system

INTRODUCTION

Hyperglycemia frequently occurs in critically ill patients both with and without previous history of diabetes (Shangraw *et al.* 1989; Van den Berghe 2000; Van den Berghe *et al.* 2006). Major patophysiological conditions underlying hyperglycemia in critical illness include enhanced hepatic gluconeogenesis, impaired insulin secretion and decreased insulin sensitivity due to anti-insulin effects of stress hormones and proinflammatory cytokines (Krinsley 2003; Van den Berghe 2000). Numerous studies have documented that increased blood glucose levels worsen morbidity and mortality in critically ill patients (Butler *et al.* 2005; van den Berghe *et al.* 2001; Vanhorebeek *et al.* 2005); and that intensive insulin therapy aimed at maintaining euglycemia markedly improves the outcome of these patients (Krinsley 2003; Van den Berghe et al. 2006; van den Berghe et al. 2001). Cardiac surgery represents major stress for the organism and leads to secretion of stress hormones (e.g. cortisol, glucagon, growth hormone, catecholamines) and proinflammatory cytokines that in turn induce insulin resistance and hyperglycemia (van den Berghe et al. 2001). We have recently demonstrated that elective cardiac operation stimulates the production of proinflammatory cytokines such as tumor necrosis factor-α, interleukin-6 and resistin in both epicardial and subcutaneous adipose tissue (Kremen *et al.* 2006). Other study reported that epicardial adipose tissue could locally interact with the adjacent myocardium and modulate the morphology and function of the heart (Iacobellis and Sharma 2007). These data suggest that endocrine production of adipose tissue may contribute to the development of insulin resistance and modulations of cardiac function not only in patients with obesity and type 2 diabetes mellitus but also in critically ill patients.

Adipose tissue produces not only proinflammatory factors and hormones (Anderlova *et al.* 2006; Housa *et al.* 2006; Housova *et al.* 2005) but also numerous other molecules including components of renin-angiotensin system (Engeli *et al.* 2003; Iacobellis and Sharma 2007; Jones *et al.* 1997). Renin-angiotensin system is traditionally recognized as an important regulator of systemic blood pressure, water and electrolyte homeostasis (Cooper *et al.* 2007; Reid *et al.* 1978). Production of components of renin-angiotensin system (angiotensinogen, angiotensin-converting enzyme, angiotensin II type 1 receptor) have been detected in a variety of tissues such as kidney, adrenal glands, brain, heart, and blood vessels (Cooper et al. 2007). Local renin-angiotensin system has been implicated in pathological changes of organ structure and function by modulation of gene expression, growth, fibrosis, and inflammatory response (Cooper 2004; Ganong 1994). The local renin-angiotensin system of the adipose tissue is being studied as a possible contributor to the developement of systemic insulin resistance (Cooper 2004; Kim *et al.* 2006). Recent studies indicate that some of the components of renin-angiotensin system (e.g. angiotensin II - a metabolically active hormone derived from the cleavage of angiotensinogen) produced within the adipose tissue may directly induce insulin resistance, increase free oxygen radicals production and decrease production of leptin and adiponectin (Ferder *et al.* 2006; Kurata *et al.* 2006). Furthemore, many clinical studies have documented that treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers decreased the incidence of type 2 diabetes (Dahlof *et al.* 2002; Hansson *et al.* 1999; Scheen 2004; Vermes *et al.* 2003; Yusuf *et al.* 2000). The molecular mechanism of these effects have been unknown until recently. Recent data suggest that increased local levels of angiotensin II directly interfere with insulin

signalling on the postereceptor level and increase local oxidative stress in the adipose tissue (Ferder et al. 2006). Experimental studies have shown that treatment with angiotensin II type 1 receptor blockers stimulate gene expression of insulin-sensitizing adipocytokine adiponectin (Moriuchi *et al.* 2007) and increase circulating adiponectin levels in murine models of obesity (Moriuchi *et al.* 2007) indicating possible involvment of modulation of endocrine function of adipose tissue in the mechanism of insulin-sensitizing effect of renin-angiotensin system inhibitors.

Here we tested hypothesis that increased production of components of renin-angiotensin system in adipose tissue is involved in the development of insulin resistance in critically ill patients. To this end we measured blood glucose, serum insulin and adiponectin levels and mRNA expression of angiotensinogen, angiotensin-converting enzyme, and receptor for angiotensin II type 1 in samples of epicardial and subcutaneous adipose tissue obtained from cardiac surgery patients at the beginning and at the end of elective operation.

PATIENTS AND METHODS

Study subjects

10 patients (6 men and 4 women, mean age 56 ± 14 years, body mass index (BMI) 27 ± 3 kg/m^2) who underwent major elective cardiac surgery (7 patients aorto-coronary bypass, 3 valvular plastic; duration of the surgery 4.6 ± 1.2 hours) were included into the study. Clinical characteristics of the patients are shown in Table 1. One patient had type 2 diabetes on the insulin therapy, 7 patients had arterial hypertension, 6 of these patients had been treated with antihypertensive drugs (two of them with angiotensin-converting

enzyme inhibitors (ramipril), none with angiotensin receptor blockers) before the beginning of the study. 5 patients had dyslipidemia and 2 of them had been treated with statins. None of the patients had malignant tumor, thyroid disease or acute infectious disease.

All patients gave their written consent before being enrolled in the study. The study was approved by the Human Ethical Commitee, 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic and performed in accordance with the guidelines of the Helsinki declaration.

Anthropometric examination and blood and tissue sampling

Anthropometric examination of the patients was performed at basal state one day before operation. All patients were measured and weighed and BMI was calculated.

Blood samples for the measurements of serum insulin levels and blood glucose were taken before the start of anesthesia and after the end of operation (at the time of admission to intensive care unit). Paired samples of the subcutaneous and epicardial adipose tissue were collected at the beginning and at the end of elective cardiac surgery from previously non-traumatized subcutaneous (thoracic region) and epicardial adipose tissue. Approximately 100mg of tissue was collected to 1ml of RNA stabilization Reagent (RNAlater, Qiagen, Germany) and stored at -80°C until further analysis.

Blood glucose was measured on ABL 700 analyzer (Radiometer Medical A/S, Copenhagen, Denmark). Serum concentrations of insulin were measured using Human serum adipokine LINCOplex Kit (panel B) on Luminex®200 instrument (Linco

Research, USA). Serum adiponectin concentrations were measured by commercial RIA kit (Linco Research, St. Charles, Missouri, USA).

Determination of mRNA expression

Total RNA was extracted from subcutaneous and epicardial adipose tissue by homogenization on MagNA Lyser Instrument with MagNA Lyser Green Beads (Roche Diagnostics GmbH, Germany) using MagNA Pure Compact RNA Isolation (Tissue) (Roche Diagnostics GmbH, Germany). The RNA concentration was determined from absorbance at 260 nm (BioPhotometer, Eppendorf AG, Germany). All samples had a 260/280 nm absorbance ratio 1.89 ± 0.1. The integrity of the RNA was checked by visualization of 18S and 28S ribosomal bands on 1 % agarose gel with an g of total RNA was used for reverse transcription to ethidium bromid. 0.1-1 synthesize the first strand cDNA using the oligo$(dT)_{18}$ primers following the instructions of the RevertAid First Strand cDNA synthesis kit (Fermentas Life Science, Lithuania). Gene expression of angiotensinogen, angiotensin-converting enzyme and angiotensin II type 1 receptor was performed on an ABI PRISM 7500 instrument (Applied Biosystems, Foster City, CA, USA) using TaqMan® Universal PCR Master Mix, NO AmpErase® UNG and specific TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA, USA).
All PCRs for each gene were amplified separately. Controls with no template cDNA were performed with each assay and all samples were run at least in duplicates. The increase in fluorescence was measured in real time and data were obtained as threshold cycle (C_T) values. To compensate for variations in input RNA amounts and efficiency of reverse

transcription, beta-2-microglobulin was used as an endogenous reference and results were normalized to these values. Relative gene expression of genes was calculated using the formula $2^{-\Delta\Delta(\text{CT cytokine-CT B2M})}$.

Statistical analysis

The statistical analysis was performed on SigmaStat software (Jandel Scientific, USA). The results are expressed as means ± standard error of mean (SEM). Changes of gene expression during the cardiac surgery were evaluated using paired t-test. Results were considered statistical significant at $p<0.05$.

RESULTS

Both blood glucose and serum insulin levels significantly increased at the end of operation indicating the development of insulin resistance (Table 2). In contrast, serum adiponectin concentrations did not significantly change (Table 2).

At baseline, angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and angiotensin II type 1 receptor (AGTR1) mRNA expression did not significantly differ between epicardial and subcutaneous adipose tissue depots (Table 2 and Figure 1). Expression of angiotensinogen mRNA in epicardial adipose tissue significantly increased at the end of the surgery compared to the baseline values, while no significant change in this parameter was found in subcutaneous adipose tissue (Table 2 and Figure 1). Neither subcutaneous nor epicardial angiotensin-converting enzyme and angiotensin II type 1 receptor mRNA expression were significantly affected by cardiac surgery (Table 2). We did not find any significant correlations between fat mRNA expression of

angiotensinogen and blood glucose, serum insulin and serum adiponectin concentrations, respectively (data not shown).

DISCUSSION

The aim of our study was to determine whether local production of renin-angiotensin system components in the adipose tissue contributes to the development of insulin resistance after cardiac operation. We have demonstrated that cardiac surgery significantly increased mRNA expression of angiotensinogen in epicardial adipose tissue suggesting its possible involvement in the induction of local and possibly systemic insulin resistance. Adipose tissue as a source of components of renin-angiotensin system has been identified previously (Engeli et al. 1999); however it has not yet been described how the local renin-angiotensin system of the adipose tissue responds to a major stress. Here we have chosen patients undergoing elective cardiac surgery as model for insulin resistance induced acutely by cardiac surgery. It has been shown previously that patients after major cardiac surgery develop insulin resistance also called „diabetes of injury“ and that normalisation of their blood glucose using intensive insulin therapy substantially decreased morbidity and mortality (van den Berghe et al. 2001). Despite marked progress in our understanding of the etiopathogenesis of this process the exact mechanism how normalization of blood glucose improves the outcome of critically ill patients still remains to be elucidated.

The results of the present study confirm that all the main components of renin-angiotensin system (i.e. angiotensinogen, angiotensin-converting enzyme and angiotensin II type 1 receptor) are expressed in both subcutaneous and epicardial adipose tissue.

Furthermore, we demonstrate that cardiac surgery increased expression of angiotensinogen mRNA in epicardial but not in subcutaneous adipose tissue. Overexpression of components of renin-angiotensin system (angiotensinogen and angiotensin II type 1 receptor) in human visceral adipose tissue relative to subcutaneous adipose tissue in normal and overweight subjects has been previously reported by Giaccheti (Giacchetti *et al.* 2002). In our study, we found an increase in angiotensinogen mRNA expression in epicardial adipose tissue in all patients independently of the previous treatment with angiotensin-converting enzyme blockers (ramipril) or statins which are both known for the beneficial effect on insulin sensitivity (Huptas *et al.* 2006; Ostergren 2007). Our data thus offer a novel insight on the adipose tissue function showing subcutaneous and visceral adipose tissues as two heterogenous organs with different gene expression, proteins production and association with cardiovascular disease and are in agreement with previously published studies showing different expression profiles in these two depots (Kershaw and Flier 2004; Mazurek *et al.* 2003). Here we did not find any significant differences in expression of angiotensin-converting enzyme and angiotensin II type 1 receptor mRNA in adipose tissue during cardiac surgery and also no differences between subcutaneous and epicardial adipose tissue with respect to production of angiotensin-converting enzyme and angiotensin II type 1 receptor. This is in contrast with Giaccheti´s study that showed angiotensin II type 1 receptor expressed significantly more abundantly in visceral than in subcutaneous adipose tissue (Giacchetti et al. 2002). However it has to be noted that in our study we used different visceral adipose tissue depot (epicardial fat) and different subcutaneous adipose tissue depot (thoracic region fat).

In this study we did not evaluate the role of angiotensinogen produced by the adipose tissue on the systemic blood pressure in postoperative period, although this effect is traditionally the most important systemic effect of renin angiotensin system. We were not able to evaluate changes of the blood pressure because of the hemodynamical instability of cardiac surgery patients in a postoperative period and frequent need of pharmacological or mechanical support (e.g. catecholamines, intraaortic baloon pump etc.).

We suggest that increased expression of angiotensinogen leads in turn to enhanced production of angiotensin II in epicardial adipose tissue. Angiotensin II, a metabolically active hormone deriving from the cleavage of angiotensinogen, can than interfere with insulin signalling cascade (Ferder et al. 2006; Kurata et al. 2006). In summary, our data show that increased production of angiotensinogen in the epicardial adipose tissue may in concert with overproduction of other proinflammatory factors contribute to the development of insulin resistance in critically ill patients.

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Table 1

Clinical characteristics of the cardiac surgery patients. Expressed as n (number of patients); and mean ± standard error of mean.

Number of subjects (male/female)	10 (6/4)
Body mass index (kg/m^2)	27 ± 3
Age (years)	56 ± 14
Duration of the surgery (hours)	4.6 ± 1.2
Dyslipidemia	5
Arterial hypertension	7
Diabetes	1

Table 2

Biochemical characteristics of the cardiac surgery patients (expressed as mean ± standard error of mean) and expression of angiotensinogen (AGT) mRNA, angiotensin-converting enzyme (ACE) mRNA and receptor for angiotensin II type 1 mRNA (expressed as mean ± standard error of mean; normalized to B2M mRNA).

Statistical significance is from paired T-test. * indicates $p < 0.05$ vs. start of operation

	Start of operation	End of operation
Blood glucose (mmol/l)	5.9 ± 1.1	*8.6 ± 1.2
Serum insulin levels (pg/ml)	130 ± 36	*341 ± 86
Serum adiponectin levels (ng/ml)	17 ± 8	12 ± 5
AGT mRNA in subcutaneous adipose tissue	0.004 ± 0.001	0.002 ± 0.0007
AGT mRNA in epicardial adipose tissue	0.006 ± 0.002	*0.011 ± 0.002
ACE mRNA in subcutaneous adipose tissue	0.011 ± 0.002	0.009 ± 0.002
ACE mRNA in epicardial adipose tissue	0.008 ± 0.001	0.007 ± 0.001
Receptor for angiotensin II typ 1 mRNA in subcutaneous adipose tissue	0.00008 ± 0.00002	0.00005 ± 0.00001
Receptor for angiotensin II typ 1 mRNA in epicardial adipose tissue	0.00005 ± 0.00001	0.00004 ± 0.00001

Figure 1

Angiotensionogen (AGT) mRNA expression in subcutaneous (SAT) and epicardial adipose tissue (VAT) at the beginning (open bars) and at the end (black bars) of the surgery. Values are mean ± standard error of mean (SEM). Statistical significance is from paired t-test: * indicates $p < 0.05$ versus VAT at the beginning of the surgery

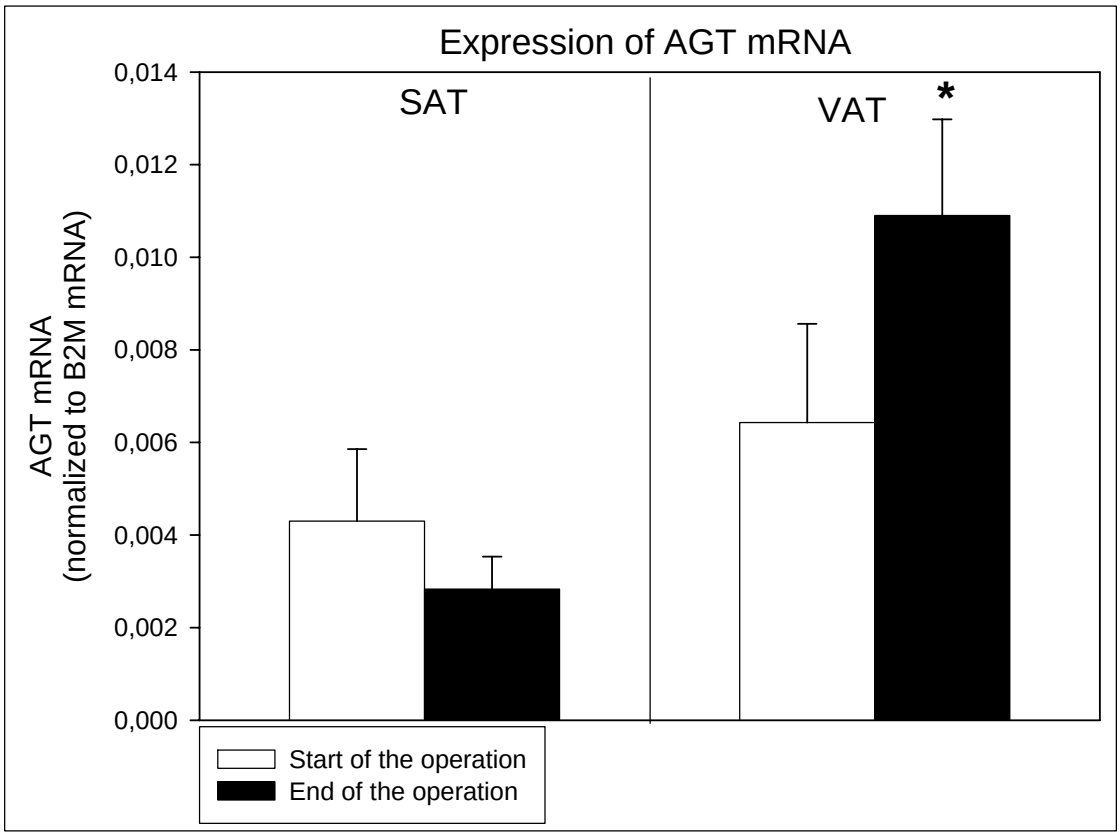

FIGURE 1