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ORIGINAL ARTICLE

Adipocytokine resistin correlates with oxidative stress and myocardial injury in patients undergoing cardiac surgery

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Abstract

OBJECTIVES: Adipocytokines are hormones regulating energy metabolism and appetite and according to recent reports also inflammatory responses including ischaemia–reperfusion injury. Based on experimental data, we hypothesized that the levels of adipocytokines adiponectin, adipsin, leptin and/or resistin would correlate with myocardial injury, inflammation and oxidative stress during cardiac surgery.

METHODS: Thirty-two patients undergoing an elective on-pump coronary artery bypass graft surgery (CABG) with cardiopulmonary bypass (CPB) were recruited into the study. Blood samples were collected after the induction of anaesthesia, and at the onset of CPB, 1 and 15 min after the removal of aortic cross-clamp and 4 and 24 h after the onset of CPB. Samples were analysed for levels of four adipocytokines (adiponectin, adipsin, leptin and resistin) and markers of oxidative stress [myeloperoxidase (MPO) and 8-isoprostane], inflammation [interleukin-6 (IL-6)] and myocardial injury [troponin T (TnT)].

RESULTS: Adiponectin and adipsin concentrations declined, while leptin and resistin levels increased significantly by 24 h after the onset of the operation. Interestingly, basal levels of resistin ($r = 0.41$, $P = 0.020$) as well as the maximal increase occurring in resistin levels during the 24-h follow-up ($r = 0.49$, $P = 0.005$) correlated positively with TnT release. In addition, the reperfusion-induced elevation in resistin levels correlated positively with oxidative stress measured as increases in MPO concentrations.

CONCLUSIONS: As an original finding, we report here that resistin levels correlate with oxidative stress and myocardial injury in patients undergoing cardiac surgery. In addition, leptin levels were increased on the first postoperative day, but only minor declines were found in adiponectin and adipsin levels. Resistin has been implicated in unfavourable metabolic, cardiovascular and inflammatory responses: it may thus serve as a useful biomarker or a drug target in conditions complicated by ischaemia–reperfusion injury.

Keywords: Adipocytokine • Cardiac surgery • Ischaemia–reperfusion injury • Myocardial injury • Oxidative stress • Resistin

INTRODUCTION

Open heart surgery utilizing cardiopulmonary bypass (CPB) and cardioplegia may induce ischaemia in the myocardium. When the aortic cross-clamp (ACC) is removed and the blood flow to the myocardium is reinitiated, further damage may incur in the myocardium due to reperfusion injury. At the beginning of reperfusion, pro-inflammatory cytokines and chemotactic agents are released from the myocardium and endothelium leading to neutrophil infiltration, inflammation and tissue injury [1].

Several methods have been postulated as helping to protect the myocardium from ischaemia–reperfusion injury related to cardiac surgery. For example, cardioplegic arrest is the established standard method for modern myocardial protection. Ischaemic preconditioning (IPC) and post-conditioning (I-post) activate the reperfusion injury salvage kinase pathway [1]. However, these methods have limited clinical use and, therefore, pharmacological interventions to mimic the effects of IPC and I-post are under investigation and development [1]. However, a more profound understanding of the mediators and mechanisms contributing to ischaemia and reperfusion will be needed if we are to develop novel therapeutic strategies to prevent and treat the consequences of ischaemia–reperfusion injury in patients undergoing cardiac surgery.

Recently, adipocytokines have been proposed as novel factors involved in the pathogenesis of ischaemia and reperfusion related to myocardial damage but there is a paucity of clinical data. Adipocytokines are hormones that were originally discovered in adipose tissue and considered to function as factors regulating energy metabolism and appetite [2]. More recently, adipocytokines have also been implicated in inflammatory responses and, interestingly, they have also been reported to be involved in regulating cardiac and vascular functions [3, 4].

There are only a few studies reporting changes in leptin, adiponectin and/or resistin levels after cardiac surgery [5, 6]. Results

from studies in cardiomyocytes and in animal models of experimentally induced ischaemia–reperfusion have indicated that leptin and adiponectin may possess cardioprotective properties but very little is known about the properties of resistin and adipsin in that respect [3]. Leptin has been shown to delay opening of the mitochondrial permeability transition pores (MPTPs) and prevent mitochondrial uncoupling and cell death [7], whereas adiponectin was reported to activate the adenosine monophosphate-activated protein kinase (AMPK) and cyclooxygenase-2 (COX-2)– prostaglandin E_2 (PGE_2) pathway suppressing TNFα production and thereby inhibiting cell death: all of these are mechanisms protecting against myocardial ischaemia–reperfusion injury [8]. The biological effects of leptin and adiponectin are mediated through each adipocytokine's specific receptors (Ob-Ra and Ob-Rb for leptin, AdipoR1 and AdipoR2 for adiponectin) which are expressed also in cardiomyocytes [3]. No specific receptors have been identified for resistin or adipsin, but a recent study revealed that the effects of resistin may be transduced through Toll-like receptor 4 (TLR4) [9] which triggers major inflammatory pathways and is also activated by bacterial lipopolysaccharide (LPS). Interestingly, activation of TLR4 has been reported to play a significant role in ischaemia–reperfusion-induced inflammation and injury (while its endogenous agonist remains unknown), and antagonists for TLR receptors are under investigation for the treatment of ischaemia–reperfusion injury [1].

Those findings led us to hypothesize that adipocytokines could be related to CPB-induced reperfusion injury and serve as biomarkers or as novel drug targets. In the present study, we tested the hypothesis in a clinical setting: We recruited patients undergoing elective on-pump coronary artery bypass graft surgery (CABG) and determined the time course of circulating levels of adipocytokines adiponectin, adipsin, leptin and resistin during surgery and for the following 24 h after the onset of CPB. Further, we assessed whether the extent of inflammatory response [interleukin-6 (IL-6)], oxidative stress [myeloperoxidase (MPO) and 8-isoprostane] and myocardial damage [troponin T (TnT)] correlated with the adipocytokine levels in the patients. We also aimed at detecting whether there was a correlation between resistin concentrations and markers of oxidative stress and myocardial injury in patients undergoing cardiac surgery.

MATERIALS AND METHODS

Patients

Thirty-two patients undergoing an elective on-pump CABG with CPB in the Tampere University Hospital Heart Center Co., Tampere, Finland, were recruited into the study. The study was approved by the Ethics Committee of Tampere University Hospital and complies with the declaration of Helsinki. All patients provided their written informed consent. Funding sources were not involved in the study design, in the collection, analysis or interpretation of the data or in the writing of the manuscript.

All patients had coronary artery disease (CAD) with at least grade II symptoms as assessed by the Canadian Cardiovascular Society (CCS) angina scale. The average CCS value in the patient group was grade III. Patients with previous malignancy, pre-existent pulmonary disease, pulmonary hypertension (pulmonary artery pressure > 40 mmHg), impaired left ventricular function (LVEF <50%), smoking or myocardial infarction during the previous 3 months; or cardiac failure period, acute coronary syndrome, infection and/or use of corticosteroids or COX-2 inhibitors within the previous month were excluded from the study. The clinical characteristics of the patients are shown in Table 1.

Anaesthesia and perfusion

Radial and pulmonary arterial lines were inserted for haemodynamic monitoring before commencing the induction of anaesthesia. Propofol (0.5–1.0 mg/kg), sufentanil (0.8–1.0 μg/kg) and rocuronium were administered for the induction of anaesthesia. Propofol infusion was continued with a rate of 50–80 μg/kg/min and sufentanil with 0.03–0.05 μg/kg/min for maintenance, supplemented with midazolam boluses as necessary. Occasional hypertensive episodes were controlled with nitroglycerine or labetalol.

Ventilation of the lungs was provided with a volume-controlled anaesthesia ventilator (Dräger Julian Plus, Dräger Medizintechnik GmbH, Germany). F_iO_2 was initially adjusted to 0.50, with a tidal volume of 7–8 ml/kg and frequency adjusted for normocarbia.

CPB was established with the standard cannulation technique, including aortic and right atrial two-stage lines. Non-pulsative perfusion flow (2.4 l/m^2) with mild hypothermia (34°C) was provided using a roller pump system (Stöckert S3, Sorin Group Deutchland GmbH, Germany). Alpha-stat blood gas management was applied during the perfusion. A tubing set with biopassive coating and hollow fibre oxygenator (D 903 Avant Phisio, Dideco S.P.A, Mirandola, Italy) was primed with 1500 ml of Ringer's acetate. Tranexamic acid was used as anti-fibrinolytic therapy. Aprotinin was not used in the present study due to its possible anti-inflammatory properties. Cardio-protection was provided with standard cold blood cardioplegia. Both antegrade and retrograde cardioplegia cannulas were inserted. The first antegrade cardioplegic infusion was given for 2 min, followed by retrograde cardioplegia for another 2 min. Subsequent retrograde

Table 1: Patient characteristics

n = 32	
Age (years), mean ± SEM	68.5 ± 1.5
Females (%)	28
BMI, mean ± SEM	28.5 ± 0.8
EuroSCORE, mean ± SEM	2.4 ± 1.4
ACC time, mean ± SEM (min)	73.6 ± 2.0
Perfusion time, mean ± SEM (min)	96.4 ± 1.8
Number of grafts, mean	4
Postoperative myocardial infarction, *n*	1
Postoperative stroke, *n*	1
Postoperative atrial fibrillation, *n*	10
Preoperative medications (%)	
Acetylsalicylic acid	80
β-Blockers	88
ACE inhibitors/ARBs	47
Calcium channel blockers	19
Diuretics	38
Nitrates	66
Statins	72
Insulin	6
Oral anti-diabetics	22

SEM: standard error of mean; ACC: aortic cross-clamp; ACE: angiotensin converting enzyme; ARBs: angiotensin receptor blockers.

cardioplegia infusions were delivered after completion of each distal anastomosis for 1 min, and final warm retrograde cardioplegia (37°C) during 3 min before aortic declamping.

Surgical approach and intensive care unit protocol

A median sternotomy was performed in all patients, and the left internal mammary artery was harvested in all cases and used for left anterior descending artery (LAD) grafting. The other bypasses were constructed using saphenous vein grafts harvested at the beginning of the operation. Parietal pleura opening was avoided if possible. Distal and proximal anastomoses were completed during single ACC period. All patients were observed in the intensive care unit with a standard protocol. They were weaned from the ventilator when they were in a state of fair mental cooperation, rewarmed and in a haemodynamically stable condition, when the chest tube drainage was <100 ml/h and when they had adequate pulmonary function (showing $PaO_2 > 9$ kPa when $FiO_2 \leq 0.45$).

Blood samples and analyses

Blood was drawn from the radial artery. The first sample was collected after the induction of anaesthesia (referred to as sample 1 or baseline in the following text and figures), and the second sample was taken at the onset of CPB (sample 2, CPB). The third sample was acquired 1 min after the removal of ACC (sample 3, ACC+1 min) and the fourth sample was drawn 15 min after the removal of ACC when the lungs were re-inflated (sample 4, ACC +15 min). The fifth sample was taken 4 h (sample 5, CPB+4 h) and the sixth sample 24 h (sample 6, CPB+24 h) after the onset of CPB. The plasma samples were stored at −80°C until analysed.

The concentrations of the adipocytokines (adiponectin, adipsin, leptin and resistin), IL-6, 8-isoprostane and MPO in plasma samples were determined by enzyme-linked immunosorbent assay (ELISA) using the following reagents: adiponectin, adipsin, leptin and resistin: DuoSet ELISA, R&D Systems Europe, Ltd, Abindgon, UK; IL-6: PeliPair ELISA, Sanquin, Amsterdam, Netherlands; 8-isoprostane: Cayman, Ann Arbor, MI, USA; and MPO: Hycult Biotechnology, Uden, Netherlands. The detection limits and interassay coefficients of variation were 15.6 pg/ml and 10.1% for adiponectin, 15.6 pg/ml and 5.6% for adipsin, 15.6 pg/ml and 4.3% for leptin, 15.6 pg/ml and 9.6% for resistin, 0.6 pg/ml and 8.9% for IL-6, 0.8 pg/ml and 6.1% for 8-isoprostane and 0.4 ng/ml and 6.8% for MPO, respectively. TnT concentrations were measured by an immunochemiluminometric method in Huslab, Helsinki University Hospital, Helsinki, Finland.

Statistical analysis

Data were analysed using PASW statistics 19.0 (IBM Corporation, Armonk, NY, USA). The results are presented as mean ± standard error of mean (SEM) or as otherwise indicated. Pearson's and Spearman's correlation analyses were used where appropriate according to the distribution of the data, and *r*-values over 0.3 and under −0.3 were considered to indicate statistically a medium-sized correlation. Differences between groups were tested by repeated measures ANOVA followed by Bonferroni multiple comparisons test. *P*-values <0.05 were considered statistically significant.

RESULTS

Adipocytokine levels during and after coronary artery bypass graft surgery

Plasma adipocytokine levels changed significantly during and after CABG surgery; adiponectin and adipsin levels declined while those of leptin and resistin increased significantly, especially after the surgery (Fig. 1).

Adiponectin and adipsin levels started to decline after the induction of anaesthesia and remained below the baseline level during the entire operation and up to the first postoperative day (Fig. 1A and 1B). Adiponectin levels were at their nadir 4 h after the onset of CPB (∼40% below the baseline). The lowest adipsin levels (∼30% below the baseline values) were found 24 h after the onset of the surgery.

After the induction of anaesthesia, leptin concentrations also decreased and remained low during the surgery. On the first postoperative day, a significant increase occurred with leptin levels becoming tripled when compared with the baseline concentrations. (Fig. 1C).

Resistin levels fell initially below the baseline level at the onset of CPB but thereafter, in contrast to the other adipocytokines, the resistin concentration started to increase, being 25% above the baseline level when measured shortly after the removal of the ACC. Thereafter, the resistin concentrations continued to increase towards the first postoperative day finally being 2.5-fold higher than the baseline level (Fig. 1D).

Myeloperoxidase, 8-isoprostane, interleukin-6 and troponin T levels during and after coronary artery bypass graft surgery

The markers of oxidative stress, MPO and 8-isoprostane, were greatly elevated during the surgery (Fig. 2A and B). MPO levels increased significantly (7-fold increase) at the onset of CPB, and the maximum concentration was reached shortly after the ACC was removed (on average 17-fold increase). After the surgery, MPO levels decreased, and were only slightly elevated at 4 and 24 h after the onset of CPB (260 and 190% of baseline levels, respectively).

The maximal concentrations of 8-isoprostane were measured at the onset of CPB and the levels remained highly elevated for the duration of the surgery. Four hours after the onset of CPB, the concentrations of 8-isoprostane had returned to the baseline levels.

IL-6 concentrations steeply increased during and after the surgery (Fig. 2C) with the peak concentration being measured at 4 h after the onset of CPB (more than 100-fold increase). On the first postoperative day, the levels were still high but started to decline (although the IL-6 levels were still 40-fold higher than at the baseline).

TnT concentrations varied from being undetectable to 31 ng/l at the baseline with the mean concentration being 7 ± 9 ng/l (Fig. 2D). On the first postoperative day, 24 h after the onset of CPB, a significant increase in TnT levels was detected with the

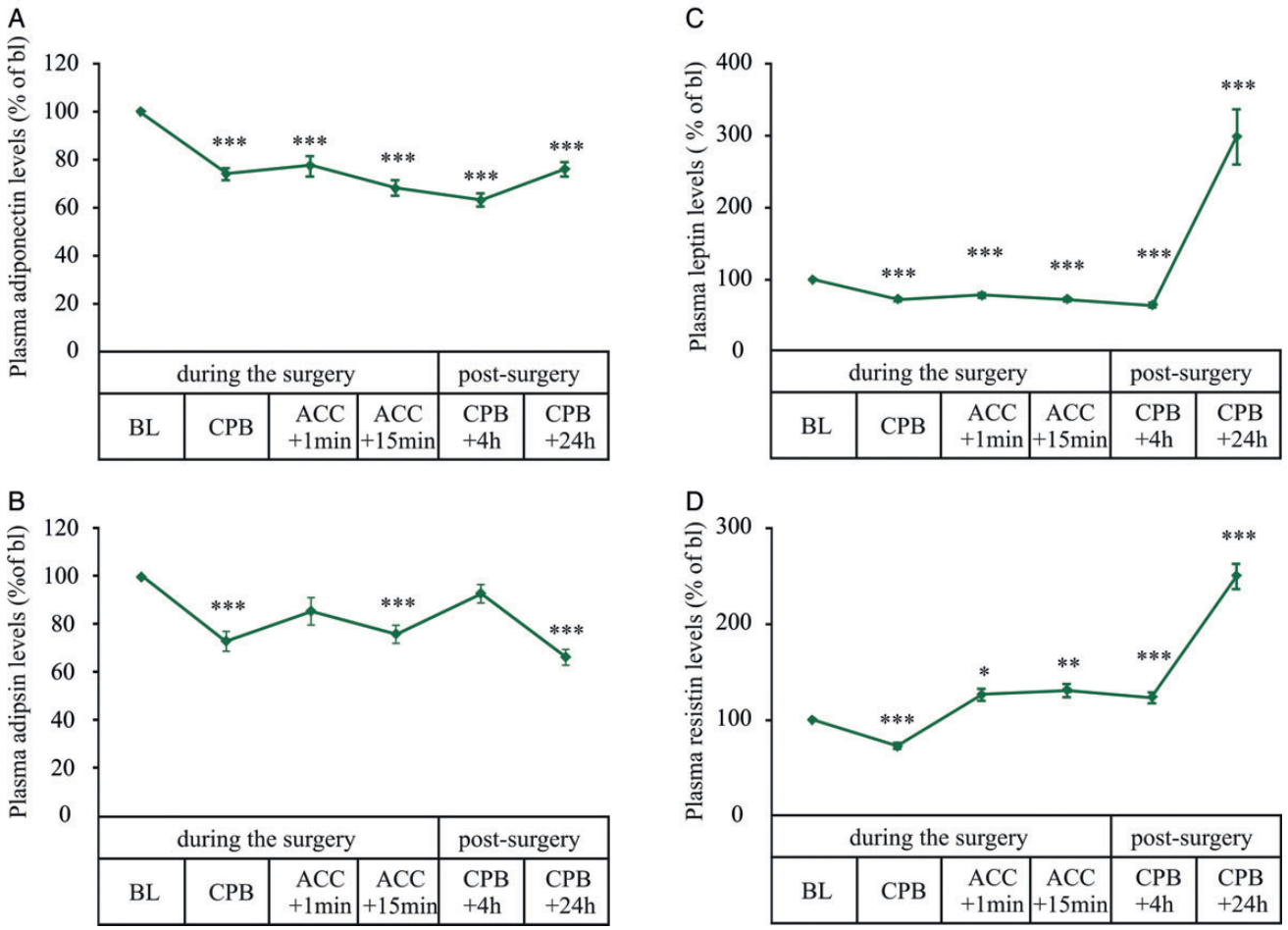

Figure 1: Adipocytokine levels in patients ($n = 32$) with CAD during and after an elective on-pump CABG with CPB. The first sample was drawn after the induction of anaesthesia [referred to as 1 or baseline (BL)], and the second sample was taken at the onset of CPB [2]. The third sample was acquired 1 min after the removal of ACC [3] and the fourth sample was drawn 15 min after the removal of the ACC [4, ACC+15 min]. The fifth sample was taken 4 h [5, CPB+4 h] and the sixth sample 24 h after the onset of CPB [6, CPB+24 h]. Results are presented as mean ± SEM. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ compared with the baseline.

mean concentration being 434 ± 17 ng/l with values ranging from 113 to 1503 ng/l (median 312 ng/l).

Correlations between concentrations of adipocytokines and biomarkers of inflammation, oxidative stress and myocardial damage

The baseline resistin levels as well as baseline leptin concentrations correlated positively with TnT release ($r = 0.408$, $P = 0.020$ and $r = 0.388$, $P = 0.028$, respectively) (Table 2). Interestingly, the maximal increase in resistin levels correlated positively with TnT release, $r = 0.489$, $P = 0.005$) (Fig. 3A), indicating that the more the resistin levels increased, the more TnT was released from the myocardium. As shown in Fig. 3A, there was extensive variation in the amounts of TnT released. Three of the patients had TnT levels that were higher than 1000 ng/l. Based on the clinical data, one of these patients suffered a myocardial infarction, which could have affected the TnT levels; but for the last 2 of those 3 patients, we could find no obvious reason that would have explained the extensive TnT increase. We analysed the data also without these 3 outlying patients and in fact, the correlation between the changes in resistin and TnT was improved from $r = 0.489$, $P = 0.005$, to $r = 0.570$, $P = 0.001$. In addition, reperfusion induced a clear elevation in resistin levels, i.e. an increase was found from baseline as well as from the sample taken at the onset of CPB to the sample collected 1 min after the removal of the ACC. Further, a positive correlation was found between the changes in resistin and MPO levels from the baseline to the sample taken after restoring the blood flow (ACC+1 min) ($r = 0.563$, $P = 0.001$) (Fig. 3B). A similar correlation between the changes in resistin and MPO levels measured in the sample taken at the onset of CPB and in the sample collected 1 min after the removal of ACC was also found ($r = 0.434$, $P = 0.0013$).

In contrast, an inverse correlation was found between the changes in the levels of adipsin and IL-6. Thus, patients with greater declines in the plasma adipsin concentrations from baseline also exhibited greater elevations in their plasma IL-6 levels ($r = -0.424$, $P = 0.015$, Table 2). There were no correlations between the levels of adiponectin and the biomarkers of inflammation, oxidative stress or myocardial damage.

DISCUSSION

This is the first study to demonstrate a clear association between resistin levels and the degrees of myocardial injury and oxidative

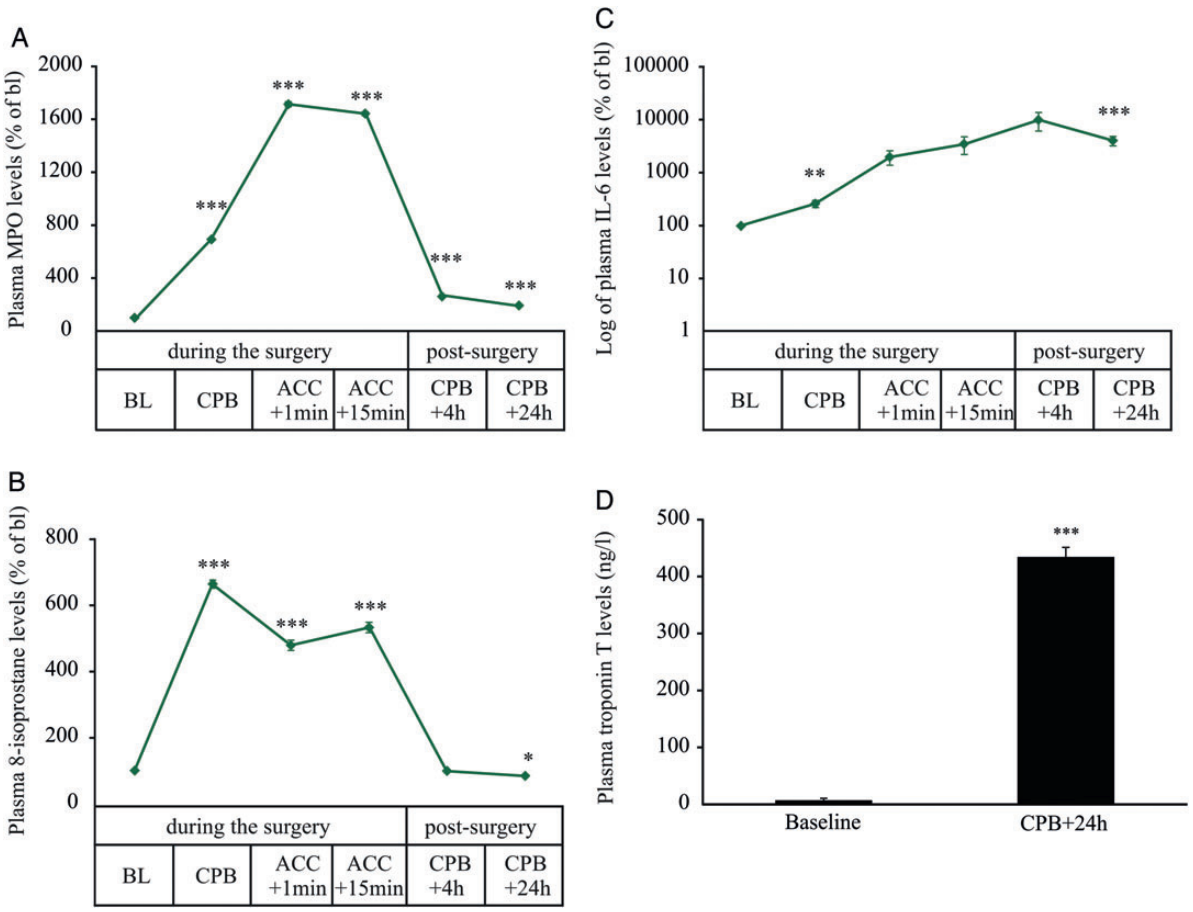

Figure 2: Markers of oxidative stress measured as MPO and 8-isoprostane, inflammation measured as IL-6 and myocardial injury measured as TnT in patients ($n = 32$) with CAD during and after elective on-pump CABG with CPB. (**A**–**C**) The first sample was drawn after the induction of anaesthesia [referred to as 1 or baseline (BL)], and the second sample was taken at the onset of CPB [2]. The third sample was acquired 1 min after the removal of ACC [3] and the fourth sample was drawn 15 min after the removal of the ACC [4, ACC+15 min]. The fifth sample was taken 4 h [5, CPB+4 h] and the sixth sample 24 h after the onset of CPB [6, CPB+24 h]. (**D**) TnT was measured after the induction of anaesthesia (baseline) and 24 h after the onset of CPB. Results are presented as mean ± SEM. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ compared with the baseline.

stress in patients undergoing open heart surgery. Our study is also the first to describe the temporal changes in resistin concentrations during on-pump CABG surgery and the possible prognostic value of baseline resistin levels for predicting the degree of myocardial injury. The changes in other studied adipocytokines, with the exception of the late elevation in the leptin concentrations, were minor and did not correlate with the extent of myocardial injury or oxidative stress.

Resistin is a pro-inflammatory adipocytokine and its expression has also been reported to be up-regulated in epicardial adipose tissue in CAD patients undergoing CABG surgery along with many other pro-inflammatory cytokines [10]. Resistin has also been linked to adverse cardiac events in patients with heart failure [11]. Accordingly, Weikert *et al.* reported that a high plasma resistin concentration was related to increased risk of acute myocardial infarction (AMI) when compared with patients with lower resistin levels [12]. In the present study, high basal resistin levels predicted a greater TnT release, and a clear association was found in the maximal increases between resistin and TnT levels following cardiac surgery, further supporting the association between resistin and myocardial injury.

Resistin was initially named due to its ability to evoke insulin resistance in rodents, but in humans, the relation between insulin resistance and resistin is less clear [2]. Our findings showed that resistin levels increased markedly during CPB and continued to rise for up to 24 h after the onset of CPB. The present study also revealed that the elevation in resistin levels induced by reperfusion correlated positively with the degrees of oxidative stress, measured by increases in MPO (which is primarily released from neutrophils). Our findings are supported by the fact that in humans, resistin is secreted mainly by inflammatory cells including neutrophils [13], and this correlation points to the possibility that part of the circulating resistin originates from activated neutrophils.

Furthermore, our data showed that those patients with the greatest increases in resistin levels displayed also the largest elevations in TnT levels. The previous evidence regarding resistin's cardiotoxic effects during experimentally induced ischaemia–reperfusion injury has been somewhat limited. Rothwell *et al.* reported an exacerbation of reperfusion injury in the myocardium in the Langendorff-perfused rat heart model when human recombinant resistin was administered prior to global ischaemia [14]. They observed an increase in levels of creatine kinase (CK), A and B-type natriuretic peptide and TNFα release and impaired myocardial contractility as opposed to the control group. This result was challenged by Gao *et al.* [15], who treated murine perfused

Table 2: Correlations between changes in the levels of adipocytokines and markers of inflammation, oxidative stress and myocardial damage

			IL-6 Maximal change	MPO Maximal change	8-Isoprostane Maximal change	TnT Maximal change
Adiponectin	Baseline	*r* =	0.256	−0.194	0.052	0.006
		P =	0.158	0.288	0.777	0.974
	Maximal change	*r* =	−0.299	0.054	−0.021	−0.036
		P =	0.096	0.771	0.908	0.843
Adipsin	Baseline	*r* =	−0.190	−0.123	−0.309	0.061
		P =	0.297	0.502	0.085	0.742
	Maximal change	*r* =	**−0.424***	−0.082	−0.071	−0.084
		P =	**0.015***	0.655	0.697	0.649
Leptin	Baseline	*r* =	0.037	0.036	0.093	**0.388***
		P =	0.842	0.844	0.612	**0.028***
	Maximal change	*r* =	−0.088	0.110	−0.150	0.208
		P =	0.631	0.549	0.413	0.254
Resistin	Baseline	*r* =	0.313	−0.050	−0.120	**0.408***
		P =	0.081	0.785	0.514	**0.020***
	Maximal change	*r* =	0.284	0.026	−0.167	**0.489***
		P =	0.122	0.888	0.371	**0.005***

Baseline represents the concentrations after the induction of anaesthesia and maximal change represents the difference between the greatest value measured and the baseline level. Correlations between parametric variables are presented as Pearson correlation coefficients and correlation between non-parametric variables as Spearman's rank correlation coefficients.
*Bold values are statistically significant.

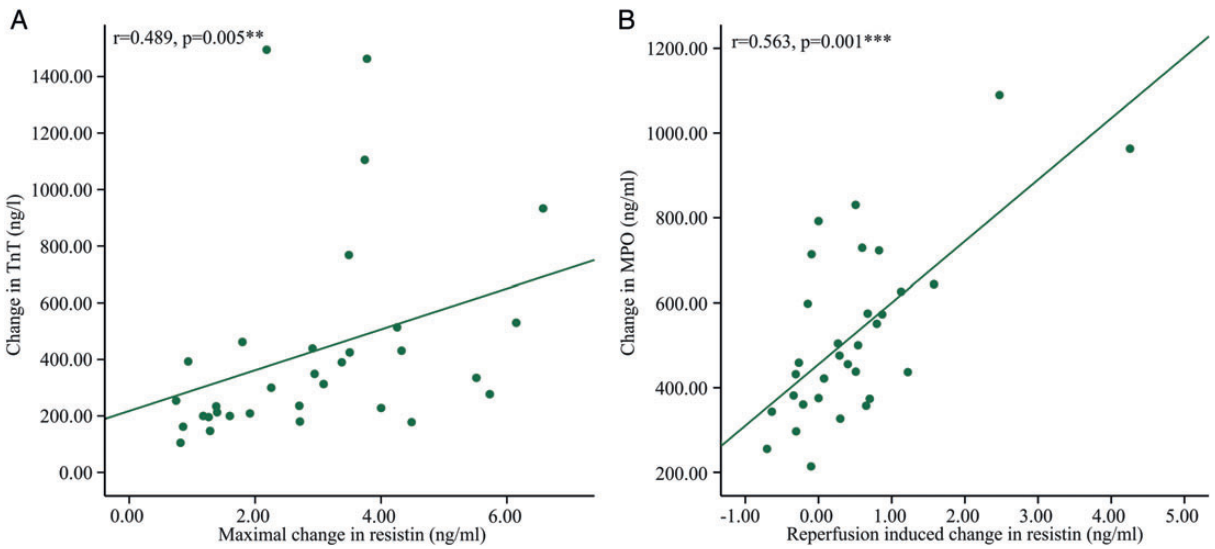

Figure 3: Correlations between increases in resistin levels and myocardial damage as measured by TnT release and oxidative stress measured by MPO release in patients ($n = 32$) with CAD during and after an elective on-pump CABG with CPB. Spearman's rank correlation coefficient was used due to the non-parametrical distribution of the variables. (**A**) Resistin levels are presented as the maximal change in resistin levels from the baseline level. The greatest change in resistin levels existed between the first and the last point of measurement in nearly all of the patients. TnT was measured after the induction of anaesthesia (baseline) and 24 h after the onset of CPB with the changes being plotted. A strong positive correlation was found between resistin levels and the extent of TnT release ($r = 0.489$, $P = 0.005$**). (**B**) Resistin levels are presented as the change in resistin levels between baseline levels and levels measured 1 min after the removal of the ACC. MPO release is presented as the maximal change from the baseline. The greatest MPO levels were reached 1 and 15 min after the removal of the ACC. A strong positive correlation was found ($r = 0.563$, $P = 0.001$***) between resistin levels and MPO release.

hearts with recombinant mouse resistin prior to ligation of the LAD. There was a marked reduction in infarct size in the resistin-treated group when compared with the control group. To add to the controversy, in the recent study of Smith *et al.* [16], resistin failed to alter the infarct size in regional ischaemia–reperfusion models. This was demonstrated in both *in vivo* mouse model and a Langendorf-perfused rat heart preparation. In addition, MPTP opening was unaffected as was the contractile recovery of human atrial trabecular cardiomyocytes.

One piece in the puzzle was added by a recent finding identificating TLR4 as a possible receptor for resistin [9], which could clearly mediate its putative myocyte-damaging effects. TLR4 has been linked to myocardial pathophysiology, and it was demonstrated that TLR4-deficient mice developed a smaller infarct size

than wild-type mice after LAD ligation [17]. In the recent whole-blood transcriptomic analysis conducted by Liangos *et al.* [18], the expression levels of TLR4 and resistin in blood cells were shown to be elevated 2 and 24 h after CABG. Antagonists of TLR receptors are under development for the prevention and treatment of ischaemia–reperfusion injury [1]. Since resistin can signal through TLR4, the present results support the hypothesis that resistin might indeed play a significant role in the post-surgery reperfusion injury and inflammation and thus evoke myocardial damage. Additional research will be needed to understand whether resistin is a useful biomarker of a poor outcome after CABG surgery. Another fruitful avenue for research may be to develop and investigate resistin-targeted treatments to prevent ischaemia–reperfusion injury. The present study found no correlation between the release of leptin and TnT but high baseline leptin levels were correlated with major elevations in the levels of TnT. Therefore, no evidence of leptin's cardioprotective role during reperfusion was obtained although there are some experimental data in this direction [19]. Leptin is a regulator of energy balance and leptin deficiency leads to hyperphagia and morbid obesity in animal models. However, the increased blood levels of leptin in obese subjects do not seem to induce the responses one would expect from high leptin values, i.e. increased energy expenditure, reduced food intake and lowering of body weight reflecting the appearance of leptin resistance [2]. The cardioprotective role for leptin was proposed in a study where leptin was administered during reperfusion to isolated mouse hearts. That treatment reduced infarct size and treatment with inhibitors of phosphoinositide 3-kinase (PI3 K) and p44/42 mitogen-activated protein kinase (MAPK) reversed this cardioprotective effect [19]. Ischaemia–reperfusion in rat hearts has also been shown to induce the expression of leptin and leptin receptor [20]. The clinical data on the putative cardioprotective effects of leptin remain, however, limited. Recently, Modan-Moses *et al.* reported a possible link between leptin and reduced troponin levels in paediatric patients undergoing open heart surgery with CPB. They detected a negative correlation between leptin and troponin levels postoperatively and in patients with larger troponin release, leptin levels fell to a greater extent during the operation and remained lower postoperatively compared with those of patients with less troponin release [6]. The study of Modan-Moses *et al.* was conducted in paediatric patients and included a variety of paediatric cardiac procedures, whereas the present study concentrated on adult patients who underwent elective CABG surgery due to severe CAD, many of them suffering also from obesity and the metabolic syndrome. In addition, the differences in age, previous disease history, pre-, peri- and postoperative medication strategies and cardioplegia protocols might also explain the different findings between these two studies although the temporal changes in leptin concentrations occurring during and after the operation were rather similar.

Adiponectin is described generally as an anti-inflammatory mediator, e.g. it is able to inhibit LPS-stimulated TNFα production and Toll-like receptor-mediated NF-κB activation and to stimulate the production of the anti-inflammatory cytokine IL-10 by macrophages. The expression of adiponectin by adipocytes is inhibited by pro-inflammatory factors, such as TNFα and IL-6, while peroxisome proliferator-activated receptor-γ agonists stimulate adiponectin secretion [2]. It has been reported that adiponectin levels are lower in obese patients and patients with impaired glucose tolerance and also in patients with CAD when compared with healthy controls [21]. Our study revealed that plasma adiponectin levels fell after the onset of CPB and remained below the baseline level even 24 h after the onset of reperfusion. The present findings are in accordance with the results of Kojima *et al.* [22], who showed that adiponectin levels remained low in patients suffering from AMI. Baker *et al.* [10] demonstrated that in patients with CAD, the expression of adiponectin in epicardial adipose tissue was reduced. Adiponectin has been shown to protect the myocardium after reperfusion via AMPK- and COX-2-dependent pathways. By acting through the AMPK-dependent pathway, adiponectin promotes glucose utilization in the myocardium, helps to diminish endoplasmic reticulum stress and prevents apoptosis [23]. Shibata *et al.* [8] subjected wild-type and adiponectin knockout (KO) mice to 30 min of ischaemia by LAD ligation and demonstrated that adiponectin deficient mice had larger areas at risk and larger infarct sizes than wild-type mice. Furthermore, left ventricular function was better in mice treated with adiponectin before, during or after ischaemia. The effect of adiponectin was proposed to be mediated through induction of COX-2 and production of prostaglandin E_2 (PGE_2) which inhibited the expression of LPS induced TNFα. Whereas high serum levels of resistin have been linked with incident congestive heart failure (CHF) in the Framingham cohort, this was not the case for low serum adiponectin concentrations [11]. Based on the existing literature, we hypothesized that adiponectin would be negatively correlated with TnT release, but this was not supported by the experimental evidence.

Adipsin (also known as complement factor D) is an enzyme involved in the alternative activation of the complement cascade [2]. As far as we are aware, there is only one study that has linked adipsin with ischaemia–reperfusion injury. After gastrointestinal ischaemia, adipsin KO mice have been reported to exhibit diminished reperfusion injury and reduced MPO and lactate dehydrogenase activity in comparison with their wild-type counterparts [24]. Our results demonstrated that adipsin levels fell slightly but in a statistically significant manner during the surgery and remained below the baseline level for at least up to 24 h after the start of the operation. The negative correlation observed between adipsin and IL-6 points to a possible link between these two variables, i.e. to anti-inflammatory properties of adipsin.

The present study has revealed interesting original data on adipocytokines in cardiac surgery and especially on the correlation of resistin with myocardial injury. However, this study is an observational pilot study because of its design and thus causality cannot be inferred based on these results. We have used the increase of TnT from the baseline to 24 h after the onset of CPB as a surrogate marker of myocardial injury, but we have no data on the course of TnT levels during the surgery. We analysed baseline values in blood samples taken after the induction of anaesthesia prior to any surgical procedures and the dilution effect induced by CPB could not be fully assessed. We found minor declines in adiponectin and adipsin levels which could be partially affected by the volume dilution related to CPB but those may also be true pathophysiological responses: The pro-inflammatory mediators, TNFα and IL-6, are known to inhibit the expression of adiponectin [2] and adipsin, as a complement factor, may be affected by the consumption of complement components related to CPB [25]. Interestingly, even though the CPB-derived dilution may have reduced the postoperative resistin concentration, we observed a significant postoperative elevation in the resistin level in this study of elective CABG patients. Further, levels of MPO, IL-6 and 8-isoprostane were already significantly elevated at the onset of CPB when compared with the baseline value. As another

limitation of the study, the possible effects of the patients' medication cannot be excluded. Even though our observational study strongly suggests that resistin is a factor in the ischaemia–reperfusion injury associated with cardiac surgery, more research will be needed to confirm whether resistin can serve as a predictive measure or biomarker of the degree of oxidative stress or the myocardial injury or if resistin does play a causative role in the pathogenesis of these detrimental responses induced by cardiac surgery.

In conclusion, resistin and leptin levels were significantly increased on the first postoperative day, while minor declines were found in adiponectin and adipsin levels. The present study introduces resistin as a factor whose levels in blood correlated with increased MPO and TnT levels, and hence with oxidative stress and myocardial injury in patients undergoing CABG surgery. Our results suggest that resistin may be a useful predictive factor for, or a biomarker of, the degree of myocardial injury and a possible drug target to prevent and treat ischaemia–reperfusion injury associated with cardiac surgery.

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