

Increased Circulating and Epicardial Adipose Tissue mRNA Expression of Fibroblast Growth Factor-21 After Cardiac Surgery: Possible Role in Postoperative Inflammatory Response and Insulin Resistance

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Summary

We studied the changes in serum fibroblast growth factor-21 (FGF-21) concentrations, its mRNA, and protein expression in skeletal muscle and adipose tissue of 15 patients undergoing cardiac surgery. Blood samples were obtained: prior to initiation of anesthesia, prior to the start of extracorporeal circulation, upon completion of the surgery, and 6, 24, 48, and 96 hours after the end of the surgery. Tissue sampling was performed at the start and end of surgery. The mean baseline serum FGF-21 concentration was 63.1 (43.03-113.95) pg/ml and it increased during surgery with peak 6 hours after its end [385.5 (274.55-761.65) pg/ml, $p < 0.001$], and returned to baseline value [41.4 (29.15-142.83) pg/ml] 96 hours after the end of the surgery. Serum glucose, insulin, CRP, IL-6, IL-8, MCP-1, and TNF-alpha concentrations significantly increased during the surgery. Baseline FGF-21 mRNA expression in skeletal muscle was higher than in both adipose tissue depots and it was not affected by the surgery. Epicardial fat FGF-21 mRNA increased after surgery. Muscle FGF-21 mRNA positively correlated with blood glucose levels at the end of the surgery. Our data suggest a possible role of FGF-21 in the regulation of glucose metabolism and insulin sensitivity in surgery-related stress.

Key words

Fibroblast growth factor-21 • Adipose tissue • Skeletal muscle • Cardiac surgery • Insulin resistance

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Introduction

Both experimental and clinical studies have suggested that fibroblast growth factor-21 (FGF-21) plays a role in the regulation of lipid and glucose metabolism and energy homeostasis (Kharitonov *et al.* 2005, Kharitonov and Shanafelt 2008). FGF-21 administration stimulated glucose uptake in mouse adipocytes and in cultures of human adipocytes (Kharitonov *et al.* 2005). Subcutaneous injection of FGF-21 improved blood glucose and lipid levels in obese mice (Kharitonov *et al.* 2005). Long-term administration of FGF-21 to diabetic rhesus monkeys decreased plasma glucose, triglycerides, insulin, and

glucagon levels (Kharitononkov *et al.* 2007).

According to previous studies, FGF-21 is expressed predominantly in the liver and to a lesser extent in fat (Kharitononkov *et al.* 2005, Mraz *et al.* 2009, Nishimura *et al.* 2000). In mice, the liver expression of FGF-21 is tightly regulated in response to nutritional status. It is increased by starvation and a ketogenic state and decreased by feeding (Badman *et al.* 2007, Inagaki *et al.* 2005, Lundasen *et al.* 2007). In humans, long-term fasting increased FGF-21 concentrations while a ketogenic diet did not (Galman *et al.* 2008). Increased FGF-21 levels were found in patients with type 2 diabetes mellitus and/or obesity relative to healthy subjects, and this was further increased by a short-term very low calorie diet (Mraz *et al.* 2009, Zhang *et al.* 2008). Elevated FGF-21 levels were also independently associated with increased risk of metabolic syndrome (Zhang *et al.* 2008).

In contrast to subjects with obesity and type 2 diabetes mellitus, patients with decreased body fat content, such as chronically malnourished patients with anorexia nervosa, were characterized by significantly lower serum concentrations of FGF-21 relative to healthy age-matched controls in one study (Dostálová *et al.* 2008), while in another study FGF-21 levels in anorexia nervosa patients were unchanged compared with healthy controls (Fazeli *et al.* 2010).

Critically ill patients represent another patient population that suffers from insulin resistance and hyperglycemia in a similar manner to those with type 2 diabetes (Van den Berghe 2002). Recent studies indicated the beneficial effect of improved glucose control after continuous intravenous insulin treatment, yet an optimal glucose range target is still unclear and differs in various types of patients (Blaha *et al.* 2009, Van den Berghe *et al.* 2001, Finfer *et al.* 2009). Although the cause of insulin resistance in critically ill patients differs from the cause in patients with type 2 diabetes mellitus, common mechanisms still exist including the development of liver insulin resistance and overproduction of proinflammatory factors by adipose tissue (Kremen *et al.* 2006, Mazurek *et al.* 2003). Since it has been suggested that FGF-21 is a possible regulator of insulin sensitivity and glucose metabolism in patients with type 2 diabetes mellitus, we hypothesized that it also may play a role in the metabolic response in critically ill patients. To this end, we measured epicardial adipose tissue, subcutaneous adipose tissue, and skeletal muscle mRNA expression of FGF-21 in patients undergoing elective cardiac surgery and

studied the dynamics of its serum concentration changes both during and after the surgery.

Methods

Study subjects

This study included 15 male patients who underwent aorto-coronary bypass surgery with extracorporeal circulation. All of the patients participating in the study had arterial hypertension, ischemic heart disease, and BMI ≥ 25 . None of the patients had diagnosis of diabetes mellitus or suffered from acute or chronic kidney injury, malignancy, thyroid disease, or acute infection. Seven patients had hyperlipidemia treated by atorvastatin (five patients) or fluvastatin (two patients), respectively. None of the study subject was treated by metformin or fibrates. **We used ATP panel III criteria for diagnosis of metabolic syndrome.**

All of the patients ate the last meal at 6 PM on the day before the surgery. The surgery was performed after an overnight fasting and it was started between 7-8 AM in all subjects. The average duration of surgery was 252 ± 31 minutes.

Three of the patients received infusion of dobutamine and norepinephrine during and after surgery with maximal dose $7 \mu\text{g}/\text{kg}/\text{min}$ and $0.2 \mu\text{g}/\text{kg}/\text{min}$, respectively with treatment duration from 14 to 31 hours. Glucose infusion was not administered in any of the patient.

All participants signed written informed consent prior to enrollment in the study. The study was approved by the Human Ethical Review Committee of the Thomayer University Hospital and Institute for Clinical and Experimental Medicine, Czech Republic and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

Anthropometric examination, blood and tissue sampling

Anthropometric examination of the patients was performed at basal state one day prior to the surgery. All subjects were measured and weighted in light clothes without shoes using standardized scales and BMI was calculated ($\text{weight (kg)} / \text{height (m)}^2$). Body surface area was calculated using the DuBois and DuBois formula that is routinely used in cardiac surgery patients. Waist circumference was measured by locating upper hip bone and placing horizontal tape measure. All anthropometric measurements were done in a standardized way by the

same study nurse.

Blood samples for hormonal measurement were taken prior to initiation of anesthesia (baseline), prior to the start of extracorporeal circulation, upon completion of the surgery, and 6, 24, 48, and 96 hours after the end of the surgery. Serum was obtained by centrifugation and the samples were subsequently stored in aliquots at -80°C until further analysis.

Samples of subcutaneous (thoracic region), visceral (epicardial) adipose tissue, and skeletal muscle (intercostal muscles) for mRNA and protein expression analysis were taken at the start and prior to the end of surgery. All of the samples (both at the start and at the end of surgery) were taken from approximately the same location in all patients. The samples were obtained from tissue that had not been previously traumatized mechanically or by cauterization in order to avoid the interference of local damage with tissue parameters. Tissue samples were collected to 1 ml of RNeasy lysis reagent (Qiagen GmbH, Hilden, Germany) and stored at -80°C until further analysis. The average time between the initial and final sampling at the end of the surgery was 244 ± 22 minutes.

Glucose control protocol and insulin administration

Undiluted arterial blood for measurement of blood glucose (BG) was drawn from an arterial line inserted for routine monitoring procedures. Whole BG was analyzed by a standard point of care testing device (Abbott Architect CI 8200, Abbott Diagnostics, Maidenhead, U.K.) every 3 hours.

No glucose infusion was administered in any of the patients during the study. No insulin was administered during the surgery. Insulin administration was initiated in postoperative intensive care unit only in patients with blood glucose exceeding 9.9 mmol/l after surgery. Insulin (Actrapid HM, Novo Nordisk, Baegsvard, Denmark) was given via central venous line as a continuous infusion. A standard concentration of 50 IU of insulin in 50 ml of 0.9 % NaCl was used. Target glucose range was 6.0-9.9 mmol/l. Normal oral food intake was started in 18 to 24 hours after surgery in all study subjects.

Hormonal and biochemical assays

Plasma samples for measurement of FGF-21 concentrations were diluted with Dilution Buffer 1:1 and measured using a commercial ELISA kit (BioVendor, Modrice, Czech Republic) which is based on the

polyclonal anti-human FGF-21 antibody and biotin-labelled polyclonal anti-human FGF-21 antibody. Diluted sera, standards, and quality controls were prepared according to the manufacturer's protocol. Absorbance was measured at 450 nm. A standard curve was constructed by plotting absorbance values against concentrations of standards (8 standards with concentration range 15-1920 pg/ml), and concentrations of samples were calculated using this standard curve. Sensitivity of the kit was 7.0 pg/ml and the intra- and inter-assay variability of the kit was 3.0-4.1 % and 10 %, respectively. Serum C-reactive protein (CRP) levels were measured by high sensitive assay (Bender Medsystems, Vienna, Austria) with a sensitivity of 3 pg/ml. The intra- and inter-assay variability of the kit was less than 5 % and less than 10 %, respectively. Serum insulin concentrations were measured by commercial RIA kit (Cis Bio International, Gif-sur-Yvette, France) with a sensitivity of 2.0 $\mu\text{IU/ml}$. The intra- and inter-assay variability of the kits was less than 5 % and less than 10 %, respectively.

Serum concentrations of interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-alpha), and monocyte chemotactic protein-1 (MCP-1) were measured using Human serum adipokine LINCOplex Kit (panel B) on a Luminex200 instrument (Linco Research, St. Charles, MO, USA). Sensitivity was 1.6 pg/ml for IL-6, 0.2 pg/ml for IL-8, 0.14 pg/ml for TNF-alpha, and 0.14 pg/ml for MCP-1, respectively. Intra- and inter-assay variability of the kits was 7.8 % and 18 % for IL-6, 7.9 % and 15 % for IL-8, 7.8 % and 16 % for TNF-alpha, 7.8 % and 16 % for MCP-1, respectively.

Determination of mRNA expression

Samples were homogenized on a MagNA Lyser Instrument using MagNA Lyser Green Beads (Roche Diagnostics GmbH, Mannheim, Germany) and QIAzol Lysis Reagent (Qiagen GmbH, Hilden, Germany). Total RNA was extracted from the homogenized sample of subcutaneous and epicardial adipose tissue using RNeasy Lipid Tissue Mini Kit (Qiagen GmbH, Hilden, Germany). Total RNA from the homogenized skeletal muscle was extracted on MagNA Pure instrument using MagNA Pure Compact RNA Isolation (Tissue) kit (Roche Diagnostics GmbH, Mannheim, Germany). The RNA concentration was determined from absorbance at 260 nm on a BioPhotometer (Eppendorf AG, Hamburg, Germany). All samples had a 260/280 nm absorbance ratio of 1.74 ± 0.016 . The integrity of the RNA was checked by

visualization of 18S and 28S ribosomal bands on 1 % agarose gel with ethidium bromide. Reverse transcription was performed using 0.25 µg of total RNA to synthesize the first strand cDNA using the random primers as per the instructions of the High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA). Measurements of FGF-21 gene expression were 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) and nuclease-free water (Fermentas Life Science, Vilnius, Lithuania). Controls with no template cDNA were performed with each assay and all samples were run in duplicate at a minimum. The increase in fluorescence was measured in real time and threshold cycle (C_T) values were obtained. To compensate for variations in the amount of RNA used and the variable efficiency of reverse transcription, the target gene C_t number was normalized to the endogenous reference beta-2-microglobulin (B2M) and the formula $2^{-\Delta C_t}$ was used to calculate relative gene expression.

Protein isolation and Western blot analysis

Approximately 80-120 mg subcutaneous adipose and muscle tissue was homogenized in ice-cold homogenization buffer [150 mM NaCl, 2 mM EDTA, 10 % glycerol, 25 mM benzamidine, 1 mM PMSF, and protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO, USA) in 10 mM Tris-HCl (pH 7.0)]. The homogenate was centrifuged at 3,000 x g for 15 min at 4 °C, the fat cake was then discarded and the homogenate was centrifuged again at 14,000 x g for 20 min at 4 °C. The supernatant was stored in aliquots at -80 °C. Total protein concentration of each sample was estimated by Bradford dye assay method using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's protocol using BSA as a standard.

For Western blot analysis, 70 µg of protein lysate were separated by 12.5 % SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) transfer membrane (Pierce Biotechnology, Inc., Rockford, IL, USA) in a semidry blotting apparatus. The membrane was blocked overnight at 4 °C with 12.5 % (w/v) nonfat milk, washed 3 times in PBST buffer, and analyzed with primary goat antibodies against human FGF-21 (BioVendor, Modrice, Czech Republic) at a final concentration of 0.2 µg/ml. The membrane was then

washed in PBST buffer and incubated for 1 h at room temperature with Polyclonal rabbit antigoat antibody with horseradish peroxidase (Dako, Glostrup, Denmark) at a 1:2000 dilution in PBST buffer. The same procedure was used for a control protein alpha-tubulin. Primary monoclonal antibody against alpha-tubulin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at dilution 1:200 and then secondary antibody ImmunoPure Goat anti-mouse IgG, (H+L), peroxidase conjugated (Pierce Biotechnology, Inc., Rockford, IL, USA) at dilution 1:5000 was used. Detection of membrane antibody binding was evaluated by enhanced chemiluminescence ECL Western Blotting Substrate (Pierce Biotechnology, Inc., Rockford, IL, USA). Signals were visualized and evaluated on a G:Box iChemi XT Bio instrument (Syngene, Cambridge, U.K.) and then analyzed and the quantifications of bands was calculated using GeneTools Software (Syngene, Cambridge, U.K.). Molecular weight of FGF-21 bands is 20.2 kDa and alpha-tubulin bands is 55 kDa.

Statistical analysis

The statistical analysis was performed on SigmaStat software (Jandel Scientific, San Rafael, CA, USA) and Matlab (Mathworks, Natick, Massachusetts, U.S.A). The results are expressed as Median and Interquartile range or Mean ± standard error of mean (SEM). Prior to analysis, all continuous variables were assessed for normality using the Kolmogorov-Smirnov test. Changes of hormonal levels, gene and protein expression during perioperative and postoperative state, respectively, were evaluated using Paired t-test or Wilcoxon Signed Rank Test, according to the normality of data. Changes of hormonal levels were adjusted for multiple comparisons by Bonferroni correction. Differences between the groups with or without insulin treatment and differences in protein expression were evaluated using unpaired T-test or Mann-Whitney Rank Sum Test, according to the normality of data. Area under the curve and unpaired T-test was used to calculate the differences in change of serum FGF-21 levels between the groups with or without insulin treatment. Correlations were evaluated using the Spearman Correlation test or Pearson Correlation test, according to the normality of data. In all statistical tests, p values <0.05 or corrected p values < 0.00625 were considered significant. Multiple regressions for data adjustment to FGF-21 levels were done.

Table 1. Clinical characteristics of the cardiac surgery patients subdivided into group with insulin treatment (IT subgroup) and group without insulin treatment (NT subgroup). Values are Median and Interquartile range or Mean \pm SEM with n=15/group. Statistical significance is from un-paired t-test or Mann-Whitney Rank Sum Test according to the normality of data distribution. NS, not significant; SS, start of surgery; T0, prior to initiation of anesthesia; T3, 6 hours after the end of the surgery; T5, 48 hours after the end of the surgery; FGF-21 AUC, area under the curve of FGF-21 serum concentration

	All subjects	IT subgroup	NT subgroup	IT vs NT
Clinical characteristics:				
No. of subjects	15	8	7	NS
Age (yr)	66 \pm 2	68 \pm 2	63 \pm 3	NS
Body mass index (kg/m ²)	29.22 \pm 0.79	27.81 \pm 0.67	30.63 \pm 1.29	NS
Body surface area (m ²)	2.02 \pm 0.04	1.96 \pm 0.05	2.08 \pm 0.07	NS
HOMA index at T0	4.31 \pm 0.71	4.39 \pm 1.04	4.24 \pm 1.07	NS
Skeletal muscle FGF-21 gene expression at SS	3.08 ⁻⁴ (0.93 ⁻⁴ - 5.45 ⁻⁴)	4.26 ⁻⁴ (3.13 ⁻⁴ - 9.52 ⁻⁴)	0.73 ⁻⁴ (0.47 ⁻⁴ - 2.94 ⁻⁴)	p = 0.040
Serum concentrations:				
HbA1c at T0 (%)	4.07 \pm 0.10	4.13 \pm 0.17	4.01 \pm 0.10	NS
Glucose at T0 (mmol/l)	6.1 \pm 0.29	6.344 \pm 0.49	5.763 \pm 0.282	NS
Glucose at T3 (mmol/l)	8.5 \pm 0.41	9.5 \pm 0.62	7.5 \pm 0.235	p = 0.005
Glucose at T5 (mmol/l)	6.3 \pm 0.24	5.8 \pm 0.29	6.85 \pm 0.303	p = 0.027
CRP at T2 (mg/l)	1.4 \pm 0.52	0.4 \pm 0.11	2.5 \pm 0.97	p = 0.009
FGF-21 at T5 (pg/ml)	130 (51 - 231)	222 (120 - 581)	66 (35 - 114)	p = 0.038
FGF-21 AUC	24673 \pm 4599	34450 \pm 6731	13498 \pm 2518	p = 0.016

Results

Clinical characteristics of the patients

Table 1 shows the clinical characteristics of the entire group of patients and the subgroups with or without insulin administration after the surgery. All patients were males with mean waist circumference 103 \pm 3.4 cm, mean baseline blood glucose 6.1 \pm 0.29 mmol/l, and mean glycated hemoglobin 4.07 \pm 0.10 %. All the statistically significant differences between insulin-treated group (IT subgroup) and group without insulin treatment (NT subgroup) are summarized in Table 1.

Serum glucose, hormonal, and cytokine concentrations

Serum FGF-21 concentration peaked 6 hours after the end of surgery, exhibiting a 6.2-fold median increase over baseline and returned to baseline level 96 hours after the end of surgery (Figure 1). Serum glucose levels peaked 6 hours after the end of the surgery and normalized 48 hours after the end of the surgery (Figure 1). Serum insulin concentration increased 2.4-fold median with the peak occurring 48 hours after the end of the surgery and remained significantly elevated for 96 hours after the end of the surgery (Figure 1). Serum

CRP concentrations peaked 24 hours after the end of the surgery, remained elevated by 24.5-fold median for 48 hours after the surgery, returning to near normal values 96 hours after the end of the surgery (Figure 1). IL-6, IL-8, TNF- α and MCP-1 concentrations peaked 6 hours after the end of the surgery, exhibiting a 55.6-, 6.7-, 1.5-, and 1.9-fold median increase over baseline, respectively (Figure 2).

To further investigate the influence of insulin administration on serum FGF-21 levels we subdivided our patient population into two subgroups: with (n=8) or without (n=7) exogenous insulin administration. At baseline no significant differences in blood glucose, serum insulin, and FGF-21 levels were found between the insulin-treated vs. insulin-untreated group. Serum FGF-21 levels tended to be higher in the insulin treated vs. insulin-untreated subgroup throughout all time-points. A significantly higher FGF-21 level in insulin-treated relative to insulin untreated patients was found 48 hours after the end of surgery [222 (120-581) vs. 66 (35-114) pg/ml, p=0.038]. Area under the curve for FGF-21 was significantly higher in insulin-treated relative to insulin untreated patients (p=0.016) (Table 1).

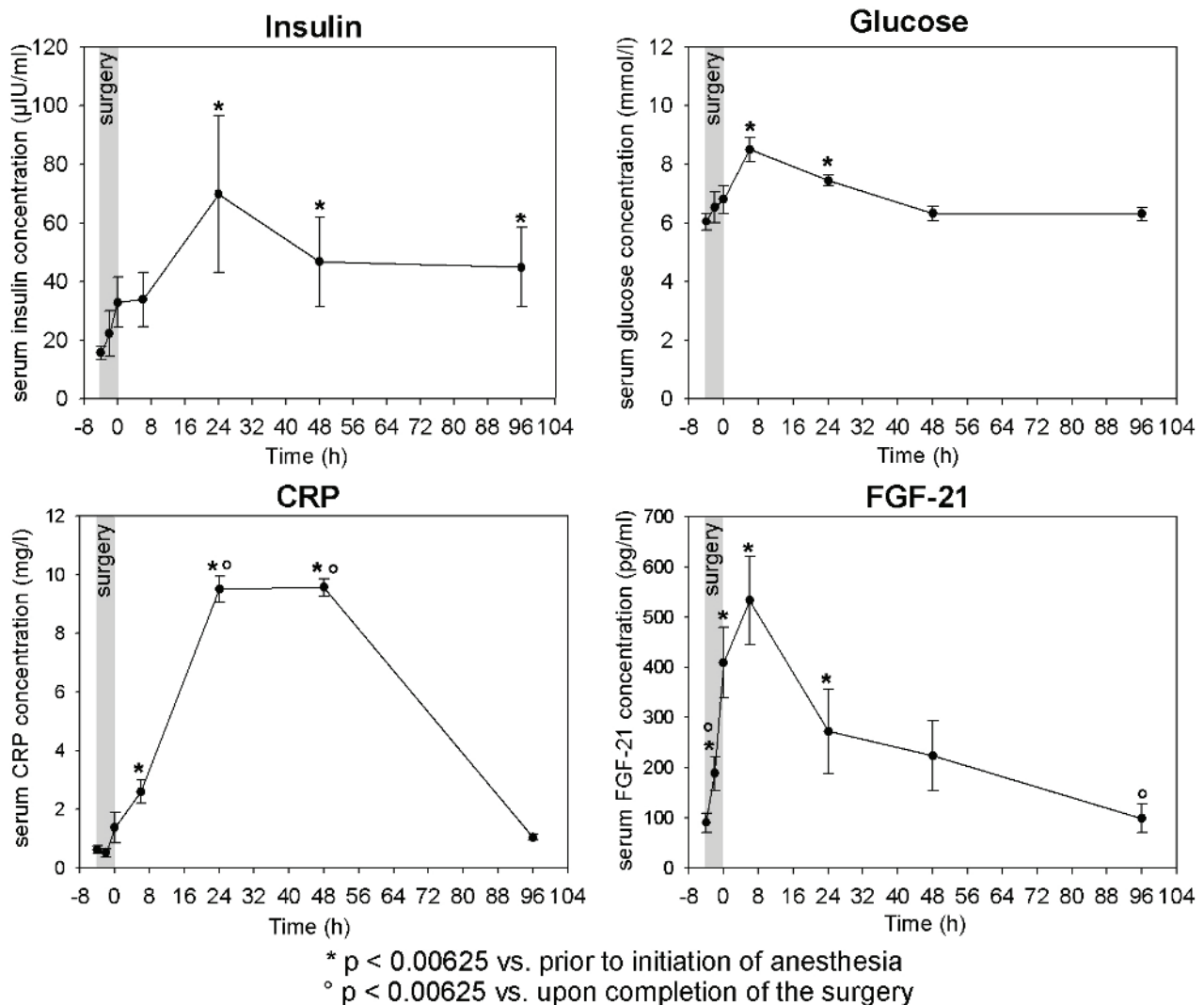


Fig. 1. Serum concentrations of insulin, glucose, CRP and FGF-21 in cardiac surgery patients prior to initiation of anesthesia, prior to the start of extracorporeal circulation, upon completion of the surgery, and 6, 24, 48, and 96 hours after the end of the surgery. Values are Mean \pm SEM with $n=15$ /group. Statistical significance is from Paired t-test or Wilcoxon Signed Rank Test, according to the normality of data. P values were adjusted for multiple comparisons by Bonferroni correction: * indicates $p < 0.00625$ vs. prior to initiation of anesthesia, ° indicates $p < 0.00625$ vs. upon completion of the surgery.

Changes of mRNA expression of FGF-21 in adipose tissue and muscle

At the start of surgery, FGF-21 mRNA expression in skeletal muscle was higher relative to both subcutaneous and epicardial adipose tissue. No significant influence of the surgery was detected concerning skeletal muscle FGF-21 mRNA expression (Figure 3C). In contrast, the surgery induced a significant increase in FGF-21 mRNA expression in epicardial adipose tissue, whereas the surgery did not significantly change FGF-21 mRNA expression in subcutaneous adipose tissue (Figure 3A, B).

Changes of FGF-21 protein expression

In skeletal muscle FGF-21 protein expression was 4.7-fold higher ($p=0.014$) than that of subcutaneous fat depot at the start of the surgery (Figure 3D).

Relationship of FGF-21 plasma levels and mRNA expression with anthropometric, biochemical, and hormonal parameters

No significant association between serum FGF-21 areas under the curve and any anthropometric or biochemical parameter was found. FGF-21 mRNA expression in skeletal muscle at the end of the surgery correlated positively with blood glucose level ($r=0.678$, $p=0.007$) at the end of the surgery.

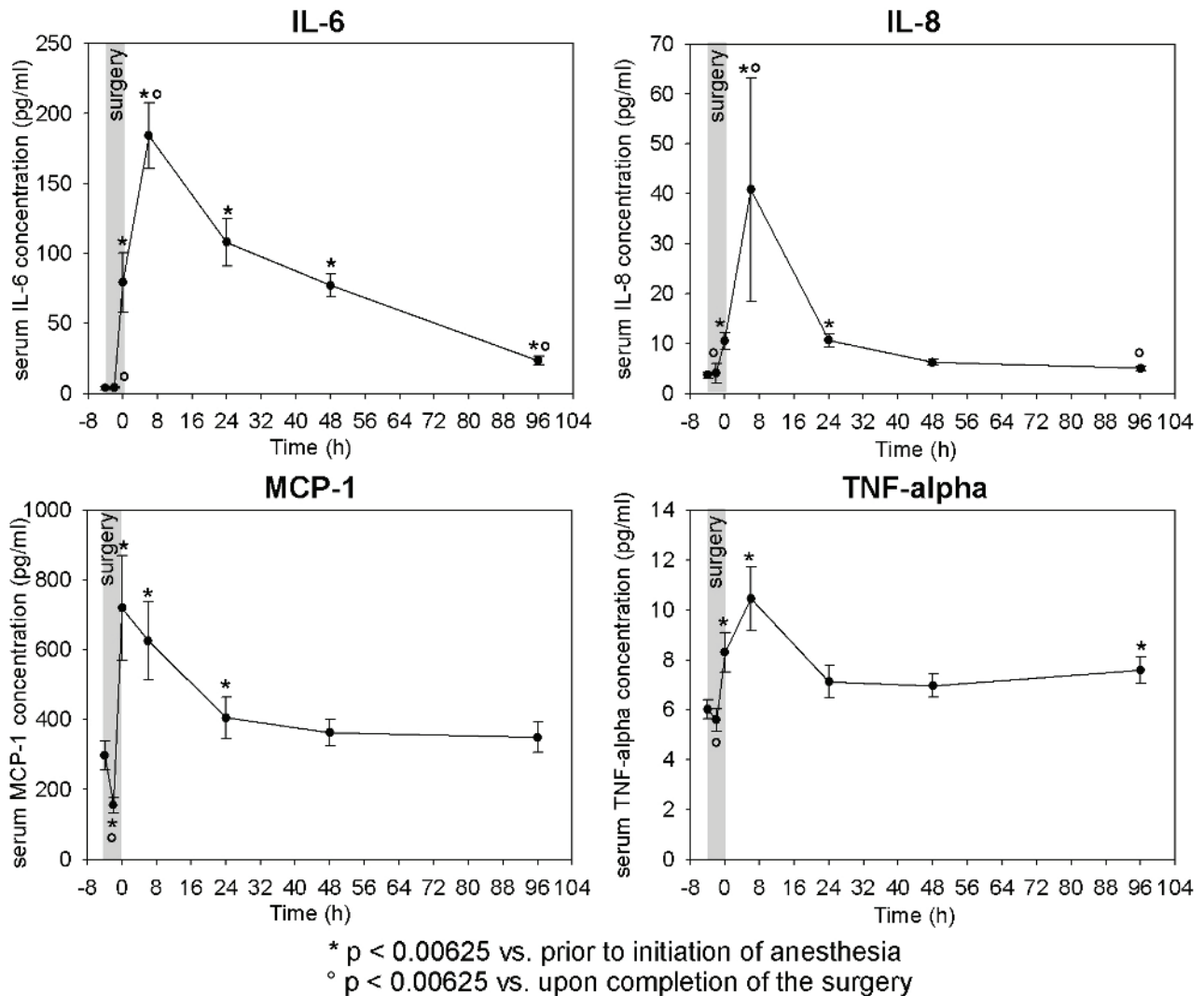


Fig. 2. Serum concentrations of IL-6, IL-8, MCP-1, and TNF-alpha in cardiac surgery patients prior to initiation of anesthesia, prior to the start of extracorporeal circulation, upon completion of the surgery, and 6, 24, 48, and 96 hours after the end of the surgery. Values are Mean \pm SEM with n=15/group. Statistical significance is from Paired t-test or Wilcoxon Signed Rank Test, according to the normality of data. P values were adjusted for multiple comparisons by Bonferroni correction: * indicates p<0.00625 vs. prior to initiation of anesthesia, ° indicates p<0.00625 vs. upon completion of the surgery.

Discussion

Our study demonstrated that serum FGF-21 concentration and its gene expression in epicardial adipose tissue significantly increased after major elective cardiac surgery. Another important finding of this study is that baseline FGF-21 mRNA gene expression and protein expression in skeletal muscle was significantly higher than in subcutaneous and epicardial adipose tissue depots suggesting that skeletal muscle could also represent an important source of this factor.

Previous studies demonstrated that FGF-21 administration, or its overexpression, exerts a strong glucose and lipid lowering effect in rodents and primates

with obesity and type 2 diabetes mellitus (Kharitonov *et al.* 2005, Kharitonov and Shanafelt 2008, Kharitonov *et al.* 2007, Dostalova *et al.* 2008). Interestingly, human studies have demonstrated that patients with obesity, both with and without type 2 diabetes mellitus, are characterized by increased FGF-21 compared to healthy lean subjects (Mraz *et al.* 2009, Zhang *et al.* 2008, Chen *et al.* 2008). FGF-21 administration also improved β -cell function in experimental studies (Wente *et al.* 2006).

In this study we demonstrate that an elective cardiac surgery that induces hyperglycemia, hyperinsulinemia and systemic inflammatory response is accompanied by marked early increase of circulating

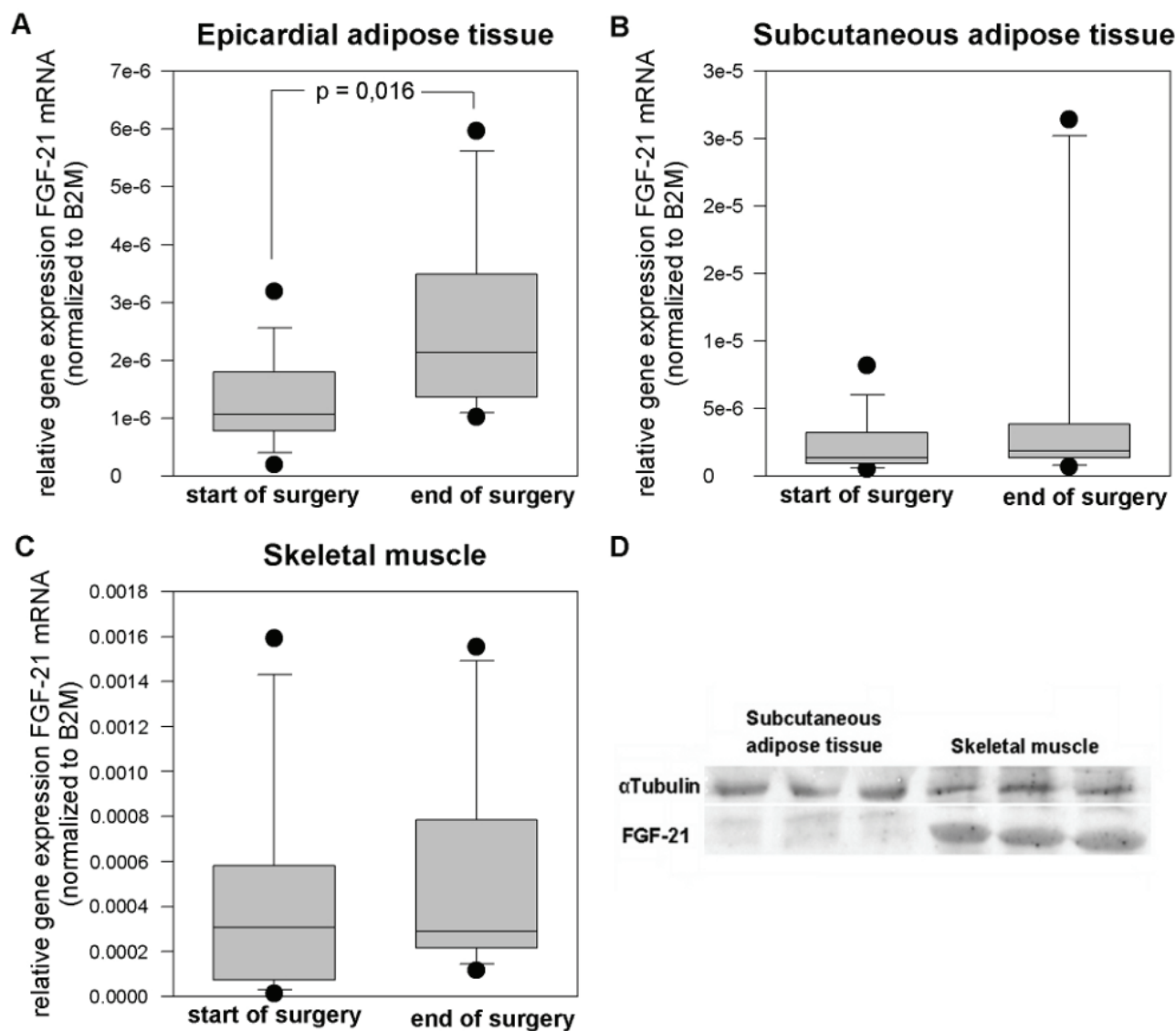


Fig. 3. Relative gene expression of FGF-21 mRNA in epicardial (A) and subcutaneous adipose tissue (B) and skeletal muscle (C). Samples were taken at the start and prior to the end of surgery. Values are Median and Interquartile range with $n=15$ /group. Statistical significance is from Paired t-test or Wilcoxon Signed Rank Test, according to the normality of data. FGF-21 Western blots of a representative subcutaneous adipose tissue and skeletal muscle at the start of surgery (D). 70 μ g of protein lysate was used at each lane and alpha-tubulin was used as control. Each sample is at 3 aliquotes.

FGF-21 levels with very similar dynamics to that of insulin and TNF-alpha level. Interestingly, after subdividing our patients into an insulin-treated vs. insulin-untreated group we found significantly higher area under the curve of circulating FGF-21 levels in the former group. The muscle FGF-21 mRNA expression at the start of surgery was also significantly higher in the group that required insulin infusion during a postoperative course. Numerous factors induced by cardiac surgery could have contributed to the increase of FGF-21 levels. Our data showing more pronounced FGF-

21 increase after surgery in patients receiving insulin suggest that either insulin treatment itself or metabolic changes associated with the need of insulin treatment could have contributed to this difference. Furthermore, a positive association between glucose concentration at the end of surgery and muscle FGF-21 mRNA expression at the end of surgery suggests a possible contribution of these factors to the regulation of muscle FGF-21 mRNA expression.

The complexity of metabolic changes and inflammatory response after cardiac surgery did not allow

us to unequivocally determine which of the metabolic changes or proinflammatory factors were responsible for increased FGF-21 concentrations. In multiple regression analysis we were not able to identify any single factor independently associated with a change of circulating FGF-21 levels. Two recent studies albeit performed under different conditions described the presence of FGF-21 mRNA gene expression in skeletal muscle in response to insulin stimulation (Izumiya *et al.* 2008, Hojman *et al.* 2009). Here we confirm that human skeletal muscle expresses significant amount of both FGF-21 mRNA and FGF-21 protein. Interestingly, in contrast to circulating FGF-21 levels muscle FGF-21 mRNA expression was not acutely affected by cardiac surgery suggesting that other tissues were responsible for increased circulating FGF-21 levels.

In original animal studies, the liver was suggested as major producer of this factor while adipose tissue exhibited rather low FGF-21 mRNA expression (Nishimura *et al.* 2000). We have previously demonstrated that liver FGF-21 mRNA expression in patients with obesity is more than 100-fold higher compared to adipose tissue, supporting its important role in FGF-21 production in humans (Mraz *et al.* 2009). Here we directly compared FGF-21 mRNA and protein expression in fat and skeletal muscle tissues and show that muscle FGF-21 mRNA and protein expression is significantly higher relative to adipose tissue.

Epicardial adipose tissue has been previously identified as a source of inflammatory mediators under basal condition (Mazurek *et al.* 2003, Baker *et al.* 2006) and cardiac surgery increased mRNA expression of pro-inflammatory cytokines in both epicardial and subcutaneous adipose tissue (Kremen *et al.* 2006). We demonstrated in this study that circulating FGF-21 levels and its mRNA expression in epicardial adipose tissue are markedly increased by cardiac surgery. It remains to be determined what is the role of increased production of FGF-21 in epicardial fat and whether it significantly

contributes to its higher systemic levels.

To date, very little information is available with respect to changes of FGF-21 in critical illness. The only study focused on this topic was performed by Johnson *et al.* (2009) in mice. In Johnson's study, the role of FGF-21 in cerulein-induced pancreatitis in control mice and transgenic mice with FGF-21 over-expression or FGF-21 knockout was explored. The authors demonstrated that acinar cell FGF-21 expression markedly increased during both cerulein-induced pancreatitis and following injury *in vitro*. The severity of cerulein-induced pancreatitis was decreased in transgenic FGF-21 overexpressing mice as demonstrated by decreased serum amylase and decreased pancreatic stellate cell activation. On the contrary, FGF-21 knockout mice had increased serum amylase and tissue damage. These experimental data suggest a possible importance of FGF-21 in experimental acute pancreatitis. Nevertheless, it is not known whether FGF-21 has any significance in critical illness of other etiology. Our data showing a marked increase in circulating FGF-21 levels during cardiac surgery indicate a possible importance for this factor also in postoperative response in humans. **Nevertheless, it should be noted that our study was performed on relatively low number of subjects and its descriptive nature does not allow us to demonstrate clearly the clinical relevance of our findings.** It needs to be further determined what factors are responsible for increased FGF-21 levels and what is the exact role for this factor under these conditions. Our data suggest that skeletal muscle may represent another important source of FGF-21 in humans.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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