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

T. KOTULÁK1, J. DRÁPALOVÁ2, P. KOPECKÝ3, Z. LACINOVÁ2, P. KRAMÁŘ1, H. ŘÍHA1, I. NETUKA4, J. MALÝ4, D. HOUSA5, J. BLÁHA3, Š. SVAČINA2, M. HALUZÍK2

1Department of Anesthesia and Resuscitation, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 2Third Department of Medicine, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic, 3Department of Anesthesia, Resuscitation and Intensive Medicine, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic, 4Department of Cardiovascular Surgery, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 5Department of Pathology, Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

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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

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 (Kharitonenkov et al. 2005, Kharitonenkov and Shanafelt 2008). FGF-21 administration stimulated glucose uptake in mouse adipocytes and in cultures of human adipocytes (Kharitonenkov et al. 2005). Subcutaneous injection of FGF-21 improved blood glucose and lipid levels in obese mice (Kharitonenkov et al. 2005). Long-term administration of FGF-21 to diabetic rhesus monkeys decreased plasma glucose, triglycerides, insulin, and...
glucagon levels (Kharitonkov et al. 2007).

According to previous studies, FGF-21 is expressed predominantly in the liver and to a lesser extent in fat (Kharitonkov 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 fibrate. 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 µg/kg/min and 0.2 µg/kg/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 (weight (kg) / 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 RNAlater 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, Baesgsvard, 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 kits 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 µIU/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 Lysier Instrument using MagNA Lysier 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 (Ct) values were obtained. To compensate for variations in the amount of RNA used and the variable efficiency of reverse transcription, the target gene Ct number was normalized to the endogenous reference beta-2-microglobulin (B2M) and the formula 2^{ΔCt} 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 semidyblotting 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 anti-goat 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 ± 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 ± 2       | 68 ± 2      | 63 ± 3      | NS       |
| Body mass index (kg/m²) | 29.22 ± 0.79 | 27.81 ± 0.67 | 30.63 ± 1.29 | NS |
| Body surface area (m²) | 2.02 ± 0.04  | 1.96 ± 0.05 | 2.08 ± 0.07 | NS       |
| HOMA index at T0   | 4.31 ± 0.71  | 4.39 ± 1.04 | 4.24 ± 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 ± 0.10 | 4.13 ± 0.17 | 4.01 ± 0.10 | NS       |
| Glucose at T0 (mmol/l) | 6.1 ± 0.29  | 6.344 ± 0.49 | 5.763 ± 0.282 | NS       |
| Glucose at T3 (mmol/l) | 8.5 ± 0.41  | 9.5 ± 0.62  | 7.5 ± 0.235 | p = 0.005 |
| Glucose at T5 (mmol/l) | 6.3 ± 0.24  | 5.8 ± 0.29  | 6.85 ± 0.303 | p = 0.027 |
| CRP at T2 (mg/l)      | 1.4 ± 0.52  | 0.4 ± 0.11  | 2.5 ± 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 ± 4599 | 34450 ± 6731 | 13498 ± 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±3.4 cm, mean baseline blood glucose 6.1±0.29 mmol/l, and mean glycated hemoglobin 4.07±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- alpha 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).
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.
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 (Kharitonenkov et al. 2005, Kharitonenkov and Shanafelt 2008, Kharitonenkov 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...
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 proinflammatory 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.

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**References**

BADMAN MK, PISSIOS P, KENNEDY AR, KOUKOS G, FLIER JS, MARATOS-FLIER E: Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metab* 5: 426-437, 2007.

BAKER AR, SILVA NF, QUINN DW, HARTE AL, PAGANO D, BONSONER RS, KUMAR S, MCERNAN PG: Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol* 5: 1, 2006.
BLAHA J, KOPECKY P, MATIAS M, HOVORKA R, KUNSTYR J, KOTULAK T, LIPS M, RUBES D, STRITESKY M, LINDNER J, SEMRAD M, HALUZIK M: Comparison of three protocols for tight glycemic control in cardiac surgery patients. Diabetes Care 32: 757-761, 2009.

CHEN WW, LI L, YANG GY, LI K, QI XY, ZHU W, TANG Y, LIU H, BODEN G: Circulating FGF-21 levels in normal subjects and in newly diagnosed patients with Type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 116: 65-68, 2008.

DOSTÁLOVÁ I, KAVÁLKOVÁ P, HALUZÍKOVÁ D, LACINOVÁ Z, MRÁZ M, PAPEZOVÁ H, HALUZÍK M: Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. J Clin Endocrinol Metab 93: 3627-3632, 2008.

FAZELI PK, MISRA M, GOLDSTEIN M, MILLER KK, KLIBANSKI A: Fibroblast growth factor-21 may mediate growth hormone resistance in anorexia nervosa. J Clin Endocrinol Metab 95: 369-374, 2010.

FINFER S, CHITTOCK DR, SU SY, BLAIR D, FOSTER D, DHINGRA V, BELLOMO R, COOK D, DODEK P, HENDERSON WR, HEBERT PC, HERITIER S, HEYLAND DK, MCArTHUR C, MCDONALD E, MITCHELL I, MYBURGH JA, NORTON R, POTTER J, ROBINSON BG, RONCO JJ: Intensive versus conventional glucose control in critically ill patients. N Engl J Med 360: 1283-1297, 2009.

GALMAN C, LUNDASEN T, KKHARITONENKOV A, BINA HA, ERIKSSON M, HAFSTROM I, DAHLIN M, AMARK P, ANGELIN B, RUDLING M: The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. Cell Metab 8: 169-174, 2008.

HOJMAN P, PEDERSEN M, NIELSEN AR, KROGH-MADSEN Y, YFANTI C, WERKSTROM T, NIELSEN S, PEDERSEN BK: Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. Diabetes 58: 2797-2801, 2009.

INAGAKI T, CHOI M, MOSCHETTA A, PENG L, CUMMINS CL, MCDONALD JG, LUO G, JONES SA, GOODWIN B, RICHARDSON JA, GERARD RD, REPA JJ, MANGELSDORF DJ, KLIEWER SA: Fibroblast growth factor 15 functions as an endohepatic signal to regulate bile acid homeostasis. Cell Metab 2: 217-225, 2005.

IZUMIYA Y, BINA HA, OUChI N, AKASAKI Y, KKHARITONENKOV A, WALSH K: FGF21 is an Akt regulated myokine. FEBS Lett 582: 3805-3810, 2008.

JOHNSON CL, WESTON JY, CHADI SA, FAZIO EN, HUFF MW, KKHARITONENKOV A, KOESTER A, PIN CL: Fibroblast growth factor 21 reduces the severity of cerulein-induced pancreatitis in mice. Gastroenterology 137: 1795-1804, 2009.

KKHARITONENKOV A, SHIYANOVA TL, KOESTER A, FORD AM, MICANOVIC R, GALBREATH EJ, SANSUSKY GE, HAMMOND LJ, MOYERS JS, OWENS RA, GROMADA J, BROZINICK JT, HAWKINS ED, WROBLEWSKI VJ, LI DS, MEHRBOD F, JASKUNAS SR, SHANAFELT AB: FGF-21 as a novel metabolic regulator. J Clin Invest 115: 1627-1635, 2005.

KKHARITONENKOV A, WROBLEWSKI VJ, KOESTER A, CHEN YF, CLUTINGER CK, TIGNO XT, HANSEN BC, SHANAFELT AB, ETGEN GJ: The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology 148: 774-781, 2007.

KKHARITONENKOV A, SHANAFELT AB: Fibroblast growth factor-21 as a therapeutic agent for metabolic diseases. BioDrugs 22: 37-44, 2008.

KREMEN J, DOLINKOVA M, KRAJICKOVA J, BLAHA J, ANDERLOVA K, LACINOV A Z, HALUZIKOVA D, BOSANSKA L, VOKURKA M, SVCINA S, HALUZIK M: Increased subcutaneous and epicardial adipose tissue production of proinflammatory cytokines in cardiac surgery patients: possible role in postoperative insulin resistance. J Clin Endocrinol Metab 91: 4620-4627, 2006.

LUNDASEN T, HUNT MC, NILSSON LM, SANYAL S, ANGELIN B, ALEXSON SE, RUDLING M: PPARalpha is a key regulator of hepatic FGF21. Biochem Biophys Res Commun 360: 437-440, 2007.

MAZUREK T, ZHANG L, ZALEWSKI A, MANNION JD, DIEHL JT, ARAFAT H, SAROV-BLAT L, O’BRIEN S, KEIPER EA, JOHNSON AG, MARTIN J, GOLDSTEIN BJ, SHI Y: Human epicardial adipose tissue is a source of inflammatory mediators. Circulation 108: 2460-2466, 2003.
MRAZ M, BARTLOVA M, LACINOVA Z, MICHALSKY D, KASALICKY M, HALUZIKOVA D, MATOULEK M, DOSTALOVA I, HUMENANSKA V, HALUZIK M: Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity. *Clin Endocrinol (Oxf)* 71: 369-375, 2009.

NISHIMURA T, NAKATAKE Y, KONISHI M, ITOH N: Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochim Biophys Acta* 1492: 203-206, 2000.

VAN DEN BERGHE G, WOUTERS P, WEEKERS F, VERWAEST C, BRUYNINCKX F, SCHETZ M, VLASSELAERS D, FERDINANDE P, LAUWERS P, BOUILLON R: Intensive insulin therapy in the critically ill patients. *N Engl J Med* 345: 1359-1367, 2001.

VAN DEN BERGHE G: Endocrinology in intensive care medicine: new insights and therapeutic consequences. *Verh K Acad Geneesk Belg* 64: 167-187, 2002.

WENTE W, EFANOV AM, BRENNER M, KHRITONENKOV A, KOSTER A, SANDUSKY GE, SEWING S, TREINIES I, ZITZER H, GROMADA J: Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. *Diabetes* 55: 2470-2478, 2006.

ZHANG X, YEUNG DC, KARPISEK M, STEJSKAL D, ZHOU ZG, LIU F, WONG RL, CHOW WS, TSO AW, LAM KS, XU A: Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes* 57: 1246-1253, 2008.