

Plasma Concentrations of Adipocyte Fatty Acid Binding Protein in Patients With Cushing's Syndrome

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Received June 30, 2009

Accepted March 26, 2010

On-line June 9, 2010

Summary

Serum adipocyte fatty acid-binding protein (FABP-4) concentrations are linked to human obesity and other features of metabolic syndrome. Patients with Cushing's syndrome (CS) develop numerous features of metabolic syndrome due to chronic cortisol excess. Here we tested the hypothesis that chronically increased cortisol levels in CS patients may alter circulating levels of FABP-4. Fourteen patients with CS, 19 patients with simple obesity (OB) and 36 healthy control subjects (C) were included in the study. Serum FABP-4 concentrations were significantly higher in both CS and OB patients relative to C group, but they did not differ between CS and OB groups. In a combined population of all groups, serum FABP-4 levels correlated positively with BMI, body fat content, serum glucose, triglycerides, HbA1c and HOMA index and were inversely related to HDL-cholesterol, resting energy expenditure and freeT3 levels. We conclude that FABP-4 levels are significantly increased in both patients with simple obesity and obese patients with Cushing's syndrome. We suggest that increased FABP-4 concentrations in CS patients are rather due to their excessive fat accumulation and related metabolic abnormalities than due to a direct effect of cortisol on FABP-4 production.

Key words

Adipocyte fatty acid binding protein 4 • Hypercortisolism • Obesity

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Introduction

Cytoplasmic fatty acid binding proteins (FABPs) are a family of proteins that have an important role in fatty acids shuttling to cellular compartments, modulation of intracellular lipid metabolism and regulation of gene expression (Binas *et al.* 1999, Levy *et al.* 2001, Wolfrum *et al.* 2001, Chmurzynska 2006). They are expressed in various tissues, including fat, and play an important role not only in lipid metabolism, but also in other metabolic regulations (Coe and Bernlohr 1998, Hertzell and Bernlohr 2000, Boord *et al.* 2002, Chmurzynska 2006). Adipocyte fatty acid-binding proteins belong to the most abundant proteins in mature adipocytes (Makowski and Hotamisligil 2004). Recent clinical studies have shown that FABP-4, produced primarily by adipocytes, is released into the circulation suggesting its possible systemic effects (Xu *et al.* 2006, 2007, Stejskal and Karpíšek 2006, Karpíšek *et al.* 2007).

It has been demonstrated that FABP-4 concentrations are increased in patients with obesity and/or metabolic syndrome and may represent a novel marker of this clinical entity (Stejskal and Karpíšek 2006, Xu *et al.* 2006, Karpíšek *et al.* 2007). Circulating levels of FABP-4 are strongly positively linked to BMI, blood glucose and glycated hemoglobin levels in patients with type 2 diabetes mellitus and have an inverse relationship to the parameters of insulin sensitivity measured by isoglycemic-hyperinsulinemic clamp (Haluzík *et al.* 2009). On the other hand, experimental studies have shown that both the knockout of adipocyte fatty acid-

binding protein gene or its inhibition by a small molecule inhibitor improved insulin sensitivity and atherosclerosis in mice (Maeda *et al.* 2005, Furuhashi *et al.* 2007).

Cushing's syndrome, a disease caused by chronic cortisol excess, is commonly accompanied by numerous features of metabolic syndrome such as development of insulin resistance, type 2 diabetes mellitus, dyslipidemia and arterial hypertension. Here we tested the hypothesis that chronically increased cortisol levels in patients with Cushing's syndrome may alter circulating levels of FABP-4. Such alterations could in turn contribute to the metabolic disturbances seen in these patients. To analyze the possible influence of concomitant obesity and related metabolic abnormalities present in patients with Cushing's syndrome, we compared FABP-4 concentrations in these patients with both lean healthy control subjects and patients with obesity and type 2 diabetes mellitus.

Methods

Study subjects

Fourteen patients with active Cushing's syndrome (12 women, 2 men, age: 45.8 ± 3.39 years, body mass index (BMI): 33.7 ± 1.42 kg/m²), nineteen obese patients (17 women, 2 men, age: 54.6 ± 3.3 years, BMI: 45.8 ± 2.42 kg/m²) and thirty six healthy controls (28 women, 8 men, age: 43 ± 2.07 years, BMI: 22.7 ± 0.26 kg/m²) were included in the study.

The diagnosis of Cushing's syndrome (CS) was based on clinical status, diminished circadian rhythm of cortisolemia (nocturnal cortisol over 150 nmol/l), unsuppressed cortisolemia in 1 mg Dexamethasone test (cortisol over 86 nmol/l) and increased free urinary cortisol excretion (urinary free cortisol over 500 nmol/day). All patients had clinical signs of abdominal fat accumulation as measured by increased waist circumference. All 14 patients with CS had arterial hypertension, 12 of them had already been treated pharmacologically, 11 of them with a combination of antihypertensive drugs (with ACE inhibitors or sartans in 10 cases, calcium channel blockers in 6 cases, beta-blockers in 8 cases, diuretics in 7 cases, imidazoline receptor antagonist in 1 case and an alpha-blocker in 1 case). Twelve patients had disturbances in serum lipid spectrum (10 patients had elevated total and LDL (low density lipoprotein) cholesterol levels, in 7 cases together with elevated triglycerides, in 3 patients also with decreased HDL (high density lipoprotein) cholesterol

levels; 2 patients had only elevated triglycerides and decreased HDL cholesterol serum concentrations). One patient had been already treated with a combination of statin and fibrate. One patient was diagnosed with impaired glucose tolerance (IGT), nine patients with type 2 diabetes mellitus (DM) – four of them had been already treated, three with antidiabetic drugs (PAD; metformin in 2 cases, gliclazide in 1 case), one with a combination of PAD (metformin) and insulin. They had had no history of renal or vascular complications.

The group of patients with obesity (OB) was characterized by BMI > 30 kg/m². Sixteen out of nineteen patients in this group had arterial hypertension, all had been already on antihypertensive treatment, thirteen of them on a combination of antihypertensive drugs (ACE inhibitors or sartans in 14 cases, calcium channel blockers in 4 cases, beta-blockers in 10 cases, diuretics in 11 cases and imidazoline receptor antagonist in 3 cases). Fifteen out of nineteen patients had pathological serum lipid spectrum (10 patients had elevated total and LDL cholesterol levels, in 2 cases together with elevated triglycerides and decreased HDL cholesterol, in 5 cases only with HDL cholesterol decrease; 2 patients had elevated triglycerides in combination with decreased HDL cholesterol and 3 patients had isolated HDL cholesterol decrease). Eight patients had already been treated, either with fibrate or statin, one patient with a combination of both. Two patients had impaired fasting glucose (IFG) levels, four patients were diagnosed with impaired glucose tolerance and nine patients had type 2 DM. All diabetic patients had been already treated either with PAD, insulin or its combination (metformin in 8 cases, gliclazide in 2 cases and rosiglitazone in 2 cases). There were no signs of renal complications in diabetic patients at the time of the examination, two patients had had a history of an ischemic stroke.

The healthy controls (C) had no history of obesity or malnutrition, arterial hypertension, glucose or lipid metabolism disturbances, malignant tumors or other severe co-morbidities. They had no regular medication. Blood tests confirmed normal blood count, biochemical and hormonal parameters.

Written informed consent was signed by all participants before being enrolled in the study. The study was approved by the Ethical Committee, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic, and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

Anthropometric examination

All subjects were asked to fast and drink only water a night long prior to the study and were examined in the morning at a basal state. All of them were weighed and measured.

A percentage of truncal body fat was assessed by body composition measurement using Dual-Energy X-Ray Absorptiometry (DEXA, Hologic Discovery, USA). Percentage of total body fat was examined by bioimpedance (Multi-frequency Bodystat QuadScan 4000, Douglas, British Isles) at body current flow of 5, 50, 100 and 200 kHz, respectively. Resting energy expenditure (REE) and respiratory quotient (RQ) were measured by indirect calorimetry (V Max Encore 29N, Viasys, Pennsylvania, USA) performed with a ventilated hood system. Oxygen consumption and carbon dioxide production were measured, and energy expenditure was calculated using the formula of Weir (1949).

Hormonal and biochemical assays

Blood samples for FABP-4, resistin, adiponectin, leptin, insulin and biochemical parameters measurement were withdrawn between 7 and 8 a.m. after 12 h of overnight fasting. Plasma was separated by centrifugation at room temperature and stored at -80°C until being assayed.

Serum FABP-4 concentrations were measured by a commercial ELISA assay (BioVendor, Brno, Czech Republic). The sensitivity was 0.1 ng/ml, and the intra- and interassay variability was <5.0 and 10.0% , respectively.

Serum insulin concentrations were measured by a commercial RIA assay (Cis Bio International, Gif-sur-Yvette, France). Sensitivity was $2.0\ \mu\text{IU/ml}$, and the intra- and interassay variability was 4.2 and 8.8% , respectively. Serum adiponectin concentrations were measured by a commercial ELISA assay (Linco Research, St. Charles, MO, USA). Sensitivity was $0.78\ \text{ng/ml}$, and the intra- and interassay variability was 3.4 and 5.7% , respectively. Serum resistin concentrations were measured by a commercial ELISA assay (BioVendor, Brno, Czech Republic). Sensitivity was $0.2\ \text{ng/ml}$, and the intra- and interassay variability was 3.1 and 6.5% , respectively. Serum leptin concentrations were measured by a commercial ELISA assay (BioVendor, Brno, Czech Republic). Sensitivity was $0.12\ \text{ng/ml}$, and the intra- and interassay variability was 1.7 and 8.0% , respectively.

Plasma levels of cortisol were measured by a commercial RIA assay (Immunotech, Prague, Czech Republic). Sensitivity was $10\ \text{nmol/l}$, and the intra- and interassay variability was 5.8 and 9.2% , respectively. Serum levels of insulin-like growth factor-1 (IGF-1) were measured by a commercial IRMA assay (Immunotech, Prague, Czech Republic). Sensitivity was $2\ \text{ng/ml}$, and the intra- and interassay variability was 6.3 and 6.8% , respectively. Thyroid stimulating hormone (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) were measured using chemiluminescence immunoassay (CLIA) on ADVIA: Centaur analyzer. Serum levels of biochemical parameters (glucose, total and HDL-cholesterol, triglycerides, urea, creatinine, total protein and albumin levels) were measured by standard laboratory methods on Hitachi analyzer, the value of LDL-cholesterol was calculated. Glycated hemoglobin was analyzed by high performance liquid chromatography (HPLC) on Variant II BioRad analyzer (with reference values $2.8\text{-}4\%$).

Statistical analysis

The statistical analysis was performed using SigmaStat software (Jandel Scientific, San Rafael, CA). Results are expressed as means \pm S.E.M. or median, upper and lower quartiles, minimum and maximum values. One way ANOVA followed by Holm-Sidak test, or ANOVA on ranks followed by Dunn's test was used for groups' comparison. The correlations between the values were estimated by Spearman correlation test. A $p<0.05$ value denoted statistical significance.

Results

Anthropometric characteristics of study subjects

Ten out of 14 patients with CS had BMI in the range of obesity, and all had signs of abdominal fat accumulation with waist circumference increased over normal values ($>80\ \text{cm}$ in women, $>94\ \text{cm}$ in men). The waist circumference of OB patients was significantly higher in comparison with CS group (Table 1). The systolic and diastolic blood pressure was significantly higher in both CS and OB groups relative to C, but it was also significantly higher in patients with CS as compared to OB group (Table 1).

The percentage of total and truncal body fat was significantly higher in CS and OB subjects relative to C. Percentage of total body fat was significantly higher in OB relative to CS group. Resting energy expenditure per

Table 1. Anthropometric, biochemical and hormonal characteristics of patients with Cushing's syndrome (CS), obesity (OB) and control subjects (C).

| | CS (n=14) | OB (n=19) | C (n=36) |
|--------------------------------------|-----------------------------|----------------|---------------|
| Age (years) | 45.8 ± 3.39 | 54.6 ± 3.3 | 43.0 ± 2.07 |
| Body mass index (kg/m ²) | 33.7 ± 1.42 * | 45.8 ± 2.42 * | 22.7 ± 0.26 |
| Waist circumference (cm) | 110.6 ± 3.38 ^{*,□} | 127.2 ± 4.26 * | 77.5 ± 1.72 |
| Systolic blood pressure (mm Hg) | 153.6 ± 5.2 ^{*,□} | 134.5 ± 3.96 * | 111.0 ± 2.92 |
| Diastolic blood pressure (mm Hg) | 91.4 ± 3.41 ^{*,□} | 77.5 ± 1.94 * | 70.0 ± 1.83 |
| % of total body fat (bioimpedance) | 40.0 ± 2.37 ^{*,□} | 51.1 ± 2.44 * | 18.8 ± 2.01 |
| % of truncal body fat (DEXA) | 44.0 ± 1.33 * | 41.2 ± 2.8 * | 20.7 ± 1.19 |
| REE/kg (kcal/day/kg) | 17.9 ± 0.42 * | 16.0 ± 0.75 * | 22.5 ± 0.47 |
| Respiratory quotient | 0.79 ± 0.02 [□] | 0.71 ± 0.01 * | 0.79 ± 0.01 |
| Glucose (mmol/l) | 6.4 ± 1.03 * | 7.8 ± 0.85 * | 4.5 ± 0.12 |
| Insulin (μU/ml) | 85.1 ± 38.66 * | 44.4 ± 10.57 * | 15.3 ± 1.4 |
| HOMA index | 20.7 ± 8.01 * | 15.3 ± 3.69 * | 3.2 ± 0.35 |
| Glycated hemoglobin A1c (%) | 5.6 ± 0.6 * | 5.7 ± 0.48 * | 3.7 ± 0.07 |
| Total cholesterol (mmol/l) | 5.7 ± 0.38 | 4.9 ± 0.21 | 5.1 ± 0.14 |
| LDL-cholesterol (mmol/l) | 3.6 ± 0.32 | 2.9 ± 0.22 | 3.1 ± 0.12 |
| HDL-cholesterol (mmol/l) | 1.2 ± 0.06 * | 1.1 ± 0.07 * | 1.5 ± 0.08 |
| Triglycerides (mmol/l) | 2.1 ± 0.27 * | 2.7 ± 0.89 * | 1.0 ± 0.07 |
| Urea (mmol/l) | 6.2 ± 0.67 | 5.9 ± 0.39 * | 4.7 ± 0.18 |
| Creatinine (μmol/l) | 74.3 ± 6.89 | 75.3 ± 5.24 | 73.7 ± 2.5 |
| Total protein (g/l) | 67.1 ± 1.58 ^{*,□} | 74.5 ± 1.24 | 72.9 ± 0.99 |
| Albumin (g/l) | 41.7 ± 0.83 * | 42.1 ± 0.82 * | 46.0 ± 0.73 |
| Leptin (ng/ml) | 60.2 ± 5.36 * | 55.7 ± 4.49 * | 9.3 ± 1.38 |
| Resistin (ng/ml) | 7.5 ± 0.65 * | 7.9 ± 1.38 | 5.4 ± 0.34 |
| Adiponectin (ng/ml) | 17.0 ± 1.84 | 17.8 ± 1.83 | 22.4 ± 1.75 |
| fT3 (pmol/l) | 4.1 ± 0.17 ^{*,□} | 5.1 ± 0.47 | 5.1 ± 0.16 |
| fT4 (pmol/l) | 13.7 ± 0.67 ^{*,□} | 16.2 ± 0.51 | 15.8 ± 0.46 |
| TSH (mIU/l) | 0.9 ± 0.19 ^{*,□} | 2.2 ± 0.33 | 2.3 ± 0.59 |
| IGF-1 (ug/l) | 282.5 ± 33.89 | 199.1 ± 20.10 | 223.0 ± 22.12 |
| Basal plasma cortisol (nmol/l) | 946.3 ± 8.2 ^{*,□} | 515.3 ± 38.4 | 611.4 ± 33.75 |
| FABP-4 (ng/ml) | 110.7 ± 18.15* | 87.3 ± 9.2* | 20.3 ± 2.22 |

* p<0.05 vs. C group, [□] p<0.05 for CS vs. OB group, values are means ± S.E.M. Statistical significance is from one-way ANOVA followed by Holm-Sidak test or ANOVA on ranks followed by Dunn's test, respectively. DEXA = Dual-Energy X-Ray Absorptiometry; REE = resting energy expenditure; HOMA = homeostatic model assessment of insulin resistance; LDL = low density lipoprotein; HDL = high density lipoprotein; fT3 = free triiodothyronine; fT4 = free thyroxine; TSH = thyroid stimulating hormone; IGF-1 = insulin-like growth factor-1; FABP-4 = fatty acid binding protein-4.

kg was significantly higher in C in comparison with CS and OB with no difference in CS vs. OB, whereas respiratory quotient was significantly lower in OB patients relative to CS and C, with no difference between CS and C subjects (Table 1).

Serum levels of hormonal and biochemical parameters

Fasting serum glucose, insulin, HOMA index (homeostatic model assessment of insulin resistance) and

glycated hemoglobin were significantly higher in both CS and OB patients relative to C, but with no significant difference between CS and OB group (Table 1). HDL-cholesterol was significantly reduced in CS and OB relative to C, but did not differ between CS and OB subjects. Triglycerides were significantly increased in CS and OB group in comparison with C. There were no significant differences in total and LDL-cholesterol levels between the studied groups (Table 1).

The average concentrations of urea and creatinine were within the normal range in all groups of patients, although the urea levels were significantly higher in OB relative to C subjects (Table 1). The situation was similar with the average concentrations of total protein and albumin levels, i.e. all of them were within the reference values. However, total protein concentrations were significantly lower in CS relative to OB and C subjects, and albumin levels were significantly lower in CS and OB relative to C subjects (Table 1).

Both fasting serum leptin and resistin were significantly higher in CS as compared to C. Leptin levels were also significantly higher in OB relative to C subjects. No significant differences were found in adiponectin levels between the examined groups (Table 1).

Basal plasma cortisol was significantly higher in CS relative to OB and C patients. TSH, free T3 and free T4 were significantly lower in CS as compared to OB and C (caused very likely by the known suppressive effect of elevated cortisol levels on TSH secretion). There were no significant differences in serum IGF-1 concentrations between the studied groups (Table 1).

Fasting serum FABP-4 levels were significantly higher in CS and OB subjects relative to C. There was no significant difference in FABP-4 levels between CS and OB patients (Fig. 1).

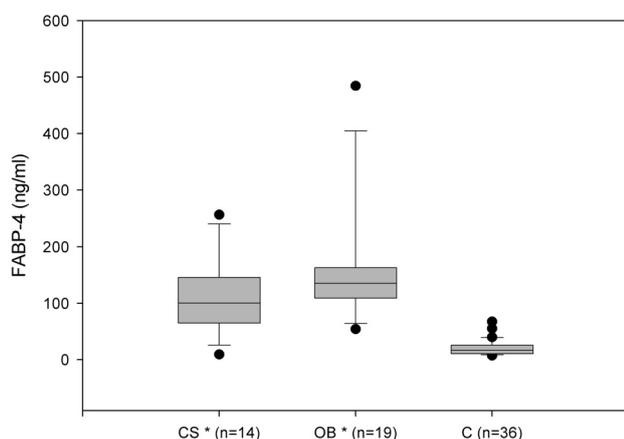


Fig. 1. Fatty acid binding protein (FABP-4) concentrations in patients with Cushing's syndrome (CS), obesity (OB) and control subjects (C). Values are median, upper and lower quartiles, minimum and maximum data. Statistical significance is from ANOVA on ranks and Dunn's test. * $p < 0.001$ vs. C group.

Relationship of FABP-4 with other anthropometric, hormonal and biochemical parameters

The relationship of FABP-4 with other studied parameters was calculated in the combined population of all three groups of patients (Table 2).

Table 2. Relationships of fatty acid binding protein (FABP-4) with anthropometric, biochemical and hormonal parameters calculated in a combined population of patients with Cushing's syndrome, obesity and lean healthy controls (n=69).

| | r | p |
|-----------------------|--------|---------|
| Age | 0.486 | <0.001 |
| Waist circumference | 0.802 | <0.001 |
| BMI | 0.776 | <0.001 |
| % of total body fat | 0.842 | <0.001 |
| % of truncal body fat | 0.888 | <0.001 |
| REE/kg | -0.666 | <0.001 |
| Respiratory quotient | -0.381 | 0.00929 |
| sBP | 0.6 | <0.001 |
| dBP | 0.501 | <0.001 |
| Total cholesterol | 0.13 | 0.289 |
| LDL-cholesterol | 0.183 | 0.158 |
| HDL-cholesterol | -0.602 | <0.001 |
| Triglycerides | 0.734 | <0.001 |
| Glucose | 0.567 | <0.001 |
| Insulin | 0.556 | <0.001 |
| HOMA index | 0.605 | <0.001 |
| HbA1c (%) | 0.603 | <0.001 |
| Urea | 0.421 | <0.001 |
| Creatinine | -0.018 | 0.886 |
| Total protein | -0.237 | 0.06 |
| Albumin | -0.632 | <0.001 |
| Free T3 | -0.612 | <0.001 |
| Testosterone | -0.403 | 0.0184 |
| Basal plasma cortisol | 0.179 | 0.249 |
| Adiponectin | -0.138 | 0.259 |
| Leptin | 0.809 | <0.001 |
| Resistin | 0.406 | <0.001 |

r = correlation coefficient; p = statistical significance. Results are from Spearman Correlation Test. BMI = body mass index; REE = resting energy expenditure; sBP, dBP = systolic, diastolic blood pressure; HDL = high density lipoprotein; LDL = low density lipoprotein; HOMA = homeostatic model assessment of insulin resistance; HbA1c = glycated hemoglobin; free T3 = free triiodothyronine.

Serum FABP-4 levels correlated positively with age, BMI, waist circumference, percentage of total and truncal fat mass, systolic and diastolic blood pressure, triglycerides and glucose levels, HOMA index, insulin, glycated hemoglobin, urea, leptin and resistin levels and were inversely related to HDL-cholesterol, albumin, REE per kg, RQ, free T3 and testosterone levels (Table 2).

No significant relationships were found among FABP-4, plasma cortisol, total and LDL cholesterol or

adiponectin levels, respectively (Table 2). No correlation has been found between FABP-4 and cortisol levels in the individual subgroups either.

Discussion

Glucocorticoids (GC) are important hormones involved in regulations of different metabolic processes. They are also important regulators of the function and distribution of adipose tissue. Patients with Cushing's syndrome caused by endogenous or exogenous excess of GC are characterized by the presence of insulin resistance and visceral obesity (Pivonello *et al.* 2005, Walker 2007). GC are also responsible for the other components of metabolic syndrome seen in CS patients due to their negative influence on glucose and lipid metabolism and direct pro-atherosclerotic effects (Wang 2005, Walker 2007).

Here we studied the relationship between cortisol and FABP-4 to test the hypothesis that elevated FABP-4 levels might be responsible for some metabolic features of Cushing's syndrome similarly as it was suggested for patients with obesity and/or type 2 diabetes mellitus (Stejskal and Karpíšek 2006, Xu *et al.* 2006, Karpíšek *et al.* 2007, Haluzík *et al.* 2009). Experimental studies have suggested that FABP-4 together with peroxisome proliferator-activated receptor gamma (PPAR γ) and other regulators influence the biological pathways regulating insulin sensitivity and body composition (Dancott *et al.* 2004), lipid metabolism (Binas *et al.* 1999, Levy *et al.* 2001) and atherogenesis (Fu *et al.* 2002, 2006).

Based on the published data, FABP-4 may represent a novel link between disturbed secretory function of adipose tissue and other metabolic abnormalities coupled within insulin resistance syndrome. Increased local cortisol production, in particular in visceral adipose tissue, is considered as one of possible mechanisms how visceral adipose tissue contributes to insulin resistance in obesity (Purnell *et al.* 2009). Higher local cortisol levels could directly induce decreased insulin sensitivity in visceral adipose tissue both through the direct effects on glucose uptake and through the changes in endocrine functions of the adipose tissue (Andrews and Walker 1999, Wang 2005).

The most important finding of this study is that serum FABP-4 levels are markedly increased in patients with Cushing's syndrome relative to healthy lean control subjects but they do not significantly differ from those of

patients with obesity and type 2 diabetes mellitus. Elevated FABP-4 levels in patients with Cushing's syndrome are in agreement with our previous findings of increased FABP-4 levels in patients with obesity and type 2 diabetes mellitus (Haluzík *et al.* 2009). Serum FABP-4 concentrations in this study strongly positively correlated with BMI, waist circumference, percentage of total and truncal fat mass, triglycerides and parameters of diabetes compensation and insulin resistance, including leptin and resistin levels and were negatively related to HDL-cholesterol levels. Interestingly, FABP-4 concentrations negatively correlated with resting energy expenditure per kg, respiratory quotient and free triiodothyronine. Such relationships suggest that in addition to possible role of FABP-4 in regulation of glucose and lipid metabolism it may also contribute to the regulation of energy homeostasis in a more complex way. The possible mechanism of such regulation, e.g. central vs. peripheral nature of these effects, needs to be further determined.

According to recent publications, FABP-4 seems to be not only an early marker of development of the metabolic syndrome (Xu *et al.* 2007), but its plasma concentrations should be taken into consideration as an early marker of kidney damage in type 2 diabetes (Cabre *et al.* 2008, Yeung *et al.* 2009). However, in our study we have found a strong positive correlation of FABP-4 with urea levels but no association between FABP-4 and creatinine concentrations (glomerular filtration rate has been not assessed in our study), although these results may be influenced by a small number of examined patients. On the other hand, this finding together with a significant negative correlation of FABP-4 with albumin levels (although with no association with total protein concentrations) opens a question of the relationship between FABP-4 and protein metabolism.

At present only few studies focused on the significance of circulating FABP-4 levels in the development of insulin resistance in humans and none of them is a prospective one. Therefore, it is currently unclear whether circulating FABP-4 is only a marker or an active player in this process. For example, Engl *et al.* (2008) demonstrated that FABP-4 concentration in morbidly obese patients increased significantly three months after gastric banding. Interestingly, FABP-4 levels one year after gastric banding with total weight loss of 24.9 kg did not significantly differ from the baseline values. Recently, Koh *et al.* (2009) described an independent association of FABP-4 concentrations with presence of non-alcoholic fatty liver disease in patients

with type 2 diabetes. On the opposite site of the nutritional spectrum, we have recently demonstrated that FABP-4 levels in patients anorexia nervosa with markedly decreased body fat content due to chronically decreased food intake do not significantly differ from those of healthy normal-weight women (Haluzíková *et al.* 2008). Our current results, however, further support the previous suggestion that FABP-4 is one of the factors most closely related to BMI, diabetes compensation and insulin sensitivity from the wide scale of adipose tissue-derived hormones, including the most commonly studied ones such as adiponectin and resistin (Weyer *et al.* 2001, McTernan *et al.* 2002, Anderlová *et al.* 2006). Nevertheless, its biological relevance remains to be further clarified.

Interestingly, respiratory quotient values in CS patients in our study were similar to control lean subjects, while it was significantly decreased in obese patients. We have no clear explanation for this interesting finding but

we believe that this might be a direct consequence of increased cortisol levels in CS patients.

In conclusion, our study shows that both simple obesity and obesity connected to hypercortisolism are accompanied by markedly increased FABP-4 levels relative to the lean controls. FABP-4 concentrations in patients with obesity were comparable to those in patients with Cushing's syndrome suggesting that obesity is the primary reason for elevated FABP-4 levels. This fact together with no significant relationship between circulating cortisol and FABP-4 levels argues against direct regulation of circulating FABP-4 levels by cortisol.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Supported by grant of IGA MHCR No. NR/9438-3 and Research Project MZOVFN2005.

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