

Plasma concentrations of adipocyte fatty acid binding protein in patients with Cushing's syndrome

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Short title: Adipocyte fatty acid binding protein 4 in Cushing's syndrome

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.

14 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 positively correlated 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

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, Hertzler 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 (Karpisek *et al.* 2007; Stejskal and Karpisek 2006; Xu *et al.* 2007; Xu *et al.* 2006).

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 (Karpisek *et al.* 2007; Stejskal and Karpisek 2006; Xu *et al.* 2006). 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 (Haluzik *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 yrs, body mass index (BMI): 33.7 ± 1.42 kg/m²), nineteen obese patients (17 women, 2 men, age: 54.6 ± 3.3 yrs, BMI: 45.8 ± 2.42 kg/m²) and thirty six healthy controls (28 women, 8 men, age: 43 ± 2.07 yrs, 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 cortisolaemia (nocturnal cortisol over 150 nmol/l), unsuppressed cortisolaemia 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 been already 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 been already 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 IGT 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, 1st 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 Weir formula (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 hours 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 µIU/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, Missouri,

USA). Sensitivity was 0.78 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 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 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 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 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 chemiluminiscence 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 2.8-4%).**

Statistical analysis

The statistical analysis was performed using SigmaStat software (Jandel Scientific, San Rafael, CA). Results are expressed as means \pm **standard error of means** (SEM) 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 value < 0.05 denoted statistical significance.

RESULTS

Anthropometric characteristics of study subjects

Ten out of fourteen 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 cm in women, >94 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. REE per kg was significantly higher in C in comparison with CS and OB with no difference in CS vs. OB, whereas RQ 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 (Figure 1).

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).

Serum FABP-4 levels significantly positively correlated with age, BMI, waist circumference, percentage of total and truncal fat mass, systolic and diastolic blood pressure, triglyceride 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 a 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 (Walker 2007; Wang 2005).

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 (Karpisek *et al.* 2007; Stejskal and Karpisek 2006; Xu *et al.* 2006, Haluzik *et al.* 2009). Experimental studies have suggested that FABP-4 together with peroxisome proliferator-activated receptor gamma (PPARgamma) and other regulators influences the biologic pathways regulating insulin sensitivity and body composition (Damcott *et al.* 2004), lipid metabolism (Binas *et al.* 1999; Levy *et al.* 2001) and atherogenesis (Fu *et al.* 2006, Fu *et al.* 2002).

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 (Wang 2005; Andrews and Walker 1999).

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 (Haluzik *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). Though, 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. demonstrated that FABP-4 concentration in morbidly obese patients significantly increased three months after gastric banding (Engl *et al.* 2008). 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. described an independent association of FABP-4 concentrations with presence of **nonalcoholic fatty liver disease** in patients with type 2 diabetes (Koh *et al.* 2009). 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 (Haluzikova *et al.* 2008). Our current results, however, further support the previously suggested finding 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 (Anderlova *et al.* 2006; McTernan *et al.*

2002; Weyer *et al.* 2001). **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 have been 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.

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REFERENCES

ANDERLOVA K, KREMEN J, DOLEZALOVA R, HOUSOVA J, HALUZIKOVA D, KUNESOVA M, HALUZIK M: The influence of very-low-calorie-diet on serum leptin, soluble leptin receptor, adiponectin and resistin levels in obese women. *Physiol Res* **55**:277-83, 2006.

ANDREWS RC, WALKER BR: Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci (Lond)* **96**:513-23, 1999.

BINAS B, DANNEBERG H, MCWHIR J, MULLINS L, CLARK AJ: Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. *Faseb J* **13**:805-12, 1999.

BOORD JB, FAZIO S, LINTON MF: Cytoplasmic fatty acid-binding proteins: emerging roles in metabolism and atherosclerosis. *Curr Opin Lipidol* **13**:141-7, 2002.

CABRE A, LAZARO I, GIRONA J, MANZARES JM, MARIMON F, PLANA N, HERAS M, MASANA L: Plasma fatty acid-binding protein 4 increases with renal dysfunction in type 2 diabetic patients without microalbuminuria. *Clinical Chemistry* **54**:181-187, 2008.

COE NR, BERNLOHR DA: Physiological properties and functions of intracellular fatty acid binding proteins. *Biochim Biophys Acta* **1391**:287-306, 1998.

DAMCOTT CM, MOFFETT SP, FEINGOLD E, BARMADA MM, MARSHALL JA, HAMMAN RF, FERRELL RE: Genetic variation in fatty acid-binding protein-4 and peroxisome proliferator-activated receptor gamma interactively influence insulin sensitivity and body composition in males. *Metabolism* **53(3)**:303-9, 2004.

ENGL J, CIARDI C, TATARCZYK T, KASER S, LAIMER M, LAIMER E, WEISS H, AIGNER F, MOLNAR C, TILG H, PATSCH JR, EBENBICHLER CF: A-FABP--a biomarker associated with the metabolic syndrome and/or an indicator of weight change? *Obesity (Silver Spring)* **16**:1838-42, 2008.

FU Y, LUO N, LOPES-VIRELLA MF, GARVEY WT: The adipocyte lipid binding protein (ALBP/aP2) gene facilitates foam cell formation in human THP-1 macrophages. *Atherosclerosis* **165(2)**:259-69, 2002.

FU Y, LUO L, LUO N, GARVEY WT: Lipid metabolism mediated by adipocyte lipid binding protein (ALBP/aP2) gene expression in human THP-1 macrophages. *Atherosclerosis* **188(1)**:102-11, 2006.

FURUHASHI M, TUNCMAN G, GÖRGÜN CZ, MAKOWSKI L, ATSUMI G, VAILLANCOURT E, KONO K, BABAIEV VR, FAZIO S, LINTON MF, SULSKY R, ROBL JA, PARKER RA, HOTAMISLIGIL GS: Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature*. **447**:959-65, 2007.

HALUZÍK MM, ANDERLOVÁ K, DOLEŽALOVÁ R, ADAMÍKOVÁ A, HALUZÍKOVÁ D, HOUSOVÁ J, SVAČINA S, HALUZÍK M: Serum adipocyte fatty acid binding protein levels in patients with type 2 diabetes mellitus and obesity: the influence of fenofibrate treatment. *Physiol Res* 2009, **58(1)**:93-9.

HALUZIKOVA D, DOSTALOVA I, KAVALKOVA P, ROUBICEK T, MRAZ M, PAPEZOVA H, HALUZIK M: Serum concentrations of adipocyte fatty acid binding protein in patients with anorexia nervosa. *Physiol Res* **58(4)**:577-81, 2009.

HERTZEL AV, BERNLOHR DA: The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol Metab* **11**: 175-180, 2000.

CHMURZYŃSKA A: The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. *J Appl Genet* **47**: 39-48, 2006.

KARPISEK M, STEJSKAL D, KOTOLOVA H, KOLLAR P, JANOUTOVA G, OCHMANOVA R, CIZEK L, HORAKOVA D, YAHIA RB, LICHNOVSKA R, JANOUT V: Treatment with atorvastatin reduces serum adipocyte-fatty acid binding protein value in patients with hyperlipidaemia. *Eur J Clin Invest* **37**:637- 42, 2007.

KOH JH, SHIN YG, NAM SM, LEE MY, CHUNG CH, SHIN JY: Serum adipocyte fatty acid-binding protein levels are associated with nonalcoholic fatty liver disease in type 2 diabetic patients. *Diabetes Care* 32:147-52, 2009.

LEVY E, MENARD D, DELVIN E, STAN S, MITCHELL G, LAMBERT M, ZIV E, FEOLI-FONSECA JC, SEIDMAN E: The polymorphism at codon 54 of the FABP2 gene increases fat absorption in human intestinal explants. *J Biol Chem* 276:39679-84, 2001.

MAEDA K, CAO H, KONO K, GORGUN CZ, FURUHASHI M, UYSAL KT, CAO Q, ATSUMI G, MALONE H, KRISHNAN B, MINOKOSHI Y, KAHN BB, PARKER RA, HOTAMISLIGIL GS: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab.* 1:107-19, 2005.

MAKOWSKI L, HOTAMISLIGIL GS: Fatty acid binding proteins--the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr* 134: 2464S-2468S, 2004.

MCTERNAN CL, MCTERNAN PG, HARTE AL, LEVICK PL, BARNETT AH, KUMAR S: Resistin, central obesity, and type 2 diabetes. *Lancet* 359:46-7, 2002.

PIVONELLO R, FAGGIANO A, LOMBARDI G, COLAO A: The metabolic syndrome and cardiovascular risk in Cushing's syndrome (CS). *Endocrinol Metab Clin North Am* 34(2):327-39, 2005.

PURNELL JQ, KAHN SE, SAMUELS MH, BRANDON D, LORIAUX DL, BRUNZELL JD: Enhanced cortisol production rates, free cortisol, and 11beta-HSD-1 expression correlate with visceral fat and insulin resistance in men: effect of weight loss. *Am J Physiol Endocrinol Metab* 296:E351-7, 2009.

STEJSKAL D, KARPISEK M: Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? *Eur J Clin Invest* 36:621-5, 2006.

WALKER BR: Glucocorticoids and Cardiovascular Disease. *Eur J Endocrinol* **157**(5): 545-559, 2007.

WANG M: The role of glucocorticoid action in the pathophysiology of the metabolic syndrome. *Nutr Metab* **2**:3-17, 2005.

WEIR JB: New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* **109**: 1-9, 1949.

WEYER C, FUNAHASHI T, TANAKA S, HOTTA K, MATSUZAWA Y, PRATLEY RE, TATARANNI PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**:1930-5, 2001.

WOLFRUM C, BORRMANN CM, BORCHERS T, SPENER F: Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors alpha - and gamma-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus. *Proc Natl Acad Sci U S A* **98**:2323-8, 2001.

XU A, TSO AW, CHEUNG BM, WANG Y, WAT NM, FONG CH, YEUNG DC, JANUS ED, SHAM PC, LAM KS: Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. *Circulation* **115**:1537-43, 2007.

XU A, WANG Y, XU JY, STEJSKAL D, TAM S, ZHANG J, WAT NM, WONG WK, LAM KS: Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* **52**:405-13, 2006.

YEUNG DC, XU A, TSO AW, CHOW WS, WAT NM, FONG CH, TAM S, SHAM PC, LAM KS: Circulating levels of adipocyte and epidermal fatty acid-binding proteins in

relation to nephropathy staging and macrovascular complications in type 2 diabetic patients. *Diabetes Care* **32(1)**:132-4, 2009.

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/m²)	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 (mmHg)	153.6 ± 5.2 ^{*,□}	134.5 ± 3.96 *	111.0 ± 2.92
Diastolic blood pressure (mmHg)	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 (μUI/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 ± SEM.

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

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

Figure legend

Figure 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

