Endocrine Journal 2018, **65** (8), 849-858

ORIGINAL

Adipokine and cytokine levels in non-functioning adrenal incidentalomas (NFAI)

Anna Babinska[1)], Mariusz Kaszubowski[2)] and Krzysztof Sworczak[1)]

[1)] *Department of Endocrinology and Internal Medicine, Medical University of Gdansk, Gdansk, Poland*
[2)] *Institute of Statistics, Department of Economic Sciences, Faculty of Management and Economics, Gdansk University of Technology, Gdansk, Poland*

Abstract. Due to the fact that overweight or obesity is accompanied by hormonally active adrenal tumors: Cushing Syndrome—(CS) and Subclinical Cushing Syndrome (SCS), it is of high interest the correlation between different adipokines and cytokines secreted by adipose tissue, with metabolic disorders and hormonal activity in this group. Even in non-functioning adrenal incidentalomas (NFAI) elevated risk for cardiovascular disease and metabolic syndrome was demonstrated. The aim of the study was to investigate plasma adiponectin, leptin, resistin, tumor necrosis factor α (TNFα), interleukin 6 (IL6) and monocyte chemoattractant protein 1 (MCP1) levels in patients with NFAIs and healthy subjects. The study included 18 NFAI patients and 18 healthy subjects. The groups were homogeneous in terms of age, sex and body mass index (BMI). Patients with NFAI showed significantly higher circulating levels of pro-inflammatory cytokines compared to healthy controls (MCP 1: $p < 0.001$; TNFα $p = 0.021$; IL6 $p = 0.012$). On the other hand, adiponectin concentration was significantly lower in the NFAI group ($p = 0.034$). The serum leptin and resistin concentrations did not differ significantly between the two groups. Acquired results were not dependent on glucocorticoid and catecholamine secretion in NFAI patients. Also, there were no clear correlations between BMI and cytokine levels. It is possible that increased risk for cardiovascular and metabolic diseases reported in NFAI patients is at least partially dependent on adipose tissue activity.

Key words: Adipokines, Cytokines, Adrenal tumors

ADRENAL INCIDENTALOMAS are an important social and economic problem. The first reports of incidentally discovered adrenal tumors were reported in 1941. In 1994 Griffing provocatively described this phenomenon as an 'endocrine epidemic' [1]. Since then, the recommendations for surgical treatment and follow-up have been changed many times [2-5].

Submitted Feb. 16, 2018; Accepted May 7, 2018 as EJ18-0066
Released online in J-STAGE as advance publication Jun. 5, 2018
Correspondence to: Anna Babinska, Department of Endocrinology and Internal Medicine, Medical University of Gdansk, ul. Dębinki 7, 80-288 Gdansk, Poland.
E-mail: a.mail@wp.pl
Correspondence to: Mariusz Kaszubowski, Institute of Statistics, Department of Economic Sciences, Faculty of Management and Economics, Gdansk University of Technology, ul. Traugutta 79, 80-233 Gdańsk, Poland.
E-mail: mkaszubo@zie.pg.gda.pl
Correspondence to: Krzysztof Sworczak, Department of Endocrinology and Internal Medicine, Medical University of Gdansk, ul. Dębinki 7, 80-288 Gdańsk, Poland.
E-mail: ksworczak@gumed.edu.pl

Adrenal incidentalomas considered hormonally inactive in the past have often been found to exhibit subclinical hormonal activity of the cortex or medulla [6-10].

Even subclinical hormonal activity, results in coexistence of metabolic diseases: Cushing Syndrome—(CS) and Subclinical Cushing Syndrome (SCS), pheochromocytoma (PHEO) [10-12] and coagulation disorders: CS, SCS [13-15]. In fact, patients with adrenal incidentalomas have a high prevalence of obesity, hypertension (HT) and diabetes mellitus (DM) [11, 12, 16, 17]. On this ground, after some consideration, this group of patients should be treated surgically.

While the risk of metabolic disorders is widely discussed in SCS/CS or PHEO patients [11, 18-20], there are few reports concerning this risk in non-functioning adrenal incidentalomas (NFAI) [21-24].

There are reports on an association between levels of cortisol secretion and severity of metabolic disorders in adrenal tumor patients [16, 22-24].

Obesity is currently a social disease. The adipose tissue secretes many adipokines as well as anti- and

Table 1 Demographic characteristics of NFAI patients and control group

Variable	Group NFAI $N = 18$	Group Control $N = 18$	p
Age [years]	54.11 ± 11.78	47.50 ± 9.48	0.073
Female/male	15/3	13/5	0.423
BMI [kg/m^2]	27.00 ± 4.24	25.92 ± 3.28	0.398
Hypertension [%]	5.56%	0.00%	0.310
DM [%]	5.56%	0.00%	0.310
Fasting glucose [mg/dL]	100.71 ± 32.02	86.50 ± 11.86	0.101
Fasting insulin [μU/mL]	9.75 ± 5.83	9.22 ± 5.81	0.789
HOMA index	2.76 ± 2.87	1.97 ± 1.29	0.310
Systolic BP [mmHg]	137.22 ± 26.97	125.11 ± 9.30	0.086
Diastolic BP [mmHg]	88.61 ± 9.04	71.11 ± 5.36	<0.001
Total cholesterol [mg/dL]	200.06 ± 39.16	209.94 ± 34.99	0.438
HDL [mg/dL]	56.29 ± 18.42	54.33 ± 9.43	0.698
LDL [mg/dL]	122.76 ± 39.25	103.67 ± 27.21	0.107
TG [mg/dL]	137.41 ± 51.58	112.11 ± 47.02	0.140

BP, blood pressure

pro-inflammatory cytokines [25-27]. The best known adipokines are adiponectin and leptin as well as pro-inflammatory resistin. Among other pro-inflammatory cytokines interleukin 6 (IL6), tumor necrosis factor alpha (TNFα) and monocyte chemoattractant protein 1 (MCP1) should be mentioned. Abnormal concentrations of pro-inflammatory cytokines was associated with increased risk of metabolic and neoplastic diseases in humans [25, 28, 29].

There are few reports on the association between adipokines and hormonal activity of adrenal tumors. Their majority concerns CS patients [30–32]. Only in a few dates authors investigated an association between adipokines and metabolic diseases as well as subclinical activity of incidentally discovered adrenal tumors [18, 21, 33].

Therefore, the authors of the current study based on their own material and experience undertook the attempt of analyzing the association of hormonal disorders that accompany only NFAI with possible oversecretion of adipokines and pro- as well as anti-inflammatory cytokines.

The authors of the study hypothesize that increased cardiovascular and metabolic disease risk among NFAI patients may be associated with adipokines oversecretion that result from slight glucocorticoid and catecholamine excess or the tumor secretion.

Subjects and Methods

Subject

The study enrolled 18 adrenal incidentaloma patients who had no hormonal activity of adrenal tumors. The subjects included 15 female and 3 male, aged 25–66 years (mean: 54.11 ± 11.78). In a group of NFAI hypertension (HT) and diabetes mellitus (DM) were present only at one case (Table 1). There were no cardiovascular events like stroke, myocardial infarction or embolism in NFAI group of patients.

Eighteen healthy subjects without adrenal lesions on abdominal imaging, comparable for sex, age and BMI were enrolled as controls (group of 13 females and 5 males aged 31–67 years with mean: 45.50 ± 9.48).

In the control group, the adrenal tumor was excluded on the basis of imaging examinations (CT) performed in the last 6 months for the reasons other than suspicion of adrenal lesions (participants' initiative or due to non-characteristic abdominal pain).

All controls were not affected by HT, DM, cardiovascular disease, chronic inflammatory diseases, chronic

Table 2 Hormonal parameters of NFAI group

Variable	Mean ± SD	Our patients ranges	Normal ranges
Morning serum cortisol [nmol/L]	354.82 ± 143.94	175.00–805.00	101.00–535.00
Midnight serum cortisol [nmol/L]	112.82 ± 37.76	53.00–178.00	79.00–478.00
Cortisol after 1 mg DXM [nmol/L]	54.82 ± 27.90	0.00–100.00	<50.00
Morning ACTH [pg/mL]	25.89 ± 29.85	10.00–132.00	5–46.00
UFC [nmol/24 h]	218.55 ± 82.12	108.00–364.40	12.00–486.00
Androstendion [ng/mL]	1.25 ± 0.8	0.5–3.20	0.70–3.60
DHEAS [ug/L]	81.67 ± 63.23	15.00–244.00	42.00–290.00
Metanephrine [µg/24h]	270.35 ± 31.65	110.00–302.00	64.00–302.00
Normetanephrine [µg/24 h]µ	173.91 ± 121.11	32.00–367.00	162.00–527.00

Cortisol after 1 mg DXM = overnight 1 mg dexamethasone (DXM) test; UFC = free urinary cortisol excretion; DHEAS - dehydroepiandrosterone sulphate.

hepatitis or known malignancies. Subjects with acute inflammatory states were also excluded from the study.

Informed consent was obtained from all participants and the study was approved by the local ethical committee.

Diagnosis of adrenal incidentaloma was based on the detection of unilateral adrenal mass (size >1 cm) in abdominal imaging performed for another reason, not due to suspected adrenal disease. All masses had radiologic characteristics of cortical adenoma. Exclusion criteria were the presence of known extra-adrenal neoplasms and PHEO.

All participants were examined on the day of blood collection, and anthropometric parameters were obtained. Height was measured with use of a wall-mounted ruler. Weight was assessed using a digital scale. Then, body mass index (BMI) was calculated according to the formula weight (kg) divided by height squared (m^2). All medications that may interfere with laboratory tests results were excluded.

All patients were admitted to our Department. Venous blood samples for biochemical and hormonal analyses were obtained by venipuncture between 7.30 and 8.30 am after requested 12-h overnight fasting.

In order to assess the cortical rhythm of blood, serum midnight cortisol was taken for analyses. All patients underwent an overnight 1 mg dexamethasone (DXM) test. Serum morning cortisol was determined under basal conditions and in daytime rhythm. Corticotropin (ACTH) levels was determined under basal conditions in the morning.

Cortisol serum concentration below 50 nmol/L (1.8 µg/dL) in the 1 mg overnight DXM suppression test, was a sufficient criterion to exclude SCS [34]. If cortisol was not fully suppressed (*i.e.* to less than 50 nmol/L); normal circadian cortisol secretion rhythm (midnight serum cortisol concentration accounts for less than 50% of morning cortisol ratio), normal morning ACTH concentration (normal ranges 5–46 pg/mL), and normal free urinary cortisol excretion (UFC) (normal ranges 12–486 nmol/24 h), were criterion to exclude SCS [35, 36]. Hormonal characteristics of NFAI was shown in Table 2.

The lack of hormonal activity means the exclusion of SCS and overproduction of metanephrine or normetanephrine, but also the absence of androstenedione or DHEAS secretion. All patients also had plasma aldosterone to plasma renin activity ratio lower than 20, excluding primary hyperaldosteronism.

Due to the lack of adrenal tumor, hormonal activity tests were not evaluated in the control group.

Both in the NFAI and control group plasma fasting glucose and insulin were measured. The insulin resistance index HOMA (homeostasis model assessment) was assessed. The HOMA index was estimated according to the following formula: fasting glucose (mmol/L) × fasting insulin level (mU/L)/22.5. The HOMA index of greater than 2,5 was considered abnormal.

In one patient diagnosed with DM, glycosylated hemoglobin (Hb A1c) was assessed.

Lipid profile, including triglycerides (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were measured in NFAI

and control groups.

No patient was taking lipid-lowering or antidiabetic medications for about 12 weeks. One patient with DM type 2 was newly diagnosed and do not on any antidiabetic treatment.

Laboratory analyses

All hormone concentrations were determined in the same laboratory using freely available kits. ACTH concentration was determined with a solid-phase, two-site sequential chemiluminescent immunometric assay Immulite 1000 ^{R}ACTH manufactured by Siemens. Serum and urinary cortisol concentrations were determined with a Chemiluminescent Microparticle Immunoassay Cortisol Reagent Kit on Abbott's Architect analyzer.

Morning serum dehydroepiandrosterone sulphate (DHEAS) and androstendione concentrations were determined with radioimmunological assay (RIA) using DHEAS commercial kits by Orion Diagnostica, androstendione commercial DRG MedTek Elisa kits, respectively.

Plasma renin activity was measured by RIA using commercial kits. The assay for aldosterone was performed with diagnostic kits by Elisa using commercial kits by DGR MedTek.

The 24-h urinary metanephrine and normetanephrines excretion determined by means of high-performance liquid chromatography—HPLC on Bio-Rad. All medications that may interfere with urinary metanephrine and normetanephrine results were excluded.

Both in the NFAI group and in the volunteer group, adiponectin, leptin, resistin, and cytokines TNFα, MCP1, IL6 were determined. Plasma adiponectin assay was performed using a Human HMW Quantikine Elisa Kit (Biokom, R&D Systems USA). Plasma leptin concentration were determined by commercial sandwich enzyme immunoassay EIA kit (DRG MedTek, DRG Instruments GmbH Germany). Resistin was measured using an Quantikine Elisa Kit (Biokom, R&D Systems USA). Plasma Human—TNFα concentration was performed using Quantikine HS Elisa (Biokom, R&D Systems USA). Serum human IL6 was determined by commercial Quantikine HS Elisa Kit (Biokom, R&D Systems USA). Plasma MCP1 concentration were determined by commercial Quantikine HS Elisa Kit (Biokom, R&D Systems USA).

Obtained adipokine and cytokine concentrations are the result of the consensus of two independent investigators performing the assay.

Statistical analyses

All raw data for each group was presented with their number and basic descriptive statistics as mean ± standard deviation. Normality of data sets was verified using the W Shapiro-Wilk test. Since not all groups met this assumption, differences between mean values in small groups were examined by Welch's *t* test (symbol *t*) and Mann-Whitney *U* test (symbol Z) at the same time. If both test were compatible the *p*-value for parametric *t*-test was presented as interpretation. Differences in fractions were checked using Fisher's exact test. To assess the interdependence between the analyzed variables, both: Pearson's and rang Spearman's correlation coefficients were calculated and for those important, supplemented with presentation of appropriate scatter plots.

The level of significance was set at $\alpha = 0.05$. All calculated *p*-values were for two-tailed tests. Statistical analysis was performed with the use of the statistical software Statistica 12.5 (StatSoft, Tulsa, OK, US).

Results

We studied 18 carefully selected patients without known hormonal activity (NFAI group) and 18 healthy volunteers (control group). Of the participants, 15 were female and 3 were male, and the mean patients age was 54.11 ± 11.78 (range: 25–66) years. The clinical features of the subjects who participated in this study were reported in Table 1. There were no significant differences between groups in terms of age, sex and BMI. Diastolic hypertension prevalence was significantly higher in NFAI group than in the control group ($p < 0.001$).

The existence of possible correlations between adipokine and cytokine levels and the anthropometric, metabolic and hormonal parameters were investigated. HT and DM were present only in patients with NFAI (Table 1). In one patient with DM the HbA1c concentration was 6.4%.

Patients with NFAI showed significantly higher circulating levels of cytokines: MCP1, TNFα and IL6 (518.00 ± 192.98 *versus* 300.93 ± 97.04 pg/mL ($p < 0.001$); 1.30 ± 0.97 *versus* 0.68 ± 0.40 pg/mL ($p = 0.021$); 3.95 ± 3.75 *versus* 1.39 ± 1.13 pg/mL ($p = 0.012$) respectively. The serum leptin and resistin concentration levels did not differ significantly between NFAI and control groups. In a NFAI group leptin serum concentration was higher than in control group, but there was not statistically signifi-

Table 3 Comparison of cytokine's levels in NFAI and control groups

Variable	*t*-test for independed samples (Mann–Whitney *U* test)			
	Group NFAI $N = 18$	Group Control $N = 18$	*t* (Z)	*p*
Leptin [ng/mL]	13.78 ± 10.46	10.21 ± 7.74	1.164 (1.232)	0.253 (0.261)
MCP1 [pg/mL]	518.00 ± 192.98	300.93 ± 97.04	4.263 (3.907)	<0.001 (<0.001)
Resistin [ng/mL]	11.15 ± 5.58	10.77 ± 5.24	0.213 (0.237)	0.833 (0.812)
TNFα [pg/mL]	1.30 ± 0.97	0.68 ± 0.40	2.484 (2.120)	0.021 (0.034)
IL6 [pg/mL]	3.95 ± 3.75	1.39 ± 1.13	2.777 (2.927)	0.012 (0.003)
Adiponectin [ng/mL]	3,954.00 ± 2,321.57	6,622.22 ± 4,479.35	–2.243 (–2.547)	0.034 (0.011)

Table 4 Differences in adipokines and cytokines in NFAI before and after surgery ($N = 5$)

Variable	Results of *t*-test for dependent samples ($N = 5$) All groups pass the normality assumption			
	Group NFAI before surgery	Group NFAI after surgery	*t*	*p*
Leptin [ng/mL]	19.49 ± 6.54	12.62 ± 3.21	3.792	0.019
MCP1 [pg/mL]	464.32 ± 197.07	203.08 ± 61.05	3.877	0.018
Resistin [ng/mL]	10.48 ± 8.73	7.73 ± 5.81	2.007	0.115
TNFα [pg/mL]	0.89 ± 0.41	0.51 ± 0.10	2.385	0.076
IL6 [pg/mL]	1.99 ± 0.95	0.79 ± 0.15	3.113	0.036
Adiponectin [ng/mL]	2,702.40 ± 541.97	4,399.80 ± 413.42	–7.408	0.002

cant differences: 13.78 ± 10.46 ng/mL *versus* 10.21 ± 7.74 ng/mL ($p = 0.253$). On the other hand, adiponectin concentration was significantly lower in NFAI group than in control (3,954.00 ± 2,321.57 *versus* 6,622.22 ± 4,479.35 ng/mL ($p = 0.034$). These results presents Table 3.

Six months after surgery in 5 cases the concentrations of adipokines and pro-inflammatory cytokines TNFα, MCP1 and IL6 were reassessed. There was a decrease in the concentration of pro-inflammatory cytokines (MCP1, IL6) and leptin; and an increase in adiponectin concentration in all of the studied cases. However, a small group of examined patients does not allow for a statistical evaluation of the test results (Table 4).

We did not demonstrate that cytokines and adipokines exhibit statistically significant correlation with glucocorticoids (UFC, morning or midnight serum cortisol, ACTH and cortisol after 1 mg of DXM) and catecholamines secretion in NFAI group.

There was no clear correlation between BMI and cytokines (no case where both: Pearson's and Spearman's coefficients statistically important). Only MCP1 and TNFα have slight negative correlation with BMI.

Mean adrenal tumor size was 4.88 ± 2.47 cm (range: 1.50–10.00 cm). No significant correlation between tumor size and adipokine and cytokine levels was detected.

Discussion

Wide use of imaging techniques results in more frequent diagnosis of adrenal lesions, which affects 4% of middle-aged patients and increases to over 10% in the elderly [4, 5, 37, 38].

The discovery of an adrenal tumor in imaging studies requires an assessment of its hormonal activity and of the risk of primary neoplasm as well as metastatic lesions of the adrenal gland [4, 5, 34, 37, 39].

Recent studies indicate that adrenal tumors in patients without clinical features of hormonal disorders may exhibit subclinical adrenal hormone secretion [10, 19, 39, 40]. Many researchers showed that incidentally dis-

covered adrenal tumors are associated with increased risk of cardiovascular diseases even in cases without any clinical signs of hormonal oversecretion [22, 24, 41]. In particular, this concerns patients with subclinical glucocorticoid and mineralocorticoid excess due to adrenal cortex tumors, and catecholamines due to adrenal medulla tumors [20, 39, 42, 43].

Nevertheless, there are no universally accepted hormonal criteria for SCS exclusion. The Endocrine Society recommended to consider 50 nmol/L serum cortisol after the 1 mg DXM suppression test as a threshold for CS diagnosis [34, 43-45]. Cardiovascular risk in adrenal incidentaloma, irrespective of secretion have been suggested in few reports [11, 18, 22-24, 33] and recently reported also in 2017 [41].

Di Dalmazi and co-authors in 15 years long term study, investigated cardiovascular events and mortality in NFAI and SCS individual. The difference in post-DXM cortisol concentrations from baseline, represents an independent risk factor for occurrence of new cardiovascular diseases [22]. Debono showed that patients with adrenal incidentalomas and cortisol over 50 nmol/L after 1 mg DXM test, have increased mortality, mainly related to cardiovascular disease and infection [23].

Androlaukis and co-authors presented results that patients harboring adrenal incidentaloma with subtle autonomous cortisol secretion are linked with atherosclerotic risk factors. Authors adopted serum cortisol 30 nmol/L as a cut-off to exclude initial cardiovascular damage on carotid artery in the NFAI [24]. In the other date by Ceccato [41] it was shown that high UFC level could also be considered as markers to stratify cardiovascular risk in patient with adrenal incidentaloma tumors.

Research presented above have shown that chronic exposure to mild hypercortisolism as well as a rise in cortisol production over time could have a substantial role in development of cardiovascular diseases [22-24, 41].

Independently from hormonal secretion of adrenal tumors, we must bear in mind that hypertension, obesity and hyperglycemia are strong cardiovascular risk factors.

The percentage of overweight and obesity in patients with Cushing's syndrome is as high as 79–95%. Also, SCS patients are overweight and obese in 43 to 67% of cases [11, 12]. Adipose tissue is known for producing many molecules with auto-, para- and endocrine effects [26, 27, 33]. Population research showed correlations between classic inflammatory markers CRP, IL6, TNFα, many adipokines (leptin, resistin, adiponectin) and the presence of carbohydrate metabolism disorders and atherosclerosis. Data indicate that not only visceral fatty tissue is a modulator of inflammatory reactions [46-49]. It was shown that also periadrenal fatty tissue is a source of pro-inflammatory cytokines [46].

The best known adipokine is adiponectin, which has an anti-inflammatory effect [25, 47]. It is known that adiponectin has a favorable effect on insulin sensitivity and has anti-inflammatory and anti-atherosclerotic actions. Low adiponectin concentration is considered a risk factor of many complications of insulin resistance [18, 20, 50]. Dogruk Unal and co-authors showed that adiponectin level was significantly lower in SCS group of patients than those of the NFAI or control group [21]. Authors suggest that low adiponectin levels in SCS patients may be important in treatment decision due to the known relation between adiponectin and cardiovascular events [21].

In our study, we evaluated the relationship between possible even subclinical hormonal secretion in NFAI and adipokines or cytokines with pro and anti-inflammatory effects that may affect the risk of cardiovascular and metabolic diseases. On the basis of strict criteria, a group of patients without subclinical hormonal activity was isolated. Adiponectin concentration was significantly lower in NFAI patients compared to controls. The role of adiponectin in subclinical hormone secretion by adrenal tumors is ambiguous. Results of *in vivo* studies suggest suppression of adiponectin by glucocorticoids [51]. Low adiponectin concentrations were also found in patients with overt CS and in healthy volunteers treated with glucocorticoids [21, 30, 31, 43]. In a study by Ermetici adiponectin concentration of adrenal tumor patients and controls did not differ [33].

It is possible that other steroids or minimal cortisol secretion may affect the lowering of adiponectin and worst metabolic profiles in the NFAI group. In presented study, an increase in adiponectin and decrease leptin concentration in 5 examined cases was demonstrated after surgery.

We investigated the association of adiponectin concentrations in NFAI patients and glucocorticoid secretion by the tumor. In our patients there were no statistically significant relationships between adiponectin concentration and secretion of glucocorticoids as well as catecholamine metabolites. However, in studies by other researchers it was demonstrated that even mild chronic subclinical hypercortisolemia of seemingly NFAI may lead to decreased anti-inflammatory adiponectin concentrations and predispose to metabolic diseases [20, 21, 33, 42].

In our study we also found that NFAI patients had significantly higher pro-inflammatory cytokine concentrations compared to healthy controls: TNFα, IL6 and MCP1.

TNFα and IL6 inhibit differentiation of preadipocytes and impair adipogenesis; TNFα increases the expression of adhesive molecules in endothelial cells and synthesis of endothelin 1 and angiotensinogen. Through this mechanism TNFα contributes to HT. Apart from pro-inflammatory effects, TNFα and IL6 lower adiponectin secretion [25, 47, 52]. Further, high IL6 concentrations constitute a risk factor of type 2 DM and myocardial infarction [52].

So far, research reports are contradictory concerning a relationship between TNFα and excess glucocorticoid secretion by the adrenal glands. *In vitro* studies showed TNFα was inhibited by glucocorticoids, while *in vivo* studies gave the opposite result. Even in patients with overt hypercortisolemia due to CS TNFα concentrations were normal [32]. Similarly to IL6, TNFα was elevated in adrenal cortex insufficiency patients [53]. In the our study we found significantly higher TNFα concentrations compared to healthy volunteers. However, we did not find a relationship between TNFα and cortisol and/or catecholamine metabolites secretion by the adrenal tumors.

In the presented study we demonstrated that in NFAI patients IL6 concentrations are significantly higher, which did not correlate with excess cortisol or catecholamine secretion. IL6 is involved in adrenal steroidogenesis [54, 55]; what is more, high IL6 mRNA expression has been demonstrated in adrenal adenomas of patients with CS [54, 55]. It seems that IL6 as well as TNFα might be produced by the adrenal glands [54].

MCP1 also favors the development of atherosclerosis and chronic circulatory failure [56]. So far, MCP1 concentrations in adrenal tumors have not been widely studied. However, *in vitro* studies it was shown that glucocorticoids inhibit MCP1 secretion [57]. In patients studied by us MCP1 concentrations were higher compared to controls. We did not find an association between MCP1 concentrations and cortisol, catecholamine metabolites secretion by the studied tumors.

There is a mutual regulation of cytokines. It seems that MCP1 increases IL6 and TNFα secretion. Perhaps elevated MCP1 concentrations can be associated with increased concentrations of other pro-inflammatory cytokines in NFAI patients.

In several cases, both IL6 and MCP1 decreased after adrenalectomy. Small group of examined patients does not allow for a statistical evaluation of the test results and requires further research.

Resistin is produced by adipocytes and macrophages; it stimulates the production of TNFα, IL1, IL6, IL12 and induces endothelin 1 release [35, 47]. Some authors have shown an increase in resistin levels in patients with adrenal tumors and oversecretion of cortisol [33]. In our study, we have not shown that the resistin secretion was significantly higher in the NFAI than in the control group. We have also failed to show its association with the possible mild subclinical hormonal activity of NFAI patients. In other *in vivo* and *in vitro* studies, a stimulating effect of glucocorticoid excess on resistin secretion has been shown [30, 51]. We believe that in patients with NFAI, the relationship between glucocorticoid secretion and resistin concentration is not strongly manifested, and SCS/CS patients need further attention and research.

The hormonal role of adipose tissue has become more noticed in recent years. Evaluation of the association of obesity and metabolic diseases with seemingly hormonally inactive adrenal tumors, will allow developing new therapeutic trends and planning of optimal follow-up.

Conclusions

Due to widespread availability of increasingly accurate imaging studies, the incidence of accidentally discovered adrenal tumors is increasing. Tumors suspected of a malignancy and those exhibiting hormonal activity are eligible for surgery. Approach to NFAIs without suspicion for malignancy is not so clear. More evidence is emerging that the incidence of cardiovascular and metabolic diseases is also higher in patients with NFAI [18, 21-24].

We showed significantly higher levels of pro-inflammatory cytokines and lower of anti-inflammatory adiponectin in the NFAI group compared with healthy volunteers. It is possible that the increased metabolic and cardiovascular risk factors reported in patients with NFAI is at least partially dependent on fatty tissue activity, possibly induced by the slight increase in glucocorticoid secretion (which is not high enough to recognize SCS). It is possible that minimal cortisol or other steroids secretion is the cause of the disorders presented by the authors.

In our study, adipokine and cytokine levels did not correlate with BMI. This may suggest excessive secretion also within the adrenal tumors or adipose tissue sur-

rounding them.

Further prospective studies will clarify the role of adipokines and cytokines in metabolic disorders in patients with adrenal tumors and their association with subclinical hormonal activity. If a clear explanation is reached, surgical treatment may be suggested for NFAI patients with cardiovascular disease risk in the future.

Authors' Contributions

AB contributed to planning and conducting the study, collecting and interpreting data, and drafting the manuscript. MK performed the statistical analysis. KS approved the final draft submitted.

Compliance with Ethical Standards

Funding

This research was supported with the funds of the Medical University of Gdansk grant number: ST-81.

Conflict of interest

The authors declare that they have no conflicting interests.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The study was confirmed by the Independent Ethics Committee of Medical University of Gdansk (NKBBN/360-98/2016).

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

1. Griffing GT (1994) AIDS: the new endocrine epidemic. *J Clin Endocrinol Metab* 79: 1530–1531.
2. Bittner JG, Brunt LM (2012) Evaluation and management of adrenal incidentaloma. *J Surg Oncol* 106: 557–564.
3. Otto M (2010) Surgical treatment of adrenal tumors. *Endokrynol Pol* 61: 716–722.
4. Bednarczuk T, Bolanowski M, Sworczak K, Gornicka B, Cieszanowski A, *et al.* (2016) Adrenal incidentaloma in adults—management recommendations by the Polish Society of Endocrinology. *Endokrynol Pol* 67: 234–258.
5. Mantero F, Terzolo M, Arnaldi G, Osella G, Masini AM, *et al.* (2000) A survey on adrenal incidentaloma in Italy. Study Group on Adrenal Tumors of the Italian Society of Endocrinology. *J Clin Endocrinol Metab* 85: 637–644.
6. Grumbach MM, Biller BM, Braustein GD, Cambell KK, Carney JA, *et al.* (2003) Management of the clinically inapparent adrenal mass ("incidentaloma"). *Ann Intern Med* 138: 424–429.
7. Bravo EL, Tagle R (2003) Pheochromocytoma: state-of-the-art and future prospects. *Endocr Rev* 24: 539–553.
8. Barzon L, Fallo F, Sonino N, Boscaro M (2002) Development of overt Cushing's syndrome in patients with adrenal incidentaloma. *Eur J Endocrinol* 146: 61–66.
9. Libe R, Dall'Asta C, Barbetta L, Baccarelli A, Beck-Pecoz P, *et al.* (2002) Long-term follow—up study of patients with adrenal incidentalomas. *Eur J Endocrinol* 147: 489–494.
10. Babinska A, Siekierska-Hellmann M, Blaut K, Lewczuk A, Wisniewski P, *et al.* (2012) Hormonal activity in clinically silent adrenal incidentalomas. *Arch Med Sci* 8: 97–103.
11. Tauchmanova L, Rossi R, Biondi B, Pulcrano M, Nuzzo V, *et al.* (2002) Patients with subclinical Cushing's syndrome due to adrenal adenoma have increased cardiovascular risk. *J Clin Endocrinol Metab* 87: 4872–4878.
12. Torlontano M, Zongrillo M, D'Aloiso L, Ghiggi MR, Di Cerbo A, *et al.* (1997) Pre-Cushing's syndrome not recognized by conventional dexamethasone suppression-tests in an adrenal "incidentaloma" patient. *J Endocrinol Invest* 20: 501–504.
13. Swiatkowska-Stodulska R, Skibowska-Bielinska A, Wisniewski P, Sworczak K (2015) Activity of selected coagulation factors in overt and subclinical hypercortisolism. *Endocrine J* 62: 687–694.
14. Swiatkowska-Stodulska R, Mital A, Wisniewski P, Babinska A, Skibowska-Bielinska A, *et al.* (2015) Assessment of platelet function in endogenous hypercortisolism. *Endokrynol Pol* 66: 207–213.
15. Swiatkowska-Stodulska R, Sworczak K (2013) Disorders of hemostasis in overt and subclinical hypercortisolism. *Exp Clin Endocrinol Diabetes* 121: 588–594.
16. Terzolo M, Pia A, Ali A, Osella G, Reimondo G, *et al.* (2002) Adrenal incidentaloma: a new cause of the metabolic syndrome? *J Clin Endocrinol Metab* 87: 998–1003.

17. Rossi R, Tauchmanova L, Luciano A, Di Martino M, Battista C, *et al.* (2000) Subclinical Cushing's syndrome in patients with adrenal incidentaloma: clinical and biochemical features. *J Clin Endocrinol Metab* 85: 1440–1448.
18. Tuna MM, Imga NN, Doğan BA, Yılmaz FM, Topçuoğlu C, *et al.* (2014) Non-functioning adrenal incidentalomas are associated with higher hypertension prevalence and higher risk of atherosclerosis. *J Endocrinol Invest* 37: 765–768.
19. Morelli V, Masserini B, Salcuni AS, Eller-Vainicher C, Savoca C, *et al.* (2010) Subclinical hypercortisolism: correlation between biochemical diagnostic criteria and clinical aspects. *Clin Endocrinol (Oxf)* 73: 161–166.
20. Elenkova A, Matrozova J, Zacharieva S, Kirilov G, Kalinov K (2010) Adiponectin- a possible factor in the pathogenesis of carbohydrate metabolism disturbances in patients with pheochromocytoma. *Cytokine* 50: 306–310.
21. Dogruk Unal A, Ayturk S, Aldemir D, Bascil Tutuncu N (2016) Serum adiponectin level as a predictor of subclinical Cushing's syndrome in patients with adrenal incidentaloma. *Int J Endocrinol* 2016: 8519362.
22. Di Dalmazi G, Vicennati V, Garelli S, Casadio E, Rinaldi E, *et al.* (2014) Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15-year retrospective study. *Lancet Diabetes Endocrinol* 2: 396–405.
23. Debono M, Bradburn M, Bull M, Harrison B, Ross RJ, *et al.* (2014) Cortisol as a marker for increased mortality in patients with incidental adrenocortical adenomas. *J Clin Endocrinol Metab* 99: 4462–4470.
24. Androulakis II, Kaltsas GA, Kollias GE, Markou AC, Gouli AK, *et al.* (2014) Patients with apparently nonfunctioning adrenal incidentalomas may be at increased cardiovascular risk due to excessive cortisol secretion. *J Clin Endocrinol Metab* 99: 2754–2762.
25. Sieminska L (2007) Adipose tissue. Pathophysiology, distribution, sex differences and the role in inflammation and cancerogenesis. *Endocrinol Pol* 58: 330–342 (In Polish).
26. Gnacinska M, Malgorzewicz S, Guzek M, Lysiak-Szydlowska W, Sworczak K (2010) Adipose tissue activity in relation to overweight or obesity. *Endokrynol Pol* 61: 160–168.
27. Gnacinska M, Malgorzewicz S, Stojek M, Lysiak-Szydlowska W, Sworczak K (2009) Role of adipokines in complications related to obesity: a review. *Adv Med Sci* 54: 150–157.
28. Midorikawa S, Sanada H, Hashimoto S, Suzuki T, Watanabe T (2001) The improvement of insulin resistance in patients with adrenal incidentaloma by surgical resection. *Clin Endocrinol (Oxf)* 54: 797–804.
29. Pittas AG, Nandini JA, Greenberg AS (2004) Adipocytokines and insulin resistance. *J Clin Endocrinol Metab* 89: 447–452.
30. Krsek M, Silha JV, Jezkova J, Hana V, Marek J, *et al.* (2004) Adipokine levels in Cushing's syndrome; elevated resistin levels in female patients with Cushing's syndrome. *Clin Endocrinol (Oxf)* 60: 350–357.
31. Libe R, Morpurgo PS, Cappiello V, Maffini A, Bondioni S, *et al.* (2005) Ghrelin and adiponectin in patients with Cushing's disease before and after successful transsphenoidal surgery. *Clin Endocrinol (Oxf)* 62: 30–36.
32. Kristo C, Godang K, Ueland T, Lien E, Aukrust P, *et al.* (2002) Raised serum levels of interleukin-8 and interleukin-18 in relation to bone metabolism in endogenous Cushing's syndrome. *Eur J Endocrinol* 146: 389–395.
33. Ermetici F, Malavazos AE, Corbetta S, Morricone L, Dall'Asta C, *et al.* (2007) Adipokine levels and cardiovascular risk in patients with adrenal incidentaloma. *Metabolism* 56: 686–692.
34. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, *et al.* (2016) Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. *Eur J Endocrinol* 175: G1–G34.
35. Mazzuco TL, Bourdeau I, Lacroix A (2009) Adrenal incidentalomas and subclinical Cushing's syndrome: diagnosis and treatment. *Curr Opin Endocrinol Diabetes Obes* 16: 203–210.
36. Terzolo M, Pia A, Reimondo G (2012) Subclinical Cushing's syndrome: definition and management. *Clin Endocrinol (Oxf)* 76: 12–18.
37. Bada M, Castellan P, Tamburro FR, Berardinelli F, Neri F, *et al.* (2016) Work up of incidental adrenal mass: state of the art. *Urologia* 83: 179–185.
38. Young WF Jr (2007) Clinical practice. The incidentally discovered adrenal mass. *N Engl J Med* 356: 601–610.
39. Babinska A, Peksa R, Wisniewski P, Swiatkowska-Stodulska R, Sworczak K (2017) Diagnostic and prognostic role of SF1, IGF2, Ki67, p53, adiponectin, and leptin receptors in human adrenal cortical tumors. *J Surg Oncol* 116: 427–433.
40. Young WF Jr (2000) Management approaches to adrenal incidentalomas: view from Rochester, Minnesota. *Endocrinol Metab Clin Notrh Am* 29: 159–185.
41. Ceccato F, Antonelli G, Frigo AC, Rerazzo D, Plebani M, *et al.* (2017) First-line screening tests for Cushing's syndrome in patients with adrenal incidentaloma: the role of urinary free cortisol measured by LC-MS/MS. *J Endocrinol Invest* 40: 753–760.
42. Fallo F, Della Mea P, Sonino N, Bertello C, Ermani M, *et al.* (2007) Adiponectin and insulin sensitivity in primary aldosteronism. *Am J Hypertens* 20: 855–861.

43. Fallo F, Scarda A, Sonino N, Paoletta A, Boscaro M, *et al.* (2004) Effect of glucocorticoids on adiponectin: a study in healthy subjects and in Cushing's syndrome. *Eur J Endocrinol* 150: 339–344.
44. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, *et al.* (2008) The diagnosis of Cushing's syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 93: 1526–1540.
45. Boscaro M, Arnaldi G (2009) Approach to the patient with possible Cushing's syndrome. *J Clin Endocrinol Metab* 94: 3121–3131.
46. Letizia C, Petramala L, Di Gioia CR, Chiappetta C, Zinnamosca L, *et al.* (2015) Leptin and adiponectin mRNA expression from the adipose tissue surrounding the adrenal neoplasia. *J Clin Endocrinol Metab* 100: E101–E104.
47. Schäffler A, Müller-Ladner U, Schölmerich J, Büchler C (2006) Role of adipose tissue as an inflammatory organ in human diseases. *Endocr Rev* 27: 449–467.
48. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, *et al.* (2003) Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation* 108: 2460–2466.
49. Michalski B, Szymczyk E, Peczek L, Nawrot B, Kupczynska K, *et al.* (2017) The role of selected adipokines and ghrelin in the prognosis after myocardial infarction in 12-month follow-up in the presence of metabolic syndrome. *Arch Med Sci* 13: 785–794.
50. Isobe K, Fu L, Tatsuno I, Takahashi H, Nissato S, *et al.* (2009) Adiponectin and adiponectin receptors in human pheochromocytoma. *J Atheroscler Thromb* 16: 442–447.
51. Fasshauer M, Paschke R (2003) Regulation of adipocytokines and insulin resistance. *Diabetologia* 46: 1594–1603.
52. Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21: 697–738.
53. Papanicolaou DA, Tsigos C, Oldfield EH, Chrousos GP (1996) Acute glucocorticoid deficiency is associated with plasma elevations of interleukin-6: does the latter participate in the symptomatology of the steroid withdrawal syndrome and adrenal insufficiency? *J Clin Endocrinol Metab* 81: 2303–2306.
54. Judd AM, Call GB, Barney M, Mcilmoil CJ, Balls AG, *et al.* (2000) Possible function of IL-6 and TNF as intraadrenal factors in the regulation of adrenal steroid secretion. *Ann N Y Acad Sci* 917: 628–637.
55. Willenberg HS, Päth G, Vögeli TA, Scherbaum WA, Bornstein SR (2002) Role of interleukin-6 in stress response in normal and tumorous adrenal cells and during chronic inflammation. *Ann N Y Acad Sci* 966: 304–314.
56. Malavazos AE, Cereda E, Morricone L, Coman C, Corsi MM, *et al.* (2005) Monocyte chemoattractant protein 1: a possible link between visceral adipose tissue-associated inflammation and subclinical echocardiographic abnormalities in uncomplicated obesity. *Eur J Endocrinol* 153: 871–877.
57. Reddy KV, Bhattacharjee G, Schabbauer G, Hollis A, Kempf K, *et al.* (2004) Dexamethasone enhances LPS induction of tissue factor expression in human monocytic cells by increasing tissue factor mRNA stability. *J Leukoc Biol* 76: 145–151.