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Fibroblast growth factor 21 in patients with mild autonomic cortisol secretion and non-functioning adrenal incidentalomas

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Abstract

Introduction: Epidemiological studies have reported a link between adrenocortical adenomas (ACA), obesity, and cardiometabolic risk. Fibroblast growth factor 21 (FGF21) is a stress-induced protein synthesised predominantly in the liver, which regulates metabolism. The aim of the current study was to evaluate the concentration of FGF21 in patients with ACA and its relationship with hypothalamic-pituitary-adrenal function, obesity, markers of cardiometabolic health, and adenoma size.

Material and methods: A total of 197 patients with ACA were included in the analysis, 82 diagnosed with mild autonomous cortisol secretion (MACS) and 115 with non-functioning adrenal adenoma incidentaloma (NFAI). MACS was defined as serum cortisol concentration post 1 mg dexamethasone test (DST) ≥ 1.8 μg/dL. In each patient weight, height, and waist circumference were measured, and body mass index (BMI) was calculated. Serum concentrations of FGF21, cortisol, dehydroepiandrosterone sulphate, adrenocorticotropic hormone (ACTH), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, glucose, and insulin were measured. The cortisol-to-ACTH ratio, homeostatic model assessment for insulin resistance index (HOMA-IR), lipid accumulation product (LAP), and cardiometabolic index (CMI) were calculated. Adrenal tumour size was evaluated from imaging procedures.

Results: Serum FGF21 concentrations were significantly higher in patients with MACS than in NFAI, which was independent of BMI. There were no differences between MACS and NFAI groups regarding HOMA-IR, LAP, and CMI. We observed a positive correlation between serum FGF21 concentration and cortisol level after DST, as well as the cortisol-to-ACTH ratio. FGF21 was negatively correlated with dehydroepiandrosterone sulphate (DHEAS). There were no significant correlations between serum FGF21 concentration and BMI, waist circumference, and HOMA-IR, but serum FGF21 levels were positively correlated with TG, LAP, and CMI. Positive relationships between adenoma size and serum FGF21 concentration were found.

Conclusions: Higher levels of FGF21 in adrenal tumours with MACS when compared with NFAI represent another pathophysiological link related to chronic glucocorticoid excess.

Key words: *FGF21; adrenal incidentaloma; mild autonomic cortisol secretion; adrenal adenoma size; cardiometabolic index; lipid accumulation product; obesity*

Introduction

The most common adrenal tumours are adrenal cortical adenomas (ACA). They are most often hormonally non-functioning adrenal incidentalomas (NFAI). However, 20–50% may present mild autonomic cortisol secretion (MACS) [1–3].

MACS is defined by European Society of Endocrinology guidelines as serum cortisol after 1 mg of dexamethasone greater than 1.8 μg/dL (50 nmol/L), in the absence of the classic features of Cushing syndrome [4]. MACS may impair both glucose and lipid metabolism and may be associated with metabolic syndrome [3, 4]. However, several studies have shown that not only patients with MACS but also patients with NFAI are at increased risk of type 2 diabetes, dyslipidaemia, and hypertension, which is potentially related to glucocorticoid (GCs) excess [4–6]. Currently, it is known that cortisol secretion should not be considered as a dichotomous condition, but rather as a wide spectrum of cortisol release, from normal range, through subtle autonomous form, then mild autonomic cortisol secretion, to the rare clinical overt adrenal Cushing syndrome [2]. It can be assumed that patients with

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NFAI may have a slight excess of GCs, which is not detected by commonly available diagnostic tests, or it occurs periodically.

The population studies have shown that the incidence of ACA is higher in people with obesity or overweight compared to people of normal weight [7]. The detailed mechanisms of mutual links between obesity and adrenal tumours are unknown. So far, it has not been clearly established whether tumours develop in the course of obesity as a consequence of insulin resistance, hyperinsulinaemia, and insulin-like growth factor (IGF) overexpression, or if obesity is secondary to mild but chronic GCs excess released by the ACA [8]. Insulin and IGF-1 have mitogenic activity, which stimulates the proliferation of adrenal cortex cells and contributes to tumour growth [9]. IGF-1 receptor (IGF-1R) and insulin receptor (IR) are located in the adrenal cortex [8,10]. Moreover, the degree of insulin resistance has been shown to be positively correlated with the diameter of ACA, and adrenalectomy improved insulin sensitivity in patients with NFAI [11, 12].

Adrenocortical growth is also controlled by growth factors such as fibroblast growth factors (FGFs). FGF receptor (FGFR) signalling is critical to maintain cortical cell growth and proliferation. The mitogenic effects of FGF on adrenal cortical cells were first observed by Gospodarowicz et al. and Hornsby and Gill [13, 14]. FGFRs have been detected in both the adrenal capsule and cortex [15].

One of the members of the FGF family, preferentially expressed in the liver, is fibroblast growth factor 21 (FGF21), which mainly presents metabolic effects, but the mitogenic influence is also recognised. This protein improves insulin sensitivity, promotes fatty acid oxidation, and increases energy expenditure. However, in humans the pathophysiological role of FGF21 is much more complex and far from understood. Despite the beneficial effects of FGF21 observed in animal models, it is difficult to confirm the favourable effect of FGF21 in obese people. In response to chronic nutritional metabolic stress, FGF21 is paradoxically upregulated, and increased serum FGF21 concentrations are observed in individuals with visceral fat accumulation, metabolic syndrome, and type 2 diabetes. People with obesity and metabolic disorders have impaired FGF21 signalling and decreased FGF21 sensitivity [16–19].

The expression and secretion of FGF21 increase in response to hepatic PPARα activation, during fasting, or high-fat, high-carbohydrate, and low-protein diets [20]. Other non-nutritional factors such as physical exercises and stress conditions also play a role [21, 22].

A complex feedback has been documented between FGF21 and hypothalamic-pituitary-adrenal (HPA) activity. FGF21 can cross the blood–brain barrier and acts directly on the hypothalamus to increase the activity of the HPA axis. This phenomenon triggers the secretion of GCs from the adrenal cortex and induces enlargement of the adrenal glands [23, 24]. Furthermore, in the adrenal gland, FGF21 has been shown to increase the expression of genes necessary for the synthesis of GCs. In addition, FGF21 increases GCs secretion in response to ACTH [26]. However, interactions between FGF21 and GCs are bilateral, and it was reported that GCs through GC receptors may induce FGF21 expression in the liver [26]. In individuals with obesity, a parallel increase in the concentration of both FGF21 and GCs in the blood was observed [19].

The reports on the role of FGF21 in adrenal tumours and cortisol secretion are scarce [27, 28]. The goal of this study was to provide information about FGF21 serum concentrations in patients with non-functioning adrenal adenomas as well as in patients with adenomas presenting mild autonomous cortisol secretion, and its relationship with hypothalamic-pituitary-adrenal function, obesity, markers of cardiometabolic health, and adenoma size.

Material and methods

We analysed the medical data of patients with incidentally discovered ACA admitted in our centre. The study was approved by the Bioethics Committee at Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch.

Diagnosis of adrenal cortical adenoma was based on computed tomography (CT) or magnetic resonance imaging (MRI). Hormonal testing was performed according to current clinical guidelines.

We excluded from the study patients with overt hypercortisolism and primary hyperaldosteronism.

Only patients with 2 subtypes — NFAI and MACS — were eligible for the study. We used the DST cut-off of 1.8 μg/dL to identify the NFAI group (serum cortisol after DST less than 1.8 μg/dL). Subjects with serum cortisol concentration post DST $\geq$ 1.8 μg/dL, without classical Cushing's signs, were classified as MACS. In cases of MACS diagnosis, ACTH independence was confirmed as suppressed or low normal ACTH concentration.

Individuals with chronic inflammatory diseases and chronic kidney failure were excluded from the study.

Finally, 197 patients were included in the analysis — 82 diagnosed with MACS and 115 with NFAI.

Each patient underwent a routine physical examination. Weight, height, and waist circumference (WC) were measured, and body mass index (BMI) was calculated. All studied participants were divided, according

to the BMI, into a group without obesity (BMI < 30 kg/m^2) or a group with obesity (BMI ≥ 30 kg/m^2).

In all patients venous blood samples were taken in the morning fasting state, and serum concentrations of FGF21, cortisol, dehydroepiandrosterone sulphate (DHEAS), plasma adrenocorticotropic hormone (ACTH), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), glucose, and insulin were measured. The cortisol to ACTH ratio was calculated. Serum FGF21 assay was performed by ELISA methods using commercial assay (Fibroblast Growth Factor 21 Human ELISA, BioVendor R&D). Cortisol, DHEAS, and ACTH were measured by chemiluminescence immunoassay analyser in the IMMULITE-2000XPi platform, Siemens. The measurement of cholesterol, HDL-C, LDL-C, TG, and glucose was performed using enzymatic methods.

As metabolic health parameters we used lipid accumulation product (LAP) and cardiometabolic index (CMI), calculated as shown below:

$$LAP = [WC\ (cm) - 65] \times TG\ (mmol/L)\ for\ men,$$
$$and\ LAP = [WC\ (cm) - 58] \times TG\ (mmol/L)\ for\ women$$

$$CMI = TG\ (mmol/L) / HDL\ (mmol/L) \times WHtR\ (waist\ to\ height\ ratio)$$

We also calculated the homeostatic model assessment for insulin resistance index (HOMA-IR), using the formula as follows:

$$Fasting\ concentration\ of\ glucose\ (mmol/L) \times fasting\ concentration\ of\ insulin\ (mIU/mL) / 22.5$$

Adenoma size was assessed by the largest diameter of the tumour. In the case of lesions in both adrenal glands, the maximal diameter of the largest mass was considered as an adenoma size. All patients were divided into the following groups depending on tumour size:
— group 1 — ACA size < 20 mm;
— group 2 — ACA size ≥ 20 mm but < 40 mm;
— group 3 — ACA size ≥ 40 mm.

Data regarding the presence of comorbidities like diabetes mellitus and hypertension were collected as binary variables (present or nor present).

Statistical analysis was performed using STATISTICA 13.3, StatSoft Inc. Data were tested for normal distribution using the W Shapiro-Wilk test. All continuous data were presented as mean values with standard deviations and medians. Groups were compared by Student's test or U Mann-Whitney test. Correlations between variables were estimated by calculating the correlation coefficient R by Spearman's method. Differences in categorical variables between groups were tested using the chi-squared test.

The Kruskal-Wallis ANOVA test was used in testing whether there was a statistically significant difference in FGF21 levels between groups 1, 2, and 3 or not.

Multiple regression analysis was performed with serum FGF21 level as a dependent variable. All results were considered as statistically significant with $p < 0.05$.

Results

Among 197 patients (148 female, 49 men), 82 (41.62%) were diagnosed with MACS (67 female, 15 male) and 115 (58.38%) with NFAI (78 female, 37 male).

There were 104 (52.79%) patients without obesity and 93 (47.21%) patients with obesity.

The characteristics of NFAI and MACS, including hormonal profiles and metabolic parameters, are presented in Table 1. Most tumours were unilateral. The prevalence of bilateral disease was higher in MACS than in NFAI. The mean BMI of all patients was 29.50 kg/m^2. The groups did not differ in terms of BMI, WC, LAP, and CMI.

The mean serum FGF21 concentration was significantly higher in patients with MACS compared to the NFAI group. Patients with MACS had similar basal cortisol, a higher cortisol-to-ACTH ratio, and lower concentrations of DHEAS and ACTH compared to subjects with NFAI.

A higher prevalence of hypertension was observed in the MACS group than in the NFAI group. There was no difference between NFAI and MACS patients with regard to diabetes. Groups did not differ in lipid profile and fasting glucose, or in terms of HOMA-IR.

The mean dimension of adrenal tumour was significantly larger in the MACS than in the NFAI group. The tumour distribution according to their size in patients with ACA is depicted in Table 1 and Figure 1. NFAI patients were more likely to have tumours with a diameter of less than 20 mm compared to MACS patients (Tab. 1, Fig. 1A). In patients with MACS, the most common tumours were 2–4 cm in size (Tab. 1, Fig. 2A).

Spearman correlation was used to assess the correlation between the analysed variables in all participants. The results are given in Table 2. We observed a positive correlation between serum FGF21 concentration and cortisol level after DST. Furthermore, correlation analyses revealed that FGF21 levels positively correlated with morning cortisol and cortisol-to-ACTH ratio. Conversely, serum FGF21 levels negatively correlated with DHEAS. There were no significant correlations between serum FGF21 concentration and body composition parameters such as BMI and WC, but serum FGF21 levels positively

Table 1. *Clinical, biochemical, and hormonal characteristics of patients with non-functioning adrenal incidentaloma (NFAI) and with mild autonomous cortisol secretion (MACS)*

Variables	NFAI (n = 115) (78F/37M)	MACS (n = 82) (67F/15M)	
Age	62.57 ± 9.92; [64.50]	64.88 ± 8.24; [65.00]	NS
BMI [kg/m^2]	29.88 ± 4.42; [29.74]	28.96 ± 5.06; [28.54]	NS
WC [cm]	104.02 ± 11.29; [104.00]	102.68 ± 12.47; [101.50]	NS
Cholesterol [mmo/L]	5.17 ± 1.21; [5.12]	5.26 ± 1.31; [5.20]	NS
HDL [mmo/L]	1.39 ± 0.32; [1.36]	1.43 ± 0.38; [1.40]	NS
LDL [mmo/L]	3.39 ± 1.14; [3.40]	3.69 ± 2.96; [3.35]	NS
TG [mmo/L]	1.40 ± 0.71; [1.27]	1.49 ± 0.70; [1.37]	NS
Glucose [mmo/L]	5.88 ± 0.96; [5.67]	6.04 ± 0.97; [5.90]	NS
HOMA-IR	2.68 ± 1.78; [2.19]	2.25 ± 0.96; [2.09]	NS
LAP	63.41 ± 43.10; [52.80]	65.77 ± 35.39; [58.28]	NS
CMI	0.71 ± 0.52; [0.54]	0.75 ± 0.53; [0.59]	NS
Cortisol [μg/dL]	10.87 ± 4.13; [10.60]	13.86 ± 11.17; [12.20]	< 0.001
ACTH [pg/mL]	15.32 ± 7.68; [13.10]	10.41 ± 7.60; [9.29]	< 0.001
Post-DST cortisol [μg/dL]	1.21 ± 0.27; [1.09]	3.44 ± 1.70; [3.04]	< 0.001
DHEAS [μg/dL]	100.59 ± 76.27; [86.40]	65.03 ± 79.12; [32.00]	< 0.01
Cortisol-to-ACTH ratio	0.89 ± 0.70; [0.68]	1.77 ± 1.63; [1.32]	< 0.001
FGF21 [pg/mL]	220.67 ± 197.44; [155.73]	459.18 ± 556.43; [331.11]	< 0.001
Hypertension, n (%)	73 [63.48]	65 [79.27]	< 0.05
T2DM, n (%)	44 [38.26]	29 [35.37]	NS
Mean adenoma size [mm]	20.75 ± 9.09 [20.00]	32.12 ± 15.92 [30.00]	< 0.001
Adenoma size categories			
< 20 mm, n (%)	58 [50.43]	16 [19.51]	< 0.001
≥ 20 mm < 40 mm, n (%)	52 [45.22]	55 [67.07]	< 0.01
≥ 40 mm, n (%)	5 [4.35]	11 [13.42]	< 0.05
Bilateral disease, n (%)	18 [15.65]	23 [28.05]	< 0.05

BMI — body mass index; WC — waist circumference; HDL — high-density lipoprotein; LDL — low-density lipoprotein; TG — triglycerides; HOMA-IR — homeostatic model assessment for insulin resistance index; LAP — lipid accumulation product; CMI —cardiometabolic index; ACTH — adrenocorticotropic hormone; post-DST — post 1 mg dexamethasone test; DHEAS — dehydroepiandrosterone sulphate; FGF21 — fibroblast growth factor 21; T2DM — type 2 diabetes mellitus; NS — non significant

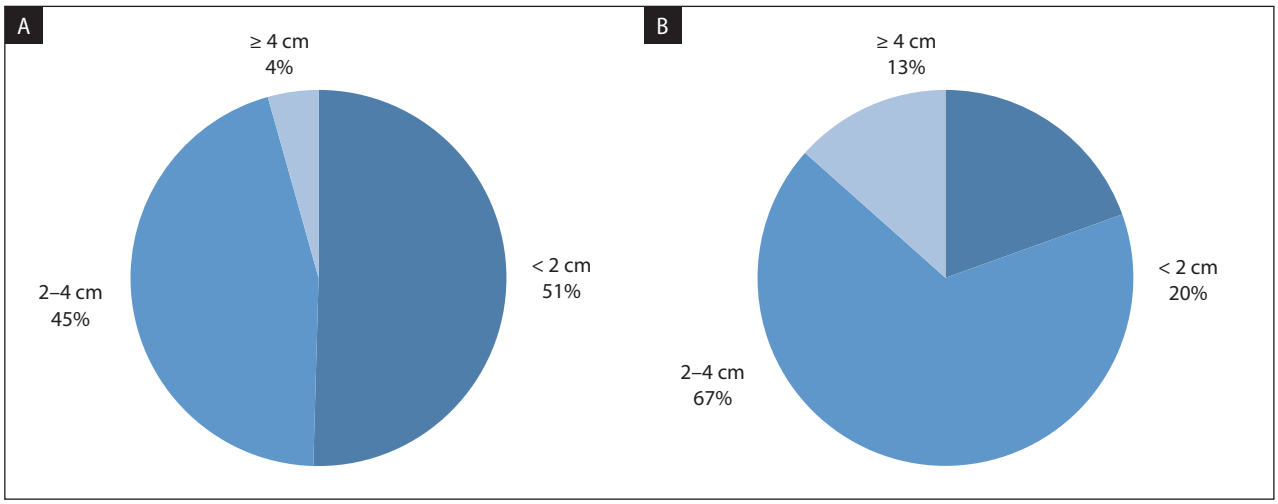

Figure 1. *Division of adrenal tumours according to tumour size.* **A.** *Size-based division of non-functioning adrenal adenoma (NFAI);* **B.** *Size-based division of mild autonomous cortisol secretion (MACS)*

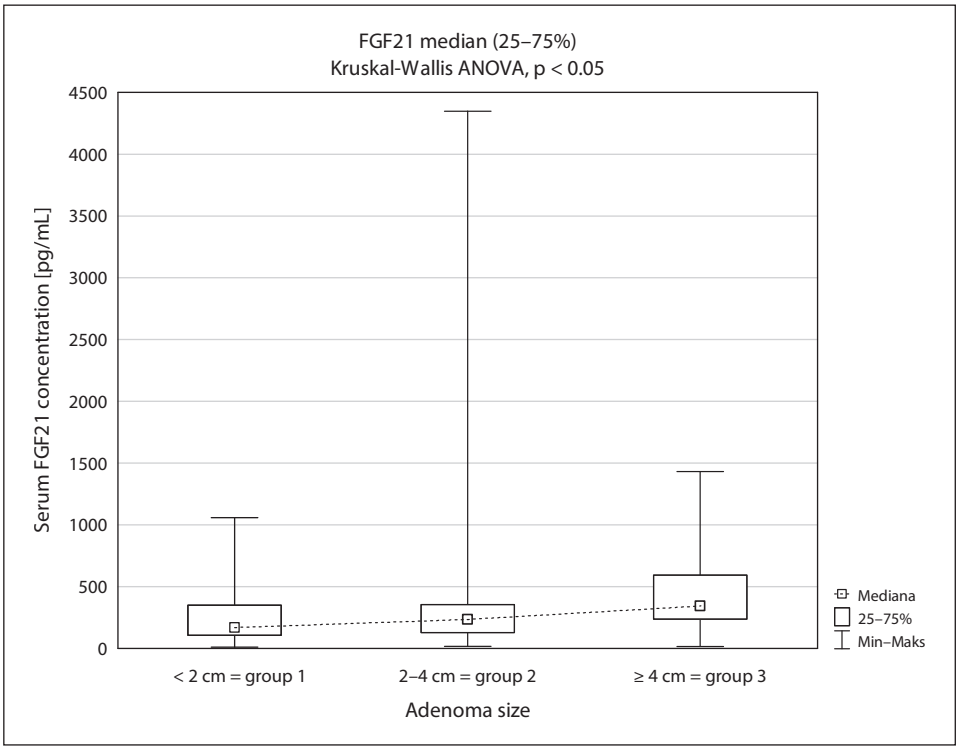

Figure 2. *Concentration of fibroblast growth factor (FGF21) in all patients with adrenocortical adenomas (ACA) divided according to tumour size*

Table 2. ***Correlations of serum of fibroblast growth factor 21 (FGF21) with anthropometric, biochemical, and hormonal variables in all patients***

Variables	R Spearman	p
FGF21 and age	0.07	NS
FGF21 and BMI	0.09	NS
FGF21 and WC	0.13	NS
FGF21 and cholesterol	0.07	NS
FGF21 and HDL	–0.14	< 0.05
FGF21 and LDL	0.05	NS
FGF21 and TG	0.31	< 0.001
FGF21 and glucose	0.23	< 0.01
FGF21 and HOMA-IR	–0.00	NS
FGF21 and LAP	0.36	< 0.001
FGF21 and CMI	0.31	< 0.001
FGF21 and cortisol	0.18	< 0.05
FGF21 and ACTH	–0.14	NS
FGF21 and cortisol after DST	0.39	< 0.001
FGF21 and DHEAS	-0.21	< 0.01
FGF21 and cortisol-to-ACTH ratio	0.26	< 0.001
FGF21 and adenoma size	0.15	< 0.05

BMI — body mass index; WC — waist circumference; HDL — high-density lipoprotein; LDL — low-density lipoprotein; TG — triglycerides; HOMA-IR — homeostatic model assessment for insulin resistance index; LAP — lipid accumulation product; CMI —cardiometabolic index; DST — 1 mg dexamethasone test; DHEAS — dehydroepiandrosterone sulphate; ACTH — adrenocorticotropic hormone; NS — non significant

correlated with TG, glucose, LAP, and CMI. There was positive correlation between FGF21 concentration and adenoma size.

Figure 2 compares FGF21 concentrations in groups 1, 2, and 3. The lowest FGF21 concentrations were detected in ACA < 2 cm and the highest in ACA ≥ 4 cm (Fig. 2).

To assess the potential effects of obesity, we performed a subgroup analysis according to the presence or absence of obesity (Tab. 3). There were no differences in the prevalence of MACS in patients with BMI < 30 kg/m^2 and BMI ≥ 30 kg/m^2 (42.31% *vs*. 40.86%). Similarly, no differences were found in the prevalence of NFAI between subjects with and without obesity.

As demonstrated in Table 3, when we compared MACS with NFAI among subjects with and without obesity separately, higher FGF21 concentrations were observed in MACS patients in both subgroups. There were no differences in FGF21 between obese NFAI and non-obese NFAI individuals, as well as between obese MACS and non-obese MACS. When all subjects were stratified by BMI, the prevalence of hypertension was 92.11% in obese MACS and 68.18% in non-obese MACS.

Variables that were found to be correlated with serum FGF21 in univariate analysis were used in the multiple regression analysis (Table 4). Only cortisol after DST remained significantly associated with FGF21.

Table 3. *Clinical, biochemical, and hormonal characteristics of obese and non-obese subjects with non-functioning adrenal adenoma (NFAI) and with mild autonomous cortisol secretion (MACS)*

	Non-obese, BMI < 30 kg/m² (n = 104)		Obese, BMI ≥ 30 kg/m² (n = 93)		Differences between			
	NFAI	MACS	NFAI	MACS	NFAI	MACS	Non-obese	Obese
	n = 60 (57.69%)	n = 44 (42.31%)	n = 55 (59.14%)	n = 38 (40.86%)	BMI < 30 *vs.* BMI ≥ 30	BMI < 30 *vs.* BMI ≥ 30	MACS *vs.* NFAI	MACS *vs.* NFAI
Age	64.23 ± 9.69 [66.00]	64.77 ± 8.22 [66.00]	60.74 ± 9.93 [62.00]	65.02 ± 8.39 [65.00]	NS	NS	NS	< 0.05
BMI [kg/m²]	26.62 ± 2.44 [27.15]	25.12 ± 2.74 [25.67]	33.32 ± 3.30 [32.34]	33.54 ± 2.88 [33.34]	< 0.001	< 0.001	< 0.01	NS
WC [cm]	97.21 ± 8.82 [97.50]	95.29 ± 9.86 [96.00]	111.58 ± 8.62 [111.00]	111.04 ± 9.53 [112.50]	< 0.001	< 0.001	NS	NS
Cholesterol [mmo/L]	5.14 ± 1.26 [5.17]	5.57 ± 1.33 [5.36]	5.21 ± 1.17 [5.09]	4.93 ± 1.23 [4.82]	NS	NS	NS	NS
HDL [mmo/L]	1.40 ± 0.32 [1.38]	1.47 ± 0.43 [1.44]	1.37 ± 0.32 [1.34]	1.38 ± 0.32 [1.35]	NS	NS	NS	NS
LDL [mmo/L]	3.39 ± 1.18 [3.43]	3.66 ± 1.35 [3.50]	3.39 ± 1.11 [3.36]	3.71 ± 4.06 [3.06]	NS	NS	NS	NS
TG [mmo/L]	1.25 ± 0.47 [1.21]	1.58 ± 0.85 [1.32]	1.56 ± 0.88 [1.32]	1.39 ± 0.50 [1.38]	NS	NS	< 0.05	NS
Glucose [mmo/L]	5.82 ± 0.90 [5.66]	5.93 ± 0.86 [5.72]	5.95 ± 1.02 [5.68]	6.17 ± 1.07 [6.14]	NS	NS	NS	NS
HOMA-IR	2.39 ± 1.76 [2.04]	2.10 ± 0.87 [1.90]	3.07 ± 1.75 [2.35]	2.48 ± 1.07 [2.31]	NS	NS	NS	NS
LAP	46.38 ± 21.54 [44.53]	60.35 ± 38.62 [53.28]	82.67 ± 52.55 [63.70]	71.99 ± 30.68 [61.95]	< 0.001	NS	< 0.05	NS

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Table 3. *Clinical, biochemical, and hormonal characteristics of obese and non-obese subjects with non-functioning adrenal adenoma (NFAI) and with mild autonomous cortisol secretion (MACS)*

	Non-obese, BMI < 30 kg/m² (n = 104)		Obese, BMI ≥ 30 kg/m² (n = 93)		Differences between			
	NFAI	MACS	NFAI	MACS	NFAI	MACS	Non-obese	Obese
	n = 60 (57.69%)	n = 44 (42.31%)	n = 55 (59.14%)	n = 38 (40.86%)	BMI < 30 *vs.* BMI ≥ 30	BMI < 30 *vs.* BMI ≥ 30	MACS *vs.* NFAI	MACS *vs.* NFAI
CMI	0.58 ± 0.35 [0.47]	0.76 ± 0.64 [0.52]	0.86 ± 0.63 [0.58]	0.74 ± 0.39 [0.65]	< 0.01	NS	NS	NS
Cortisol [μg/dL]	11.19 ± 3.90 [10.95]	14.58 ± 14.51 [11.75]	10.53 ± 4.37 [9.99]	13.02 ± 4.92 [13.50]	NS	NS	NS	< 0.05
ACTH [pg/mL]	15.96 ± 7.72 [13.25]	9.64 ± 6.25 [8.58]	14.70 ± 7.67 [12.65]	11.30 ± 8.92 [10.00]	NS	NS	< 0.01	NS
Post-DST cortisol [μg/dL]	1.24 ± 0.30 [1.10]	3.21 ± 1.77 [2.70]	1.18 ± 0.25 [1.06]	3.73 ± 1.59 [3.33]	NS	NS	< 0.001	< 0.001
DHEAS [μg/dL]	105.33 ± 84.56 [86.95]	62.38 ± 75.86 [34.50]	95.57 ± 66.86 [85.80]	68.02 ± 83.61 [29.60]	NS	NS	< 0.01	NS
Cortisol/ACTH ratio	0.83 ± 0.46 [0.73]	2.05 ± 2.07 [1.31]	0.95 ± 0.87 [0.67]	1.45 ± 0.81 [1.40]	NS	NS	< 0.001	< 0 .01
FGF21 [pg/mL]	218.74 ± 207.60 [149.99]	433.61 ± 655.29 [307.63]	222.82 ± 187.42 [189.08]	488.79 ± 420.81 [353.95]	NS	NS	< 0.05	< 0.001
Hypertension, n (%)	32 [53.33]	30 [68.18]	44 [80]	35 [92.11]	< 0.01	< 0.01	NS	NS
T2DM, n (%)	11 [18.33]	11 [25.00]	16 [29.09]	18 [47.37]	NS	NS	NS	NS
Mean adenoma size [mm]	19.94 ± 8.78 [20.00]	29.04 ± 9.93 [28.50]	21.64 ± 9.43 [21.00]	35.78 ± 20.49 [32.00]	NS	NS	< 0.001	< 0.001

BMI — body mass index; WC — waist circumference; HDL — high-density lipoprotein; LDL — low-density lipoprotein; TG — triglycerides; HOMA-IR — homeostatic model assessment for insulin resistance index; LAP — lipid accumulation product; CMI —cardiometabolic index; ACTH — adrenocorticotropic hormone; post-DST — post 1 mg dexamethasone test; DHEAS — dehydroepiandrosterone sulphate; FGF21 — fibroblast growth factor 21; T2DM — type 2 diabetes mellitus; NS — non significant

Table 4. *Multiple regression analysis for fibroblast growth factor 21 (FGF21) serum. Dependent variable: FGF21*

Independent variable	Regression coefficient	p
CMI	0.21	NS
LAP	0.03	NS
DHEAS	0.04	NS
Cortisol after DST	0.20	< 0.05
Adenoma size	–0.01	NS

CMI — cardiometabolic index; LAP — lipid accumulation product; DHEAS — dehydroepiandrosterone sulphate; DST — 1 mg dexamethasone test

Discussion

In epidemiological studies, adrenal incidentalomas have been associated with obesity and metabolic syndrome [4, 6, 7, 29–31].

In our study, among all patients with ACA, the prevalence of obesity was 47.21%. Other authors observed a similar incidence of obesity in patients with adrenocortical tumours [31–33]. In the retrospective cross-sectional study conducted at Poznan University of Medical Sciences, among 2005 patients with adrenal tumours, 37.5% of subjects had overweight, while 38.3% had obesity [31]. In another group of 300 Polish people with adrenal tumours, 40% had overweight and 39.4% had obesity [32]. In a study conducted among 305 patients with NFAI in the Spanish population, obesity was diagnosed in 39.3% of the subjects [33].

This frequency is higher than estimated by statistical data in the general population. Currently, the prevalence of obesity among adults aged 18 years or older in the global population is estimated at 16%, while in the European region it is no more than 25% [34, 35].

Relationships between adrenal tumours and obesity are bidirectional. ACA development in associations with obesity can be attributed to hyperinsulinaemia, hyperleptinaemia, insulin resistance, increased production of IGF-1, and different inflammatory cytokines. However, tumours may produce subtly higher amounts of cortisol, which are not detectable by current diagnostic methods [5]. This could explain why NFAIs are at increased risk of type 2 diabetes, dyslipidaemia, and hypertension [36–38].

The persisting excess of GCs has been shown to induce lipogenesis and adipogenesis in visceral depots. Moreover, it has been demonstrated that GC receptors are more abundant in visceral adipose tissue than in subcutaneous fat [39]. Furthermore, GCs increase the expression of lipogenesis enzymes in hepatocytes and cause brown adipose tissue dysfunction [40]. In addition, GCs can directly affect appetite and may be a cause of overeating [41].

It is worth noting that in humans, FGF21 regulates the same processes as GCs: energy homeostasis, glucose, and lipid metabolism [42]. Moreover, like GC, FGF21 modulates eating behaviour and shows similar circadian rhythms [43, 44].

In our study, we have shown that the FGF21 concentration was higher in the MACS group compared to NFAI patients.

The underlying mechanisms are unknown, but our findings are consistent with the observations of other researchers [27, 28]. A previous cross-sectional study found that patients with autonomous adrenal and pituitary cortisol secretion had significantly higher FGF21 concentrations than healthy controls [28].

It is not clear whether the increase of FGF21 in MACS is only a consequence of chronic GC excess, which promotes the accumulation of visceral fat, or whether elevated concentrations of FGF21 are related to the direct influence of GCs on FGF21. The results of the study by Ďurovcová et al., including 14 patients with Cushing syndrome and 36 control subjects, suggested that increased FGF21 concentrations in patients with overt hypercortisolism were more due to obesity and metabolic abnormalities than to a direct effect of hypercortisolaemia on FGF21 secretion [28].

Evidence for the importance of GCs in the development of central obesity comes from experimental studies in animals. Adrenalectomy prevents the development of obesity in Zucker rats, while corticosterone administration leads to fat accumulation in central depots [45]. In another experimental animal model, ovariectomised (OVX) mice developed abdominal obesity with simultaneous increases in GC concentrations. Interestingly, this effect disappeared in OVX FGF21 knockout mice [46]. This model suggests that FGF21 may be the link between GCs and obesity.

In our study, the NFAI and MACS groups were comparable for BMI and WC, but even for similar anthropometric parameters the FGF21 concentrations were still significantly higher in MACS than in NFAI. Furthermore, when we divided all subjects into 2 subgroups, 104 subjects without obesity and 93 with obesity, with the analysis performed separately in each group, the results showed that FGF21 was still significantly higher in MACS, eliminating obesity as the main factor that influences serum FGF21 level.

In vivo and *in vitro* experimental studies have shown that GCs and FGF21 regulate their production in a feedback loop. FGF21 acts as a neuroendocrine signal that activates the HPA axis and increases adrenocortical function [23–25]. Furthermore, the study by Patel et al. has shown that in the adrenal gland, FGF21 increases the expression of genes necessary for GC synthesis and favours GC secretion in response to ACTH [26].

However, it seems that in humans, FGF21 does not directly stimulate adrenocortical steroidogenesis, because negative correlations between FGF21 and DHEAS were documented in our study. This observation argues against the hypothesis that FGF21 is a pivotal inducer in the relationships between FGF21 and GCs. Therefore, it seems more likely that an increase in FGF21 is a consequence of a slight excess of GC secretion.

In our study, we found a striking positive relationship between FGF21 and cortisol after DST. Furthermore, FGF21 has been shown to correlate positively with the cortisol-to-ACTH ratio and negatively with ACTH and DHEAS. ACTH and DHEAS are usually measured when autonomous cortisol secretion is suspected, and it may be useful for estimating the presence of MACS. In turn, it is assumed that the cortisol-to-ACTH ratio reflects the state of the HPA axis, and that a higher ratio may be a useful indicator of the subclinical form of hypercortisolaemia in patients with adrenal tumours [47].

Our findings demonstrating the associations between GCs and FGF21 are in line with the results of Al-Aqil et al., who reported that treatment with dexamethasone, prednisolone, and budesonide increased liver FGF21 mRNA in experimental animals [48]. Similarly, Vispute et al. documented that dexamethasone can directly regulate the expression of FGF21 in mouse liver and human hepatoma cells, in a dose- and time-dependent manner [49].

It is well documented that the FGF21 concentration also rises in metabolic syndrome [50, 51]. It is not elucidated whether elevated serum levels of FGF21 result from resistance to its action or from compensatory increased secretion [19].

Several studies have shown that serum FGF21 levels are increased in obesity [52–54]. In our study we did not observe a positive correlation between FGF21 concentration and BMI nor WC. Likewise, in the study encompassing more than 100 pairs of twins, subjects with high FGF21 concentrations had similar measures of overall adiposity (body mass index, body fat percentage) as subjects with lower FGF21. Moreover, in the monozygotic twin group higher liver fat but not subcutaneous or intraabdominal fat content was found in subjects with high FGF21 [55]. Similarly, in the study by Crudele et al. there was no positive correlation between BMI and FGF21 levels [56]. It is suggested that in humans the relationship between circulating FGF21 and BMI remains more complicated. Such discrepancies may be due to subject characteristics or interindividual variations of serum FGF21 concentrations [57]. In our study, the mean BMI was relatively low: in the NFAI group 29.88 and in the MACS group 28.96, which may affect the results.

However, our study showed a positive correlation between FGF21 and LAP and CMI, which are metabolic indexes dependent on obesity parameters (WC, WHtR) but also on TG concentration. In the study of Tyynismaa et al. it was shown that high liver fat and TG rather than overall adiposity are associated with high FGF21 levels [55]. Similarly, Lee et al. confirmed that serum FGF21 concentrations were significantly associated with lipid profiles, and especially positively correlated with the TG level, which is in line with our findings [57]. FGF 21 decreases hepatic lipogenesis and suppress white adipose tissue lipolysis, which results in lowering TG concentration [50]. It is not clear if FGF21 increment in hypertriglyceridaemia state is compensatory excessive secretion or, for example, a consequence of impaired FGF21 reactivity in adipocytes [58]. Upon these findings, there are ongoing trails concerning the use of FGF21 analogues to diminish circulating TG and consequently to reduce liver fat fraction [50].

FGF21 has emerged as an important beneficial regulator of not only lipid homeostasis but also glucose metabolism, with its levels abnormally increased in insulin-resistant states in rodents and humans [59]. The principal glucose-lowering effect of FGF21 depends on enhancing peripheral glucose disposal in brown adipose tissue, accomplished by an increase in peripheral insulin sensitivity [60, 61]. Surprisingly, we did not observe a positive correlation of FGF21 concentration and HOMA-IR. Nevertheless, it is worth pointing out the relatively low mean HOMA-IR in our study: in the NFAI cohort 2.68 and in MACS group even lower 2.25, which may not be enough to provoke a compensatory increment of FGF21 according to the preserved (to some extent) sensitivity to this regulatory protein.

In our study, we also observed a higher incidence of hypertension in patients with MACS. Our results are in agreement with several recent studies that showed increased prevalence of hypertension in patients with MACS compared with NFAIs [62, 63]. The cause of hypertension is the increased activity of the renin angiotensin aldosterone system (RAAS), which results in sodium retention and increased plasma volume. Visceral obesity may also contribute to the development of hypertension. The role of FGF21 in the pathogenesis of hypertension in patients with MACS is not entirely clear. In animal experimental studies, FGF21 directly and dose-dependently increased angiotensin-converting enzyme 2 (ACE2) and angiotensin 1–7 (ANG-[1–7]) production in adipocytes and renal cells, and consequently protected animals against ANG II-induced hypertension [64]. However, clinical studies have shown that the concentration of FGF21 in peripheral

blood in hypertensive patients is significantly higher, and despite high circulating FGF21 levels a beneficial effect on blood pressure does not occur [50].

Apart from observed in our study correlation between FGF21 concentration and GC excess, we have also shown associations between FGF21 concentration and adenoma size. FGF/FGFRs signalling has been shown to play a role in both early adrenal development and later adrenal cortical neoplasia [65, 66]. Recent experimental animal studies have shown that FGF21 is involved in adrenal growth and hypertrophy [28]. Furthermore, increased expression of the FGF21 gene has been demonstrated in both adrenocortical adenoma and carcinoma, with significant up-regulation in advanced forms of cancer [66]. Higher circulating levels of FGF21 have been documented in different neoplasms [67–69]. These observations suggest that FGF21 promotes tumour development. To date, researchers have focused primarily on elucidating the role of FGF/FGFRs signalling in the pathogenesis of adrenocortical carcinoma [66], but the importance of this pathway in benign adrenocortical tumours remains largely unexplored. Because ACA has FGFRs, it could be speculated that FGF21 affects adrenal tumourigenesis [66].

Conclusions

Higher levels of FGF21 in adrenal tumours with mild autonomous cortisol secretion when compared with non-functioning adrenocortical adenomas represent another pathophysiological link related to chronic glucocorticoid excess.

Data availability statement

The original data are available upon request from the corresponding author.

Ethics statement

Informed consent was obtained from all subjects involved in the study.

Author contributions

Conceptualisation: S.L..; data curation: D.A., S.K..; formal analysis: S.L., S.K.; funding acquisition: S.L..; investigation: S.L., K.B.A.; project administration: S.L..; resources: S.L.; supervision: S.L., K.B.A..; visualisation: S.K.; writing — original draft: S.L., S.K., K.B.A.

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None

Conflict of interest

The authors declare no conflicts of interest.

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