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ORIGINAL ARTICLE

Serum adiponectin levels in adolescents and young adults with growth hormone deficiency

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Abstract

INTRODUCTION: Adiponectin (APN) is adipose tissue-derived hormone influencing energy metabolism. Growth hormone deficiency (GHD) may contribute to the development of disturbances in the hormonal function of adipose tissue (AT), and many disorders observed in untreated patients with GHD coincides with these contributed to low serum APN levels. **OBJECTIVES:** The assessment of serum adiponectin levels in adolescents and young adults with severe or partial GHD and analysis of relationships between serum APN and GH/IGF-1 axis function impairment as well as cardiometabolic risk factors. **DESIGN AND SETTING:** Based on the results of insulin tolerance test (ITT) patients were qualified for one of the following groups: 1) severe GHD – SGHD (26 patients; 8 women and 18 men); 2) partial GHD – PGHD (22 patients, 7 women and 15 men); 3) normal GH status – NGHS (28 patients, 9 women and 19 men). The fourth examined group consisted of healthy individuals – H (46 participants; 15 women, 31 men). Anthropometric measurements (height, weight, BMI), analysis of body composition and serum glucose, lipids, insulin, IGF-1 and APN assays were performed in all participants. **RESULTS:** There were no significant differences in the concentrations of APN between groups. After calculation of the total APN content in extracellular fluids per unit of fat tissue mass (TAPN/FM), these values were significantly lower in the SGHD ($p<0.001$) and correlated with the degree of impairment of the GH/IGF-1 axis functioning. In patients with GHD positive correlations between APN and serum HDL cholesterol (r=0.39, $p<0.05$) have been demonstrated. In the subjects with normal GH secretion serum APN correlated positively with

serum HDL cholesterol (r=0.28; $p<0.05$), and negatively with fasting blood glucose (r=–0.31; $p<0.05$). **CONCLUSIONS:** Severe, but not partial growth hormone deficiency impairs adiponectin production in the adipose tissue that is compensated by the increase of fat mass. The degree of GH/IGF-1 axis disruption is related to the TAPN/FM. This parameter may be potentially useful in diagnosing severe growth hormone deficiency in the adults.

Abbreviations:

ANOVA	- analysis of variance
APN	- adiponectin
CO-GHD	- childhood onset growth hormone deficiency
ECW	- extracellular water volume
ELISA	- enzyme-linked immunosorbent assay
FaGHRKO	- Fat GHR Knockout mice
FM	- fat mass
FM%	- percentage of fat mass
GH	- growth hormone
GHD	- growth hormone deficiency
GHR	- growth hormone receptor
GHRH	- growth hormone releasing hormone
H	- healthy controls
HDL-Chol	- high density lipoprotein cholesterol
HMW	- high molecular weight polymers
IGF-1	- insulin growth factor 1
LDL-Chol	- low density lipoprotein cholesterol
NGHS	- normal growth hormone status
PGHD	- partial growth hormone deficiency
rhGH	- recombinated human growth hormone
SAT	- subcutaneous adipose tissue
SGHD	- severe growth hormone deficiency
TAPN/FM	- total adiponectin content in the extracellular fluid per unit of fat tissue mass
T-Chol	- total cholesterol
WHR	- waist-to-hip ratio
VAT	- visceral adipose tissue

INTRODUCTION

Adiponectin (APN) is adipose tissue-derived hormone, which is thought to influence energy metabolism and might predict susceptibility to cardiovascular disease (Trujillo & Scherer 2006). It is produced predominantly by adipocytes and differentiating preadipocytes, but also found in the brown adipose tissue, cardiomyocytes, skeletal muscle, smooth muscle, brain, liver, osteoblasts, placenta and pituitary (Scherer *et al.* 1995; Fujimoto *et al.* 2005; Fu *et al.* 2016). Visceral fat compartments are largely responsible for the secretion of adiponectin, when compared to subcutaneous deposits (Bruun *et al.* 2003). Expression and secretion of this hormone is regulated by nutrition, hormones, inflammatory cytokines and post-translational modifications (Hebbard & Ranscht 2014).

Adiponectin is a multimeric protein that exists in different, biologically active isoforms. The basic 30 kD monomeric subunits which consists of an N-terminal collagenous domain and a C-terminal globular domain may form trimers (~67 kDa), hexamers (~120 kDa), and high molecular weight polymers (HMW, greater than 300 kDa, 18–36 monomer units) in the endoplasmic reticulum prior to secretion. Both full-length and globular isoforms circulate in the body, but HMW-adiponectin is the dominant form in the plasma and considered to be physiologically most relevant (Pajvani *et al.* 2005; Simpson & Whitehead 2010).

The role of adiponectin is mediated by receptors known as adiponectin receptors 1 and 2 (AdipoR1 and AipoR2), which have seven transmembrane portions. Their distribution may vary from tissue to tissue but, in general, both types of adiponectin receptors are expressed simultaneously (Yamauchi *et al.* 2007).

APN is the most abundant adipokine in the plasma (3–30 μg/ml), accounting for 0.01% of total plasma protein which is comparatively higher than that of other hormones and growth factors (Scherer *et al.* 1995). The serum adiponectin level is reduced in obesity and paradoxically increased in lean individuals (Arita *et al.* 1999; Guenther *et al.* 2014).

Many reports have revealed that this adipokine has an unique anti-diabetic, anti-atherosclerotic and anti-inflammatory properties and may be one of the key molecules involved in metabolic syndrome (Simpson & Whitehead 2010).

Adolescents and young adults with childhood onset growth hormone deficiency (CO-GHD) who discontinued treatment with recombinant growth hormone at completion of linear growth reveal reduced muscle mass and strength (Rutherford *et al.* 1991; Hulthen *et al.* 2001; Koranyi *et al.* 2001), lower bone mass (Boot *et al.* 2009), increased visceral adiposity (Colle *et al.* 1993; Johannsson *et al.* 1999), abnormal lipid profile (Capaldo *et al.* 1997; Johannsson *et al.* 1999; Mukherjee *et al.* 2004; Follin *et al.* 2006), cardiac impairment (Colao *et al.* 2002; Follin *et al.* 2006) and increased cardiovascular risk (Colao *et al.* 2002; Follin *et al.* 2006).

Reassessment of GH secretion in adults treated in childhood for GHD showed that it persists in 12.5–90% of them (Erfurth 2005). One of the reasons for such a large discrepancy is the lack of evidence-based guidelines for diagnosis and treatment of GHD in the so called transition period, extending from late puberty to full adult maturity (i.e., from mid- to late teenage years until 6–7 years after achievement of final height) (Clayton *et al.* 2005). It is suggested that these patients should be re-evaluated and further indications for reinstitute GH treatment should be established then, but there is no general agreement according to the choice of the test and the cut-off values to be used (Clayton *et al.* 2005; Ho 2007; Cook *et al.* 2009; Bonfig *et al.* 2008; Maghnie *et al.* 2005). Moreover, some population of adolescents is not severely GH deficient, but fails to attain normal GH status (peak GH on ITT >10 ng/ml) on re-evaluation. This condition is defined as partial growth hormone deficiency (Taubert *et al.* 2003; Clayton *et al.* 2005; Shalet 2010).

There is some data that GHD may contribute to the disturbances of adipokines secretion (Lanes *et al.* 2006; Yamaza *et al.* 2007; Wang *et al.* 2007; Chiba *et al.* 2008; Oliveira et al.; 2009; Lopez-Siguero *et al.* 2011; List *et al.* 2013; Recinella *et al.* 2013; Li *et al.* 2014). Additionally, many metabolic and cardiovascular abnormalities found in the untreated GHD patients correspond to symptoms of APN deficiency, but little is known regarding the impact of the GH secretory state on the regulation of its serum concentrations. The results of the animal in vitro and in vivo studies are scarce and divergent (Lam *et al.* 2004; Nilsson *et al.* 2005; Yamaza *et al.* 2007; Wőlfing *et al.* 2008; Chiba *et al.* 2008; List *et al.* 2013; Recinella *et al.* 2013). Also human research on serum adiponectin levels in GHD adults and children yielded inconsistent and contrasting findings (Fukuda *et al.* 2004; Giavoli *et al.* 2004; Lanes *et al.* 2006; Ciresi *et al.* 2007; Joaquin *et al.* 2008; Oliveira et al.; 2009; Lopez-Siguero *et al.* 2011; Li *et al.* 2014)

There is no research on serum adiponectin levels in adolescents and young adults with CO-GHD during the transition period in the available literature. Either, the relationship between the degree of GH deficiency and adiponectin is not well established. Therefore, the aim of this study is the assessment of serum adiponectin levels in adolescents and young adults with different grade of GH deficiency (severe or partial) and analysis of relationships between serum APN and the degree of GH/IGF-1 axis function impairment as well as cardiometabolic risk factors in these patients.

MATERIAL AND METHODS

Subjects

The study involved 76 participants aged 16–25 years (24 females and 52 males), who had stopped the treatment with recombinant growth hormone due to CO-GHD at least 6 months before and 46 healthy controls (15 females, 31 males) at similar age. The exclusion criteria were: Turner syndrome or other chromosomal aberrations, skeletal dysplasia, chronic diseases of the heart, liver, kidney, cystic fibrosis, coeliac disease, arterial hypertension, hyperlipidemia), type 1 or 2 diabetes, primary hypothyroidism, Addison's disease or hypergonadotrophic hypogonadism, insufficient treatment of multihormonal pituitary deficiency (pituitary hypothyroidism, adrenal insufficiency, hypogonadism or diabetes insipidus), low birth weight, pregnancy in women.

The adequacy of the treatment of multihormonal pituitary insufficiency was assessed on the basis of medical history, physical examination and laboratory tests (serum fT_4, cortisol, serum and urine osmolality, estradiol in women or testosterone in men).

There were 16 patients with multihormonal pituitary insufficiency in SGHD group and 1 – in PGHD group. All participants with current normal growth hormone secretion had idiopathic growth hormone deficiency in childhood. No subject had neoplasm or neurosurgery in the past history.

According to the results of insulin tolerance test (ITT) the subjects were included to the one of three studied groups: 1) severe growth hormone deficiency (SGHD) (26 subjects – 8 females, 18 males); 2) partial growth hormone deficiency (PGHD) (22 subjects – 7 females, 15 males); 3) normal growth hormone status (NGHS) (28 subjects – 9 females, 19 males).

The insulin tolerance test was performed according to Biller *et al.* (2002) and the peak GH cut-off levels for SGHD group was <5.0 ng/ml and >10.0 ng/ml for NGHS (Clayton *et al.* 2005; Bonfig *et al.* 2008). The patients with results between these values were included to the PGHD group.

The fourth group were healthy controls (H) (46 subjects; 15 females, 31 males) with normal height and body mass according to the normal ranges (Palczewska & Niedźwiedzka 2001) in whom ITT was not performed

The clinical characteristics of the examined groups are given in Table 1.

Methods

In all participants anthropometric measurements (height, body weight, BMI, 3 skinfolds thickness, waist and hip circumferences) were performed and blood samples were collected in the morning between 7:00 AM and 8:30 AM after after a 12-hour fast and passing urine.

The body weight was measured on medical scales and height using Harpander type anthropometer. In all participants BMI (body weight [kg]/height [m^2]) and WHR (waist-to-hip ratio) were calculated. The thickness of subscapular, triceps and biceps skinfolds was measured by calibrated calipers (GPM, Zurich, Switzerland) and summed. The body composition was analyzed using biompedance (BIA-101, Akern, Italy) in the tetrapolar system to assess fat mass (FM), percentage of fat mass (FM%) and extracellular water volume (ECW).

Serum fasting glucose, total-, HDL- and LDL-cholesterol, triglycerides and insulin were assayed using Cobas 6000 with Cobas c 501 module (Roche Diagnostics, Basel, Switzerland) and HOMA-IR values were calculated.

IRMA *(immunoradiometric assay)* method (Bio-Source Europe, Nivelles, Belgium) was used to evaluate serum growth hormone concentrations in the samples obtained during the ITT. The limit of detection for the kit was 0.12 ng/ml. The intra- and inter-assay coefficients of variation were 4.4% and 8.17%, respectively. Serum insulin growth factor-1 concentrations were evaluated using ELISA *(enzyme-linked immunosorbent assay)* kit (Bio-Line, Brussels, Belgium) with the limit of detection 1.1 ng/ml. The intra- and inter-assay coefficients of variation were 5.6% and 11.5%, respectively. Serum adiponectin levels were evaluated using commercially available ELISA kit (Millipore, Massachusetts,

Tab. 1. Clinical characteristics of the examined groups.

	mean ± SEM (range)				
	[1] SGHD (n=26; F=8; M=18)	[2] PGHD (n=22; F=7; M=15)	[3] NGHS (n=28; F=9; M=19)	[4] H (n=46; F=15; M=31)	*p*-value
age [years]	20.93±0.54 (16.7–25.1)	19.05±0.43 (17.1–24.8)	20.01±0.49 (15.6–25.0)	20.44±0.30 (16.8–24.2)	ns
body weight [kg]	62.77±4.05 (31.8–105.2)	57.76±1.98 (33.2–79.3)	57.85±1.78 (41.4–75.2)	63.58±1.60 (46.5–88.4)	ns
height [cm]	164.89±2.44* (149.3–184.2)	163.01±1.79 (141,30–175.30)	166.48±1.39 (149.40–176.70)	170.06±1.28 (157.0–184.7)	*1/4 <0.05
BMI [kg/m^2]	22.55±0.78 (13.76–33.58)	21.74±0.73 (16.63–30.84)	20.82±0.53 (16.03–27.15)	21.86±0.27 (18.11–30.11)	ns
duration of rhGH treatment [months]	56.73±9.77* (12.4–170.4)	37.13±5.72 (12.9–143.1)	27.15±3.26 (3.4–67.8)		*1/3 <0.01
time from the end of GH treatment [months]	57.38±8.89 (7.1–136.2)	46.77±4.60 (6.3–78.6)	41.87±4.35 (13.1–86.4)		ns

F – female; M – male; SGHD – severe growth hormone deficiency; PGHD – partial growth hormone deficiency; NGHS – normal growth hormone status; H – heathy controls; SE – standard error of the mean; rhGH – recombinated human growth hormone; ns – non significant

USA). The threshold of detection and the intra- and interassay coefficients of variations were 0.78 mg/ml, 7.4% and 8.4%, respectively.

On the basis of serum adiponectin and the results of body composition analysis total adiponectin content in the extracellular fluid per unit of fat tissue mass (TAPN/FM) using the following formula TAPN/FM [mg/kg] = (APN [mg/l] x ECW [l]) / FM [kg], where APN – serum adiponectin concentration; ECW – the volume of extracellular water; FM – fat mass (Majewska 2008).

This study was approved by the Bioethics Committee at the Medical University of Silesia in Katowice, Poland (no. L. dz. KNW-6501-63/I/08) and written informed consent was obtained from all examined participants and their parents or legal guardians if the participant is younger than 18 years of age before their involvement.

Statistical analysis

The database was prepared using Excel 2000 (Microsoft Corporation). Statistical analysis was carried out with Statistica 6.0 software (StatSoft Inc., Tulsa, Oklahoma, USA). Results are presented as means ± standard error of the mean (SEM).

Normal data distribution was assessed using the Shapiro-Wilk test; the homogeneity of variance was computed using Levene's test. Comparisons between the examined groups were performed using the analysis of variance (ANOVA) and *post-hoc* RIR Tukey's multiple comparison test for different sample sizes or Kruskal-Wallis test if data distribution was not normal. Correlations were analyzed by Pearson's linear correlation test or Spearman's test if data distribution was not normal.

Receiver operating curves (ROC) were constructed for TAPN/FM values to discriminate SGHD patients from participants with normal growth hormone secretion and the optimal cut-off value for maximal efficiency of the test was calculated.

All results were considered statistically significant at $p<0.05$.

RESULTS

There were no significant differences between the examined groups according age, body weight, BMI and time from the end of GH treatment. Height was statistically significantly ($p<0.05$) higher in H (170.06±1.28 cm) in comparison to SGHD (164.89±2.44 cm) and PGHD (163.01±1.79 cm) groups. The duration of the treatment was the longest in the SGHD group (Table 1).

Peak serum GH during ITT as well as IGF-1 concentrations were significantly lower in SGHD group than in NGHS. These values in PGHD subjects were significantly higher than in SGHD, but decreased when compared to NGHS (Table 1).

Mean FM and FM% as well as the sum of 3 skinfolds in SGHD patients were significantly higher than in NGHS and H groups, but did not differ between PGHD, NGHS and H subjects. Mean WHR was higher in SGHD patients in comparison to NGHS and H. Mean ECW values were similar in all examined groups (Table 2).

Mean serum total cholesterol (T-Chol), LDL and triglycerides concentrations were significantly higher ($p<0.05$; $p<0.01$; $p<0.0005$, respectively) in SGHD than in PGHD, NGHS and H subjects. On the other hand, mean serum HDL-cholesterol (HDL-Chol) in SGHD

patients was significantly (p<0.05) lower in comparison to NGHS group. There were no signifivcant differences according to mean T-Chol, HDL-Chol, LDL-Chol and triglycerides between PGHD, NGHS and H participants. HOMA-IR values and mean serum fasting glucose, insulin and adiponectin were similar in all examined groups (Table 2).

Mean TAPN/FM value (18.7±1.8 mg/kg) was significantly lower (p<0.001) in the SGHD than in PGHD, NGHS and H groups (30.1±3.5; 27.9±2.4 and 24.6±0.9 mg/kg, respectively). There were no statistically significant differences according to TAPN/FM between PGHD, NGHS and H participants (Figure 1).

Receiver operating curves enabled to establish that the optimal cut-off point for TAPN/FM is <18.0 mg/kg. This value discriminates with the 63% sensitivity and 90% specificity the group of adolescents and young adults with severe growth hormone deficiency (SGHD) from participants with normal somatotropin secretion (NGHS and H subjects taken together) (Figure 2).

Analysis of correlations showed statistically significant correlations between TAPN/FM and peak GH concentrations during ITT (r=0.54, p<0.0001) as well as serum IGF-1 levels (r=0.45, p<0.05) in GHD (SGHD and PGHD groups taken together), but not in subjects with normal somatotropin secretion.

Tab. 2. Results of analysis of body composition and biochemical and hormonal parameters in the examined groups.

	mean ± SEM (range)				
	[1] SGHD (n=26; F=8; M=18)	**[2] PGHD (n=22; F=7; M=15)**	**[3] NGHS (n=28; F=9; M=19)**	**[4] H (n=46; F=15; M=31)**	***p*-value**
FM [kg]	18.72±1.92* (6.64–41.50)	13.82±1.71 (3.19–37.4)	12.46±0.42 (4.40–19.30)	13.53±0.47 (8.10–24.20)	*1/3; 1/4 <0.05
FM% [%]	29.00±1.42* (13.00–39.50)	22.95±2.24 (9.60–35.70)	21.14±1.49 (10.80–39.80)	21.43±0.62 (12.60–31.60)	*1/2; 1/3; 1/4 <0.05
ECW [l]	15.93±1.14 (6.50–27.20)	16.15±0.49 (10.70–19.90)	15.49±0.42 (11.60–19.30)	16.63±0.43 (12.10–22.50)	ns
Sum of 3 skinfolds thickness [mm]	59.50±5.25* (31.5–99.0)	44.48±4.93 (19.5–98.5)	35.91±3.61 (19.0–86.0)	39.01±1.47 (16.4–57.2)	*1/2; 1/3; 1/4 <0.05
WHR	0.82±0.02* (0.72–0.99)	0.78±0.01 (0.70–0.92)	0.73±0.01 (0.68–0.85)	0.73±0.01 (0.65–0.83)	* 1/3; 1/4 <0.001
Peak GH in ITT [ng/ml]	1.37±0.27* (0.19–4.98)	7.79±0.34* (5.15–9.98)	15.82±0.45 (11.91–20.13)	–	*1/2; 1/3; 2/3 <0.0005
IGF-1 [ng/ml]	75.98±15.60* (12.53–287.92)	157.93±11.88 (54.13–239.34)	135.24±9.84 (69.38–225.67)	146.01±7.85 (61.72–316.54)	*1/2; 1/3; 1/4 <0.0005
T-Chol [mmol/l]	5.37±0.17* (4.45–6.92)	4.30±0.11 (3.37–5.44)	4.56±0.16 (3.41–6.14)	4.60±0.10 (3.02–6.12)	*1/2;1/3;1/4 <0.05
LDL-Chol [mmol/l]	3.27±0.18* (2.30–5.40)	2.51±0.10 (1.60–3.60)	2.58±0.14 (1.80–4.10)	2.51±0.10 (1.20–4.10)	*1/2; 1/3; 1/4 <0.01
HDL-Chol [mmol/l]	1.31±0.10* (0.60–2.23)	1.41±0.05 (0.87–1.99)	1.65±0.10 (0.97–2.41)	1.69±0.06 (0.97–2.84)	*1/3; 1/4 <0.05
TG [mmol/l]	1.43±0.16* (0.53–2.65)	0.81±0.06 (0.48–1.54)	0.83±0.05 (0.50–1.28)	0.98±0.06 (0.36–2.49)	*1/2; 1/3; 1/4 <0.0005
Fasting glucose [mg/dl]	90.56±2.91 (63.9–117.5)	90.27±1.66 (70.5–103.4)	87.36±0.73 (63.00–118.4)	87.72±1.10 (74.1–104.9)	ns
Insulin [mU/ml]	8.76±1.76 (0.47–29.39)	8.30±0.76 (1.48–19.16)	7.61±0.73 (2.69–19.16)	8.58±0.59 (2.05–22.78)	ns
HOMA-IR	2.04±0.43 (0.08–7.44)	1.84±0.16 (0.33–3.92)	1.64±0.15 (0.52–3.66)	1.86±0.13 (0.45–4.52)	ns
Adiponectin [mg/ml]	19.8±0.5 (15.8–24.5)	19.8±0.45 (17.5–24.8)	19.2±0.28 (16.8–23.6)	19.3±0.3 (15.4–22.8)	ns

F – female; M – male; SGHD – severe growth hormone deficiency; PGHD – partial growth hormone deficiency; NGHS – normal growth hormone status; H – heathy controls; SE – standard error of the mean; FM – fat mass; ECW – extracellular water volume; ITT – insulin tolerance test; T-Chol – total cholesterol; TG – triglycerides; ns – non significant

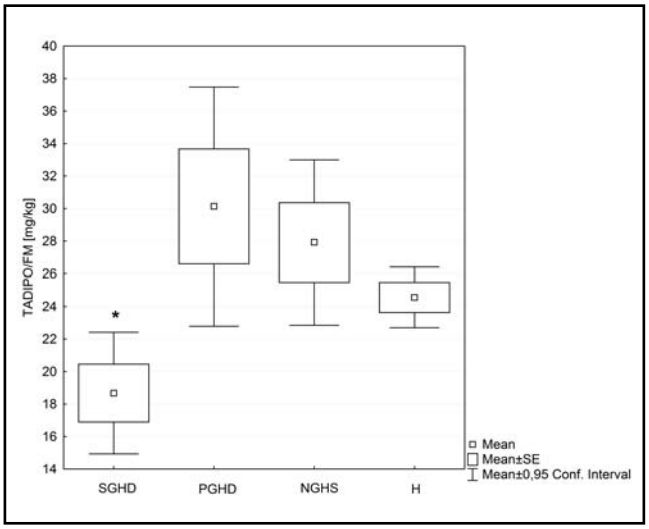

Fig. 1. Total adiponectin content in extracellular fluid per unit of fat tissue mass (TAPN/FM) in the examined groups. *p<0.01 SGHD vs. PGHD, NGHS and H; SGHD – severe growth hormone deficiency; PGHD – partial growth hormone deficiency; NGHS – normal growth hormone status; H – heathy controls.

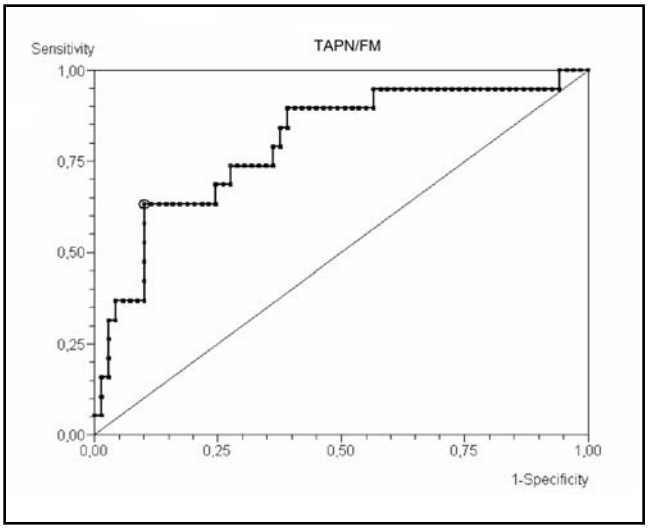

Fig. 2. ROC curve for total adiponectin content extracellular fluid per unit of fat tissue mass (TAPN/FM). cut-off point 18.0 mg/kg; sensitivity 0.63; specificity 0.90; efficiency 0.80.

Tab. 3. Analysis of correlations between serum adiponectin and results of body composition analysis and biochemical and hormonal parameters in patients with growth hormone deficiency and normal somatotropin secretion.

Parameter / Group	BMI [kg/m^2]	FM [kg]	FM% [%]	Sum of 3 skinfolds thickness [mm]	WHR	T-Chol [mmol/l]	LDL [mmol/l]	HDL [mmol/l]	TG [mmol/l]	fasting glucose [mg/dl]	INS [mU/ml]	HOMA-IR
SGHD+PGHD	r=0.03 ns	r=0.03 ns	r=0.37 p<0.05	r=0.46 p<0.01	r=–0.17 ns	r=0.23 ns	r=0.29 ns	r=0.39 p<0.05	r=0.23 ns	r=0.21 ns	r=–0.03 ns	r=–0.03 ns
NGH+H	r=–0,39 p<0.001	r=–0.47 p<0.0005	r=–0.30 p<0.01	r=–0.26 p<0.05	p=–0.34 p<0.005	r=0.10 ns	r=0.08 ns	r=0.28 p<0.05	r=–0.19 ns	r=–0.31 p<0.01	r=–0.12 ns	r=–0.05 ns

F – female; M – male; SGHD – severe growth hormone deficiency; PGHD – partial growth hormone deficiency; NGHS – normal growth hormone status; H – heathy controls; FM – fat mass; T-Chol – total cholesterol; TG – triglycerides; INS – insulin; ns – non significant

Serum adiponectin correlated negatively with BMI, FM, FM%, the sum of 3 skinfolds and WHR only in subjects with normal somatotropin secretion (NGHS and H analyzed together). In contrast, a positive significant correlation between serum APN nad FM% as well as the sum of 3 skinfolds was noted in patients with GHD. Serum adiponectin levels correlated with HDL-cholesterol in growth hormone deficient patients (SGHD and PGHD groups taken together) as well as in subjects with normal somatotropin secretion (NGHS and H analyzed together) (Table 3). In the latter group serum adiponectin also correlated negatively with fasting glucose (Table 3).

DISCUSSION

The novel approach to the interpretation of the serum adiponectin concentrations using body composition analysis enabled adjusting the obtained results for differences in fat mass and the volume of extracellular water, and hence the height and weight. Although adiponectin levels were not significantly different between the examined groups, calculation of its total content in the extracellular fluid per unit of adipose tissue mass revealed significantly lower values in SGHD subjects compared to the results obtained in the other groups. This may indicate disturbed hormonal activity of adipose tissue in patients with severe GH deficiency, which shows a positive correlation with the degree of impairment of GH/IGF-1 axis function. On the other hand, no differences in adiponectin concentrations between the groups suggest that young patients with GHD develop adaptive mechanisms to preserve normal serum ADIPO values despite a significant increase in fat mass.

In some *in vitro* and *in vivo* animal studies the effect of GH deficiency on APN expression and secretion has been demostrated, although the results of these experiments are contradictory.

Lam *et al.* (2004) in cell culture of 3T3-L1 murine adipocyte did not observed the effect of GH addition in concentration of 1mM on the adiponectin mRNA expression. In contrast, addition of insulin (1.5 mM)

or IGF-1 (1 mg ml) to the culture substrate reduced APN production. Similar results were obtained also by other authors (Houseknecht *et al.* 2000). On the other hand, Wőlfing *et al.* (2008) in the same murine cell line culture observed that the secretion of adiponectin is stimulated by GH under normo- and hyperglycaemia suggesting that also glucose and insulin have significant and interfering effects on its production. In human subcutaneous adipose tissue (SAT) derived adipocytes GH inhibits adiponectin secretion, what was established by this hormone concentration measurements in the culture medium (Nilsson *et al.* 2005).

In vivo experiments in transgenic rats with supressed GH/IGF-1 axis Chiba *et al.* (2008) demonstrated, that adiponectin mRNA expression in white adipose tissue is similar to wild type animals and was not influenced by caloric restrictions. However, the other study showed increased APN serum concentrations in dwarf rats with GHD, which normalized after rhGH administration (Yamaza 2007). Contrary to it, Nilsson *et al.* (2005) observed decreased adiponectin serum concentrations in GH receptor (GHR) deficient mice and as could be expected, transgenic animals with GH receptor overexpression showed higher APN levels than in wild-type ones (Wang *et al.* 2007). Interestingly, $GHR^{-/-}$ animals have a significantly higher percent body fat throughout their lifespan with a disproportionate amount of fat deposition in the SAT (Berryman *et al.* 2010).

The results of above-mentioned animal studies indicate that the effect of GH on adiponectin expression and secretion is species-dependent, complex and indirect, but modulated by nutritional status, glycaemia, insulin and other factors. This hypothesis is confirmed by List *et al.* (2013), who selectively disrupted GHR in adipose tissue to produce Fat GHR Knockout (FaGHRKO) mice. Like $GHR^{-/-}$, FaGHRKO animals are obese with increased total body fat and increased adipocyte size, but their adiponectin levels are similar to controls (or slightly decreased) unlike the increased levels found in $GHR^{-/-}$ mice, suggesting that GH *in vivo* does not regulate these adipokines directly in adipose tissue (List *et al.* 2013).

If we assume that TAPN/FM may reflect diminished APN expression/production in SGHD patients, our data are in line to the results obtained in an animal model of isolated GHD by Recinella *et al.* (2013). In particular, they showed that in homozygous mice carrying a targeted ablation of the GHRH (growth hormone releasing hormone) gene adiponectin mRNA expression in both subcutaneous and visceral adipose tissue was diminished, whereas serum levels of this adipokine were increased in comparison to hetereogenous littermates. This may suggest the existence of complex post-transcriptional control mechanisms for circulating adiponectin levels. Increased percentage fat mass could be also a compensatory mechanism due to the lack of the GHRH action (Recinella *et al.* 2013).

In most studies conducted in the adult patients with GHD serum adiponectin concentrations are similar to those obtained in healthy subjects (Fukuda *et al.* 2004; Giavoli *et al.* 2004; Joaquin *et al.* 2008). Also in prepubertal pediatric population there were no significant differences between children with GHD and control group (Ciresi et al.; 2007; Capalbo *et al.* 2014). These results are in line with our findings. However, there are also some studies on serum adiponectin levels that yielded contradictory results. Namely, Oliveira *et al.* (2009) reported elevated serum APN levels in 20 adult individuals with congenital isolated GHD. Also in the study by Li *et al.* (2014) serum adiponectin concentrations were increased in 40 patients with GHD in comparison to the age- and BMI matched controls. The same findings were obtained by others in prepubertal children with GHD (Lopez-Siguero *et al.* 2011; Meazza *et al.* 2013). On the other hand, Lanes *et al.* (2006) showed that in a group of adolescents with GHD deficiency, who had never received treatment with rhGH, serum APN concentrations were decreased in comparison to the control non GH-deficient children, as well as treated GHD group.

Also the effects of growth hormone administration in GHD on serum adiponectin levels are various and depend on population studied, dose and treatment duration.

Svensson *et al.* (2006) administrated the relatively high dose of rhGH (9.5 mg/kg/day) in adults with GH deficiency for a week and observed no significant differences in the serum adiponectin before and after drug administration. Similarly Giavoli *et al.* (2004) did not show any changes of serum APN during IGF-1 generation test (rhGH 0.025 mg/kg/day for 7 days), however they demonstrated their increase after a year of rhGH at a dose of 0.3 mg/day administration in GHD patients, which may result from the significant BMI and FM% reduction induced by long-term treatment (Gavioli *et al.* 2004). However, the other researchers who have applied similar treatment protocol, found no significant changes in adiponectin, despite the observed decrease in fat mass induced GH therapy (Hana *et al.* 2004).

Yuen *et al.* (2005) investigated the effect of treatment of GHD adults with different doses of rhGH on serum adiponectin levels. In one group of patients, administration of low, constant doses of GH (0.1 mg/day) improved their insulin sensitivity without affecting the body composition. In the other group, where higher – individually adjusted based on serum IGF-1 doses (standard dose of 0.48 mg/day) were used, a signficant reduction of body fat in the trunk was noted. The treatment did not influenced serum adiponectin levels in both groups, which values were similar to those in the untreated group (Yuen *et al.* 2005).

Also in GHD children there were no effect of rhGH treatment on serum APN, although their BMI and HOMA-IR increased during 12 months of continuous GH replacement therapy (Ciresi *et al.* 2007;

Lopez-Siguero *et al.* 2011). Similar results in the adults were published by Joaquin *et al.* (2008). On the other hand, Capalbo *et al.* (2014) demonstrated a significant increase in serum adiponectin levels after 2 years of rhGH therapy in GHD children, which were accompanied by significant WHR reduction.

The large variability in serum adiponectin concentrations in GHD may result from different fat tissue content, its distribution as well as insulin levels and sensitivity. In our study, we did not observed significant differences in body weight and BMI in SGHD patients. Also insulin, fasting glucose and HOMA-IR were similar in all examined individuals. Contrary to it, FM and FM% were higher in growth hormone deficient patients in comparison to the values obtained in NGHS and H groups.

Analysis of correlations between serum adiponectin concentrations and the results of anthropometric and body composition measurements (BMI, FM, FM%, WHR and the sum of 3 skinfolds thickness) confirmed their widely described in the literature negative relationship in the group of patients with growth hormone secretion (NGHS and H groups analyzed together) (Weyer *et al.* 2001; Cnop *et al.* 2003; Yannakoulia *et al.* 2003). The lack of correlations between serum APN and BMI or WHR in GHD individuals may result from their disturbed body composition. Therefore, BMI cannot serve as a good marker of obesity in this group. Interestigly, in our GHD patients some parameters of adiposity (the sum of 3 skinfolds thickness and FM%) correlated positively with serum APN.

Our findings may support the hypothesis, that young patients with GHD may reveal protective compensatory mechanisms to prevent the reduction of serum adiponectin levels. It is suggested, that visceral obesity is associated with lower APN secretion due to low-grade inflammation and excessive oxidative stress in hypertrophic and overloaded with lipids adipocytes (Aguillar-Valles *et al.* 2015; Chang *et al.* 2015; Paniagua 2016). Indeed, a potent proinflammatory cytokine – TNF-alpha inhibits APN secretion in adipose tissue leading to insulin resistance (Suganami *et al.* 2005). However, there are some reports that in inflammation, under the influence of aggressive metabolic and oxidative factors the production of adiponectin outside adipose tissue (e.g. in skeletal muscle) can be increased, creating a local mechanism preventing damage caused by free radicals and insulin resistance development (Delaigle *et al.* 2004).

It cannot be excluded, that diminished APN secretion in visceral adipose tissue (VAT) in GHD patients is compensated by its increased production in SAT. It is known that adiponectin gene expression is dependent on the size of fat cells, and is higher in young, immature adipocytes (Meier & Gressner 2004; Sharma & Staels 2007). In the case of positive energetic balance subcutaneous adipocyte hypertrophy occurs, and the number of small, insulin-sensitive fat cells increases (Kajita *et al.* 2013). Such mechanism is suggested by the positive relationships between serum APN and adiposity (FM% and sum of 3 skinfolds thickness).

This hypothesis is also supported by the results by Berryman *et al.* (2004), who demonstrated significantly positive correlations between serum APN and fat mass or insulin sensitivity.

In our study as well as in reports by Giavoli *et al.* (2004) and Ciresi *et al.* (2007) HOMA-IR and/or QUICKI values were similar as in healthy controls. On the other hand, Fukuda *et al.* (2004) observed lower insulin sensitivity in their GHD group.

Serum APN levels are decreased in type 2 diabetes and metabolic syndrome and correlate negatively with insulin resistance (Ghomari-Boukhatem *et al.* 2017; Li et al.,2017). However, there are also reports where such relationship was not confirmed (Silha *et al.* 2003).

In children with GHD, treated as well as non-treated with recombinated growth hormone Lanes *et al.* (2006) showed the negative relationship between serum adiponectin and insulin. The same results in the adults were presented by Fukuda *et al.* (2004). Contrary to it, the others did not revealed significant correlations between serum APN and HOMA-IR in the adult GHD patients (Hana *et al.* 2004; Svensson *et al.* 2006). The possible source of the observed discrepancies may be differences regarding the content of the different molecular forms of adiponectin in the assayed samples. Pajvani *et al.* (2005) suggests that, in the regulation of insulin sensitivity, the total serum adiponectin concentration is less important than the ratio of its oligomeric forms HMW to LMW. In the above mentioned reports, as well as in our study, only total serum adiponectin was assayed.

Analysis of the relationship between serum APN and determined biochemical parameters revealed its significant positive correlations with serum HDL-cholesterol concentrations both in the growth hormone deficient patients ($r=0.39$; $p<0.05$), and subjects with normal pituitary growth hormone (NGHS and H analyzed together) ($r=0.28$; $p<0.05$). In the latter, a negative correlation ($r=-0.31$; $p<0.01$) between the serum APN fasting blood glucose was noted. These findings are also confirmed by others (Cnop *et al.* 2003; Lanes *et al.* 2006).

On the basis of our results we can conclude that severe, but not partial growth hormone deficiency impairs adiponectin production in the adipose tissue that is compensated by the increase of fat mass. The degree of GH/IGF-1 axis disruption is related to the total content of adiponectin in the extracellular fluid per unit of adipose tissue mass. This parameter may be potentially useful in diagnosing severe growth hormone deficiency in the adults.

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