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Analysis of ghrelin, leptin, and interleukin-6 salivary concentration among children aged 7–10 years and its relationship with nutritional status and some anthropometric data

Analiza stężenia w ślinie greliny, leptyny oraz interleukiny 6 wśród dzieci w wieku 7–10 lat wraz z oceną zależności ich stężenia od stanu odżywienia i wybranych danych antropometrycznych badanych

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Abstract

Introduction: Obesity is a complex condition with multifactorial aetiopathogenesis. Adipose tissue is reservoir of many adipokines which play a great role in proinflammatory response in obesity.

Aim of the study: Comparative assessment of ghrelin, leptin, and interleukin-6 (IL-6) salivary concentration among children having proper and excess of body mass. Analysis of the interrelationship between the obtained concentrations of substances and selected anthropometric parameters and blood pressure values in the studied children.

Material and methods: The study group comprised 102 children aged 7–10 years. The nutritional status of children was assessed by the use of the BMI index. The control group (n = 74) comprised children with proper body mass, and the study group (n = 28) contained children having overweight/obesity. Saliva samples were taken from all children at school. Subsequently, some anthropometric parameters and blood pressure values of the children were measured. The laboratory assessment of substances was made by ELISA method. Next, statistical analysis of all obtained results was performed using professional software.

Results: Salivary ghrelin, leptin, and IL-6 concentrations were statistically significantly higher in the study group than in the control group (p = 0.001). The study revealed a positive correlation between salivary ghrelin concentration and BMI in the whole study population (p = 0.001), and between ghrelin concentration and body weight, waist circumference, hip circumference, and waist-to-hip ratio in all subjects. In the study group, the BMI value was positively correlated only with IL-6 saliva concentration (p = 0.005).

Conclusions: The study revealed significant differences between saliva ghrelin, leptin, and IL-6 concentration between the control group and the study group. The above findings can be a good predictor with which to detect co-existing metabolic alternations in obese patients.

Key words:
children, interleukin-6, leptin, saliva, ghrelin.

Streszczenie

Wprowadzenie: Otyłość jest złożonym schorzeniem o wieloczynnikowej etiopatogenezie. Tkanka tłuszczowa jest rezerwuarem wielu adipokin, które odgrywają istotną rolę w odpowiedzi prozapalnej w otyłości.

Cel pracy: Porównawcza ocena stężenia greliny, leptyny i IL-6 w ślinie dzieci z prawidłową i nadmierną masą ciała. Analiza zależności między uzyskanymi stężeniami tych substancji a wybranymi parametrami antropometrycznymi i wartościami ciśnienia tętniczego krwi badanych dzieci.

Materiał i metody: W badaniu wzięło udział 102 dzieci w wieku 7–10 lat. Stan odżywienia dzieci oceniono za pomocą wskaźnika BMI. Do grupy kontrolnej (n = 74) należały dzieci z prawidłową masą ciała, a do grupy badanej (n = 28) dzieci z nadwagą lub otyłością. Od wszystkich dzieci w szkole pobrano próbki śliny. Następnie zmierzono niektóre parametry antropometryczne i wartości

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ciśnienia tętniczego krwi dzieci. Ocenę laboratoryjną substancji przeprowadzono metodą ELISA. Następnie przeprowadzono analizę statystyczną wszystkich uzyskanych wyników przy użyciu profesjonalnego oprogramowania.
Wyniki: Stężenie greliny, leptyny i IL-6 w ślinie jest istotnie statystycznie wyższe w grupie badanej niż w grupie kontrolnej ($p = 0{,}001$). Wykazano dodatnią korelację między stężeniem greliny w ślinie a BMI w całej badanej populacji ($p = 0{,}001$) oraz między stężeniem greliny a masą ciała, obwodem talii, obwodem bioder i wskaźnikiem talia–biodro u wszystkich badanych. W badanej grupie wartość BMI była dodatnio skorelowana jedynie ze stężeniem IL-6 w ślinie ($p = 0{,}005$).
Wnioski: Badania wykazały istotne różnice pomiędzy stężeniem greliny, leptyny i IL-6 w ślinie w grupie kontrolnej i badanej. Powyższe wyniki mogą być dobrym prognostykiem do wykrywania współistniejących zaburzeń metabolicznych u pacjentów z otyłością.
Key words:
dzieci, leptyna, ślina, interleukina 6, grelina.

Introduction

Obesity is a complex condition generally considered to be the disequilibrium of energy intake and expenditure with multifactorial aetiopathogenesis. Worldwide the obesity prevalence has nearly tripled since 1975, having reached epidemic proportions these days [1]. The increased incidence of obesity among children is particularly alarming. In 2016, 50 million girls and 74 million boys aged 5–19 years were obese worldwide [2]. Body mass index (BMI) assessment is the generally accepted and widely utilized screening method to evaluate nutritional status both among children and adults. Body mass disorders among children implicate devastating psychological consequences as well as serious medical morbidities throughout the life course. Unfortunately, many parents still ignore the gravity of overweight and obesity in children. Some standard blood tests are performed to monitor health status and the earlier onset of complications associated with excess body weight, but the assessment of the inflammatory process that causes complications associated with excess body weight is less predictable. Adipose tissue is the reservoir for this type of substance. As well as its energy storage function, it is responsible for secretion regulation. The regulatory function of energy expenditure is so important that adipose tissue (mainly visceral) is now considered to be an endogenous organ [3]. In the event of excess adipose tissue, there is a predominance of proinflammatory adipokines like interleukin-6 (IL-6), tumour necrosis factor α (TNF-α), leptin, visfatin, and resistin. They are involved in the pathophysiology of chronic sustained systemic inflammation, insulin resistance, adipogenesis, and the development of atherosclerosis [4]. The elevated concentrations of proinflammatory adipokines in saliva are frequently encountered in the inflammatory state, metabolic disease, and cardiovascular risk associated with obesity. Conversely, adiponectin appears as the main anti-inflammatory adipokine [5].

Leptin as a 'satiety hormone' is one of the key hormones regulating food intake. Leptin as an afferent satiety signal plays a crucial role in regulating body mass via a negative feedback mechanism between adipose tissue and the hypothalamus. Altogether, leptin appears to generally regulate energy homeostasis, decreasing energy intake and increasing energy expenditure [6]. Its circulating concentration in the body is proportional to the amount of adipose tissue. Although leptin should decrease weight when circulating at high levels, many typical cases of obesity demonstrate that increased expression of leptin and its receptor predisposes to leptin resistance and a proinflammatory state. It also promotes the development of obesity-related complications [7, 8]. Leptin is likely to increase Th-1 cytokine secretion, mainly interferon γ (IFN-γ), and to inhibit Th-2 response [9]. It is documented that in the hunger state, which is correlated with low leptin blood concentration, there is also decreased proinflammatory cytokines synthesis [10]. Other functions of leptin are reproductive functioning, the angiogenesis process, and bone metabolism [11, 12]. In carbohydrate economy, leptin decreases insulin production and its secretion [13].

Ghrelin, the so-called 'hunger hormone', is a polypeptide hormone produced mainly by X/A neuroendocrine cells in the stomach, but also by other organs such as salivary glands. Its physiological action is to enhance appetite and, along with leptin, it is one of the most important substances responsible for regulating appetite. The physiological role of ghrelin is made by its acetylated form containing an octanyl group, which has the potential to activate GHS-R1a receptor. To the most important factors that regulate ghrelin secretion are as follows: nutritional state, glucose and insulin blood concentration, and 'lifestyle' [14]. Hunger state is the greatest ghrelin secretion trigger. On the other hand, hyperglycaemia and hyperinsulinaemia decrease the ghrelin concentration. An increase in BMI value reduces the ghrelin concentration, and a decrease of BMI is linked with an increase in ghrelin secretion [15, 16]. Besides food intake regulation, ghrelin takes part in the digestion process. It can alter the secretion of other hormones like prolactin, cortisol, and ACTH. Ghrelin is involved in carbohydrate economy, the cardiovascular system, bone formation, and cell proliferation. It has also an anti-inflammatory and anticachectic action. In addition, ghrelin modulates the secretion of other hormones (like protactin, cortisol, ACTH), and influences carbohydrate metabolism, pancreatic secretory function, and the cardiovascular system. It exhibits an anti-inflammatory effect, as well [17–19].

Interleukin 6 is a cytokine with pleiotropic activity. It affects the metabolism of many organs, such as adipose tissue, liver, skeletal muscle, pancreas, or the central nervous system [20]. In physiology, during fasting or exercise IL-6 released from skeletal muscle communicates with adipose tissue to facilitate lipid mobilization [21]. In obesity IL-6 secretion and concentration increase, acting like a main proinflammatory mediator. It has

been described in the development of obesity-associated derangements, taking part in signalling pathways of insulin sensitivity, regulation of lipoprotein lipase, triglyceride synthesis, and modulation of expression of some adipose tissue-specific genes [22].

In recent years, saliva has been studied as an alternative biological fluid suitable for simple, safe, non-invasive, and relatively cheap diagnosis. Extensive research is currently focused on using saliva as a diagnostic matrix. On the other hand, there are some limitations associated with saliva-based diagnostics, which relate primarily to the correct clinical interpretation of the result obtained. Currently, there is lack of recognised laboratory standards for the determination of substances in saliva. Concentrations of substances in saliva are not always compatible with their concentrations in blood. Furthermore, the content of substances in saliva may depend on the method of sampling and/or the degree of stimulation of saliva secretion and its pH. Proteolytic enzymes in saliva may affect the stability of markers present. It can also significantly impede the assay itself [23]. Current medical diagnostic tools use this biological fluid for testing in the area of toxicology, infectious diseases, or endocrinology [24].

The development of new, non-standard, non-invasive methods to analyse other parameters previously correlated with obesity pathophysiology is of great interest, especially in patients of young age. Leptin and ghrelin were found to have a strong correlation between blood and salivary concentrations [25].

Aim of this study

The aim of this study is to analyse salivary concentrations of the hormones: ghrelin and leptin as well as cytokine IL-6, among school children aged 7–10 years with normal body mass and those suffering from overweight or obesity. The study aims to determine the interrelationships between the obtained salivary concentrations and the nutritional status of children as well as selected anthropometric parameters (weight, body height, waist circumference, hip circumference, waist-hip ratio, birth weight), and blood pressure values of children.

Material and methods

General assumptions of the study

The study was conducted only in primary schools where the approval of the Head Teacher was obtained. Moreover, the statutory representative of a child was given an information questionnaire prior to the study, including the main assumptions and its purpose. It also contained data about the authors of the study and a consent form for the child's guardian to participate in the study. The examination was a one-time event, i.e. saliva was only taken once from the subject, also with child anthropometric measurements. During the examination, the parents (if they wanted) had the opportunity to ask questions.

The study has ethical approval of the Bioethics Committee of the Medical University of Silesia in Katowice (Approval No. KNW/0022/ KB1/94/I/18/19).

Study group

A total of 107 children took part in the study following written parental consent. Children were randomized to the study. Underweight children (n = 3) were excluded from the analyses and 2 outliers were removed. Simultaneously children having acute or chronic diseases were excluded from the study (Table I). Final calculations were based on the results obtained from 102 children aged 7–10 years. The mean age in the entire treatment group was over 8 years (M = 8.49). The number of girls (n = 50) was comparable to the number of boys (n = 52). The nutritional state was assessed by the BMI value interpretation. The BMI values were plotted on growth charts, appropriate to the children's sex and age, developed by the WHO for children aged 5–19 years [26]. According to the results, 72.5% of the children taking part in the study had proper body mass and 27.5% of the children suffered from excess of body mass (overweight or obesity).

The determining factor for the division of the subjects into the control and study groups was nutritional status expressed as the BMI. The control group (n = 74) comprised children having proper body mass, and the study group (n = 28) contained children having overweight or obesity.

Examination procedure

Between January 2019 and June 2019, approximately 3 ml of saliva was taken from children aged 7–10 years. The material was taken with Sarstedt® Salivettes. Saliva was taken in the morning and/or afternoon. Children were advised to abstain from food for a minimum of one hour prior to material collection. Moreover, children were asked to avoid physical activity from the beginning of the day of examination. Prior to saliva collection, the children underwent a physical examination by one of the authors of this study. After the saliva collection process, the children's basic anthropometric data (body weight, body height, waist circumference, hip circumference, arm circumfer-

Table I. Inclusion criteria for the study

Sex	Age	Health condition	Nutritional status
Girls and boys	7–10 years	No signs of infection on physical examination	Normal weight, overweight, and obese children
		No chronic diseases: • progressive neurodegenerative diseases • congenital metabolic disorders • autoimmune diseases • requiring cardiorespiratory support	

ence) were measured. All the procedures took place in a hygienic room at the school. Blood pressure was also measured a few minutes after anthropometric measuring in the sitting position by the use of a paediatric sphygmomanometer (model TC1079). The final part of the examination was aimed at the parents of the examined children. It included the authors' own questionnaire containing basic data on the perinatal period of the child (birth weight, date of delivery – given in weeks of gestation, assessment of the child after birth in the Apgar scale in the 1st minute) as well as the presence of co-morbidities and regular medications taken.

Once collected, the test material was transported under appropriate conditions to the Department of Molecular Biology at the Medical University of Silesia in Katowice for analysis. The material was stored in the fridge at 4°C.

Analysis of the salivary concentration of substances

Concentrations of selected substances in saliva were analysed using the immunoenzymatic method (enzyme-linked immunosorbent assay – ELISA) with commercially available test kits suitable for salivary determinations. A test from Cloud-Clone Corp was used to determine the salivary ghrelin concentration (USA, cat. no. CEA99 1Hu). The leptin concentration was assessed using the Bio-Vendor LLC-Labolatorini medicína a.s. test. (Czech Republic – Human Leptin ELSA, Clinical Range, cat. no. RD 191001100). The IL-6 concentration was assessed using the Thermo Fisher Scientific test (USA, cat. no. BMS213HS). Individual substances were labelled according to the instructions of the respective test manufacturer. A calibration curve was prepared to determine concentrations of the test samples using standards provided in the kit. Absorbance readings were carried out using the μQuant Universal Microplate Spectrophotometer from BIO-TEK INC (Bio-Tek World Headquarters, California, USA) at a wavelength of 450 nm, and for the determination of ghrelin concentration – a reference wavelength of 630 nm. The results were prepared using KCJunior software (Bio-Tek, USA). The sensitivity of the kits used is shown in Table II.

Statistical analysis

The statistical analysis of the results obtained was performed using specialized software: IBM SPSS Statistics version 25. It was used to calculate descriptive statistics using Kolmogorov-Smirnov (for groups $n \geq 100$) and Shapiro-Wilk (for groups $n < 100$) normality tests of distribution for all quantitative variables included in the project. Subsequently, comparative analyses were performed using Kruskal-Wallis and Mann Whitney U non-parametric tests. In contrast, correlation analysis was performed using the *Pearson* correlation coefficient (Pearson's *r*) (parametric coefficient) and the Spearman's rank correlation coefficient (non-parametric coefficient). In this study, a p-value ≤ 0.05 marks the level of statistical significance, while p-value results within the range 0.05–0.1 were considered to indicate the significance of the test statistic at the level of a statistical trend.

The non-parametric Mann-Whitney U test was used for analyses of salivary hormone concentrations.

Table II. Sensitivity of the kits used for the salivary determination of individual substances

	Ghrelin	Leptin	IL-6
Kit sensitivity	49.5 pg/ml	0.2 ng/ml	0.03 pg/ml

Results

Characteristics of the control and study groups

The results obtained indicate that the analysed groups differed significantly in all measured anthropometric parameters. As shown by the data in Table III, children in the study group scored significantly higher on body weight, body height, waist circumference, hip circumference, and waist-to-hip ratio compared to the values of these variables in the control group. For birth weight, there were no statistically significant differences between the 2 groups. The study also revealed that the study group had statistically significantly higher blood pressure scores in both systolic and diastolic blood pressure compared to the children in the control group (Table IV).

Ghrelin, leptin, and interleukin-6 concentrations depending on nutritional status

The salivary ghrelin concentration was statistically significantly higher in the study group than in the control group (145.08 pg/ml vs. 179.00 pg/ml; $p < 0.05$). The scale of differences between the groups can be assessed as moderate. With regard to leptin and IL-6 concentrations, the study also indicated their statistically significantly higher concentrations in the study group than in the control group (Table V).

Correlations of ghrelin, leptin, and interleukin-6 concentrations with selected anthropometric measurements

All subjects

The study revealed that among all subjects, the salivary ghrelin concentration was positively correlated with body weight and BMI values ($p = 0.001$). No such relationship was observed for leptin and IL-6 concentrations.

Positive relationships were also observed between the ghrelin concentration and body weight, waist circumference, hip circumference, and calculated waist-to-hip ratio in all subjects. With regard to leptin and IL-6 concentrations, no such relationships were found. In contrast, birth weight appeared to be negatively correlated only with the leptin concentration. The results of the analyses are presented in Table VI.

Control group vs. study group

The BMI value in the study group correlated positively with the IL-6 concentration ($p = 0.005$). Ghrelin and leptin concentrations in the study group did not depend statistically signif-

Table III. Mean scores, standard deviations, and significance of differences in anthropometric measurements in the study and control groups (* indicates statistical significance)

Parameter	Control group (n = 74)		Study group (n = 28)		Z	p	r
	M	SD	M	SD			
BMI	16.23	1.57	22.27	2.82	–7.71	0.001*	–0.99
Body mass	29.02	5.13	45.04	7.52	–7.29	0.001*	–0.94
Body height	1.33	0.08	1.42	0.07	–4.66	0.001*	–0.60
Waist circumference	63.30	6.38	81.86	7.83	–7.35	0.001*	–0.94
Hip circumference	70.22	5.84	86.00	7.14	–7.21	0.001*	–0.92
Waist-to-hip ratio	0.90	0.05	0.95	0.04	–5.00	0.001*	–0.64
Birth weight	3333.07	533.69	3495.71	543.73	–1.09	0.277	–0.14

Table IV. Mean scores, standard deviations, and significance of differences in systolic and diastolic blood pressure in both groups (* indicates statistical significance)

Parameter	Children with normal body weight (n = 74)		Overweight/obese children (n = 28)		Z	p	r
	M	SD	M	SD			
Systolic blood pressure	103.78	9.43	118.21	13.07	–4.93	0.001*	–0.61
Diastolic blood pressure	70.14	10.88	82.14	11.66	–4.19	0.001*	–0.52

Table V. Mean results, standard deviations, and significance of differences in concentration levels of ghrelin, leptin, and IL-6 concentrations in the control and study groups (* indicates statistical significance)

Parameter	Control group (n = 74)		Study group (n = 28)		Z	p	r
	M	SD	M	SD			
Ghrelin concentration	145.08	22.94	179.00	44.63	–4.35	0.001	–0.56
Leptin concentration	5.64	1.64	6.18	1.53	–2.20	0.028	–0.28
IL 6 [ng/ml]	7.02	2.00	8.09	1.99	–2.85	0.004	–0.37

icantly on the BMI value in the study group. In contrast, the control group showed a positive relationship between the BMI value and the leptin concentration.

The body weight in the study group increased statistically significantly with the IL-6 concentration, whereas in the control group, body weight was negatively correlated with the IL-6 concentration. Salivary ghrelin and leptin concentrations were not dependent on body weight in the study group.

The study showed no statistically significant relationship between the waist circumference measurement and the waist-to-hip ratio and salivary concentrations of the tested substances in the study group. The control group demonstrated a negative correlation between body height and salivary leptin and IL-6 concentrations. Hip circumference, in turn, was positively correlated with the IL-6 concentration in the study group, while the control group showed a negative correlation in this regard.

The control group demonstrated a negative correlation between birth weight and the leptin concentration. With regard to birth weight, no statistically significant correlations were found in the study group. When analysing the Pearson's *r* coefficient values, it can be assumed that increasing the number of subjects in a given group would contribute to revealing significant relationships between the waist-to-hip ratio and the ghrelin concentration (positive, moderate correlation) and between the waist circumference and the IL-6 concentration (positive, moderate correlation). All correlations are shown in Table VII (control group) and Table VIII (study group).

Correlations of salivary substance concentrations with blood pressure values

The study revealed that in the whole study group, systolic and diastolic blood pressure values were statistically significantly positively correlated with the ghrelin concentration. With regard to leptin and IL-6 concentrations, there were no significant blood pressure-dependent statistical differences (Table IX). The control group also showed no statistically significant relationships between systolic and diastolic blood pressure values and salivary concentrations of the tested hormones (Table X). In contrast, in the study group, the results obtained show that the

Table VI. Correlations of ghrelin, leptin, and IL-6 concentrations with anthropometric variables in the whole group (n = 102) (* indicates statistical significance)

Parameter	Ghrelin concentration	Leptin concentration	IL-6 [ng/ml]
BMI			
Pearson's *r*	0.46	0.21	0.23
Significance	0.001*	0.031	0.019
Body mass			
Pearson's *r*	0.38	0.11	0.19
Significance	0.001*	0.285	0.053
Body height			
Pearson's *r*	0.14	–0.12	0.03
Significance	0.173	0.241	0.780
Waist circumference			
Pearson's *r*	0.42	0.19	0.19
Significance	0.001*	0.056	0.054
Hip circumference			
Pearson's *r*	0.37	0.14	0.17
Significance	0.001*	0.157	0.096
Waist-to-hip ratio			
Pearson's *r*	0.32	0.19	0.14
Significance	0.001*	0.060	0.154
Birth weight			
Pearson's *r*	–0.03	–0.27	0.11
Significance	0.779	0.007*	0.284

Table VII. Correlations of ghrelin, leptin, and IL-6 concentrations with anthropometric measurements in the control group (n = 74) (* indicates statistical significance)

Parameter	Ghrelin concentration	Leptin concentration	IL-6 [ng/ml]
BMI			
Pearson's *r*	0.15	0.24	0.18
Significance	0.198	0.039*	0.126
Weight			
Pearson's *r*	0.06	–0.01	–0.32
Significance	0.626	0.958	0.005*
Body height			
Pearson's *r*	–0.04	–0.23	–0.28
Significance	0.763	0.049*	0.017*
Waist circumference			
Pearson's *r*	0.06	0.13	–0.17
Significance	0.583	0.252	0.145
Hip circumference			
Pearson's *r*	0.05	0.09	–0.29
Significance	0.664	0.461	0.012*
Waist-to-hip ratio			
Pearson's *r*	0.05	0.08	0.09
Significance	0.694	0.473	0.454
Birth weight			
Pearson's *r*	–0.20	–0.35	–0.02
Significance	0.081	0,003*	0.876

Table VIII. Correlations of ghrelin, leptin, and IL-6 concentrations with anthropometric measurements in the study group (n = 28) (* indicates statistical significance)

Parameter	Ghrelin concentration	Leptin concentration	IL-6 [ng/ml]
BMI			
Pearson's *r*	0.20	0.05	0.52
Significance	0.317	0.810	0.005*
Weight			
Pearson's *r*	0.04	–0.03	0.45
Significance	0.834	0.865	0.018*
Height			
Pearson's *r*	–0.19	–0.15	0.11
Significance	0.340	0.452	0.574
Waist circumference			
Pearson's *r*	0.20	0.08	0.34
Significance	0.309	0.691	0.079
Hip circumference			
Pearson's *r*	0.07	–0.06	0.38
Significance	0.725	0.768	0.044*
Waist-to-hip ratio			
Pearson's *r*	0.32	0.30	0.01
Significance	0.098	0.116	0.987
Birth weight			
Pearson's *r*	0.03	–0.14	0.11
Significance	0.872	0.468	0.584

Table IX. Correlations between selected hormones and systolic and diastolic blood pressure in the whole group (n = 102) (* indicates statistical significance)

Parameter	Systolic blood pressure	Diastolic blood pressure
Ghrelin concentration		
Pearson's *r*	0.44	0.32
Significance	0.001*	0.001*
Leptin concentration		
Pearson's *r*	0.07	–0.03
Significance	0.488	0.754
IL-6 [ng/ml]		
Pearson's *r*	0.13	0.04
Significance	0.190	0.724

Table X. Correlations between ghrelin, leptin, and IL-6 concentrations and systolic and diastolic blood pressure in the control group (n = 74) (* indicates statistical significance)

Parameter	Systolic blood pressure	Diastolic blood pressure
Ghrelin concentration		
Pearson's *r*	–0.04	–0.01
Significance	0.762	0.908
Leptin concentration		
Pearson's *r*	0.02	–0.08
Significance	0.850	0.514
IL-6 [ng/ml]		
Spearman's rank correlation coefficient	–0.19	–0.18
Significance	0.102	0.117

value of systolic pressure is statistically significantly positively correlated with the concentration of ghrelin and IL-6. Diastolic blood pressure, on the other hand, depends most significantly on ghrelin (positive correlation). All correlations obtained in the study group are given in Table XI.

Discussion

Obesity, which has been dubbed the epidemic of the 21st century, has been of interest to scientists and practitioners for many years. New diagnostic methods are still being sought to assess the risk of developing obesity in populations at risk. The so-called 'markers' of obesity are being sought, whose fluctuations in concentration are observed well before the clinical consequences of obesity are manifested. To this end, intensive research is underway into the hormonal activity of adipose tissue. It is believed that when there is an excessive amount of adipose tissue, a low-grade chronic inflammation develops there, which further intensifies the release of proinflammatory mediators secreted by adipose tissue [27]. In recent years, saliva has been

Table XI. Correlations between ghrelin, leptin, and IL-6 concentrations and systolic and diastolic blood pressure in the study group ($n = 28$) (* indicates statistical significance)

Parameter	Systolic blood pressure	Diastolic blood pressure
Ghrelin concentration		
Pearson's *r*	0.58	0.40
Significance	0.001*	0.036*
Leptin concentration		
Pearson's *r*	–0.08	–0.20
Significance	0.681	0.315
IL-6 [ng/ml]		
Pearson's *r*	0.42	0.14
Significance	0.025*	0.485

a very popular biological material for the assessment of adipocytokine concentrations. According to Chromańska *et al*., there is sufficient scientific evidence to support different saliva compositions among obese individuals compared to slim individuals [28]. The subject of this study was the analysis of salivary concentrations of 2 proinflammatory substances, i.e. cytokine IL-6 and leptin. The study also analysed salivary concentrations of ghrelin – a hormone characterized by anti-cachectic and anti-inflammatory potential. There are studies concerning this issue, but scientific data are much more limited for the paediatric population. This study is one of the few to assess selected substances in saliva in children.

Goodson *et al.* conducted a similar study to ours, but among a much wider paediatric population. The study included 744 children of one age (11 years). Salivary concentrations of 20 different substances were analysed, and their results were interpreted in relation to the nutritional status of children. According to the results obtained, the concentration of *C*-reactive protein in saliva was 6 times higher, and leptin and insulin concentrations 3 times higher in obese children, compared with a group of normal-weight children ($p < 0.0001$). Conversely, adiponectin concentrations were almost one-third lower in the group of obese children than in the group of normal weight children ($p < 0.0001$). Additionally, the study indicated an increase in all tested pro-inflammatory parameters in saliva in almost three-fourths of obese children. In the remaining 13% of obese children, the determined high insulin concentration was not associated with an increase in other inflammatory mediators. The further 11% of obese children were also characterized by a high insulin concentration with a concomitant reduction in the adiponectin concentration. Very importantly, the authors of that study emphasize that based on the obtained concentration profile of selected biomarkers, about 40% of children with normal body weight may be at risk of obesity [29]. The results of the study by Goodson *et al*. are somewhat consistent with the results of our study, which showed that a group of overweight and obese children had higher salivary concentrations of selected proinflammatory cytokines. The results of the study by Pîrsean *et al.* are also in agreement with the results of our report. Salivary concentrations of IL-6 and leptin in relation to the nutritional status of the children were analysed, as well. In addition, basic blood laboratory tests were performed in all children in the study, which covered the assessment of lipid profile, blood glucose, and liver transaminases. The results obtained indicate that children diagnosed with overweight and obesity (study group) did not differ in blood lipids and blood glucose compared to the control group, i.e. children with normal body weight. Additionally, the study did not demonstrate a correlation of the body mass index with blood laboratory test results. On the other hand, with regard to salivary IL-6 and leptin concentrations, the results showed significantly statistically higher concentrations of these parameters among children in the study group compared to the control group. It was also demonstrated that the salivary IL-6 concentration was positively correlated with children's BMI. This study indicates that several pathophysiological changes in the body are underway in overweight and obese patients, which promote the development of obesity at a time when abnormalities in basic blood laboratory tests are yet to be seen [30].

Our study also demonstrated that the IL-6 salivary concentration increases with the BMI among children with overweight and obesity. It is worth mentioning once more that in our study the children avoided physical activity (like physical activity lessons) before examination. This is very important because during physical activity skeletal muscle releases large amounts of IL-6. It contributes to fasting- or exercise-induced lipid mobilization from adipose tissue. In contrast, chronically elevated IL-6 levels are linked with pathological conditions [31]. In transgenic mice chronically elevated IL-6 serum levels were associated with glucose intolerance and hepatic insulin resistance [32]. Additionally, in humas with rheumatoid diseases, administration of an IL-6R antagonizing antibody was found to improve whole-body insulin sensitivity [33]. From the results mentioned above, IL-6 concentration varies depending on metabolic context and reflects its adaptive role for short-term energy allocation. It can act like an anti-inflammatory as well as a proinflammatory cytokine [34]. Another interesting report comes from 2016, authored by Doğan *et al*. The study analysed the concentration of 2 cytokines with opposite action, namely IL-6 and IL-10, among individuals diagnosed with gingivitis. The results showed statistically significantly higher concentrations of IL-6 among obese subjects than among those with normal body weight ($p = 0.002$). Moreover, the statistical analysis of all subjects demonstrated that the IL-6 concentration was positively correlated with the BMI of the subjects ($p = 0.019$), whereas the IL-10 concentration was negatively correlated with the value of the so-called Gingival index (GI) ($p = 0.003$), which determines oral health [35]. This study also pointed to similar correlations between the IL-6 concentration and the BMI and body weight in

the study group. This may indirectly support the theory of the so-called low-grade inflammation among the obese. The study by Ramírez-De Los Santos also indicated that elevated salivary IL-6 and IL-15 concentrations were reported in a group of obese children both with and without co-occurring caries [36]. These findings highlight a large effect of obesity on changes in salivary concentrations of proinflammatory cytokines. A Polish study published in 2010 also pointed to increased salivary concentrations of other proinflammatory substances, i.e. calprotectin, matrix metalloproteinase-2 (MMP-2), and cytokine IL-8 among obese women compared with lean individuals. Significantly positive correlations were also observed between the BMI and TNF-α, IL-8 and MMP-2 concentrations. Similarly, total fat content (expressed in kg and %) correlated positively with TNF-α, IL-8, and MMP-2 concentrations. The waist-to-hip ratio calculated in the study was also found to be positively correlated with TNF-α and MMP-2 concentrations. In addition, insulin concentrations increased with increasing TNF-α, which may somehow confirm the co-occurrence of other metabolic disorders in obesity [37]. A very interesting study was conducted by Abdalla *et al*., which assessed the potential relationship between the salivary leptin concentration and selected anthropometric parameters. That study comprised young men aged 18-25 years who were divided into control (BMI < 24.9 kg/m^2) and study (BMI > 25 kg/m^2) groups. The study results show that the control group was found to have the hip circumference, waist circumference, and waist-to-hip ratio as good predictors of the salivary leptin concentration. However, in the study group, it was found that body height was the most important independent variable that affected the leptin concentration [38]. The results obtained in this study are slightly different, i.e. in the study group there were no statistically significant differences between the leptin concentration and the measured anthropometric parameters, while in the control group it was shown that body height and BMI affected the salivary leptin concentration to the greatest extent. Another study among type 2 diabetics demonstrated salivary leptin concentrations more than 2 times higher in this group. There were also significantly higher salivary concentrations of IL-6, TNF-α, insulin, and MCP-1 (monocyte chemoattractant protein-1) in these patients. The leptin concentration was also directly proportional to the insulin concentration [39]. Furthermore Perez *et al*. demonstrated that the concentration of salivary IgA (s-IgA) is significantly higher in the group of overweight and obese children than in the group of normal weight children, reaching the highest concentration among obese children ($p < 0.05$) having body fat content expressed as percentage of total body composition ($p < 0.05$) [40].

While ghrelin is mainly known as a hunger hormone, causing increased appetite and weight gain, it is also characterized by many other functions such as reducing insulin resistance or having a proproliferative effect as well as the previously mentioned anti-inflammatory activity [11, 41]. The relatively good correlation between the ghrelin concentration in blood and saliva, proven in studies, makes saliva a convenient and easily accessible biological material in which to determine the ghrelin concentration. This is of particular importance in studies among the paediatric population. The present study showed a significantly higher salivary ghrelin concentration among overweight and obese children than among children with normal body weight. Similar results were also obtained in the study by Li *et al*. conducted among almost 200 children living in China. According to the results obtained, it was found that salivary and serum ghrelin concentrations increased as the BMI of the children increased. The study also demonstrated a positive correlation between the salivary and serum ghrelin concentration ($p < 0.01$) [42]. The study by Abdalla *et al*. covering obese men also showed a positive relationship between the salivary ghrelin concentration and the BMI, hip circumference, and waist-to-hip ratio. The study also indicated that only in the group of obese men were there significant correlations between the salivary ghrelin concentration and total body fat, the amount of subcutaneous adipose tissue, as well as muscle mass, although the latter parameter had lower significance than the parameters related to adipose tissue. These relationships were not found in other groups [43]. In the study covered by this paper, the ghrelin concentration was not dependent on the measured anthropometric variables in the study group, but in the entire study population, a positive relationship between the ghrelin concentration and the nutritional status and body weight of the subjects was shown. The above may be attributable to the insufficient size of the study group.

A positive relationship between the ghrelin concentration and parameters characterizing adipose tissue obtained in the previously cited study [43] is also an interesting relationship. Unfortunately, our study did not measure body fat itself among children. Nevertheless, measuring the waist-to-hip ratio can provide indirect information on visceral fat. While no statistically significant correlation between this parameter and the ghrelin concentration was found in the study group, a positive correlation between the ghrelin concentration and the waist-to-hip ratio as well as between waist and hip circumference was found among the entire study population. Increasing values of these parameters are related to the amount of subcutaneous and visceral adipose tissue; thus, according to the authors, this relationship is also of great importance. In the study by Sondergaard *et al*., conducted among premenopausal women, visceral adipose tissue is identified as one of the strongest variables determining the blood ghrelin concentration [44]. The stimulatory effect of ghrelin on adipogenesis is not without significance either [45]. What is more, a report by Vanderwall *et al*. documented a weak relationship between the BMI value in relation to body fat-related parameters among children [46]. Additionally, in this study, a significantly higher salivary ghrelin concentration was obtained in the study group than in the control group. In contrast, a report by Benedix *et al.* pointed to contrary results. There were no significant differences in the salivary ghrelin concentration between a group of healthy individuals, those with morbid obesity, and those at an advanced stage of neoplastic disease [47]. The lack of differences in the obtained concentrations of ghrelin between groups may be influenced by the fact that nutritional status is one – but not the only – factor determining the concentration of ghrelin in the body. There are also many central and peripheral signals such as blood glucose, insulin, and oth-

er hormone concentrations like leptin, which affect the ghrelin concentration. Another study also reported significant changes in the salivary ghrelin concentration among patients undergoing surgical treatment for obesity. Six months after the surgery, significant differences in the salivary ghrelin concentration were revealed depending on the type of bariatric surgery [48].

The analysis of the dependence of blood pressure values on salivary concentrations of adipokines included in this paper is also rather unusual in other studies. There is one report demonstrating a hypotensive effect following ghrelin administration in healthy individuals [49]. Other reports support the above-mentioned ability of ghrelin to lower blood pressure. The mechanism by which the hypotensive effect is obtained is explained by the effect of ghrelin on the autonomic nervous system, the vasodilatory capacity of ghrelin, and the effect on diuresis [50]. Admittedly, this analysis showed a positive relationship between the salivary ghrelin concentration and blood pressure values. Nevertheless, according to the authors of this paper, the relationships obtained may be largely unreliable because the children were very excited during the testing process itself. The relatively small size of the study group is also to be taken into account.

Limitations

According to the authors, the study lacks the analysis of these substances in blood along with the measurement of basic biochemical parameters determined routinely. Such measurements would give much more accurate information about the severity of already co-existing clinical metabolic implications of obesity. Moreover, in our study there is lack of comparative analysis of saliva and blood concentration of measured substances, which could further authenticate the validity of the diagnosis of these substances in saliva.

Conclusions

Leptin, ghrelin, and IL-6 are easily measured in saliva associated with low stressful circumstances can be applied to distinguish the status of proper body mass vs. obese children. It is also a promising method to evaluate the advancement of progress of selected hormonal and inflammatory disorders in childhood obesity. Nevertheless, further studies should focus their attention not only on the relationship of leptin, ghrelin, and IL-6 in saliva to anthropometric parameters but also to the metabolic status of obese children.

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