EXPERIMENTAL STUDY

Selected hormones levels in individuals with endothelial dysfunction and insulin resistance

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Abstract

Aim: **To determine possible differences in selected serum hormones levels related to endothelial function and insulin sensitivity.**
Methods: **Ghrelin, insulin, serotonin, growth hormone, IGF-1, leptin and adiponectin serum levels were determined in a group of 83 adults (40 women, 43 men) with a mean age 49.4±4.6 years. Total ghrelin, insulin and serotonin levels were measured using RIA, growth hormone and leptin using IRMA and human adiponectin was measured using ELISA. Results were associated with BMI, calculated as kg/m2, endothelial function, determined by ultrasound measured flow mediated vasodilatation of brachial artery, and with insulin resistance, calculated by IR HOMA index.**
Results: **We found no difference in age comparing subjects with (ED+) and without (ED-) endothelial dysfunction, neither comparing subject with (IR+) and without (IR-) insulin resistance. In individuals ED+ and IR+ a higher BMI, serum leptin and insulin levels and lower ghrelin, growth hormone and adiponectin levels were found. Subject with ED presented with a higher serum serotonin level compared to subjects without ED. This difference was not found in individuals with and without IR.**
Conclusion: **Lower ghrelin, growth hormone and adiponectin levels along with higher insulin and leptin levels may contribute to the progression of endothelial dysfunction and insulin resistance.** ***(Tab. 2, Ref. 24.)***
Key words: hormones serum, endothelial dysfunction, insulin resistance, ghreline, growth hormone, adiponectin, serotonine, leptin.

Atherosclerosis and its complications represent a serious cause of premature morbidity and mortality. The first event, yet reversible sign of future atherosclerosis, is endothelial dysfunction. Endothelial dysfunction manifests itself as a decrease in endothelial-derived NO production as well as a decreased response of the vessel wall to NO stimulation. Moreover, the permeability of the vessel wall is increased and the balance between vasoactive and haemocoagulative mechanisms is impaired. These changes result in predominance of vasoconstrictive and prothrombotic processes.

Endothelial dysfunction may be proved even before the presence of vessel muscle cells proliferation.

Many recent studies suggest some relation between endothelial function and levels of certain hormones. The major objective of our study was to find out the changes in the concentrations of hormones, which affect the vascular endothelium. We focused not only on one hormone, but simultaneously more hormones were studied. Individuals with confirmed endothelial dysfunction were compared with individuals without endothelial dysfunction.

Subjects and methods

The levels of ghrelin, adiponectin, insulin, leptin, growth hormone (GH), IGF-1 and serotonin levels were measured in a group of 83 adults (40 women, 43 men) with the mean age of

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Tab. 1. Endothelial function and results.

Parameters	ED undetected n=48 x±SD	ED detected n=35 x±SD	p
Age	42.5±14.2	46.8±10.4	NS
BMI kg/m^2	26.6±5.8	33.7±6.6	<0.01
Ghrelin total pg/ml	1746.5±547	1379.9±404	<0.01
RH mIU/l (0.1-7.0)	2.01±3.7	0.28±0.4	<0.05
IGF-1 ng/ml (130-354)	252.3±187.1	116.6±88.3	<0.01
Leptin ng/ml	21.2±18.9	35.9±25.1	<0.01
Insulin IU/ml (2.0-17)	6.6±4.8	11.5±1.5	<0.01
Adiponectin ng/ml	28.1±11.4	23.4±10.6	<0.05
Serotonin ng/ml (80-450)	380±228	442±228	NS

Tab. 2. Insulin resistance and results.

Parameters	IR undetected n=61 x±SD	IR detected n=22 x±SD	p
Age	44.3±15.4	46.8±8.4	NS
BMI kg/m^2	27.1±5.7	35.7±5.8	<0.01
Ghrelin total pg/ml	1662.4±527	1457.2±538	NS
RH mIU/l (0.1-7.0)	1.68±3.4	0.40±1.2	<0.01
IGF-1 ng/ml (130-354)	217.6±181.9	137.7±81.9	<0.05
Leptin ng/ml	20.1±17.3	44.9±23.1	<0.01
Insulin IU/ml (2.0-17)	5.6±2.9	15.1±7.9	<0.01
Adiponectin ng/ml	28.7±11.6	17.8±5.6	<0.01
Serotonin ng/ml (80-450)	403.7±228	369.5±176	NS

49.4±4.6 years. There was no significant difference between the age of women and men (48.9/45.2). Each subjects underwent following examinations: blood tests, blood pressure measuring using the mercury-filled sphygmomanometer, anthropometric measurements (height, weight). The data from patients medical history were recorded, focusing on diabetes, hypertension, dyslipidemia treatment and smoking.

Total ghrelin, insulin, serotonin and IGF-1 serum levels were measured by RIA method, GH and leptin levels by IRMA method and adiponectin levels by ELISA methods. The obtained results were evaluated in relation to endothelial function, which was measured by ultrasonographic method „flow mediated vasodilatation“ using 7 MHz linear array transducer on brachial artery, 5–10 cm over the cubital fossa. This method is used to estimate the dynamics of endothelial function based on changes in dilatation of arteria brachialis, which follows a change of the blood flow after previous ischemization (endothelial-dependent dilatation). We measured basal diameter (media-media) and post-ischemia diameter. The average of three consecutive measurements and formula “reactive diameter-basal diameter/basal diameter x 100 (H-B/B x 100)” were fundamental. The results were expressed in percents (10,26). Response of dysfunctional endothelium to identical stimuli is slow or none (the upper arm cuff was inflated to suprasystolic pressure (220 mmHg) for five minutes, then was the occlusion released). Endothelial dysfunction was determinates when dilatation following ischemia was under 10 %.

Serum hormone levels were associated with insulin sensitivity, they correlated with BMI and mutually. Insulin sensitivity was determinated from fasting glucose and insulin levels using index IR HOMA(Go x Io/22.5). Insulin resistance was diagnosed when a value higher than 1.88 was obtained. We used dissertation of the authors Radikova-Cervenakova UEE-SAV, 2003 (22).

The results were analysed using basic descriptive statistics. Following statistical methods were used for: unpaired *t* test, non-parametric equivalent to this method – Mann–Whitney test, analysis of variance (ANOVA) or non-parametric equivalent to this method (when differences between variances or few values occured), regression analysis and correlation analysis.

Results

Endothelial dysfunction (ED+) was found in 35 out of 83 studied individuals (42.2 %). There was no difference in the age between ED+ (46.8±10.4) and individuals without endothelial dysfunction (ED-) (42.5±14.2).

Risk factors of endothelial dysfunction were found more frequently in the ED+ group.

12 individuals in the ED+ group (34.3 %) and 6 individuals in the ED- group (12.5 %) received long-term treatment by medicaments positively affecting endothelial function (ACEI, statins).

Individuals with proven endothelial dysfunction had a higher BMI compared to BMI in the ED- group (33.7±6.6/26.6±5.8) ($p<0.01$), higher serum leptin (35.9±25.1/21.2±18.9) ($p<0.01$) and insulin level (11.5±1.5/6.6±4.8) ($p<0.01$), lower serum ghrelin (1379.9±404/1746.5±547) ($p<0.01$), GH (0.28±0.4/2.01±3.7) ($p<0.05$), IGF-1 (116.6±88.3/253.3±187.1) ($p<0.01$) and adiponectin (23.4±10.6/28.1±11.4) ($p<0.05$) levels.

There was only a slight difference in serum serotonin levels between ED+ group (442±228) and ED- group (380±228) (Tab. 1).

Insulin resistance (IR) was proved in 22 subjects (26.5 %).

There was no difference in the age between individuals with proved IR and individuals without IR (46.8±8.4/44.3±15.4).

Individuals with proved IR had a significantly higher BMI (35.7±5.8/27.1±5.7) ($p<0.01$), leptin (44.9±23.1/20.1±17.3) ($p<0.01$) and insulin levels (15.1±7.9/5.6±2.9) ($p<0.01$), lower GH (0.40±1.2/1.68±3.4) ($p<0.01$), IGF-1 (137.7±81.9/217.6±181.9) ($p<0.05$) and adiponectin levels (17.8±5.6/28.7±11.6) ($p<0.01$).

There were no significant differences in serum ghrelin and serotonin levels (Tab. 2).

A positive correlation between BMI and serum leptin ($r=0.780$, $p<0.01$) and insulin ($r=0.730$, $p<0.01$) levels was found. Serum levels of GH, ghrelin and adiponectin were decreased, while BMI was increased, but correlation coefficients were low (-0.407, -0.276, -0.293). Serum leptin level correlated significantly positively with serum insulin level ($r=0.560$, $p<0.01$). We have found an indirect relation between leptin and

adiponectin, leptin and GH, leptin and ghrelin, but correlation coefficients were again low (-0.262, -0.205, -0.024).

We could not prove a relation between GH and insulin (r=-0.168, p=0.2), GH and ghrelin (r=0.092, p=0.5), and GH and adiponectin (r=-0.145, p=0.2). We neither proved a relation between adiponectin and insulin (r=-1.180, p=0.1) in our studied group of individuals.

Discussion

Changes in serum levels of some hormones are associated with known risk factors of atherosclerosis such as obesity, dyslipidemia and insulin resistance. In this study we focused on determination of serum levels of hormones having direct effect on the vascular endothelium. These are ghrelin, adiponectin, insulin, GH, leptin and serotonin.

Ghrelin influences the level of endothelial NO synthase and thereby the level of NO and vasodilatation of the vessel wall. Ghrelin has an antiproliferative effect, too. It inhibits proinflammatory cytokines (IL-6, TNF, IL-13) produced by monocytes and thereby modulates vessel wall inflammatory reaction. Decrease of ghrelin level may contribute to endothelial dysfunction and development of atherosclerosis (24). We have proved the association between ghrelin level and endothelial function. Serum ghrelin level was significantly lower in individuals with ED compared to individuals without ED.

Adiponectin interferes with the process of atherosclerosis, too. Adiponectin stimulates NO production, inhibits adhesion of monocytes to endothelial cells, suppresses lipid accumulation in monocyte-macrophage cells and also their fagocytic activity. Furthermore, adiponectin suppresses expression of growth factors in stimulated endothelial cells and proliferation of vessel smooth muscle cells (9, 13, 28). Our study had also proved a significant increase of adiponectin level in individuals with ED.

Insulin acts as an endothelial-dependent vasodilatator in physiological levels, but hyperinsulinemia or insulin resistance lead to loss of NO bioactivity in the vessel wall and thereby to ED (6, 33, 34). Insulin is a physiological modulator of ghrelin, too. Increased insulin levels decrease level of ghrelin (18, 20).

Both ghrelin and adiponectin regulate metabolic processes. Ghrelin considerably regulates metabolism of carbohydrates, negatively correlates with BMI and insulin resistance (8, 18, 20, 21, 31). Adiponectin increases free fatty acids deposits, suppresses gluconeogenesis, decreases body weight, glycemia and increases insulin sensitivity (16). Decrease of ghrelin and adiponectin may be related not only to ED, but obesity, insulin resistance and diabetes as well, and may increase the risk of known factors of cardiovascular disease even in younger (1, 16). Higher adiponectin level decreases heart attack risk (11) and enhances insulin sensitivity mainly in liver (13, 15, 32). We have found higher insulin and lower ghrelin and adiponectin levels in our studied group of individuals with ED and insulin resistance, too.

Higher leptin levels boost risk of atherogenesis (23). Leptin directly influences endothelium by stimulating inflammation, an important factor of atheroma formation (29). There is a mutual interaction between leptin, insulin and angiotensin II. We have found and proved a positive correlation between leptin and insulin (27).

Growth hormone influences the body weight, lipid profile and has a suppressive effect on leptin (30). GH deficit is associated with obesity, atherogenic lipid profile and a higher production of leptin in adipocytes, which is a result of a reduced central leptin sensitivity. Ghrelin shows a low influence on GH (17, 19, 31). Serum inflammatory markers, IL-6, CRP are increased not only in subjects with atherosclerosis, but in subjects with GH deficit as well. That implicates the progression of vessel wall inflammation (29), an another factor playing an important role in the development of atherosclerosis.

IGF-1 also stimulates endothelial-derived NO production and its decrease may play an important role in the development of atherosclerosis in individuals with GH deficit. IGF-1 decrease leads not only to endothelial cells activation, but to hyperinsulinemia and insulin resistance as well (2). IGF-1 decrease enhances cardiovascular risk in younger (7, 14).

We have proved a negative influence of decreased GH and IGF-1 serum levels on endothelial function and on insulin sensitivity, as well.

Serotonin in the condition of normal endothelial function acts as a vasodilatator. In case of ED, serotonin permeates through the vessel wall and initiates contraction of vascular smooth muscle cells, which leads to vascular vasoconstriction. This is the way serotonin negatively influences microcirculation and reduces blood flow especially in coronary arteries. Higher serotonin levels in individuals with ED represent an another risk factor which worsens atherosclerosis and its manifestations (4, 5, 25). Although we did not find any significant difference in serum serotonin levels, individuals with ED had this level higher compared to individuals without ED.

Lower levels of ghrelin, adiponectin, GH, IGF-1 and higher leptin and insulin levels may be accompanied by obesity, insulin resistance and diabetes. Having a direct effect on the vascular endothelium and regulation of metabolic processes, they enhance the effect of known risk factors, contribute to development of ED and accelerate the process of atherosclerosis.

Our results show the necessity to continue the growth hormone therapy in adults already treated by growth hormone during childhood in whom growth hormone deficit remains. In these adults the growth hormone therapy using low doses means a prevention of premature development of atherosclerosis, predominantly coronarosclerosis.

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