By definition, essential hypertension (EH), also called primary, or idiopathic hypertension, is a persistent elevation of blood pressure which is not caused by renovascular disease, pheochromocytoma, aldosteronism, Cushing’s syndrome or other causes of secondary hypertension. However, a number of factors increasing blood pressure (BP) must be considered as implicated in pathophysiology of EH, including positive family history, obesity (especially abdominal obesity), insulin resistance, low potassium and calcium intake, high salt intake, high alcohol consumption, aging (via arteriosclerosis), stress and sedentary lifestyle (1). There are many hypotheses on complexity of pathophysiologic mechanisms of EH. Some of them refer to the abnormalities in the vasculature including endothelial dysfunction, increased oxidant stress and vascular tone, that may be affected by variety biological factors.

Ghrelin, a 28-amino acid peptide, released by cells in the stomach, small and large bowel and other tissues, has been initially identified as an appetite stimulatory peptide that regulates energy balance and is one of the major stimulators for growth hormone secretion (2). The major biological functions of ghrelin are due to its peripheral and central activity, where ghrelin may affect the hypothalamic arcuate nucleus including signalling in the tyrosine hydroxylase positive neurons (3). Two major forms of ghrelin are found in plasma: n-octanoyl-modified acyl ghrelin (A Ghhr) that combines with growth hormone secretagogue receptor (GHSR-1a) and desacyl ghrelin (DGhr) which, until recently, was considered biologically inactive. Based on many reports DGhr is now suggested to work through non-GHSR1a pathway as a separate hormone that may act together with active AGhr, antagonize AGhr or exhibit AGhr independent effects (4). The data consistently suggest that ghrelin itself, apart from appetite regulation and growth hormone releasing activity, is involved in metabolic, immune, reproductive and cardiovascular functions of the body (5, 6). In animal models, reported cardiovascular protective activities of ghrelin include: regulation of atherosclerosis, protection against myocardial injury, cardiac hypertrophy, and hypertension, however, conflicting data also exist (7-10).

In humans, ghrelin may have beneficial effects in patients with cardiovascular diseases including of left ventricular dysfunction and cardiac cachexia (10). Moreover, intravenous ghrelin administration significantly improves cardiac output, cardiac contractility, stroke volume, and decreases mean arterial pressure and peripheral vascular resistance in normal subjects (11). Endothelial dysfunction is one of the primary factors affecting development of atherosclerotic plaques and arterial stiffness. Thus, the protective endothelial activity of ghrelin, supported by clinical trials, could be one of the mechanisms by which ghrelin might modulate blood pressure (12).

We examined an association between ghrelin, including its major isoforms, interleukin-6 (IL-6), body mass index (BMI), and mean arterial pressure (MAP) in male overweight patients with essential hypertension. Twenty hypertensive male patients with newly diagnosed essential hypertension (EH) before starting drug treatment and 22 age-matched healthy controls were enrolled in the study. Fasting total plasma ghrelin (T Ghhr), acyl ghrelin (A Ghhr), des-acyl ghrelin (D Ghhr) and IL-6 were determined and correlations between studied parameters were calculated. We found significantly lower total plasma ghrelin and higher plasma IL-6 in hypertensives when compared with the control. In patients with hypertension the negative correlations were found: between T Ghhr and BMI, D Ghhr and BMI, T Ghhr and MAP, and between D Ghhr and MAP. IL-6 positively correlated with BMI and MAP in hypertensive subjects. No correlations between all forms of ghrelin and IL-6 were noted. The changes in plasma ghrelin and IL-6 contribute independently to the elevated blood pressure in essential hypertension. Negative correlation of D Ghhr and MAP may suggest its hemodynamic involvement in regulation of blood pressure.

Key words: ghrelin, interleukin-6, blood pressure, hypertension, body mass index, tumor necrosis factor-alpha
It has been shown that interleukin-6 (IL-6) is one of the inflammatory markers associated with peripheral endothelial dysfunction and increased risk of coronary artery disease. A body of evidences suggest that vascular inflammation is also implicated in the pathophysiology of any type of hypertension. IL-6, as a central stimulator in mediating inflammation, induces CRP production, opposes synthesis of anti-inflammatory markers (IL-1 and tumor necrosis factor alpha (TNF-α)), attenuates nitric oxide (NO) endothelial formation and prolongs endothelial dysfunction affecting vascular tone and possibly leading to hypertension (13).

Based on these recent studies, it appears that both ghrelin and IL-6 might have role in development of primary hypertension. Therefore, we decided to investigate an association between ghrelin (total, acyl and des-acyl isoforms) and IL-6 in hypertensive patients and to evaluate the correlations between BMI, MAP, ghrelin and IL-6 in normotensive and hypertensive subjects with newly diagnosed, non-complicated EH, before the treatment started.

MATERIALS AND METHODS

Patients

The study was conducted in the Department of Internal Medicine, Metabolic Disorders and Hypertension in Poznan University of Medical Sciences. The study population consisted of 20 hypertensive male patients with newly diagnosed essential hypertension (EH) before starting drug treatment and 22 age-matched healthy controls. All the subjects underwent pre-study screening by standard physical examination and routine clinical laboratory tests. The diagnosis of EH was made, according to the American Society of Hypertension (ASH) guidelines. Following a 10-minute resting blood pressure readings were taken from the right arm in a sitting position using mercury sphygmomanometer. Three blood pressure measurements were averaged for the analysis, and subjects with systolic blood pressure of ≥140 mm Hg and/or diastolic pressure of ≥90 mm Hg were considered to be hypertensive. The exclusion criteria were based on the presence of any of the following: secondary hypertension (after careful clinical and biochemical examination), acute cardiovascular event within last 6 months, surgery, acute infection or allergic reaction within last 4 months, chronic inflammatory disease, neoplasm, treatment with anti-inflammatory drugs for a period longer than 4 weeks, renal and/or hepatic failure, diabetes. For the parallel control group, normotensive healthy voluntaries were recruited.

All the patients gave their written consent with the study and the protocol was approved by the Institutional Ethical Committee (Poznan University of Medical Sciences, Poland).

Study design

Venous blood samples were taken from each individual after 12-hour overnight fast. To avoid any diurnal variation in IL-6, and ghrelin samples were collected in the mornings of the experimental days between 6.30 and 7.00 a.m. Blood samples were put into tubes containing potassium EDTA (50 µl liquid/ml blood), centrifuged (10 min, 5000 rpm, 4°C) and the supernatant plasma was separated for further storage at –20°C. Plasma concentrations of ghrelin and IL-6 were measured in duplicate using commercially available assays: total ghrelin plasma concentration (T Ghhr) was estimated by enzyme immunoassay ghrelin (human) Elisa (Cat. No ELIA 3706, DRG Inc.), acylated form of ghrelin (AGhr) using enzyme immunoassay Active Ghrelin Elisa (Cat. No ELIA 4190, DRG Inc.), and plasma IL-6 was measured with high-sensitivity immunoassay (Quatikine human IL-6, Cat No HS600B, R&D Systems). Levels of des-acyl ghrelin (DGhr) were calculated as the difference between total ghrelin and acylated ghrelin. To keep high specificity and sensitivity of the tests, the protocols provided by manufacturers were not modified. The sensitivity of the assay for IL-6 was 0.01 pg/ml. The intra-assay coefficient of variation was 8%. IL-6 levels from all patients were above the detection limit. To exclude patients with diabetes blood glucose and HbA1c were estimated and appeared to be within normal range.

Statistics

Statistical analysis of data was performed using the STATISTICA statistical programme, version 9. Numerical variables are presented as means ± S.D. The Mann-Whitney U test

Table 1. Baseline characteristics of the study participants and plasma levels of ghrelin and IL-6.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 20)</th>
<th>Control (n = 22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67 ± 11.2</td>
<td>61 ± 8.4</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83 ± 11.2</td>
<td>74 ± 11.4</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168 ± 7.3</td>
<td>164 ± 8.6</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.4 ± 3.9</td>
<td>26.4 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>162 ± 12</td>
<td>122 ± 8</td>
<td>0.022</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>105 ± 6</td>
<td>77 ± 5</td>
<td>0.018</td>
</tr>
<tr>
<td>MAP</td>
<td>124 ± 9</td>
<td>92 ± 6</td>
<td>0.02</td>
</tr>
<tr>
<td>T Ghhr (pg/mL)</td>
<td>603.1 ± 29.5</td>
<td>772.2 ± 40.1</td>
<td>0.025</td>
</tr>
<tr>
<td>A Ghhr (pg/mL)</td>
<td>113.0 ± 52.0</td>
<td>125 ± 46.1</td>
<td>NS</td>
</tr>
<tr>
<td>D Ghhr (pg/mL)</td>
<td>482 ± 48.3</td>
<td>644 ± 58.9</td>
<td>0.02</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>1.98 ± 0.52</td>
<td>0.89 ± 0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8 ± 1.5</td>
<td>5.0 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>% HbA1c</td>
<td>5.55 ± 0.33</td>
<td>5.81 ± 0.28</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.D. M - males; F - females, BMI - body mass index, SBP - systolic blood pressure, DBP - diastolic blood pressure, MAP - mean arterial pressure, TG - total ghrelin, AG - acyl ghrelin, DG - desacyl ghrelin, NS - not statistically significant. BMI was calculated as weight (kg)/(height (m))². MAP was calculated as DBP + 1/3(SBP-DBP).
was used for unpaired analyses. Pearson’s linear correlation coefficients were calculated to assess associations between variables, $P < 0.05$ were considered statistically significant.

**RESULTS**

General characteristics and biochemical parameters of the subjects are summarized in *Table 1*. We examined baseline plasma levels of total ghrelin and IL-6 in all study participants. When compared with the control, fasting plasma ghrelin levels were significantly lower in hypertensive patients ($P = 0.025$), and plasma IL-6 levels were significantly higher ($P = 0.015$). Additionally, we estimated the correlations (presented in *Table 2 and Table 3*) between ghrelin and BMI, and IL-6 and BMI in entire study population as well as in groups of hypertensive and normotensive subjects. When entire population was analyzed we found negative correlations: between TGhr and MAP (not statistically significant), TGhr and BMI, and between DGhr and BMI. In patients with hypertension the negative correlations were found: between TGhr and BMI, DGhr and BMI, TGhr and MAP, and between DGhr and MAP. AGhr showed positive correlation with BMI, which was very weak and not statistically significant. IL-6 positively correlated with BMI and MAP in hypertensive subjects. In all study participants no correlations between any form of ghrelin and IL-6 were noted.

**DISCUSSION**

Only a few studies have examined association between markers of inflammation, body weight and blood pressure in newly diagnosed hypertension in humans. Our work extends earlier investigations to ghrelin and its isoforms in highly homogenous study population (males, nonsmokers, non-complicated and newly diagnosed EH). An important methodological issue of this study is that our hypertensive subjects did not receive any antihypertensive drugs before an experiment.

Ghrelin has been postulated to lower blood pressure by vasodilatation and endothelium linked pathways, or affect central suppression of sympathetic activity and activation of baroreceptor afferents, but the exact mechanisms are still not well understood, and nihilistic and even contradictory findings exist (14-18). When compared with the control, we found low TGhr and DGhr levels in hypertensive subjects and we agree with Poykko et al. who demonstrated an association between low fasting total plasma ghrelin and high blood pressure in the population of hypertensive patients. They suggested that the effect of ghrelin is independent of BMI and other most commonly recognized risk factors for hypertension (19). Their findings, however, cannot discern between plasma isoforms of ghrelin and, with respect to MAP in essential hypertension, the relevance between AGhr and DGhr has been assessed by us for the first time. When we considered entire study population,
TGhr was negatively correlated with MAP indicating that generally decreased plasma ghrelin might be implicated in the pathophysiology of EH when its compensatory ability to reverse increase in BP is “turned off”. Lower DGhr in hypertensive subjects, as observed in our study, and its significant negative correlation with MAP (Fig. 2) suggest that deficiency of this isoform might be due to lost or damaged protective vascular effects of ghrelin. Studies on animals have shown that DGhr may have hemodynamic activities and may contribute to the regulation of cardiovascular control at the central and peripheral level. Microinjection of DGhr into rat nucleus tractus solitarii elicited decrease in the mean arterial blood pressure and heart rate (20). As suggested by Kleinz et al. DGhr possesses vasodilatatory endothelium independent effects similar in potency to those observed for total ghrelin. Both of them are involved in paracrine regulation of vascular tone in humans (21), and both of them might work through the novel, distinct from GHSR-1a receptor in the cardiovascular system (22). DGhr does not exhibit endocrine activities in human but, as shown in our study, it may have specific functions distinct from those of acylated ghrelin.

It is not clear whether low TGhr and DGhr levels predispose to hypertension, or rather are the consequence of already elevated blood pressure and irreversible changes in blood vessels and/or baroreflex failure that results in sustained stimulation of the sympathetic nervous system. Theoretically, both mechanisms are possible, but this requires further investigation.

It has been shown that arterial stiffness and vascular smooth muscle tone, as a primary factors of EH, are closely related with vascular inflammation and endothelial changes (23). Our hypertensive subjects had significantly higher level of IL-6 than controls. This finding is in line with the study indicating association between IL-6, as a key marker of systemic inflammation, and hypertension in humans. As suggested by authors, the mechanism probably involved relationship between angiotensin II (AII) and IL-6, since IL6 increased significantly in response to AII infusion (24). Elevated in hypertensive subjects IL-6 levels indicate endothelial dysfunction and may lead to angiotensin II mediated vasoconstriction (25). Recent data describe ghrelin as a peptide coupling the metabolic axis to the immune system that exerts inhibitory effect on proinflammatory cytokine production and expression by human endothelial cells, monocytes and T cells (26, 27). The inhibition of basal and TNF-alpha induced chemotactic cytokine production and mononuclear cell adhesion in HVEC culture was observed at physiological range of ghrelin and was due to its acylated form, suggesting that anti-inflammatory activity of ghrelin in endothelial cells is mediated through growth hormone.
secretagogue receptor (GHSR-1a) present on the immune cells (27). Thus, reduced ghrelin could potentially contribute to the proinflammatory state and further development of cardiovascular complications. On the other hand, IL-6 at high concentration has been shown to inhibit ghrelin mRNA and proteins expression (28). Considering vascular pathomachinery of EH we hypothesized that lower levels of ghrelin might be correlated with elevated IL-6 levels in hypertensive patients. Contrary to our expectations, no significant correlations were noted. We conclude that although IL-6 is elevated and both TGhr and DGhr are lowered in EH, the levels of circulating IL-6 and ghrelin do not track one another.

We have also noticed that in patients with EH, plasma ghrelin and IL-6 were correlated with BMI. Our results support previous observations describing adiposity as an important determinant of ghrelin and IL-6 concentrations (29, 30). We confirm that plasma IL-6 is elevated with increased BMI, and that ghrelin secretion is downregulated with a positive energy balance (our patients were classified as overweight; 29 > mean BMI value > 25). Definitely, overweight inhibits protective vascular activity of ghrelin by changing its plasma concentration and additionally serves as a risk factor for development of endothelial inflammation and dysfunction. High BMI is accompanied with relative DGhr deficiency and DGhr levels could be regulated by body weight (31). It is also important to acknowledge, as shown by Zhao et al., that lower plasma ghrelin levels are observed in lean hypertensive subjects (14). Therefore, overweight/obesity is not the only one factor that may potentially inhibit vascular protective effects of ghrelin, and there must be an additional mechanism by which ghrelin level/activity is downregulated in hypertensive subjects. Studies of Skoczylas et al. have shown that monotherapy with one of the angiotensin converting enzyme inhibitors (ACEI) markedly increases plasma ghrelin concentration and this effect is not directly related to the resultant lowering of blood pressure (17). The effective long-term control of blood pressure by ACEI with resulting increase in plasma ghrelin was also reported by Meric et al. (32). Together, these and our findings suggest the existence of reciprocal relationship between the level of circulating TGhr and DGhr (reduced) and blood pressure (elevated) when blood pressure is no more under homeostatic control.

Some of the limitations of this study include small number of study participants. So any interpretation should be made with caution. However, it is hard to find the patients without comorbidities at the time of diagnosis. Moreover, our intention was to exclude as many factors affecting tested variables as possible. Thus, we’ve been looking for nonsmoking males without any serious complications. As reported by Marques-Vidal et al., male sex, increased BMI and smoking are strongly associated with greater IL-6 levels (33). Examining women could be problematic because of gender differences in basal ghrelin levels (lower in females). BP measurements depending on standardized phase of the menstrual cycle, and lower BP support by autonomic nervous system plus less effective baroreceptor buffering of BP, which means different conditions for possible cardiovascular action of ghrelin (34, 35). Additionally, it is possible that unmeasured variables e.g. endothelin-1, intercellular adhesion molecule 1 (ICAM-1), vWF, serum urocortins, or severity (grade) of hypertension, may account for the relationships observed (36, 37). Despite these limitations, our results may bring a new insights into pathogenesis of EH.

In summary, the two most important findings of this study include: 1) significant negative correlation between TGhr and MAP and between DGhr and MAP in hypertensives that may indicate hemodynamic effects of DGhr implicated in the regulation of blood pressure in humans, 2) independent association of low plasma ghrelin and high plasma IL-6 with elevated blood pressure (in males with EH), and with BMI (in hypertensive and normotensive overweight male subjects).

We believe that our work will accomplish a practical applications as an additional scientific background to develop better treatment and prophylaxis of EH and obesity.

Acknowledgments: This research was funded by the Department of Physiology Poznan University of Medical Sciences grant number 502-0101-1251-8404-401.

Conflict of interests: None declared.

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Received: September 29, 2014 Accepted: March 23, 2015

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