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RENAL MICROCIRCULATORY BLOOD FLOW CHANGES IN METOPRINE-INDUCED REVERSAL OF HAEMORRHAGIC HYPOTENSION IN RATS

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A b s t r a c t
Background and aim: The histaminergic system is involved in the central cardiovascular regulation. An increase in central histamine concentration after inhibition of histamine N-methyltransferase (HNMT) activity leads to a long-lasting pressor effect, with an increase in survival rate, in haemorrhage-shocked rats. The study was undertaken to examine renal cortical microcirculatory changes associated with HMNT antagonist metoprine-induced resuscitating effect in rats.

Methods: Study was performed in ketamine/xylazine-anaesthetised male Wistar-Kyoto rats subjected to irreversible haemorrhagic shock with mean arterial pressure (MAP) 20-25 mmHg. Animals were treated with metoprine (15 mg/kg; intraperitoneally [ip]) or saline (0.5 ml; ip). Haemodynamic (MAP, heart rate [HR], renal microcirculatory blood flow [RMBF]) and biochemical (renal cortical histamine concentration) parameters were measured.

Results: Metoprine given peripherally evoked a long-lasting pressor effect with an increase in HR and RMBF. Renal cortical microcirculatory changes were associated with a lower local histamine concentration.

Conclusion: Inhibition of HNMT activity with metoprine administered intraperitoneally in anaesthetised haemorrhage-shocked rats produces a resuscitating effect with an increase in renal microcirculatory blood flow. The effect can be associated not only with central histamine-induced activation of compensatory mechanisms, but also with peripheral histamine-mediated vasodilatation.

Key words: metoprine, histamine, haemorrhagic shock, microcirculation, rats

INTRODUCTION
There are two phases of neural and humoral response to acute blood loss in mammals – an initial sympathoexcitatory phase, which results from a reflex reaction from arterial baroreceptors, and the second – sympathoinhibitory phase, which is associated with the Bezold-Jarisch reflex (1). Despite the activation of humoral compensatory mechanisms, such as the release of arginine vasopressin, catecholamines and proopiomelanocortin (POMC)-derived peptides, as well as the activation of the renin-angiotensin system, there is a fall in mean arterial pressure (MAP) in the second phase of cardiovascular regulation (1, 2). The effect is related to extremely decreased total blood volume and inhibition of the sympathetic system activity (1).

Our previous studies clearly demonstrate that exogenous histamine (10-100 nmol) given intracerebroventricularly ([icv], acting via central H1 receptors, is able to reverse the critical haemorrhagic hypotension and to increase the survival rate at 2 h in a rat model of irreversible pressure-controlled haemorrhagic shock (3). Similar effects are induced by endogenous central histamine, after loading with histamine precursor L-histidine (4) or inhibition of histamine N-methyltransferase (HNMT; EC 2.1.1.8) activity with SKF 91488 (10-100 μg; [icv]) (5) or metoprine (5-20 μg; [icv]) (6). Interestingly, MAP and heart rate (HR) changes elicited by histamine in the sympathoinhibitory phase of haemorrhagic hypotension are long-lasting and significantly higher compared to those in normovolaemic animals (3-6).

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Our recent studies demonstrate that metoprine, which easily penetrates the blood-brain barrier and increases central histamine concentrations, administered peripherally also induces a resuscitating effect (7). Since metoprine action concerns not only central, but also peripheral tissue HNMT activity, the purpose of the present study was to examine renal microcirculatory and histamine concentration changes, which are associated with metoprine-induced resuscitating effect in rats.

METHODS

Experiments were performed on anaesthetised (ketamine/xylazine, 100/10 mg/kg; intramuscularly) male Wistar-Kyoto rats (215-278 g, 5-6 months old). The animals were housed five per cage, in controlled conditions of temperature (20-22°C), humidity (60-70%), lighting (12 h light/dark cycle) and provided with food and water ad libitum. The Ethical Committee of Medical University of Silesia approved all procedures (Notification No 5/03).

The rats were implanted with catheters in the right femoral artery and vein. MAP and HR were measured using the pressure transducer RMN-201 (Temed, Zabrze, Poland) and the electrocardiograph Diascope 2 (Unitra Biazet, Bialystok, Poland), respectively. Renal microcirculatory blood flow (RMBF; arbitrary units [a. u.]) was measured in the middle part of the right kidney using laser flowmeter (Laser Flow type BRL-100, Bio Research Center Co., Ltd, USA) (8). All measurements of blood flow were started after a 30 min adaptation period to avoid influences of probe implantation. Concentrations of histamine in renal cortical tissue (middle parts of both kidneys) were measured spectrofluorometrically (9).

Irreversible haemorrhagic shock was produced by intermittent blood withdrawal from the catheter inserted into the right femoral vein over a period of 15-25 min, until MAP stabilised at 20-25 mmHg (10). To study cardiovascular effects of HNMT inhibition, metoprine (15 mg/kg, 0.5 ml of solution; intraperitoneally [ip]) or saline (0.5 ml; ip) were injected in two groups of rats (n = 6) at 5 min of critical hypotension. Body temperature was monitored by a rectal thermometer and maintained at 36.5-37.5°C using the heating lamp throughout the experiment. All the experiments were performed between 8 a.m. and 2 p.m.

The following drugs were used: metoprine (Burroughs Wellcome Co., Research Triangle Park, NC, USA), xylazine hydrochloride (Research Biochemicals Inc., Natick, MA, USA), ketamine (Gedeon Richter, Budapest, Hungary), lactic acid, NaHCO₃ (POCh, Gliwice, Poland) and heparin (Polfa, Warszawa, Poland). All solutions were isoosmolar to the plasma. Osmolarity was measured using osmometer Marcel OS 3000 (Marcel, Bialystok, Poland) based on the freezing-point method. Metoprine was dissolved in 0.5% lactic acid and was neutralised with NaHCO₃. All drug solutions were prepared freshly on the day of the experiment.

All values are given as means ± standard deviation with P < 0.05 considered as the level of significance. Statistical evaluation was performed by analysis of variance (ANOVA) and the post-ANOVA test of Neuman-Keul. The Fisher’s exact test was used to examine significant differences in survival rates.

RESULTS

The pre-haemorrhage MAP, HR and RMBF values in the control saline-treated group were 78.4 ± 7.6 mmHg, 351 ± 19 beats/min and 211 ± 27 a. u., respectively, with no significant differences between the two groups.

The total bleeding volume for the induction of critical hypotension in all animals was 2.46 ± 0.21 ml/100 g b.w. In the control saline-treated group, a decrease in MAP to 22.3 ± 1.8 mmHg was associated with a decrease in HR (Table 1) and RMBF (Fig. 1) and death of all animals within 30 min.
Fig. 1. Influence of metoprine (15 mg/kg; ■) and saline (❑) administered at 0 min (arrow) on renal microcirculatory blood flow (RMBF) in haemorrhage-shocked rats. Data are presented as mean ± SD, six animals per group. *

Table 1. Mean arterial pressure (MAP) and heart rate (HR) changes in rats before and after haemorrhage and after metoprine (15 mg/kg; ip) and saline treatment. Data are presented as mean ± SD, five animals per group.

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
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<tbody>
<tr>
<td></td>
<td>metoprine</td>
<td>saline</td>
</tr>
<tr>
<td>Before bleeding</td>
<td>81.2 ± 6.3</td>
<td>78.4 ± 7.6</td>
</tr>
<tr>
<td>After bleeding</td>
<td>22.3 ± 1.8</td>
<td>23.2 ± 1.6</td>
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<tr>
<td>5 min after treatment</td>
<td>26.3 ± 2.5*</td>
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<td>10 min after treatment</td>
<td>29.5 ± 3.1*</td>
<td>22.9 ± 3.1</td>
</tr>
<tr>
<td>30 min after treatment</td>
<td>33.1 ± 4.2*</td>
<td>0</td>
</tr>
<tr>
<td>60 min after treatment</td>
<td>43.7 ± 4.7*</td>
<td>0</td>
</tr>
<tr>
<td>90 min after treatment</td>
<td>50.6 ± 4.2*</td>
<td>0</td>
</tr>
<tr>
<td>120 min after treatment</td>
<td>53.4 ± 5.3*</td>
<td>0</td>
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</table>

* P < 0.05 vs. the control group (ANOVA and the post-ANOVA test of Neuman-Keul)

Metoprine (15 mg/kg; ip) administered to rats bled to a critical hypotenion evoked long-lasting rises in MAP, HR (Table 1) and RMBF (Fig. 1) which started within 10 min after treatment. The effect was accompanied by a 100% survival rate at 2 h (P < 0.05 vs. the control saline-treated animals; Fisher's exact test).

Renal cortical histamine concentration in metoprine-injected group was lower than in control animals (0.42 ± 0.14 vs. 2.03 ± 0.64 Kμg/g of wet tissue; P < 0.01) as measured 20 min after treatment.
RESULTS OF THE PRESENT STUDY CONFIRM OUR PREVIOUS FINDINGS THAT HNMT INHIBITOR METOPRINE ADMINISTERED PERIPHERALLY EVOKES A LONG-LASTING PRESSOR EFFECT IN ANAESTHETISED HAE-MORRHAGE-SHOCKED RATS (7). IN ADDITION, WE DEMONSTRATE FOR THE FIRST TIME THE ASSOCIATION BETWEEN RENAL TISSUE MICROCIRCULATORY BLOOD FLOW CHANGES AND LOCAL HISTAMINE CONCENTRATIONS.

OUR RESULTS SHOW THAT HEMODYNAMIC EFFECTS OF PERIPHERALLY ADMINISTERED METOPRINE IN RATS SUBJECTED TO HEMORRHAGIC SHOCK ARE SIMILAR TO THOSE PRODUCED BY HNMT INHIBITORS SKF 91488 AND METOPRINE INJECTED CENTRALLY (5, 6). IN BOTH CASES, INCREASES IN ENDogenous CENTRAL HISTAMINE CONCENTRATIONS IN THE HYPOTHALAMUS, MEDULLA OBLOYGATA AND CEREBRAL CORTEX ARE ACCOMPANIED BY LONG-LASTING RISES IN RENAL, MESENTERIC AND HINDQUARTERS BLOOD FLOWS, WITH A DECREASE IN PERIPHERAL VASCULAR RESISTANCE. THEREFORE, IT IS SUGGESTED THAT THE CENTRAL HISTAMINERGIC SYSTEM BELONGS TO NEURONAL SYSTEMS RESPONSIBLE FOR THE MAINTENANCE OF CIRCULATORY HOMEOSTASIS IN CONDITIONS OF HEMORRHAGIC HYPOTENSION (10). THE HYPOTHESIS IS CONFIRMED BY THE STUDY OF PHILIPPU ET AL., WHO SHOWED FOR THE FIRST TIME THAT A DECREASE IN BLOOD PRESSURE IS ASSOCIATED WITH THE ACTIVATION OF HISTAMINERGIC NEURONES IN THE HYPOTHALAMUS (11).


HEMORRHAGIC SHOCK IS ACCOMPANIED BY INCREASED LEVELS OF HISTAMINE IN BLOOD PLASMA (4, 16) AND SKELETAL MUSCLES (17). AS WE PREVIOUSLY SUGGESTED, AN INCREASE IN PLASMA HISTAMINE CONCENTRATION CAN RESULT FROM ITS RELEASE FROM BOTH CIRCULATING BASOPHILS AND TISSUE MAST CELLS (17). INTERESTINGLY, OUR PREVIOUS STUDY DEMONSTRATES A DECREASE IN SKELETAL MUSCLE HISTAMINE CONCENTRATIONS AFTER METOPRINE ADMINISTRATION IN HEMORRHAGE-SHOCKED RATS (18). PRESENT RESULTS ARE IN LINE WITH THESE FINDINGS AND DEMONSTRATE THAT RENAL CORtical TISSUE HISTAMINE CONCENTRATION IS DECREASED. Thus, after ischaemia and reperfusion, peripheral tissues can be the source of circulating histamine.

PRESENT RESULTS CLEARLY DEMONSTRATE AN INCREASE IN RMBF IN METOPRINE-TREATED GROUP. SIMILAR EFFECTS IN SKELETAL MUSCLE MICROcirculatory BLOOD FLOW WERE OBTAINED EARLIER IN THE SAME EXPERIMENTAL MODEL (18). Thus, we suggest that metoprine-induced resuscitating effect, with a rise in MAP and peripheral tissue perfusion, could be associated not only with central histamine-induced activation of compensatory mechanisms, but also with peripheral histamine-mediated vasodilatation.

IN CONCLUSION, THE PRESENT RESULTS DEMONSTRATE THAT INHIBITION OF HNMT ACTIVITY WITH METOPRINE ADMINISTERED INTRAperitonealy IN ANAESTHETISED HEMORRHAGE-SHOCKED RATS PRODUCES A RESUSCITATING EFFECT WITH AN INCREASE IN RENAL MICROcirculatory BLOOD FLOW. MOREOVER, OUR RESULTS SUGGEST A POSSIBLE INVOLVEMENT OF PERIPHERAL HISTAMINE IN RENAL HEMODYNAMICS.
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DUAL EFFECT OF L-NAME ON AIRWAY HYPERREACTIVITY

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A b s t r a c t
The airway hyperreactivity is a tendency to sudden narrowing of the air passages of the lung in response to various stimuli (exogenous irritants in the air, allergens, emotional shock etc). This symptom is typical for various respiratory diseases including asthma. The pathomechanism of this attribute is as yet unknown but some experimental studies presuppose that nitric oxide (NO) plays an important role in this process.

The aim of our study was to investigate the position of nitric oxide in toluene and allergen-induced hyperreactivity by using non-selective NO-synthase inhibitor L-NAME.

Guinea pigs were used in our experiment. The animals received L-NAME in a dose of 40 mg/kg b.w. during two therapeutic regimens (acute or chronic) in toluene or allergen-induced hyperreactivity. We observed after L-NAME pre-treatment the tracheal and lung tissue reactivity changes to histamine and acetylcholine in vitro conditions.

Statistical analysis was done using one-way ANOVA and the comparisons of baseline values between groups were analyzed by Student’s two-sided t-test.

We recorded significant decrease of tracheal smooth muscle reactivity after acute L-NAME pre-treatment in toluene-induced hyperreactivity. We found the opposite effect – an increase of tracheal smooth muscle reactivity in allergen-induced hyperreactivity after acute and chronic L-NAME pre-treatment. The lung tissue reactivity was reduced after acute and chronic L-NAME pre-treatment in toluene-induced hyperreactivity but changes in allergen-induced hyperreactivity were non significant.

In summary, our study demonstrates that the effect of L-NAME on the airway reactivity changes was dependent on the hyperreactivity provoking factor and type of therapeutic regimen.

Key words: Nω-nitro-L-arginine methyl ester, nitric oxide, toluene/allergen-induced airway hyperreactivity

INTRODUCTION

Airway hyperreactivity (AHR) is a typical feature of asthma and other respiratory diseases (chronic obstructive pulmonary disease). It is defined as excessive bronchial narrowing and it is manifested itself as an exaggerated bronchoconstrictive response of the airways to various stimuli e.g. cold air, environmental pollutants, chemicals or mediators (1). It is often connected with mucus hypersecretion, small airway plugging and with the infiltration of inflammatory cells, usually eosinophils (2). Many other cells (epithelial, endothelial, vascular or bronchial smooth muscle cells) that produce a wide array of mediators (histamine, cysteinyl leukotrienes, prostaglandins, adenosine, acetylcholine, substance P, neurokinin A, nitric oxide) participate in the pathogenesis of airway hyperreactivity. It is known that high nitric oxide (NO) level is significant in this process but the specific position of NO in airway hyperreactivity is unclear at present (3).

Nitric oxide is important mediator and neurotransmitter that attends in many various physiological and pathological processes in the human organism. It is generated from semi-essential amino acid L-arginine in different cell types (nervous, endothelial, epithelial, vascular, inflammatory or bronchial smooth muscle cells) by three isoforms of nitric oxide synthases (NOS). Nitric oxide is the main neurotransmitter of inhibitory non-adrenergic non-cholinergic nervous system (nNANC) in the airways and it controls the bronchial smooth muscle tone together with acetylcholine. It participates furthermore in the airways in vascular smooth muscle tone regulation, surfactant production, antimicrobial defence,
mucous production and other functions (4, 5). These, mainly physiological effects are connected with optimal nitric oxide production mostly by constitutive NOS (cNOS) that generates NO in small, picomolar amounts. The free radicals production is an important ability of NO predominantly in the pathological conditions that is connected with the activity of inducible NO synthase (iNOS). This isoform produces NO in high, nanomolar amounts active during pathological conditions (e.g. inflammation).

The pathophysiological significance of endogenous NO is controversial in the conditions of hyperreactivity (6). It is interesting that activities of NOS isofoms are dependent on the type of hyperreactivity-inducing factors. Toluene and allergen exposure increased the experimental airways reactivity to various mediators of bronchoconstriction e.g histamine, meta-choline, bradykinine (7, 8). Toluene is a reactive agent and an exposure to this solvent or its derivatives (toluene diisocyanate) is connected with tracheal epithelial damage, airway inflammation, bronchial hyperreactivity, eosinophilic and lymphocytes infiltration in lung parenchyma and emphysema (8, 9, 10). In addition, these substances can evoke a free radicals production in the airways coupled with various cell damage that is associated with the increase of NO production by inducible NO synthase (11). Jang et al. (12) on the other side present that ozone exposure increases endothelial NOS (eNOS) and neuronal (nNOS) activity and decreases iNOS activity although ozone has similar tissue effect as a toluene. The repeated exposure to allergen evoked changes as an eosinophilic inflammation, the production of reactive nitrogen species and hyperreactivity after allergen sensitization (3). The expression and activity of iNOS is increased in these conditions but the effect of allergen on cNOS is different. Samb and co-workers observed that allergen inhalation decreased the expression and activity of nNOS in respiratory system but eNOS activity was not affected in these conditions (13, 14). The other literature data about cNOS position in allergen-induced hyperreactivity are very different and up to now unclear and need further specification.

We were interested in our study in the relationship between the decrease of NO level and airway hyperreactivity in two different experimental conditions. Therefore, we administered non-selective NOS inhibitor L-NAME (N-nitro-L-arginine methyl ester) in the same dose in the different therapeutic regimens (acute or chronic) to determine the changes of AHR in toluene and allergen-induced airway hyperreactivity.

MATERIAL AND METHODS

Study design
Outbred specific pathogen-free male Trik guinea pigs (180-250g) were used in our experimental study. Guinea pigs were housed in commercial cages in climate-controlled animal quarters. Water and food were provided ad libitum.

We demonstrated in our previous studies that toluene inhalation and allergen sensitization evoked the airway hyperreactivity in our conditions (15, 16). Now, we observed changes of tracheal and lung tissue smooth muscle reactivity in toluene or allergen AHR after L-NAME administration. We divided animals into eight groups – four experimental and four control groups. All animals of experimental groups received non-selective NOS inhibitor L-NAME (Sigma Aldrich) in a dose of 40 mg/kg b.w. during two different therapeutic regimens (acute and chronic) in the toluene and allergen hyperreactivity conditions. The agent was dissolved in Aqua pro injectione and administered once a day in the constant time.

The first experimental group (n=8) received L-NAME intraperitoneally 30 minutes before toluene exposure during three consecutive days (acute pre-treatment).

The second experimental group (n=8) received L-NAME 17 days intraperitoneally (chronic pre-treatment), during last three days 30 minutes before toluene exposure, too.

The third experimental group (n=8) obtained L-NAME by inhalation 30 minutes before last allergen exposure (acute pre-treatment).
The fourth experimental group (n=8) received L-NAME by intraperitoneal injection during all time of sensitization – 14 days (chronic pre-treatment).

The control groups received Aqua pro injectione (1ml/kg b.w) by intraperitoneal injection in the same therapeutic regimen and hyperreactivity conditions.

All protocols described in this study were approved by the Ethical Committee of Jessenius Faculty of Medicine in accordance with internationally accepted recommendations.

Provocative techniques

Toluene exposure
The method in vivo exposure of animals to the toluene vapours described by Strapková et al. (9) was used in this study. The guinea pigs were spontaneously breathing toluene vapours in a special Plexiglass exposure chamber. The chamber consists of compressor, flow-meter, vaporizer and exposure cage. The device was situated in the fume-cupboard at 22°C. Toluene vapours were delivered into cage with constant flow of 4 l/min. The average concentration of the toluene was 6 mg/l (1600 ppm). The concentration of toluene was monitored continuously. The duration of exposure was two hours in each of three consecutive days.

Allergen sensitization
Guinea pigs were sensitized with ovalbumin (OVA, Sigma Aldrich). Animals received 100 μg ovalbumin dissolved in 1ml saline subcutaneously (0,5 ml - skin on the neck) and intraperitoneally (0,5 ml) on the first day. On the third day received OVA in the equal dose intraperitoneally only. Guinea pigs inhaled 0.1% OVA solution during 5 minutes on the fourteenth day.

The reactivity changes measurements
Airway responsiveness was measured as response to cumulative doses (10⁻⁸ – 10⁻³ mol.l⁻¹) of histamine or acetylcholine (Sigma Aldrich) in in vitro conditions. Animals were sacrificed 24 hours after last toluene or allergen exposure. The trachea and lungs were removed and small thin strips from these organs were prepared and placed into the organ bath with Krebs-Henseleit solution (110.0 mol/l NaCl, 4.8 mol/l KCl, 2.35 mol/l CaCl₂, 1.20 mol/l MgSO₄, 1.20 mol/l KHPO₄, 25.0 mol/l NaHCO₃ and 4g glucose in glass-distilled water). The solution was continuously aerated with mixture of 95% O₂ and 5% CO₂ at pH 7.5 ± 0.1 and temperature of 36 ± 0.5°C. The strip endings were connected to a force transducer and an amplifier (RES s.r.o, Martin, Slovak Republic). The changes of tension were recorded on a computer with specific software (RES s.r.o, Martin, Slovak Republic). The tissue strips were exposed initially to the tension of 4 g (30 minutes - loading phase). Thereafter, the tension was reduced to a baseline of 2 g (30 minutes - adaptive phase). Krebs-Henseleit solution was changed every 10 minutes. We recorded the changes of trachea and lung tissue strips reactivity to cumulative doses of both bronchoconstriction mediators after one hour of tissue incubation. A cumulative concentration-response curve to 10⁻⁸-10⁻³ mol.l⁻¹ histamine or acetylcholine was determined for every strip.

Statistical analysis
Statistical analysis was performed using one-way analysis of variance (ANOVA). The comparisons of baseline values between groups were analyzed by Student’s two-sided t-test. All statistical analyses were done with Microsoft Excel and Microcal Origin 7.0 (OriginLab, Data analysis and Graphing Software). Differences were considered statistically significant when p-value was below 0.05. All results are expressed as mean ± SEM.

RESULTS

Tracheal smooth muscle reactivity changes after acute and chronic L-NAME administration in toluene-induced hyperreactivity
We observed a decrease of tracheal smooth muscle response to both mediators of bronchoconstriction in animals that received L-NAME intraperitoneally during three days 30 minutes before irritant exposure in comparison with the control group. The amplitude of tracheal smooth muscle contraction was significantly reduced at concentration 10^{-5} mol.l^{-1} - 10^{-3} mol.l^{-1} of histamine and 10^{-6} mol.l^{-1} - 10^{-3} mol.l^{-1} of acetylcholine (Fig 1.). L-NAME administered during 17 days did not evoke any statistically significant changes of tracheal smooth muscle reactivity to histamine and acetylcholine (Tab.1).

![Fig. 1](image)

The comparison of the tracheal smooth muscle reactivity changes in animals after toluene exposure (grey columns – control group) and animals with acute (3 days) L-NAME pre-treatment (crosshatch columns) to histamine and acetylcholine. The acute pre-treatment with L-NAME significantly decreased the amplitude of tracheal smooth muscle contraction to both mediators of bronchoconstriction. The columns represent the average values of the contraction amplitude with mean average S.E.M. Axis x – the concentration of histamine or acetylcholine in log M, axis – the amplitude of contraction in mN. ** P <0.01, *** P <0.001.

<table>
<thead>
<tr>
<th>tissue</th>
<th>mediator</th>
<th>L-NAME in toluene induced hyperreactivity</th>
<th>L-NAME in ovalbumin induced hyperreactivity</th>
</tr>
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<tr>
<td></td>
<td></td>
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↑ increase of reactivity  ↓ decrease of reactivity  —— unaltered

Table 1. Changes of airways reactivity after acute and chronic L-NAME pre-treatment in toluene and ovalbumin-evoked hyperreactivity conditions. The changes depend on the type of hyperreactivity trigger (toluene, ovalbumin), on the regimen of L-NAME pre-treatment (acute, chronic), the mediators of bronchoconstriction (histamine, acetylcholine) and the level of respiratory system (trachea, lung tissue). When L-NAME effect was the statistically significant we use symbols ↑ - increase of reactivity or ↓ - decrease of reactivity. The insignificant changes we marked as a unaltered - ——.
Lung tissue reactivity changes after acute and chronic L-NAME administration in toluene-induced hyperreactivity

We recorded a small depression of lung tissue reactivity after acute (3-days) L-NAME administration. The changes were statistically significant only to 10^{-3} mol.l^{-1} of histamine. Chronic L-NAME administration reduced lung tissue hyperreactivity to histamine at concentration of 10^{-6} - 10^{-3} mol.l^{-1} in toluene-induced hyperreactivity (Fig. 2). The changes of lung tissue contraction amplitude to acetylcholine after acute and chronic L-NAME administration were similar to control group. The decrease of lung tissue reactivity to acetylcholine was not statistically significant (Tab.1).

Fig. 2 The comparison of the lung tissue reactivity changes in control (toluene) group (grey columns) and animals with acute (3 days) and chronic (17 days) L-NAME pre-treatment (crosshatch columns) to histamine. The pre-treatment with L-NAME in both therapeutic regimens significantly decreased the amplitude of lung tissue contraction to histamine. Data are the mean ±S.E.M. *P <0.05, ** P <0.01.

Tracheal smooth muscle reactivity changes after acute and chronic L-NAME administration in allergen-induced hyperreactivity

We recorded that acute L-NAME administration in allergen-induced hyperreactivity evoked the expressive enhancement of tracheal smooth muscle reactivity. L-NAME inhalation after last OVA administration generated an increase of tracheal smooth muscle contraction amplitude to histamine at concentration of 10^{-6} - 10^{-5} mol.l^{-1} and acetylcholine at concentration of 10^{-6} - 10^{-3} mol.l^{-1} (Fig. 3). L-NAME applied during longer therapeutic regimen (17 days) evoked similar reactivity changes as an acute L-NAME administration. We recorded statistically significant increase of tracheal smooth muscle reactivity at concentration of 10^{-6} - 10^{-4} mol.l^{-1} histamine and 10^{-6} - 10^{-3} mol.l^{-1} acetylcholine (Fig. 4).
Fig. 3 The comparison of the tracheal smooth muscle reactivity changes in animals after ovalbumin sensitization (grey columns - control group) and animals with acute (one-shot) inhalation L-NAME pre-treatment (crosshatch columns) to histamine and acetylcholine. The acute pre-treatment with L-NAME in ovalbumin-induced hyperreactivity conditions significantly enhanced the amplitude of tracheal smooth muscle contraction to histamine and acetylcholine in comparison to control group. Data are the mean ± S.E.M. *P  < 0.05.

Fig. 4 The comparison of the tracheal reactivity changes in control (ovalbumin) group (grey columns) and animals with chronic (17 days) L-NAME pre-treatment (crosshatch columns) to histamine and acetylcholine. The chronic pre-treatment with L-NAME significantly increased the amplitude of lung tissue contraction to both of bronchoconstrictor mediators. Data are the mean ± S.E.M. *P  < 0.05, ** P < 0.01.

**Lung tissue reactivity changes after acute and chronic L-NAME administration in allergen-induced hyperreactivity**

We recorded any significant lung tissue reactivity changes after acute and chronic L-NAME administration in allergen-induced hyperreactivity. The lung tissue reactivity changes to both used mediators of bronchoconstriction were similar as a reactivity changes in control groups (Tab.1).
DISCUSSION

The airway hyperreactivity is a typical symptom present in various respiratory diseases when the airways respond both too much and too easily to various stimuli (17). The exact pathomechanism of this hallmark is unknown for this time. It is presumed that small amount of cNOS-derived NO has a protective role in the conditions of hyperreactivity. This assumption is confirmed in studies that demonstrate that an inhibition of constitutive NOS with the non-selective NOS inhibitor L-NAME enhances the airways reactivity to contractile agonists, such as histamine and methacholine both in in vivo and in vitro conditions (13, 18). This agent blocks largely cNOS but has small inhibitory effect on the inducible NOS (19). We suppose that an inhibition mainly of cNOS will increase the airways hyperreactivity evoked by two different triggers – chemical (toluene) and biological (ovalbumin). We observed airway reactivity changes in our study after the decrease of NO level by using a non-selective NOS inhibitor L-NAME in conditions of experimental hyperreactivity.

We presented the dual effect of L-NAME on the tracheal and lung tissue smooth muscle reactivity. We recorded mainly beneficial effect of this NOS inhibitor in toluene-induced hyperreactivity. We observed the predominant influence of L-NAME after acute administration on tracheal smooth muscle reactivity. On the other side, the effect of L-NAME in allergen-induced hyperreactivity was predominantly detrimental. We also observed more expressive action on tracheal smooth muscle reactivity. We can say that the changes of the airways reactivity are dependent on the type of hyperreactivity trigger (toluene, OVA), on the regimen of L-NAME pre-treatment (acute, chronic), the mediators of bronchoconstriction used in in vitro conditions (histamine, acetylcholine) and the level of respiratory system (trachea, lung tissue).

We demonstrated in our previous experiments that toluene exposure increases the airways smooth muscles reactivity in guinea pigs (9, 15). This increase can be associated with an elevation of free radicals production (20) and with pathological changes in the respiratory tract because toluene-induced hyperreactivity simulates the pulmonary oxidative stress. It is known that the beneficial role of NO in the optimal conditions is probably a result of its ability to scavenge free radicals and with the ability to reduce lipid peroxidation, too (21). Further reason may be the deficiency of L-arginine that may result in the switching of NOS from NO production to superoxide formation and cellular injury (13) or may cause the inhibition of constitutive NOS. This situation activates inducible NOS that begin the production of the detrimental amount of nitric oxide (22) that can be partly modulated by L-NAME. It is possible that in toluene-induced hyperreactivity may modulate the activity of antioxidant mechanisms that determine the final response of the airways smooth muscle.

We can speculate that toluene exposure evoked the increase of cNOS expression with predominantly pro-hyperreactive effect. This effect may be connected with the changes in iNOS expression, too. Enhanced smooth muscle reactivity may be probably evoked by high amounts of NO synthetised by cNOS and iNOS. Jang et al. (12) mentioned the increase of both cNOS (neuronal and endothelial) activity and the decrease of iNOS activity in ozone-exposed mice. Ozone induces hyperreactivity by the oxidative injury (23). This chemical irritant may damage the structure of cell membrane (mainly phospholipide bi-layer), various ion channels, enzymes (NOS) or can change the specificity of receptors for endogenous substrates (histamine). The effect of toluene is similar and toluene exposure may evoke damage of epithelial cells, release of different mediators including NO and generation of free radicals (e.g. superoxide anion). These conditions are harmful for tracheal and lung tissue.

We observed mainly protective effect of L-NAME in toluene-induced hyperreactivity that is also in accord with results of McDowell et al. (24). This study describes the protective effect of L-NAME in acute lung injury (after nickel aerosol inhalation). The animals that received L-NAME show lower nitrogen metabolites production, the decrease of eNOS activity, the decrease of cytokine expression and production and low appearance of inflammatory pro-
cesses. The selective inhibitor of iNOS activity - aminoguanidine does not effect in these conditions. The effect of NOS inhibition with L-NAME in toluene exposure can be due to functional impairment of signalling mechanisms mediating the physiological effects of NO in airways function (25).

We observed the opposite effect of L-NAME in allergen-induced hyperreactivity. The acute and chronic administration of L-NAME evoked the statistically significant increase of tracheal smooth muscle reactivity to histamine and acetylcholine. The changes of lung tissue reactivity were not significant. The mechanisms of airway hyperreactivity genesis and NO participation are probably different in these conditions. The inflammatory cells and their mediators have the most important role in this process. Hamid et al. (26) and Koarai et al. (3) show that inhalation of ovalbumin increases the iNOS immunoreactivity in the airway epithelial and some inflammatory cells. Inducible NOS induced by inflammatory cytokines such as tumour necrosis factor (TNF-α), interleukin (IL-1β) or interferon (IFN-γ) produced much larger amounts of NO that may act prohyperreactively. Schuling et al. (27) present that iNOS-derived NO may show both beneficial and detrimental effects on allergen-induced airway hyperreactivity to histamine. Iijima et al. (28) present that increased NO production is connected with activity of constitutive - mainly neural NOS isoform. Our results are conformable with Schuling et al. (29) who described that the deficiency cNOS derived NO contributes to allergen-induced airway hyperreactivity. The effect of L-NAME on lung tissue reactivity in these conditions is not significant and the reactivity changes were almost identical with the control group.

The effects of L-NAME in our condition can be dependent on the dose also, on the time and duration of application or on other possible mechanisms of L-NAME action. The participation of other mediators regulating the bronchomotoric tone, mainly vasoactive intestinal peptid can be one of the other possible explanations. It is known that NO is the principal relaxant mediator in human bronchus but in guinea pigs trachea supply only half iNANC response (4, 30). Nitric oxide inhibits more widely the higher than the smaller airways contraction. NO neurons are mostly presented in proximal than in distal airways. The results that we present are to validate these facts, because tracheal responses were more expressive as changes of lung tissue responses in our conditions. It is necessary to take into consideration possible participation of the different vascularization of trachea and lung tissue, too.

Our results confirm that NO is involved in the regulation of the airways reactivity changes, depending on the hyperreactivity triggers and on the activity of individual NOS isoforms. Therefore, it is necessary to continue research of the NO role in the respiratory system that may bring some new therapeutic approaches in the treatment of many respiratory diseases with airway hyperreactivity.

REFERENCES

ABSTRACT

Obesity and arterial hypertension are a serious risk factor for insulin resistance patients leading to diabetes and other disorders. The aim of this study was to compare average levels of the homeostatic indices HOMA and QUICKI in obese children compared to healthy and hypertonic children in order to find convenient markers for insulin sensitivity in clinical pediatric practice. 21 obese, 29 healthy and 28 hypertonic children were selected. The average level of HOMA in obese children was 3.6; in healthy children 1.7 and in the group of hypertonic children the level was 2.65. The average level of QUICKI in obese children was 0.33; in healthy children 0.36 and in hypertonic children 0.34. The results demonstrate the possibility of insulin sensitivity assessment using these indices in pediatric practice. QUICKI has a narrower confidence interval and thus a lower variability.

Key words: obesity in children, insulin resistance, hypertension in children, HOMA, QUICKI

INTRODUCTION

In recent years, the increasing number of children in the Czech Republic manifests the first signs of disorders which earlier only used to be seen predominantly in adults, for example a high blood pressure and the insulin resistance syndrome leading to diabetes type 2 (DT2). These illnesses are connected to risk factors, one of which is overweight, obesity or hypertension. Obesity itself needs not always mean overweight but an accumulation of fatty tissue. In childhood, it is obvious that the continuous increase in weight is not merely caused by the increase of fat tissue but also by the development of the body frame and the muscle mass. The share of this component differs according to the individual age group and gender.

Child’s obesity in the Czech Republic is a serious epidemiological problem: 20% of children aged 6-12 and 11% of children aged 13-17 years are already overweight or obese. These data were provided by the study of the Czech Obesity Association entitled „Life Style and Obesity 2005“. Obesity naturally is one of the most important risk factors in the development of insulin resistance and other disease conditions which are connected with this syndrome(1).

Hypertension.

Based on studies by the American Academy of Pediatrics (AAP), 2004, hypertension in children is defined as a mean systolic and diastolic pressure above 95th percentille depending on sex, age and a height of children.

During the course of a child’s growth the blood pressure increases and its level also depends on the gender. Therefore, it is necessary to evaluate the measured levels by taking into account the age, gender and the height of a child. Percentile graphs for values of blood
pressure in children have been compiled by measuring blood pressure in tens of thousands of healthy children. The graphs currently in use by most doctors in developed world are those published by an American group for a child's hypertension in 1987 and published as the "Report of the Second Task Force".

The update of this Report is the Third Report of the year 1996 and also the Fourth Report of 2004. The output of the Fourth Report are tables according to which the blood pressure is examined not only by judging the age and the gender of a child. The third criteria became to be the child’s height.

The aim of the study was to establish average levels of homeostatic indices in a group of children with primary hypertension and with obesity and to compare them to the indices of healthy children with the aim of establishing the usefulness of the indices for prediction of insuline resistance development.

**MATERIAL AND METHODS**

Twenty one obese boys of an average age of 16.5 were being given an advice on losing weight at the Department of Clinical Psychology, Faculty Hospital in Olomouc. 28 hypertensive boys with mean systolic blood pressure above 95th percentilee according to their height and sex formed the patient group. Another 29 children (24 boys and 5 girls) of an average age of 16.2 who showed normal physiological parameters, were used as the control group of healthy children. The following parameters were measured in every child: blood pressure, body mass index (BMI) and laboratory concentrations of total cholesterol, triglycerides, HDL and LDL cholesterol, glycemia and insulineaemia. The values of glucose and insulin concentration were used to calculate homeostatic indices, HOMA and QUICKI.

The homeostatic index for insulin resistance, HOMA IR was calculated according to the homeostatic model (2,3) as:

\[
HOMA \text{ IR} = \frac{\text{insulin fasting (μIU/ml)} \times \text{glycemia fasting (mmol/l)}}{22.5}
\]

Homeostatic index insulin resistance QUICKI was calculated according to Katz et al (4)

\[
\text{QUICKI} = \frac{1}{\log \text{insulin fasting (μIU/ml)} + \log \text{glycemia fasting (mg/100ml)}}
\]

Insulin resistance is defined as values of HOMA index greater than or equal to 2.68 and QUICKI as values less than or equal to 0.34.

Statistical evaluation was done using the programme Statistics version 6.0.

**RESULTS**

In Table 1 are shown the results of the examination in the control group of children as means and SD. The results could be calculated as physiological parameters for each following value. In table 2 are shown the results of the obese group for each parameter. Individuals in this group also showed normal physiological values. Only the insulin resistance was higher. In table 3 there are results of hypertensive children.
In figures 1 and 2 are depicted mean values for homeostatic indices with their 95% confidence intervals. Fig. 1 shows mean values for the HOMA index in healthy children, which was 1.7 while in obese children it was 3.6. Fig. 2 shows mean values for QUICKI in health-

### Table 1. Results of parameters in healthy children

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<thead>
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<th>Standard deviation</th>
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<td>HOMA</td>
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<tr>
<td>QUICKI</td>
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### Table 2. Results of obese children

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### Table 3. Results of hypertensive children

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hy children, which was 0.36 while in obese children it was 0.33. Values for QUICKI in obese children may indicate a shift towards the insulin resistance. From figures 1 and 2 it seems clear that the levels of a control group and an obese group are different (HOMA p=0.000036). It is because confidence intervals do not overlap. Confidence intervals of control group and hypertensive group overlap only partly (HOMA p=0.9693).

**Fig. 1.** Means and 95% confidence intervals of HOMA.

![Fig. 1. Means and 95% confidence intervals of HOMA.](image1)

**Fig. 2.** Means and 95% confidence intervals of QUICKI.

![Fig. 2. Means and 95% confidence intervals of QUICKI.](image2)
DISCUSSION

The methods currently used for the diagnosis of the insulin resistance are very demanding to be carried out and completely unsuitable for the investigation of large number of people. Other approaches are therefore sought, such as the easy way to assess homeostatic relations between insulin and glycaemia. An increase in concentration of insulin can be a physiological terms guide to glycaemia and vice versa. Disorders in this relationship show insufficient activity of insulin and are the basis of the homeostatic model used to appraise the insulin resistance. Among indices and results, the „clamping technique“ has repeatedly verified the highly significant correlation (5,6).

In the table of the results, it can be seen that the mean value for the HOMA index which can characterise the insulin resistance, in healthy non-obese children of mentioned age, it is 1.7 +0.98 while the QUICK1 value in these children is 0.36+0.02. These values can be considered as a standard in healthy children (7).

Obesity in adolescence may be a result of growth in the infantile age. The majority of studies on infant size has shown that infants who were recognised as „obese“ or who were in the higher range of the body mass index were more inclined to the development of obesity in their childhood, adolescence or an early adulthood in comparison to other children. The infants who grew quickly were more inclined to obesity in their childhood, puberty and an early adulthood than other children. A large size or a rapid phase of growth in the intervals between the first and the second year of their life are predictive of an inclination to later obesity (8). In groups of obese or hypertensive, children changed HOMA and QUICK1 indices, which was the sign of the possible development of the insulin resistance. At the current moment no visible increase in other metabolic parameters apart from insulinemia was found. If these children experienced negative changes in metabolic parameters, the development of the insulin resistance could be accelerated in the near future.

CONCLUSIONS

The results show the possibility of evaluating insulin sensitivity from the levels of glycaemia and insulemia with the help of the homeostatic indices HOMA and QUICK1 which is for the use in pediatric practice. In reality, index QUICK1 which has narrower confidence intervals and hence less variability may be more useful. Healthy children have values of QUICK1 index around 0.36. Obese and hypertensive children have lower values.

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METABOLIC SYNDROME AND HORMONES OF ADIPOSE TISSUE

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A b s t r a c t

Background: Metabolic syndrome (MS) is associated with increased risk of cardiovascular diseases and type 2 diabetes mellitus (DM2). Insulin resistance is one of the major mechanisms of developing of MS, but an equally important role in its pathogenesis is played by the white adipose tissue and substances it produces – the adipokines. The aim of our study was to investigate the influence of major factors of the metabolic syndrome on plasma levels of adipokines (adiponectin, leptin) and gastrointestinal hormone ghrelin.

Methods: We examined 153 patients (87 men) with average age of 58.9 years. In each patient the presence of MS components was assessed according to modified NCEP ATP III criteria, which include impaired glucose tolerance (IGT) or DM2, obesity, hypertriglyceridaemia and elevated blood pressure. Hormone plasma concentrations were measured using standard RIA (radioimmunoassay) methods. The influence of several MS components on these concentrations was then statistically analysed.

Results: In our study we confirmed that a tendency to decreased adiponectin levels was associated with the presence of DM2 and elevated BMI, although these differences were not statistically significant. In males its values depended more on BMI and in females especially on DM2. Leptin levels were significantly increased in individuals with glucose metabolism disorders and this relation was more stressed in men. The values of ghrelin tended to rise according to BMI, especially in non-diabetic individuals.

Conclusion: Levels of investigated hormones showed a relation to the major components of MS – total obesity and glucose metabolism disorders connected with insulin resistance. Statistical significance of this relation was influenced by great interindividual variability, presumably caused by other factors of MS. Intersexual differences could most likely be contributed to different distribution of body fat – gluteofemoral vs. abdominal obesity.

Key words: metabolic syndrome – adipokines – obesity – type 2 diabetes mellitus

INTRODUCTION

The metabolic syndrome (MS) includes several metabolic and non-metabolic abnormalities which significantly increase the risk of developing cardiovascular diseases or type 2 diabetes mellitus (DM2) (1). It was first mentioned by Reaven in 1988 as the “Syndrome X” that at that time included insulin resistance (IR), hyperinsulinaemia, impaired glucose tolerance (IGT) or DM2, increased VLDL and low HDL lipoproteins and arterial hypertension (2). In 1993 Reaven revised his definition in the way that the primary disorder is IR, with which DM2, hypertriglyceridaemia and hypertension are tightly coupled. Other disorders like coronary heart disease, android obesity and haemostatic defects are only loosely connected with this state (1). The present concept of MS comprises the central role of both insulin resistance with compensatory hyperinsulinaemia and central obesity in the etiopathogenesis of this syndrome, which nowadays includes impaired glucose metabolism associated with IR, central and ectopic obesity, dyslipidaemia (elevated triglycerides and low HDL lipoproteins) and arterial hypertension (1). Several definitions of MS criteria have been proposed so far, of which the revised NCEP ATP III definition from 2003 is the most used:

1. Fasting glucose ≥ 6.1 mmol/l or DM2.
2. Waist circumference > 102 cm in males and 88 cm in females,
3. Plasma triglycerides > 1.7 mmol/l.

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E-mail: peter-kruzlik@post.sk; galajda@lefa.sk
4. Plasma HDL < 1.0 mmol/l in males and 1.3 mmol/l in females, 
5. Blood pressure > 130/85 mmHg (3).

The white adipose tissue and its secretory products – adipokines play one of the key roles in the development of MS (1, 4-6). The adipose tissue cells produce a great variety of substances which take part in the regulation of a wide range of physiological and pathological processes – from energy balance and expenditure to atherosclerosis and haemostasis (4-6). The most important adipokines in the pathogenesis of MS according to the present state of knowledge seem to be adiponectin and leptin.

Adiponectin was independently discovered by 4 research groups in 1995 – 96. In 2001 it was proved to have, in contrast to other adipokines, an insulin-sensitizing effect (7). Several studies have showed that the complete adiponectin molecule enhances the inhibition of glucose secretion in hepatic cells induced by insulin. Adiponectin acts also in skeletal muscle, where especially its C-terminal fragment stimulates the oxidation of fatty acids (6, 7). According to experiments in mice, where the adiponectin gene was specifically knocked-out, it is now obvious that this adipokine not only improves insulin sensitivity, but also suppresses the formation of atherosclerotic changes in vessel walls. This effect can be explained by the inhibition of some adhesive molecules and inflammatory cytokines expression (7). Adiponectin plasma levels are influenced by a lot of factors: they are decreased in obese individuals or in higher concentrations of insulin, TNFα and IL-6, and raised due to PPARγ receptors activation (5-7).

Being the first adipokine to be discovered (1994), leptin meant a real revolution in the field of adipokines and white adipose tissue function (4). The inactivation of leptin or his receptor has proved to be responsible for the phenotype of 2 mice models (ob/ob and db/db) used in obesity research for over 20 years (9). The loss of effect of leptin caused severe insulin resistance and obesity in these animals. Leptin decreases body mass by modulating the synthesis of various hypothalamic proteins. It especially stimulates the forming of anorexigenic and inhibits the synthesis of orexigenic proteins, which leads to decreased appetite and food intake (4, 5, 9). In blood leptin enhances the activity of hormone sensitive lipase (HSL) (4). In healthy individuals these functions of leptin serve to prevent excessive accumulation of body fat. But since levels of this adipokine in a large number of obese are elevated, resistance to central effects of leptin is supposed to be present in these persons (4, 5, 8, 9). This resistance seems to play an important role in the pathogenesis of MS.

In the present study we also assessed levels of the gastrointestinal hormone ghrelin, another important regulator of food intake. This hormone is produced mainly in neuroendocrine cells in stomach. Ghrelin especially acts on hypothalamus, where it, on the contrary to leptin, stimulates the synthesis of orexigenic and inhibits the formation of anorexigenic peptides, which leads to increased appetite and food intake. Plasma levels of ghrelin are elevated before meals and fall down postprandially, but the postprandial lowering is significantly less pronounced in obese compared to lean individuals (10, 11).

The aim of our study was to evaluate the influence of the main components of MS on plasma levels of adiponectin, leptin and ghrelin.

**METHODS**

Patients with at least one component of MS according to the modified NCEP ATP III criteria were involved in the study. The presence of glucose metabolism disorder (impairment of glucose tolerance or type 2 diabetes mellitus) was assessed according to anamnestic or oral glucose tolerance test. Body mass index (BMI) was calculated from the patient’s weight in kilograms and height in meters using standard formula. Blood triglycerides and cholesterol...
were assessed with the help of common biochemical methods. Systolic and diastolic blood pressure was measured with the Riva-Rocci sphygmomanometer. The levels of investigated hormones (leptin, adiponectin, ghrelin) were assessed from peripheral venous blood using standard RIA (radioimmunoassay) kit by LINCO Research Inc. Probands were divided into groups according to the presence or absence of glucose metabolism disorder or obesity with BMI more than 30. In these groups mean values of investigated hormones, their standard deviations and medians were calculated and compared using unpaired Student t-test. This procedure was then repeated for the subgroups of men and women. Statistical analyses were performed with the help of Microsoft Excel statistical tools.

RESULTS

The study cohort consisted of 153 patients with average age of 58.9 years, among them 87 men (58.8 years) and 66 women (59.0 years). Leptin was assessed in the whole 153 subjects, adiponectin in 113 persons (60 men) and ghrelin in 146 probands (80 men).

Adiponectin levels in the whole group were increased in persons without diabetes than with DM2 – 26.31 vs. 21.15 μg/ml – but the difference was not statistically significant. Similar situation could be seen in non-obese probands (BMI < 30) when compared to the obese ones (BMI > 30) (23.28 vs. 21.80 μg/ml, n.s.). In the female subgroup adiponectin levels were significantly increased in non-diabetic persons compared to diabetics (34.30 vs. 16.43 μg/ml, p < 0.05). Obese women showed a trend to a bit higher levels of this adipokine than the non-obese ones (21.83 vs. 19.76 μg/ml, n.s.). In males trend to higher adiponectin was observed in diabetic (26.27 vs. 21.11 μg/ml, n.s.) and lean individuals (25.56 vs. 22.52 μg/ml, n.s.).

Leptin levels were significantly increased in diabetic probands in the whole group (11.86 vs. 8.65 ng/ml, p < 0.05) and even more in the male subgroup (10.29 vs. 5.79 ng/ml, p < 0.05). In females there was almost no difference between the diabetic and non-diabetic group (13.52 vs. 14.12 ng/ml, n.s.). In individuals with BMI > 30 only slight and non-significant trend to increased leptin levels was observed in the whole group (10.69 vs. 10.42 ng/ml, n.s.) and the male subgroup (8.45 vs. 7.99 ng/ml, n.s.). In women the situation was reversed (12.49 vs. 14.91 n/ml, n.s.).

Ghrelin levels were non-significantly decreased in the presence of glucose metabolism disorder in the whole group (614.4 vs. 730.1 pg/ml, n.s.) and especially in females (573.5 vs. 840.1 pg/ml, n.s.). The male subgroup showed only a minor difference between individuals with and without DM (660.8 vs. 664.6 pg/ml, n.s.). There was also trend to increased ghrelin levels together with the elevation of BMI in the whole study group regardless of gender (637.3 vs. 703.0, n.s. in the whole group, 631.0 vs. 751.4, n.s. in males, 649.5 vs. 675.5 pg/ml, n.s. in females). Complete results with calculated statistical data are showed in Table 1, 2 and 3.
Table 1: Results – whole group

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<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>87</td>
<td>59</td>
</tr>
<tr>
<td>Average</td>
<td>614.4</td>
<td>730.1</td>
</tr>
<tr>
<td>Median</td>
<td>499.7</td>
<td>443.2</td>
</tr>
<tr>
<td>SD</td>
<td>442.3</td>
<td>895.1</td>
</tr>
<tr>
<td>Disp</td>
<td>195675.9</td>
<td>801217.4</td>
</tr>
</tbody>
</table>

DM2 – type 2 diabetes mellitus, IGT – impaired glucose tolerance, BMI – body mass index, SD – standard deviation, Disp - dispersion

Table 2: Results – males

<table>
<thead>
<tr>
<th>Adiponectin</th>
<th>DM/IGT</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Average</td>
<td>26.27</td>
<td>21.11</td>
</tr>
<tr>
<td>Median</td>
<td>19.37</td>
<td>13.46</td>
</tr>
<tr>
<td>SD</td>
<td>18.5</td>
<td>17.4</td>
</tr>
<tr>
<td>Disp</td>
<td>340.6</td>
<td>291.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leptin</th>
<th>DM/IGT</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>45</td>
<td>42</td>
</tr>
<tr>
<td>Average</td>
<td>10.29</td>
<td>5.79</td>
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<tr>
<td>Median</td>
<td>5.40</td>
<td>2.6</td>
</tr>
<tr>
<td>SD</td>
<td>11.2</td>
<td>7.9</td>
</tr>
<tr>
<td>Disp</td>
<td>124.3</td>
<td>62.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ghrelin</th>
<th>DM/IGT</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>Average</td>
<td>660.8</td>
<td>664.6</td>
</tr>
<tr>
<td>Median</td>
<td>504.9</td>
<td>371.2</td>
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<tr>
<td>SD</td>
<td>567.6</td>
<td>747.1</td>
</tr>
<tr>
<td>Disp</td>
<td>322141.4</td>
<td>558199</td>
</tr>
</tbody>
</table>

DM2 – type 2 diabetes mellitus, IGT – impaired glucose tolerance, BMI – body mass index, SD – standard deviation, Disp - dispersion
DISCUSSION

In our study we confirmed that adiponectin levels depended more on the presence or absence of glucose metabolism disorder than on the amount of body mass assessed by BMI. This effect was more stressed in females where the difference between diabetic and non-diabetic subjects showed statistical significance. These findings support the role of high adiponectin levels as a protective factor against diabetes (5, 7). The fall of adiponectin concentrations with growing BMI in the whole group and in males is also consistent with already published data (4, 5, 6, 7). The slight adiponectin rise in obese females compared to lean subject might be contributed to the more frequent occurrence of gluteofemoral fat in these women in contrast to men with prevailing abdominal adiposity, but this hypothesis could not be proved, because measures of abdominal obesity were not taken.

Leptin levels also seemed to be more influenced by DM2 or IGT than BMI. Especially in males without glucose metabolism disorders leptin reached significantly lower values than in other groups. This speaks for the role of elevated leptin in the pathogenesis of DM2. In females leptin levels were in general higher than in males, but the influence of DM2 was rather small.

Decreased ghrelin values were associated with the presence of glucose metabolism disorder, especially in female probands. In men elevated ghrelin levels depended more on a higher BMI and the same tendency could be to a smaller extent seen in the whole group. These findings are contrary to most published data and could be caused by other metabolic and non-metabolic factors which influence ghrelin levels independently on BMI, like HDL-cholesterol, physical activity, smoking and alcohol intake (12) and which were not assessed in our study. Great variety of obtained ghrelin values in each subgroup could contribute to this result as well.

<table>
<thead>
<tr>
<th>Table 3: Results – females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
</tr>
<tr>
<td>(μg/ml)</td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Leptin</td>
</tr>
<tr>
<td>(ng/ml)</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ghrelin</td>
</tr>
<tr>
<td>(pg/ml)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

DM2 - type 2 diabetes mellitus, IGT – impaired glucose tolerance, BMI – body mass index, SD – standard deviation, Disp - dispersion

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In conclusion, we suppose that levels of adiponectin, leptin and ghrelin were influenced by major components of MS (DM/IGT and obesity), which suggests a possible role in the pathogenesis of MS for these hormones. One of the limitations of our pilot study was the absence of abdominal obesity measurement in most participants, but we suppose that intersexual differences in adipokine levels were caused by different distribution of body fat in males and females – abdominal vs. gluteofemoral fat. Great interindividual variability of measured adipokine concentrations might be contributed to the presence of other factors of MS, which should become target of our further investigations.

REFERENCES


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6. **Discussion**: Emphasize the new and important aspects of the study, link the conclusions with the presented goals of the study, relate the results to other relevant studies with a short summary of results at the end.

7. **References**: All publication cited in the text should be presented in references. References have to be numbered consecutively in the order in which they are first mentioned within the text. Identify references in the text by Arabic numerals in parentheses. Use abbreviations of the journals according to Index Medicus (List of Journal Indexed in Index Medicus, http://www.nlm.nih.gov).

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