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Metabolic characteristics of soleus muscle in relation to insulin action in the offspring of hypertensive parents

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Abstract

Insulin resistance affecting skeletal muscle metabolism is present in the prehypertensive state. The aim of our study was to test the hypothesis that blood pressure value is related to skeletal muscle composition, measured by ^{31}P magnetic resonance (MR) spectroscopy, and to insulin sensitivity in the offspring of hypertensive parents (OH) and healthy controls. Study groups consisted of 10 healthy young lean OH with normal glucose tolerance, confirmed with oral glucose tolerance test, and 13 controls matched for age, sex, and body mass index. Insulin action was estimated as glucose disposal (M), glucose metabolic clearance rate (MCR), and insulin sensitivity index (M/I) during a 10-hour hyperinsulinemic euglycemic clamp. The sum of immunoreactive insulin values from the oral glucose tolerance test was calculated. ^{31}P MR spectroscopy was performed on a whole-body MR scanner (Siemens Vision, Erlangen, Germany) operating at 1.5 T and equipped with actively shielded gradient coils. There were no differences in common metabolic and anthropometric parameters between OH and controls except for the blood pressure, which was in the range of normal to high-normal level in OH. Mean blood pressure was significantly higher in OH (95.73 ± 4.39 vs 83.76 ± 3.95 mm Hg; $P < .001$). Trend toward insulin resistance was registered in OH with significantly lower M/I (0.74 ± 0.47 vs 1.42 ± 0.65 mg · kg^{-1} · min^{-1} · mIU^{-1} · L^{-1}; $P < .05$). There were no significant differences in total serum magnesium (sMg) levels between OH and controls, although a positive correlation exists between sMg and insulin sensitivity expressed as M ($r = 0.63$, $P < .01$), MCR ($r = 0.54$, $P < .01$), and M/I ($r = 0.51$, $P < .05$). No differences in signal intensities of phosphocreatine (PCr), phosphomonoesters, phosphodiesters, inorganic phosphates (Pi), adenosine triphosphates (Patp and βATP), and calculated concentrations of intracellular ionized magnesium (Mgi) and H^+ ions between the groups were detected. Systolic blood pressure correlates positively with PCr/Patp ($r = 0.43$, $P < .05$), Pi/Patp ($r = 0.413$, $P < .05$), and Pi/βATP ($r = 0.48$, $P < .05$). Diastolic blood pressure correlates positively only with the ratio Pi/βATP ($r = 0.42$, $P < .05$). The sum of immunoreactive insulin values correlates with PCr/βATP ($r = 0.53$, $P < .01$) and with Pi/βATP ($r = 0.6$, $P < .01$). In conclusion, increase in blood pressure and insulin resistance were confirmed in offspring of OH. Insulin sensitivity is related to sMg and the elevation of blood pressure is associated with the activation of energy metabolism in skeletal muscle. The relationship between muscle energetic characteristics and markers of insulin resistance suggests that the alteration of energy metabolism may be present in early stages of metabolic syndrome.

1. Introduction

Elevated blood pressure represents a component of the metabolic syndrome, clustered with insulin resistance, hyperinsulinemia, hyperlipidemia, and glucose intolerance, and hypertension is often observed as the first clinical manifestation of the syndrome. Although the pathogenesis of the metabolic syndrome is uncertain, the role of insulin resistance as the unifying underlying pathology of the syndrome is proposed by some investigators [1]. Decrease in insulin sensitivity has already been shown in normotensive individuals with a family history of hypertension [2-6], and insulin resistance has been proposed to play a role in the progression of hypertension and subsequent development of cardiovascular complications [7].

Several concepts as to the pathophysiology of hypertension have already been presented, and alterations in ion homeostasis affecting natrium, potassium, calcium, or magnesium could play an important role in the process. Ionic

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abnormalities, especially those of magnesium metabolism, are supposed to constitute the link between the pathophysiology of hypertension and diabetes [8,9]. Skeletal muscle, bioenergetically similar to smooth muscle in vessels involved in the regulation of blood pressure, is one of the most important sites of insulin resistance in glucose metabolism.

Phosphorus 31 magnetic resonance spectroscopy (^{31}P MRS) can be used to determine skeletal muscle energy metabolism and intracellular free ionized magnesium Mg^{2+} concentration (Mgi). In vivo, the ^{31}P spectrum (Fig. 1) comprises peaks of phosphomonoesters (PMEs), phosphodiesters (PDEs), phosphocreatine (PCr), inorganic phosphate (Pi), and adenosine triphosphate (Patp). The PME peak is generated by signals from lipid metabolism precursors, ie, phosphoethanolamine and phosphocholine, and the PDE peak from molecules of glycerol-3-phosphocholine and glycerol-3-phosphoethanolamine. Patp peak is formed by 3 signals (α, β, and γATP); out of them βATP peak with the minimal contamination of other signals reflects best the total content of ATP molecules. The concentration of Mgi can be calculated from chemical shifts of αATP and βATP [10,11], and the intracellular pH can be calculated from chemical shifts of Pi and PCr [12]. PCr serves as an energy reservoir and can provide phosphate molecules for ATP regeneration in case of energy demand. Pi molecules arising after the ATP utilization are used for the formation of new PCr and close the energetic cycle in muscle. Every biochemical process taking place in the cell is reflected by changes in energy status. Skeletal muscle cells are supposed to be an important target for insulin action and pathologic biochemical processes that occur inside result in different energy handling. ^{31}P MRS enables one to display these differences without, however, their precise specification.

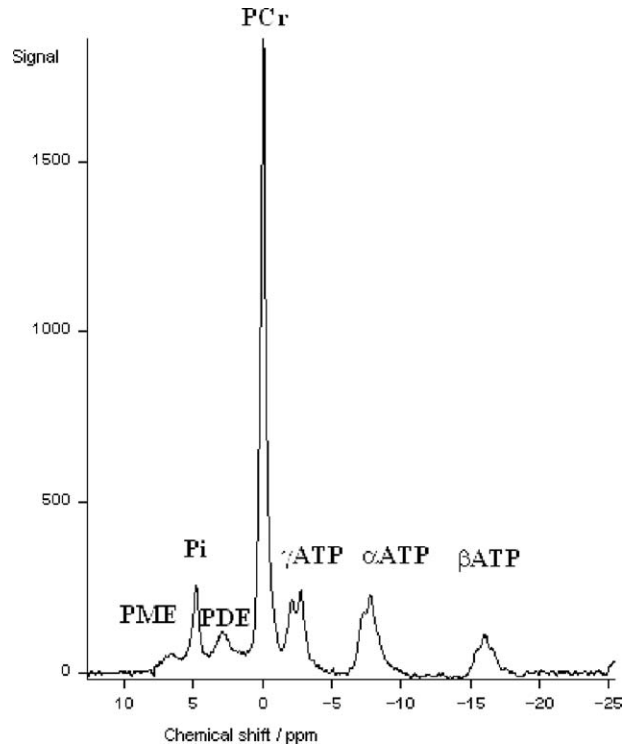

Fig. 1. Typical ^{31}P MR spectrum of musculus soleus.

Attention has recently been focused mostly on myocardium metabolism in hypertension, diabetes, and other forms of heart dysfunction [13], but differences in the signal intensity ratios of high-energy phosphates in skeletal muscle cells have already been shown in patients with primary juvenile hypertension [14].

The aim of the study was to compare the metabolic characteristics of skeletal muscle (musculus soleus) in the offspring of hypertensive parents (OH) and healthy volunteers and (1) to determine whether metabolic differences are present at an age when blood pressure is technically not in the hypertensive range, (2) to determine whether said metabolic differences contribute to hypertension, and (3) to specify the particular role of insulin resistance in these determinations. A hyperinsulinemic-euglycemic clamp lasting 10 hours was used for estimation of insulin sensitivity and precise measurement of the dynamics of insulin action.

2. Materials and methods

Two groups of subjects were examined: offspring of hypertensives (OH) and healthy controls. Common metabolic and anthropometric characteristics of patients were assessed. To determine insulin sensitivity, a hyperinsulinemic-euglycemic clamp was performed. ^{31}P MRS was used to assess the composition of the musculus soleus.

2.1. Subjects

The OH group consisted of 10 young, lean men, with one or both parents treated pharmacologically for hypertension. They were matched for sex, age, and body mass index (BMI) to a group of 13 healthy controls without a family history of hypertension, diabetes mellitus, obesity, and coronary heart disease. All subjects were in good health, as assessed by medical history, physical examination, and routine laboratory testing, and they were not taking any medication. All had normal serum glucose levels as confirmed by an oral glucose tolerance test (oGTT) using criteria defined by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [15], and 3 values of blood pressure as determined on 3 different days were classified as normal or high normal according to the European Society of Hypertension criteria [16]. Written informed consent was obtained from each participant after the purpose, nature, and potential risks of the study were explained to them. Women were excluded to eliminate potential variations in glucose disposal related to ovarian function. Healthy subjects were recruited from hospital staff and OH were recruited from among the offspring of our hypertensive patients.

Table 1
Characteristics of the study groups

	Offspring of hypertensives (n = 10)	Controls (n = 13)	P
Age (y)	27.4 ± 3.8	26.6 ± 4.8	NS
BMI (kg/m^2)	25.1 ± 3.4	24.4 ± 1.5	NS
WHR	0.89 ± 0.1	0.85 ± 0.1	NS
BPs (mm Hg)	129.7 ± 6.0	115.6 ± 4.0	*
BPd (mm Hg)	78.8 ± 4.7	67.8 ± 5.1	*
BPm (mm Hg)	95.7 ± 4.4	83.8 ± 4.0	*
HbA_{1c} (%)	5.14 ± 0.2	5.08 ± 0.3	NS
Chol (mmol/L)	4.65 ± 1.2	4.46 ± 1.1	NS
HDL (mmol/L)	1.22 ± 0.2	1.11 ± 0.2	NS
LDL (mmol/L)	2.68 ± 1.1	2.6 ± 0.8	NS
TAG (mmol/L)	1.76 ± 0.9	1.76 ± 0.8	NS
sMg (mmol/L)	0.78 ± 0.1	0.83 ± 0.1	NS

Data are means ± SD. NS indicates not significant; BPs, systolic blood pressure; BPd, diastolic blood pressure; BPm, mean blood pressure; Chol, total serum cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

* $P < .001$.

The clinical and laboratory characteristics of the 2 groups are summarized in Table 1.

2.2. Procedures

2.2.1. Oral glucose tolerance test

The oGTT was performed by giving the subjects 75 g of glucose dissolved in water. Blood for measuring plasma glucose level, immunoreactive insulin (IRI) level, and C peptide concentrations were taken from antecubital veins before and at 30-, 60-, and 120-minute intervals after the glucose load.

2.2.2. Hyperinsulinemic-euglycemic clamp

Subjects were examined on an outpatient basis after an overnight fast. They were instructed to avoid alcohol consumption, tobacco smoking, and vigorous exercise the day before examination. A hyperinsulinemic-euglycemic clamp study taking 10 hours was performed to quantify insulin sensitivity as previously described [17].

Briefly, 2 indwelling catheters were inserted, one into an antecubital vein in the right arm for infusions of insulin and glucose and another in a wrist vein for sampling of arterialized venous blood (the hand was placed in a heated box with a temperature of 65°C). After a priming dose of insulin, the rate of the continuous insulin infusion (Actrapid HM, 100 U/mL, Novo Nordisk, Copenhagen, Denmark) was kept at 1 mU · kg^{-1} · min^{-1} (resulting in constant hyperinsulinemia of approximately 75 μU · mL^{-1}). The rate of the 15% glucose infusion was adjusted to maintain fasting levels (about 5 mmol · L^{-1}) based on plasma glucose measurements that were performed every 5 to 15 minutes from arterialized venous blood. Blood samples for the IRI assessment were taken twice in each steady-state period.

2.2.3. Calculations

The sums of the oGTT values, ie, ∑Gly, ∑IRI and ∑Cpep, were calculated by adding the respective glycemia, IRI, and C peptide values obtained at 0, 30, 60, and 120 minutes.

Whole-body insulin sensitivity was calculated from the mean glucose infusion rate needed to maintain euglycemia in 6 steady-state periods during the clamp, divided by body weight and expressed as glucose disposal (M1, minutes 100-120; M2, minutes 180-200; M3, minutes 280-300; M4, minutes 360-380; M5, minutes 480-500; M6, minutes 580-600). The metabolic clearance rate (MCR) was calculated by dividing M by the average glycemia during the respective clamp period. The insulin sensitivity index (M/I) was calculated by dividing each M by the average of 2 IRI concentrations during the respective intervals. A maximal M/M1 index was calculated to assess the dynamics of insulin action. We assumed that hepatic glucose production was completely suppressed during euglycemic hyperinsulinemia at the achieved level of plasma insulin concentration. The glucose infusion rate then reflected the total insulin-stimulated glucose metabolism during hyperinsulinemia.

2.3. Laboratory measurements

Plasma glucose was measured immediately during the clamp by using the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, CA). Plasma IRI concentration was determined by radioimmunoassay using an insulin IRMA kit (Immunotech, Prague, Czech Republic). C peptide was determined by a C peptide IRMA kit (Immunotech). Total serum cholesterol, high-density lipoprotein cholesterol, and serum triglycerides (TAG) were measured by an enzymatic method using CHOD-PAP tests (Hoffmann-LaRoche, Basel, Switzerland). Low-density lipoprotein cholesterol was calculated by using a modified version of the Friedewald formula. Glycosylated hemoglobin (HbA_{1c}) concentrations were measured by ion exchange high-performance liquid chromatography using a Bio-Rad Hemoglobin A_{1c} Column Test

Table 2
Results of oGTT and clamp studies

	Offspring of hypertensives (n = 10)	Controls (n = 13)	P
Gly 0 min (mmol · L^{-1})	4.99 ± 0.5	5.01 ± 0.3	NS
∑Gly (mmol · L^{-1})	23.7 ± 3.1	22.2 ± 2.9	NS
IRI 0 min (mIU · L^{-1})	10.0 ± 4.4	8.85 ± 2.8	NS
∑IRI (mIU · L^{-1})	199.3 ± 97.4	129.8 ± 36.2	NS
Cpep 0 min (pmol · mL^{-1})	0.69 ± 0.2	0.6 ± 0.2	NS
∑Cpep (pmol · mL^{-1})	9.78 ± 0.7	7.24 ± 0.7	*
∑M (mg · kg^{-1} · min^{-1})	62.12 ± 13.3	75.58 ± 16.8	NS
∑MCR (mL · kg^{-1} · min^{-1})	68.38 ± 16.0	82.17 ± 18.3	NS
∑M/I (mg · kg^{-1} · min^{-1} · mIU^{-1} · L^{-1})	0.74 ± 0.47	1.42 ± 0.65	*

Data are means ± SD. Gly indicates glycemia; C pep, C peptide; M, glucose disposal; M/I, insulin sensitivity index.

* $P < .05$.

Table 3
^{31}P MRS parameters

	Offspring of hypertensives (n = 10)	Controls (n = 13)	P
PCr/Pi	7.05 ± 1.21	7.63 ± 1.07	NS
PCr/Patp	1.63 ± 0.08	1.38 ± 0.08	NS
PCr/PME	19.7 ± 8.1	18.84 ± 4.19	NS
PCr/PDE	8.4 ± 3.9	7.58 ± 1.93	NS
PCr/βATP	6.9 ± 1.3	6.47 ± 0.77	NS
Pi/PME	2.75 ± 1.02	2.49 ± 0.56	NS
Pi/PDE	1.21 ± 0.57	1.02 ± 0.27	NS
Pi/βATP	1.00 ± 0.21	0.86 ± 0.15	NS
Pi/Patp	0.24 ± 0.05	0.21 ± 0.03	NS
PDE/βATP	1.03 ± 0.49	0.93 ± 0.24	NS
PDE/PME	2.47 ± 0.77	2.59 ± 0.77	NS
PME/βATP	0.42 ± 0.22	0.36 ± 0.08	NS
Patp/βATP	4.25 ± 0.79	4.08 ± 0.38	NS
Patp/PME	12.03 ± 4.97	11.88 ± 2.54	NS
Patp/PDE	5.11 ± 2.45	4.78 ± 1.18	NS
Mgi	608.8 ± 81.2	632.4 ± 123.9	NS
pH	7.07 ± 0.02	7.06 ± 0.03	NS

Data are means ± SD.

(Bio-Rad Laboratories, Munich, Germany). Serum magnesium concentration was measured by using photometry with xylidin blue.

2.4. Magnetic resonance examination

Magnetic resonance examination was performed after an overnight fast on a whole-body Siemens Vision (Erlangen, Germany) MR scanner operating at 1.5 T. A standard head coil and commercial dual ^{1}H/^{31}P surface coil were used for proton imaging and ^{31}P MRS of musculus soleus. All patients were examined while in the supine position. The MR imaging protocol consisted of multislice transversal turbo spin-echo T1-weighted images with a repetition time (TR) of 500 milliseconds, echo time (TE) of 12 milliseconds, flip angle of 90°, 10 slices with a thickness of 6 mm, 200-mm field of view (FOV); and T2-weighted images with a TR of 5400 milliseconds, TE of 90 milliseconds, flip angle of 180°, 10 slices with a thickness of 6 mm, 200-mm FOV.

For ^{31}P MRS, simple one-pulse sequence free induction decay spectra with nuclear overhouse effect (NOE) enhancement was used (TR, 5 seconds; number of acquisitions, 16; number of points, 512; spectrum width, 4000 Hz). Three spectra were obtained by using standard Siemens Numaris software. The following steps of spectral evaluation were performed for all measured spectra: zero filling, exponential apodization, Fourier transformation, zero- and first-order phase correction, time domain baseline correction, and deconvolution. Deconvolution was performed step by step on each peak to obtain chemical shifts and signal areas. The starting point for deconvolution was a dominating signal of PCr, which was kept at 0.0 ppm. Again, the signal intensities are proportional to the metabolite concentrations. The method was evaluated and tested separately [14]. Intracellular pH and the concentration of free magnesium Mg^{2+} (Mgi) were calculated according to the equations listed in Appendix A [10,11].

2.5. Statistical analysis

Values are reported as means ± SD. Simple and stepwise multiple linear regression analyses were performed to evaluate the associations between anthropometric and metabolic parameters. The 2 study groups were compared using Student t test. A P value ≤.05 was considered statistically significant. Analysis of variance with repeated measures and grouping factor was used to compare the dynamics of insulin action between the groups.

3. Results

The clinical and biochemical characteristics of the 2 groups are presented in Table 1. There were no differences

Table 4
Correlations between selected laboratory and anthropometric parameters and muscle characteristics

	∑Cpep	∑IRI	TAG	Chol	BPs	BPd	WHR	BMI
PCr/Pi	−0.2	−0.27	0.22	0.04	−0.26	−0.15	−0.27	−0.07
PCr/Patp	0.14	0.06	−0.33	−0.27	0.43*	0.08	−0.33	−0.04
PCr/βATP	0.51*	0.53**	0.27	0.05	0.33	0.37	−0.03	0.45*
Pi/Patp	0.23	0.21	−0.34	−0.17	0.413*	0.18	0.11	0.02
Pi/βATP	0.54**	0.60**	−0.02	−0.02	0.48*	0.42*	0.17	0.408
Patp/βATP	0.52*	0.58**	0.44*	0.14	0.21	0.39	0.09	0.54**
PDE/PME	0.27	0.15	0.45*	0.25	−0.28	−0.1	0.03	0.38
Mgi	−0.21	0.1	−0.22	−0.42*	0.1	0.05	−0.44*	0.12
BMI	0.61**	0.68***	0.43*	0.47*	0.28	0.37	0.08	–
WHR	0.36	0.32	0.47*	0.58**	0.15	0.14	–	–
BPs	0.5*	0.51*	−0.08	0.14	–	0.75***	–	–
BPd	0.5*	0.62**	0.13	0.2	0.75***	–	–	–
Chol	0.43*	0.43*	0.66***	–	–	–	–	–
TAG	0.33	0.414*	–	–	–	–	–	–
∑IRI	0.84***	–	–	–	–	–	–	–
∑Cpep	–	–	–	–	–	–	–	–

* $P < .05$.
** $P < .01$.
*** $P < .001$.

in age, BMI, waist-to-hip ratio (WHR), and lipid, HbA_{1c}, and total serum magnesium (sMg) levels. As expected, both systolic and diastolic blood pressures were higher in the OH group, although within a normal or high-normal range ($P < .001$).

Results obtained during oGTT and clamp studies are shown in Table 2. All values were physiologic, but a slight trend toward insulin resistance in OH was evident. In the OH group, lower M/I ($P = .011$) during clamp, and higher $\sum$C peptide ($P = .015$) and $\sum$IRI values ($P = .055$) during oGTT were found. No differences in the dynamics of insulin action were revealed. No significant differences between the groups were observed in the concentrations of metabolites as examined using ^{31}P MRS. Marginally significantly different are the ratios Pi/βATP between the groups ($P = .08$). The data are shown in Table 3.

When linear regression analysis was performed, several significant relationships between clinical and laboratory parameters and phosphate metabolites were noted (Table 4). Systolic blood pressure correlates positively with the ratios PCr/Patp, Pi/Patp, and Pi/βATP. Diastolic blood pressure correlates with the ratio Pi/βATP as well, and a trend toward significant correlation was also registered with the ratio PCr/βATP. $\sum$IRI and $\sum$C peptide values correlate with PCr/βATP and with Pi/βATP. BMI correlates with PCr/βATP, a marginally nonsignificant correlation was observed with Pi/βATP ($r = 0.408$), weight correlates with PCr/βATP ($r = 0.51$; $P < .05$; not shown), and waist circumference correlates with Pi/βATP ($r = 0.44$; $P < .05$; not shown). The PDE/PME ratio correlates positively with weight ($r = 0.454$; $P < .05$; not shown), with C peptide 0′ value ($r = 0.45$; $P < .05$; not shown), and TAG ($r = 0.49$; $P < .05$). Negative correlations between Mgi and WHR or waist circumference ($r = -0.426$; $P < .05$; not shown) and serum cholesterol were found. A close positive correlation was shown between sMg and insulin sensitivity expressed as M ($r = 0.63$; $P < .01$) (Fig 2), MCR ($r = 0.54$; $P < .01$), and M/I ($r = 0.51$; $P < .05$). In stepwise multiple regression analysis, sMg depended predominantly on M ($r = 0.63$). No significant correlations were observed between these parameters and muscle characteristics. A borderline negative correlation was noted between diastolic blood pressure and M ($r = -0.42$; $P < .05$), and a significant negative correlation with M/I ($r = -0.54$; $P < .01$).

In stepwise multiple regression analysis with phosphate metabolites (Table 3) as independent variables entered into the equation, systolic and diastolic blood pressure both depend significantly on Pi/βATP.

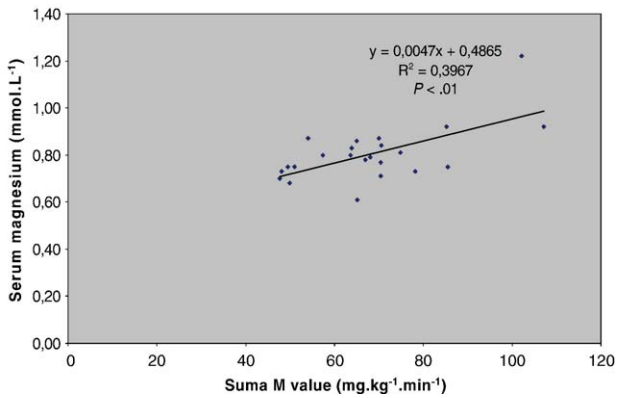

Fig. 2. Relationship between serum magnesium level and insulin sensitivity (M) in the whole group (n = 23).

4. Discussion

Correlations among the metabolic syndrome characteristics such as $\sum$IRI, WHR, BMI, blood pressure, or TAG were shown in our sample. This typical clustering has already been reported in young healthy OH by our group [6]. $\sum$IRI and $\sum$C peptide values obtained in the oGTT seem to be the strongest predictors for predisposition to insulin resistance in individuals with normal glucose tolerance, and these values also closely correlate with blood pressure.

Our data do not show any significant difference in sMg between the OH group and controls, but we found a close positive correlation with insulin sensitivity (M), MCR, or M/I regardless of the predisposition for hypertension. We did not observe any differences in Mgi or any correlation with blood pressure; only weak negative correlations between Mgi and WHR or waist circumference and total serum cholesterol were shown. To the best of our knowledge, no study evaluating this parameter in the offspring of hypertensives has been conducted to date. Although we have not found any differences in sMg or Mgi concentration between the groups, the correlations we have shown are in accordance with expected trends and with experimental and human studies.

Hypomagnesemia is a common feature of diabetes mellitus [18], but only a reduction in Mgi has been reported by some authors [19]. As for intracellular pools, magnesium depletion has been found in erythrocytes [20-24], thrombocytes [24], and lymphocytes [25] in hypertension [20,23,25], type 2 diabetes mellitus [19-22,24,25], obesity [21,24], and even with increasing age [19]. In most cases, the blood pressure level correlated inversely with intracellular magnesium level [20,21,25,26].

Whether hypertension and diabetes act simultaneously in the decrease in Mgi and amplify each other is not clear. A study by Corica et al [24] showed the greatest reduction in Mgi in platelets in the combination of hypertension and diabetes; by contrast, other studies considered diabetes the essential condition in magnesium depletion with no additive effect of hypertension [25]. A differentiation between hypertension as part of the metabolic syndrome and representing a separate disease entity would probably answer the question. Contrasting data were obtained in the study by Sasaki [27], who did not report any reduction in Mgi in the lymphocytes of spontaneously hypertensive rats (SHR).

More relevant to the study of hypertension are the findings of magnesium depletion in vascular smooth muscle cells in the aorta and skeletal muscle of SHR [28-30]. By contrast, Sebekova et al [31] did not find any differences in Mgi levels in skeletal muscle cells in insulin-resistant patients with impaired kidney function, and Hajek et al [14] did not demonstrate any significant difference in Mgi levels in skeletal muscle in hypertensive patients.

Results from studies with myocardial cells in SHR are also inconsistent. The study by Jelicks et al [32] confirmed Mgi depletion in heart muscle cells, whereas the study by Kisters et al [30] did not demonstrate any difference. Conditions with reduced aortal distensibility, which represent a prerequisite for the development of hypertension, are also associated with a decrease in Mgi in skeletal muscle cells [33]. Pressurization of porcine carotid segment leads to an increase in Mgi in arterial smooth muscle [34].

Diabetes and insulin sensitivity substantially affect intracellular magnesium concentrations. Incubation of erythrocytes with insulin increases Mgi in normotensive but not in hypertensive patients [23]. Magnesium depletion can be induced after incubation of normal erythrocytes in a solution with high glucose levels (15 mmol L^{-1}) [23,35], and this treatment results in the blunting of the Mgi response to insulin [20]. Erythrocytes from hypertensive patients do not respond to hyperglycemia by a reduction in Mgi [36]. Hyperglycemia reached after an oral glucose load leads to a reduction in Mgi in red blood cells as well, and Mgi negatively correlates with integrated insulin response obtained in the test [37].

All examined serum magnesium levels in our study were within the physiologic range, but the correlation with insulin sensitivity was still apparent. Serum magnesium levels do not always reflect total body stores. Presumably, magnesium depletion is enhanced with time and may progress from an extracellular form with a "low physiologic level," being already a marker for a future pathology, to an intracellular form. In theory, the intracellular pool should be preferentially preserved while providing most of its biological roles. On the contrary, correlations of Mgi with WHR and waist circumference reported in our study suggest that Mgi might be affected as one of the first parameters during the development of the metabolic syndrome, similarly to the other two characteristics. The effects of other parameters including blood pressure might become apparent with later advance of the underlying disease. A relationship between intracellular magnesium depletion and clinically manifested disease only is supported by the findings by Matuura et al [38], who reported the reversibility of diminished Mgi in SHR by calcium channel blockers in rats, and normal Mgi levels in treated subjects were presented in humans as well [26].

A limitation to our study is that we did not evaluate calcium ion concentration and serum ionized magnesium fraction in parallel, which might better reflect intracellular magnesium pools [18] and probably explain the discrepancy between our Mgi and sMg levels. Because magnesium acts as a cofactor in many enzymatic systems including those involved in insulin and glucose homeostasis, pathologic interference with its action may occur in several regulatory levels. Variations in magnesium handling, either intracellularly or extracellularly, may appear in different stages of the disease.

Detailed studies concerning this topic are needed. The issue of magnesium supplementation may be important for therapeutic potential because it has been shown to reduce blood pressure in rats [39] and improve insulin sensitivity in humans [40].

Skeletal muscles are not directly exposed to the elevation of blood pressure the way smooth muscles in vessels are, but it is generally accepted that they reflect changes occurring in the involved structures. To the best of our knowledge, this is the first report evaluating muscle metabolism by ^{31}P MRS in OH. We have not detected any differences in energetic metabolism between the groups, but we have found several correlations of the muscle energy parameters with the level of blood pressure and with other metabolic characteristics that reflect a higher energy turnover in those likely to develop hypertension. Interpretation of the results is not definite because the method used does not allow the direct assessment of absolute concentrations of the phosphate molecules. The parameters PCr/Patp, PCr/βATP, Pi/βATP, or Pi/Patp reflect muscle energetic status. Our data suggest that an increase in blood pressure is associated with an increased activation of muscle energetics, as is shown by positive correlations with PCr/Patp, Pi/Patp, and Pi/βATP. The most conclusive correlation is that between blood pressure and Pi/βATP, suggesting that an elevation in blood pressure is associated with increased ATP utilization or slower, eventually diminished resynthesis and subsequent Pi accumulation. The effect of systolic blood pressure seems to be substantial. ATP consumption followed by Pi accumulation should be accompanied by a PCr decrease. Our data show a positive correlation between systolic blood pressure and both Pi/βATP and Pi/Patp, but also with PCr/Patp, whereas PCr/Pi does not correlate with blood pressure. PCr pool serves as an energy reservoir and the first compensatory reaction to the chronic loading in the group of young and still prehypertensive subjects may be the neoformation of PCr to keep stable or even higher level of energy substrates as a reserve. A similar mechanism was described in the group of professional cyclists who displayed higher PCr concentration in skeletal muscle as a reaction to intense exercise [41].

Concomitant correlation between PCr/βATP and $\sum$IRI, $\sum$Cpep, BMI, and weight and, analogically, between Pi/βATP and $\sum$IRI, $\sum$Cpep, and waist circumference may point to the alteration in energetic metabolism that is connected with the metabolic syndrome per se, although it may represent not the cause but a consequence of the primary disorder. Our data are in agreement with recent findings by Petersen et al [42], who confirmed impaired

mitochondrial oxidative phosphorylation activity in connection with insulin resistance in elderly patients and in the offspring of patients with type 2 diabetes mellitus [43].

As for the energetic metabolism, most attention has recently been focused on myocardium and clear negative correlations between the PCr/Patp ratio and myocardial dysfunction in connection with dilated cardiomyopathy, valve disease, or ischemic disease, and their partial reversibility with treatment were shown [13]. Neubauer et al [44] showed a decrease in PCr/Patp ratio in subjects with aortic valve disease only in symptomatic patients in New York Heart Association class III or IV. On the contrary, similar changes are detectable in the presence of diabetes even in asymptomatic patients only in connection with diastolic dysfunction [45-47], which again suggests the presence of primary energetic alteration in connection with insulin resistance.

Diastolic dysfunction in hypertensive heart disease in humans is associated with decrease in PCr/Patp ratio as well [48]. Similar results were obtained in studies evaluating cardiac metabolism in SHR and hypertensive baboons [32,49-52] presenting either a decrease in PCr or an increase in Pi, thus confirming an activation of energetic metabolism with a lower ability to resynthetize PCr. However, a study by Beer et al [53] determining absolute concentrations of PCr and ATP in human hypertensive hearts did not demonstrate any differences in PCr and ATP concentrations.

It is not clear whether these findings can be extrapolated to skeletal muscle metabolism, which represents a different muscle type and is not directly exposed to blood pressure elevation. In patients with untreated hypertension, lower PCr levels in skeletal muscle were determined at rest, whereas exercise induced a greater decrease in and a slower regeneration of PCr and also a greater decrease in and slower regeneration of ATP/Pi similarly to the heart metabolism [54]. A study by Hajek et al [14] showed an increase in PCr/Pi, PCr/βATP, PCr/Patp, and PDE/βATP ratios and a decrease in Pi/PDE, PCr/PME, and Pi/Patp ratios in the skeletal muscle of hypertensive individuals, and Sebekova et al [31] showed a lower ATP/Pi ratio and a higher PCr/ATP ratio in the skeletal muscle of insulin-resistant patients with impaired kidney function. Lower phosphate content was found in the aortic smooth muscle cells in SHR [30].

Experiments designed to evaluate the role of insulin in muscle phosphate metabolism have been conducted: Taylor et al [55] showed an increase in Pi after insulin infusion in human skeletal muscle cells and a decrease in plasma, whereas Fisher et al showed an increase in PCr and a decrease in Pi concentration when perfusing rat bladder with insulin [56]. Mgi levels have a direct impact on the concentrations of energy macromolecules in SHR, a low magnesium perfusion rate of the rat heart resulted in decreased Mgi, decreased pH, ATP, and PCr, and in increased Pi and, hence, a reduced intracellular phosphorylation potential and a decreased cardiac output in rats [57]. An altered energy metabolism is present in other tissues in hypertensive and diabetic patients because lower ATP levels were shown in erythrocytes as well [58].

PDE levels rise with age and reflect the replacement of myofibers with fat tissue [59], whereas PME peak strikes early in life and gradually disappears [60]. The correlation of PDE/PME with weight, C peptide 0′ value, and TAG indicates that it is probably not only age that participates in the process.

Our data are in a preliminary form and need further confirmation. The incidence of hypertension will be assessed in our group in a prospective study. Despite no significant differences found between OH and controls, several significant relationships between insulin sensitivity, blood pressure, sMg, and skeletal muscle characteristics were found, suggesting an alteration in energetic metabolism in skeletal muscle in those likely to develop hypertension. We conclude that energy metabolism, insulin resistance, and Mg homeostasis appear to be linked in OH subjects with the pathogenesis of hypertension.

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Appendix A

The chemical shifts of PCr and Pi were used to calculate intracellular pH according to the equation [12]: $pH = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$, where δ (in parts per million) refers to the chemical shift of the Pi peak relative to PCr.

The concentration of free magnesium Mg^{2+} (Mgi) was calculated by using the following equation [10,11], based on the difference of chemical shifts between α and β signals of ATP: $Mgi = K_D \{(\delta_{\alpha\beta obs} - \delta_{\alpha\beta ATP})(1 + [H^+]/K_H) + [H^+]/K_H (\delta_{ATP} - \delta_{\alpha\beta ATPH})\}/(\delta_{ATPMg} - \delta_{\alpha\beta obs})$, where $\delta_{\alpha\beta obs}$ is the chemical shift difference of α_{ATP} and β_{ATP} signals, and $\delta_{ATP} = 10.6$ ppm, $K_H = 3.4 \times 10^{-7}$ mol/L, $K_D = 9.0 \times 10^{-5}$ mol/L, $\delta_{ATPMg} = 8.165$ ppm, and $\delta_{\alpha\beta ATPH} = 11.66$ ppm (constants taken from the literature).

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