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Wst[^]p

Wyrazamy nadziej[^], ze zroznicowany program **Journal of Education, Health and Sport formerly Journal of Health Sciences** b[^]dzie odpowiadal Panstwa oczekiwaniom. Wierzmy, ze **Journal of Education, Health and Sport formerly Journal of Health Sciences** przyczyni si[^] do podnoszenia wiedzy, kwalifikacji i umiej[^]tnosci lekarzy, rehabilitantow, fizjoterapeutow, piel[^]gniarek, psychologow, biologow, praktykow i badaczy zainteresowanych ochron[^] zdrowia pracowników rehabilitacji, fizjoterapii, turystyki i rekreacji.

Journal of Education, Health and Sport formerly Journal of Health Sciences, odpowiadaj[^]ca na wspolczesne swiatowe wyzwania zdrowotne, gromadzi artykuly specjalistow z tych dziedzin z wiod[^]cych, renomowanych osrodkow zagranicznych i krajowych. Wielu z nich przedstawia state of art w swojej dziedzinie. B[^]dzie to szczególnie cenne dla mlodych lekarzy w trakcie specjalizacji, oraz studentow.

Mile widziani do zapoznania si[^] z t[^] problematyk[^] wszystkich zainteresowanych zagrozeniami i ochron[^] zdrowia, zycia i bezpieczenstwa w pracy w turystyce, rekreacji, rehabilitacji, fizjoterapii, piel[^]gniarstwie organizaj bezpiecznej pracy i misji w tych warunkach, wpływem warunkow srodowiska na stan zdrowia publicznego.

Autorzy z zagranicy i kraju przedstawia[^] przegl[^]d wspolczesnych wyzwan i proponowanych rozwi[^]zan w tych dziedzinach. Problematyka tekstow prac dotyczy szeroko rozumianego zdrowia czlowieka, turystyki, rekreacji, fizjoterapii, piel[^]gniarstwa, odnowy biologicznej i rehabilitacji, rowniez ekonomiki ochrony zdrowia.

Zawartosc tego czasopisma jest obj[^]ta licencj[^]
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INFLUENCE OF EXPERIMENTAL METABOLIC SYNDROME ON BIOIMPEDANCE SPECTROSCOPY INDICATORS OF RAT BODY

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Abstract

The objective of this experiment was to establish sex-related differences in the parameters of bioimpedance spectroscopy of the Wistar rat's bodies in the experimental metabolic syndrome (endocrine-salt model).

Sexual dependencies of bioimpedance measurements in intact animals have been determined for the first time: males have higher amount of total fluid, but lower one of total fat. The intra/extracellular fluid balance in males is characterized with ratio 2:1, while in females is the other one - 1:1.

For the first time the formation of the metabolic syndrome has already been determined on the seventh day in females which lead to decreasing in the percentage of total fat and to changing of the intra / extracellular fluid balance to 'male' type - 2:1. The last one should be considered as a sign of intracellular hyperhydration. In males the examined parameters have been being within the control values throughout 21 days of experiment.

Key words: bioimpedance spectroscopy; sex-related differences; metabolic disorders; corticosteroids; rats.

ВПЛИВ ЕКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛІЧНОГО СИНДРОМУ НА ПОКАЗНИКИ БІОІМПЕДАНСНОЇ СПЕКТРОСКОПІЇ ТІЛА ЩУРІВ

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Метою цієї роботи стало встановлення статевих відмінностей показників біоімпедансної спектроскопії тіла щурів ліній Wistar при експериментальному метаболічному синдромі (ендокринно-сольова модель).

Для реалізації поставленої мети, а саме встановлення статевих відмінностей показників біоімпедансної спектроскопії тіла щурів ліній Wistar при експериментальному метаболічному синдромі (ендокринно-сольова модель) був проведений комплекс досліджень, який включав: дослідження показників маси тіла, артеріального тиску і частоти серцевих скорочень у інтактних тварин; визначення показників маси тіла, артеріального тиску і частоти серцевих скорочень у тварин з змодельованим метаболічним синдромом; дослідження показників біоімпедансної спектроскопії (обсяги загальної, внутрішньо-і позаклітинної рідини, маса вільного жиру) у інтактних тварин; дослідження показників біоімпедансної спектроскопії (обсяги загальної, внутрішньо-і позаклітинної рідини, маса вільного жиру) у тварин з змодельованим метаболічним синдромом.

Вперше визначено статеві залежності показників біоімпедансметрії у інтактних тварин: у самців більш високі показники загальної рідини, але більш низькі загального жиру. Баланс внутрішньо/позаклітинної рідини у самців характеризується співвідношенням як 2:1, тоді як у самиць як 1:1.

Вперше визначено формування метаболічного синдрому, вже на 7-у добу у самиць призводить до зниження відсотку загального жиру та до змін балансу внутрішньо/позаклітинної рідини за «чоловічим» типом -2:1, що слід розглядати як ознаку внутрішньоклітинної гіпергідратації. У самців - досліджувані показники залишаються в межах контрольних значень протягом всього дослідження до 21 -ї доби.

Ключові слова: біоімпедансна спектроскопія; статеві відмінності; метаболічні порушення; кортикостероїди; щури.

ВЛИЯНИЕ ЭКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛИЧЕСКОГО СИНДРОМА НА ПОКАЗАТЕЛИ БИОИМПЕДАНСНОЙ СПЕКТРОСКОПИИ ТЕЛА КРЫС

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Целью этой работы стало установить половые различий показателей биоимпедансной спектроскопии тела крыс линий Wistar при экспериментальном метаболическом синдроме (эндокринно-солевая модель).

Впервые определены половые зависимости показателей биоимпедансметрии у интактных животных: у самцов более высокие показатели общей жидкости, но более низкие общего жира. Баланс внутренне / внеклеточной жидкости у самцов характеризуется соотношением как 2:1, тогда как у самок как 1:1.

Впервые определено формирование метаболического синдрома, уже на седьмой день у самок приводит к снижению процента общего жира и к изменениям баланса внутренне / внеклеточной жидкости за «мужским» типом - 2:1, что следует рассматривать как признак внутриклеточной гипергидратации. У самцов - исследуемые показатели остаются в пределах контрольных значений в течение всего исследования до 21 суток.

Ключевые слова: биоимпедансная спектроскопия; половые различия; метаболические нарушения; кортикостероиды; крысы.

Background and rationale. Bioelectrical impedance analysis of body composition is widely used in sports and clinical medicine now. This method is based on the ability of tissues to conduct electric current. Tissue electrical resistance depends on their water content. So, tissues with a high degree of hydration (muscle) act as current conductors, whereas less hydrated (adipose tissue) are isolators [1]. Bioimpedance analysis of body composition provides an opportunity to monitor the state of lipid, protein and water metabolism, assess the risk of metabolic syndrome development and estimate the total volume of body fluid [5]. This technique is able to assess changes in water balance, body fluid redistribution and medicines choice for cardiovascular disease patients [6].

According to the WHO statistics, such diseases as type 2 diabetes mellitus (DM), obesity, atherosclerosis, hypertension and metabolic Syndrome X are widespread among

Ukrainian population today, while the number of patients has continued to rise steadily and their age is getting younger [2].

Today, the links between glucocorticoids and the diseases listed above, regardless of the nosology, remains debatable [2]. Scientists from Fondazione Policlinico and the University of Milan found that cortisol secretion is increased in patients with type 2 DM due to complications and impaired metabolic control [9]. Analysis of the obtained results has revealed an increase in hypothalamic-pituitary-adrenal activity in patients with complicated DM, and the increased level of cortisol secretion was associated with the presence and number of chronic complications [9]. The role of cortisol as a factor for chronic complications development in DM deserves highlighting. The risk factors for vascular complications include not only specific ones like hyperglycemia and hyperinsulinemia, but also common for the population - arterial hypertension, dyslipidemia, obesity, heredity, age, and so on.

According to Dr. Biley M., hypertension is known to be multifactorial in etiology and increases mortality from cardiovascular diseases, type 2 DM, and cancer as a component of metabolic syndrome [8]. The prevalence of these comorbidities is about 25-30% in the world, so hypertension in the metabolic syndrome is a global healthcare issue. Clinical and preclinical studies provide evidence that glucocorticoid excess is an underlying cause of metabolic syndrome due to increase in active tissue glucocorticoids.

Another determining factor of DM development is obesity. The role of glucocorticoids in fat metabolism disorders has been long known. According to the experiment results of researchers from Anhui Medical University, the level of hair cortisol concentration was directly proportional to the child weight and girls aged 6-9 years had a higher level of hormone concentration than their opposite-sex peers [7].

The scientific literature review clearly shows that a detailed study of the sex-related differences in the bioimpedance spectroscopy (BIS) of the Wistar rats body in experimental metabolic syndrome (endocrine-salt model) would provide answers to the following questions: 1. Are there any differences in the body BIS between male and female rats? 2. How the indicators of water balance and fat metabolism will change in the metabolic disorders? Thus, the purpose of our work was to identify sex-related differences in the BIS indicators of Wistar rats body in the experimental metabolic syndrome (endocrine-salt model).

Materials and methods

A total of 40 Wistar rats (20 males and 20 females), aged 8 months and weighing 180-200 g were used.

A complex study was carried out to achieve the goals and included:

1. Study of the BW, BP and HR indicators in intact male and female Wistar rats aged 8 months.
2. Determination of the BW, BP and HR indicators in male and female Wistar rats aged 8 months with modeled metabolic syndrome.
3. Study of BIS parameters (total, intracellular and extracellular fluid volumes, free fat mass) in intact male and female Wistar rats aged 8 months.
4. Study of BIS parameters (total, intracellular and extracellular fluid volumes, free fat mass) in male and female Wistar rats aged 8 months with modeled metabolic syndrome.

The animals were divided into 4 groups: the groups 1 and 2 were control males and females Wistar rats, respectively, (10 animals in each). The similar groups 3 and 4 were with experimental metabolic syndrome. Body weight (BW), heart rate (HR) and blood pressure (BP) were measured and body BIS was performed on day 7 and 21 of the experiment.

Experimental model of metabolic syndrome. From the 1st day of hormonal and metabolic disorders modeling, animal were fed a standard chow rich in hydrogenated vegetable fats (margarine) in the amount of 5% of the total mass. Tap water was changed to 20% fructose solution as the water source on alternate day. A two-stage dexamethasone administration was performed via subcutaneous injection at a dose of 0.125 mg/kg body weight at 8 p.m. from the 1st to 7th days and from the 13th to 20th days of the experiment [3].

To assess the state of carbohydrate metabolism in the animals fasted for 10 - 14 hours, plasma glucose level was measured using a glucometer Glucocard II (ARKRAY Inc., Japan) by glucose oxidase-based test strips in drops of blood taken from the tail vein on days 1, 7 and 21 of the experiment.

The study of BP and HR in rats. The non-invasive blood pressure analyzer BP-2000 Blood Pressure Analysis System™ (Visitech Systems, USA) was used for BP and HR measurements. The rats were placed in individual restraint holders on a thermostatic platform at 37-39⁰C on days 1, 7 and 21. The average time of BP reading was 3-7 minutes in order to measure 3-5 preliminary cycles followed by 3-5 effective measurements with the average values of HR, systolic and diastolic pressure calculation.

BIS measurements. BIS was performed using the ImpediVet BIS1 system (ImpediMed Limited, Australia) on days 1, 7 and 21. The weight of rats and body length measured from the tip of the nose to the base of the tail were entered into the BIS analysis system for automatic calculation of body mass index (BMI). Animals were anesthetized

(thiopental sodium 45 mg/kg) for the electrodes insertion via hypodermic needles according to the standard scheme. After the matching color-coded leads were attached, 3-5 consecutive impedance measurements were taken with the average value calculation [4].

Statistical analysis. All experimental data were processed using the VIDAS-2.5 application and statistical software package (Kontron Elektronik, Germany) and EXCEL 7.0 (Microsoft Corp., USA). All indicators were presented as mean(M) ± standard error of the mean (m) and its variance. Student's t-test was used in order to evaluate the significance of differences between the study results of the experimental and control groups of rats, and then the probability of the difference in samples (p) and the confidence interval of the mean were determined according to the Student's distribution tables. A p value < 0.05 was considered statistically significant.

Results and discussion. The study of the sex characteristics of body composition (bioimpedancemetry), BW and physiological parameters (systolic BP (Ps), diastolic BP (Pd), HR) has revealed significant differences in Pd and HR of both male and female control groups of animals (Table 1).

Table 1 - BW, BP and HR indicators of the experimental rats at day 7 of metabolic syndrome modeling (M ± m)

Experimental groups	BW, g	BP, mmHg		HR, bpm
		Ps	Pd	
Control group, males (n=10)	197.7±1.96	117.2±3.07	60.9±2.13	334±2
Control group, females (n=10)	193.6±1.15	118.7±2.84	68.7±1.78 ¹	353±2 ¹
Metabolic syndrome, males (n=10)	218.0± 1.33 ²	143.1±1.64 ²	80.0±2.38 ²	366±4 ²
Metabolic syndrome, females (n=10)	225.1±1.14 ¹²	136.7±2.19 ^{1,2}	86.1±1.38 ¹²	384±5 ^{1,2}

Notes: 1. (1) significant difference (p < 0.05) between indicators in the female and male groups;

2. (2) significant difference (p < 0.05) between indicators when compared to the sex-matched control group.

At the same time, despite the fact that indicators of HR and Pd were within normal (physiological) range, Pd and HR were 12.8% (p < 0.05) and 5.6% (p < 0.05) significantly higher, respectively, in female than in male rats (Table 1).

Metabolic syndrome formation in the female and male rats was characterized on day 7 by a significant increase in all the studied parameters: BW, BP and HR. Thus, in males with metabolic syndrome an increase in BW by 10.6% (pst <0.05), Ps by 22% (pst <0.05), Pd by 31.3% (pst <0.05), HR by 9.5% (pst <0.05) was noted. An increase in all studied parameters was also found in the group of female rats on day 7 of metabolic syndrome modeling compared to their matched control group: BW increased by 16.3% (pst <0.05), Ps by 15.2% (pst <0.05), Pd by 25.3% (pst <0.05), HR by 8.7% (pst <0.05). When comparing the studied parameters in female and male rats with metabolic syndrome on day 7, it was observed that male rats were more likely to have increased Ps, whereas female rats - increased Pd, HR and BW (see Table 1).

BIS of the animal body (water balance and fat free mass) revealed significant sex differences in the control group rats. It was found that total fluid volume in males was significantly 38.3% (pst <0.05) higher than that in female rats. Measurement of the intracellular and extracellular fluid balance showed an important trend. Thus, an almost double increase in intracellular fluid was detected in males, whereas female rats represented a larger percentage of extracellular fluid, and the extracellular to intracellular fluid volume ratio was about 1:1. The percentage of total body fat mass reached almost half of BW in females, whereas it was no more than 30% in male rats (Table 2).

Table 2 - Bioimpedance spectroscopy indicators of the experimental rats at day 7 of metabolic syndrome modeling (M ± m)

Experimental groups	Total body fluid, %	Intracellular fluid, %	Extracellular fluid, %	Total body fat mass, %
Control group, males (n=10)	53.97±5.21	64.21±3.76	36.78±3.78	27.63±7.12
Control group, females (n=10)	39.0±4.76 ¹	47.51±6.29 ¹	52.48±6.29 ¹	46.71±5.15 ¹
Metabolic syndrome, males (n=10)	51.93±3.65	53.6±2.03	45.39±2.03 ²	29.04±4.99
Metabolic syndrome, females (n=10)	53.48±4.93 ²	62.39±3.64 ^{1,2}	36.6±3.64 ^{1,2}	29.66±3.39 ²

Notes: 1. (¹) significant difference (pst <0.05) between indicators relative to the sex-matched control group;

2. (²) significant difference (pst <0.05) between indicators relative to the opposite-sex group.

On the 7th day of metabolic syndrome development in male rats, the ratio of water balance parameters continued at the same level, the total body fluid and intracellular fluid did

not significantly differ from the control indicators, the percentage of extracellular fluid was 23.4% higher than in the control group. Nevertheless, the water balance remained constant with the intracellular fluid prevalence over the extracellular fluid (see Table 2).

The changes in water balance parameters of female rats on day 7 of metabolic syndrome formation were somewhat surprising. An inversion of the internal and extracellular fluid indicators was observed. Instead of the extracellular fluid prevalence, there were reliable signs of intracellular hyperhydration due to a significant 31.3% increase in the percentage of intracellular fluid and a 43.3% decrease in the extracellular fluid (see Table 2).

The total body fat mass on the 7th day of metabolic syndrome modeling in male rats did not significantly change, whereas it was significantly 60.8% decreased in female rats (see Table 2).

BW, BP and HR remained continuously increased by the end of experimental metabolic syndrome modeling (on the 21st day) in both male and female rats. At the same time, BW, Ps, Pd and HR were increased in male rats by 26.7% (pst <0.05), 44.7% (pst <0.05), 67.6% (pst < 0.05) and 9.6% (pst <0.05), respectively, compared to the matched control. In addition, the same indicators were increased in female rats by 25.8% (pst <0.05), 30.7% (pst <0.05), 62.2% (pst <0.05) and 8.4% (pst <0.05), respectively, (Table 3).

Table 3 - BW, BP and HR indicators of the experimental rats at day 21 of metabolic syndrome modeling (M ± m)

Experimental groups	BW, g	BP, mmHg		HR, bpm
		Ps	Pd	
Control group, males (n=10)	195.01±2.67	115.1±2.96	62.8±1.93	333±3
Control group, females (n=10)	196.3±3.94	117.4±3.35	69.4±1.97 ²	356±4 ²
Metabolic syndrome, males (n=10)	247.1±1.72 ¹	166.6±2.77 ¹	105.3±2.26 ¹	365±3 ¹
Metabolic syndrome, females (n=10)	254.2±1.08 ^{1,2}	153.5±1.47 ^{1,2}	112.6±1.85 ^{1,2}	385±5 ^{1,2}

Notes: 1. (¹) significant difference (pst <0.05) between indicators relative to the sex-matched control group;

2. (²) significant difference (pst <0.05) between indicators in the female and male groups.

Comparative analysis of BW indicators and functional parameters (Ps/Pd, HR) in male and female rats with the developed metabolic syndrome on day 21 of modeling showed that male rats had significantly higher Ps than female rats, while the females showed higher indicators of BW, Pd and HR (see Table 3).

BIS demonstrated that all the studied parameters stabilized at the control group level, the water balance indicators returned to normal values in male rats at the end of metabolic syndrome modeling on day 21. Whereas in female rats the total body fluid decreased compared to day 7, and the extracellular to intracellular fluid volume ratio remained unchanged, moreover, it was entirely consistent with the distribution in male rats (Table 4).

Table 4 - Bioimpedance spectroscopy indicators of the experimental rats at day 21 of metabolic syndrome modeling (M ± m)

Experimental groups	Total body fluid, %	Intracellular fluid, %	Extracellular fluid, %	Total body fat mass, %
Control group, males (n=10)	52.88±4.99	63.19±3.69	35.98±3.45	27.45±6.14
Control group, females (n=10)	39.4±4.67 ¹	46.99±5.3 ¹	52.85±5.9 ¹	48.01±4.47 ¹
Metabolic syndrome, males (n=10)	52.98±4.66	64.0±3.87	37.99±3.87	33.59±2.57
Metabolic syndrome, females (n=10)	47.27±5.33 ²	62.14±2.23 ²	37.85±2.23 ²	31.0±5.81 ²

Notes: 1. (¹) significant difference (p < 0.05) between indicators relative to the sex-matched control group;

2. (²) significant difference (p < 0.05) between indicators relative to the opposite-sex group.

Conclusions. 1. BW, BP and HR indicators are within normal (physiological) range but influenced by sex in rats of the control groups without metabolic disturbances: Pd and HR is 12.8% and 5.6% higher, respectively, in female rats than these indicators in male rats.

2. Metabolic syndrome development due to corticosteroid excess in rats of both sexes is characterized by a dynamic (from day 7 until day 21) increase in BW, BP and HR, but changes in studied parameters are sex-associated: male rats have more significant increase in Ps, while female rats - BM, Pd and HR.

3. Bioimpedance spectroscopy measurements of the water balance and body fat mass have showed that the control male rats have higher indicators of total body fluid, but lower values of total body fat mass. The extracellular to intracellular fluid volume ratio is 2:1 in male rats, whereas it is 1:1 in female rats.

4. Metabolic syndrome development on study day 7 is characterized by decrease in body fat mass in female rats and changes in the extracellular to intracellular fluid volume ratio following the "male" pattern - 2:1, which should be considered as a sign of intracellular hyperhydration. The studied parameters remain within the control values in male rats throughout the entire study.

Prospects for the further development in this regard. The next stage of our research would be aimed at studying the molecular mechanisms of gasotransmitters CO and H₂C action in pancreatic tissues in the development of experimental metabolic syndrome.

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