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REVIEW / PRACA POGLĄDOWA

Rafał Donderski, Magdalena Grajewska, Elżbieta Marcinkowska, Jacek Manitius

FUNCTIONAL AND STRUCTURAL CHANGES OF PERITONEAL MEMBRANE IN PERITONEAL DIALYSIS PATIENTS. CONTEMPORARY THERAPEUTIC APPROACH

ZABURZENIA FUNKCJI I ZMIANY STRUKTURY BŁONY OTRZEWNOWEJ U CHORYCH DIALIZOWANYCH OTRZEWNOWO. AKTUALNE MOŻLIWOŚCI TERAPEUTYCZNE

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S u m m a r y

Peritoneal dialysis is a contemporary form of renal replacement therapy. Prolonged peritoneal dialysis program is associated with functional and structural alterations of peritoneal membrane, related mainly to the use of bioincompatibile fluids. It poses an important clinical issue in peritoneal dialysis patients. Conventional peritoneal fluids can cause ultrafiltration failure and lower dialysis efficiency. In this review, we focused on mechanisms of peritoneal damage. Moreover, we presented contemporary therapeutic approach prolonging peritoneal membrane viability.

S t r e s z c z e n i e

Dializa otrzewnowa jest stosowaną współcześnie formą leczenia nerkozastępczego. Niezwykle ważnym problemem klinicznym u chorych dializowanych otrzewnowo są zmiany struktury i funkcji błony otrzewnowej zachodzące m.in. pod wpływem ekspozycji na bioniezgodne płyny dializacyjne. Powoduje to spadek ultrafiltracji otrzewnowej i obniża efektywność dializoterapii. W pracy przedstawiono mechanizmy prowadzące do uszkodzenia błony otrzewnowej. Omówiono także aktualne możliwości terapeutyczne pozwalające na wydłużenie jej żywotności.

Key words: peritoneal dialysis, dialysis fluids, functional and structural abnormalities in peritoneal membrane
Słowa kluczowe: dializa otrzewnowa, płyny dializacyjne, zaburzenia struktury i funkcji błony otrzewnowej

INTRODUCTION

Peritoneal dialysis is a contemporary form of renal replacement therapy (RRT). Results of peritoneal dialysis treatment are satisfactory in the early 2-3 years from its commencement, but after 4-5 years almost 50% of patients require commencement of hemodialysis therapy, because of deterioration of peritoneal membrane and peritoneal ultrafiltration failure. Other crucial reasons for discontinuation of peritoneal dialysis are recurrent peritonitis and progressive residual function loss. Moreover, standard peritoneal dialysis fluids are highly bioincompatible.

This contributes to progressive peritoneal membrane damage. Standard peritoneal dialysis fluids are characterized by low pH, high glucose concentration, and high level of toxic glucose degradation products (GDPs) [1, 2, 3]. The consequences of long term peritoneal dialysis procedures and prolonged peritoneal membrane exposure to high glucose concentration fluids are functional and structural derangements of peritoneal membrane. This may lead to gradual increment of membrane permeability to small solutes, osmotic

gradient loss and ultrafiltration failure with clinical signs of overhydratation. Besides peritonitis, ultrafiltration failure seems to be one of the main problems in chronic peritoneal dialysis patients [1, 3].

STRUCTURAL AND FUNCTIONAL PERITONEAL MEMBRANE DERANGEMENTS AND MECHANISMS LEADING TO ITS INJURY

Peritoneal membrane is a natural dialysis membrane. When it comes to uremic toxins and water elimination, the efficiency of peritoneal dialysis depends on structural and peritoneal membrane integrity. The loss of peritoneal integrity and its natural function is related to the sort of dialysis fluids. It should be mentioned that peritoneal membrane damage leading to hyperpermeability is observed in early stages of kidney disease (in predialysis phase) and in fact it precedes damage related to the exposure to dialysis fluids. Peritoneal membrane, as well as other serous membranes permeability increases, is observed not only in chronic kidney disease patients on conservative treatment but also in peritoneal and hemodialyzed patients.

Peritoneal membrane abnormalities appear in uremic environment. Moreover, they are related to recurrent peritonitis episodes, bioincompatible standard fluids usage, and others factors and mechanisms. Chronic inflammatory state (closely related to bioincompatible peritoneal fluids), oxidative stress, and generation of advanced glucose and lipid degradation end products, that can accumulate in peritoneal membrane play a significant role in peritoneal membrane damage. Due to prolonged peritoneal fluids storage and heat sterilization, accumulation of many GPDs like formaldehyde, glyoxal, methylglyoksal takes place. These chemical substances increase local AGEs and VEGF (vascular endothelial growth factor) production. AGEs in turn exacerbate inflammation, neoangiogenesis, increase transperitoneal protein loss, intensify proliferation of vascular smooth muscle and peritoneal NO and TGF-β production which in turn enhance peritoneal fibrosis. AGEs also decrease mesothelial cells vitality. Incidence of bacterial peritonitis, peritoneal bleeding may also contribute to the pathogenesis of peritoneal membrane damage. In the course of acute bacterial peritonitis due to NO, some proimflammatory cytokines (IL-1β, TNFα, IL-6) and prostaglandins are produced and increment in effective peritoneal membrane area and its hyperpermeability is observed. Recurrent peritonitis can lead to hyperpermeability of peritoneal membrane and some morphological changes such as submesothelial fibrosis and neoangiogenesis can be revealed. Early morphological changes in peritoneal membrane may occur 8-10 months after commencement of peritoneal dialysis. They can be described as: microvilli and intracellular junction loss. Subsequently loss of mesothelial cells is observed and some parts of peritoneal membrane surface can be denuded of these cells (it may be reported even 3 months after commencement of peritoneal dialysis). Besides mesothelial cell loss, after 12 months or more from peritoneal dialysis commencement, thickening of mesothelial basement membrane, transition of mesothelial into myofibroblasts (and also into endothelial, muscle, bone and cartilage cells) and submesothelial layer changes are reported [4, 5, 6]. These changes include: submesothelial layer thickening with extracellular matrix and collagen IV accumulation and its local oedema. The thickness of this layer may vary from 180 μm (if patients are dialyzed <24 months) to even 600 μm in cases of patients dialyzed for over 8 years. Normal peritoneal membrane thickness in healthy adults is 40 μm. Moreover, neongiogenesis and progressive oblitherative vasculopathy is observed [5, 6, 7]. These structural changes correlate with time on dialysis. Predominantly, myofibroblasts are involved in fibrosis (myofibroblasts originate from mesothelial cells), they are considered to be main extracellular matrix regulators. From morphological point of view there are 2 types of peritoneal membrane fibrosis: simple peritoneal sclerosis (reported in most patients) and sclerosis encapsulating peritonitis, which is a rare, but serious peritoneal dialysis complication, that may occur in approximately 2.5% of patients [8]. Because of morphological and functional changes in peritoneal membrane, enhanced peritoneal transfer of small solutes may occur. It is related to enhanced effective peritoneal vascular area as a consequence of small vessels proliferation. At the same time, it is the main reason for ultrafiltration failure and overhydration signs in these patients. This situation leads to the necessity of change from peritoneal dialysis to hemodialysis therapy.

CELLULAR LEVEL ASPECTS OF PERITONEAL MEMBRANE DAMAGE. CYTOKINES AND GROWTH FACTORS ROLE

Numerous locally synthesized cytokines, chemokines and growth factors play the essential role in peritoneal membrane degeneration. They all lead to the increase of effective vascular area and fibrosis. Substantial role in these processes is played by produced by mesothelial and endothelial cells proangiogenic factors – VEGF and proinflammatory

cytokines produced by peritoneal macrophages, neutrophils. Increased eNOS activity – main vascular tone regulator and hyperpermeability factor is also detected. High glucose concentration in peritoneal fluids exert proinflammatory and profibrotic effect (via stimulation of TGF-β1 and protein kinase C), increases free radicals generation and local angiotensin II production intensifying fibrosis. The role of the produced by mesothelial cells profibrogenic factors i.e. TGF-β1 (transforming growth factor-beta1), bFGF (basic fibroblastic growth factor), fibronectin, PGF, (platlet growth factor), IGF-1 (insulin-like growth factor-1), HGF (hepatocyte growth factor), EGF (epidermal growth factor), is well documented in many papers [9]. Mesothelial cells generate some extracellular matrix components such as: fibronectin, cytokeratin, vimentin, actin, laminin, collagen and many others. The interaction between AGEs and its receptor RAGE is responsible for mesothelial cell activation and TGF-β production. TGF-β plays substantial role in fibroblast activation, collagen deposition, forced fibrosis by matrix metalloproteinase inhibition and it is responsible for mesothelial into myofibroblasts transdifferentiation and neoangiogenesis [9, 10]. TGF-β is considered to be VEGF, PAI-1, and extracellular matrix synthesis regulator.

In pathogenesis of peritoneal membrane damage many cytokines such as VEGF play a role. VEGF causes increased pathological angiogenesis and vessels hyperpermeability. Increased VEGF expression on endothelial cells is reported and main stimuli for VEGF release are: local hypoxia, proinflammatory cytokines and growth factors. VEGF interacts with other active substances on endothelial cell surface, activates eNOS and causes release of bFGF which also plays a role in pathological angiogenesis. VEGF is detected in peritoneal eluent and its concentration correlates with peritoneal membrane permeability to small solutes and ultrafiltration loss [7, 10, 11, 12].

The role of local and systemic renin-angiotensin-system (RAS) in peritoneal fibrosis and neoangiogenesis should be noted. Angiotensis II is involved in VEGF, angiopoetin, TGF-β synthesis and release [12]. In vitro studies on losartan showed its inhibition of TGF-β expression on mesothelial cells exposed to high glucose concentration. It seems that pharmacological inhibition of RAS is a valuable therapeutic option that allows for longtime peritoneal membrane protection. Deficiency of natural antioxidants (low level of glutathione and vitamin C) can exacerbate oxidative stress and peritoneal damage. An important role in pathological angiogenesis and fibrosis numerous cytokines, such as IL-1β and TNF-α play [12, 13]. Suggested mechanisms of peritoneal membrane damage are presented in figure 1.

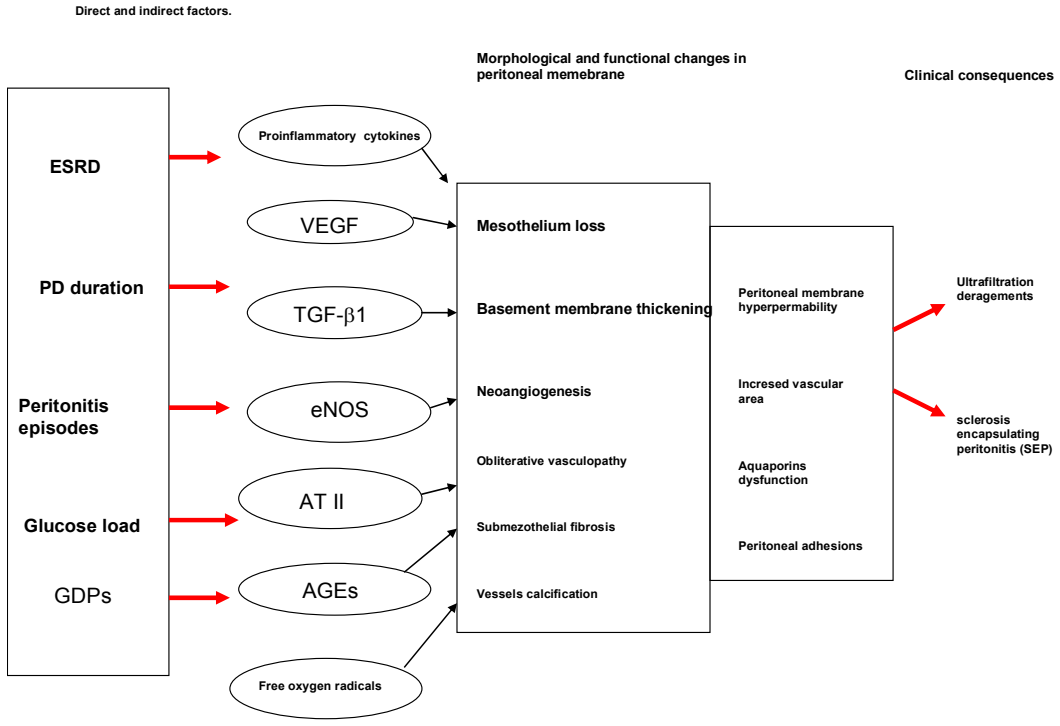

Fig. 1. *Peritoneal membrane damage mechanisms and their consequences*

GENETIC FACTORS IN PERITONEAL MEMBRANE PERMEABILITY DISTURBANCES. THE GENE THERAPY POSSIBILITIES

Without any doubt, recent years have brought development in biotechnology and genetic engineering. The gene identification, gene transcellular transfer and genetic manipulation that can improve cellular function became possible. As it was previously mentioned, peritoneal fibrosis and angiogenesis are related to dialysis fluids used but they are observed in predialysis period as well. The level of these changes determine peritoneal dialysis therapy success in future. It seems plausible that progression of peritoneal membrane deterioration may be related to selected gene expressions [2, 5]. Individual level of some profibrogenic and angiogenic gene expression evaluated before PD commencement may decide of future success of this kind of RRT. From the clinical point of view it may be interesting to compare gene expression in selected groups of patient with varying of etiology of chronic kidney disease e.g. diabetic vs non-diabetic patients or to look into the pharmacological therapy in predialysis period in this aspect. (most importantly is to detect the differences in medicines that can interfere with RAS and express some antifibrotic characteristics).

Many attempts have been made to use gene therapy in peritoneal dialysis patients to inhibit peritoneal damage and to stop local - intraperitoneal 'microinflammation' and progressive fibrosis. This therapy may be useful in severe bacterial peritonitis. Genetic recombination enables modified mesothelial cells to produce IL-1 receptor antagonist with decreased ability for activation by IL-1 that may be useful in inflammatory processes inhibition. Mesothelial cells are relatively susceptible to genetic manipulation. In ex vivo strategy mesotehelial cells isolated from a patient are genetically transfered – genome modification using viral vector or DNA liposome wrapped and then reimplanted to the patient. Mesothelial cells intake and cellular culture installation may be done during peritoneal catheter insertion. Culture of genetically modified cells may be stored in liquid nitrogen. When dealing with peritonitis it may be possible to perform reimplantation of these cells to limit post-inflammatory peritoneal damage or to accelerate its healing. In vivo strategy genetic material can be delivered intraperitoneally to patients and genetic modification performed in situ. Target cells in this situation are: mesothelial cells, neutrophils and peritoneal macrophages [14]. Some DNA sequences in modified mesothelial cells responsible for synthesis of TGF-β1 and other profibrogenic factors may be switched off. In this way, for example the expression of TGF-β1 in peritoneal membrane will be very low. Moreover, genetic engineering makes introducing selected genes into mesothelial cells possible. In mouse model of end stage renal disease introducing epo gene into mesothelial cells caused correction of renal anemia.

SELECTED THERAPEUTIC STRATEGIES OF IMPROVING PERITONEAL MEMBRANE FUNCTION IN PERITONEAL DIALYSIS PATIENTS

A crucial role in peritoneal membrane damage prevention plays limiting standard glucose based peritoneal fluids. Successful long term therapy depends on more prevalent application of icodextrin or aminoacids and most of all fluids with decreased GDPs and at physiological pH, manufactured in double chamber bags, as osmotic agents. It seems that the regimen consisting of these fluids will become the standard of peritoneal dialysis. Several experimental studies indicate reversible character of structural and functional changes after conversion from standard to low GDPs fluids [15]. There are some attempts of systemic and local intraperitoneal administration of vasoactive substances to preserve peritoneal membrane integrity and function.

In animal model of chronic kidney disease and peritoneal dialysis (and in vitro study with cell culture) a successful influence of intraperitoneal administration of aminoacids and dipeptides on peritoneal membrane was reported. Aminoguanidine, carnosine (B-alanylo L-histidine), homocarnosine, anserin, (β-alanylo L-1 methylhistidine) inhibit carbonic radicals formation (components of GDPs). In animal model, it has been shown that aminoguanidine as a GDPs scavenger, reduces toxic AGEs formation and NO synthesis as well as related vasodilatation [16]. A potential therapeutical option in this case, is dialysis with addition of aminoguanidine. Carnosine also diminished AGEs synthesis in peritoneal membrane [17]. AGEs receptor antagonists and bone morphogenetic protein 7 (BMP-7) prevent mesothelial into fibroblasts transdifferentiation [18].

It is worth mentioning the beneficial effects of glycosaminoglycans (hyaluronian, sulodexide) in

peritoneal dialysis patients. They inhibited peritoneal fibroblast proliferation and decreased TGF-β1, bFGF, IL-1, IL-6 expression and extracellular matrix synthesis. As a results of the glycosaminoglycans administration peritoneal ultrafiltration increased and diminished of transperitoneal albumin loss was observed [19, 20].

Peroxisome proliferator-activated receptor-γ (rosiglitazone) added to standard peritoneal fluid improved peritoneal membrane morphology and function, increased peritoneal ultrafiltration, but decreased VEGF expression (animal model) [21]. In several experimental studies advantages of intraperitoneal low molecular weight heparin was proven [22, 23]. It is summarized in table I.

Table I. *Beneficial effects of low molecular-weight heparin in peritoneal dialysis*

Low molecular weight heparin and its clinical significance
1. Inhibition of factor Xa activity.
2. Angiogenesis inhibition (VEGF inhibition and others).
3. Diminished local intraperitoneal inflammation.
4. Improvement of intraperitoneal blood flow hemodynamics.
5. Prevention of peritoneal fibrosis.

Other pharmacological agents which show beneficial effects on peritoneal membrane integrity and function are angiotensin converting enzyme inhibitors (ACE-I) and angiotensin II receptor antagonists. Kolesnyk and co-workers demonstrated that angiotensin II receptor antagonists administration decreased neoangiogenesis, peritoneal fibrosis, peritoneal small solutes transports and exerted beneficial influence on method survival [24]. In animal model studies enalapril given orally stopped mesothelial cells remodeling via inhibiting TGF-β synthesis, reduce transperitoneal protein loss and increase Ca-125 antigen level in peritoneal effluent [25]. Both captopril, enalapril, and losartan in in vitro model diminish VEGF production by mesothelial cells [26]. Both angiotensin converting enzyme inhibitors (ACE-I) and angiotensin II receptor antagonists maintain residual renal function in PD patients improving patients survival rate.

In animal model of peritoneal dialysis calcium channel blocker – diltiazem suppressed collagen type I, type III and mesothelial TGF-β synthesis [27]. Taken together, both ibersartan and spironolactone show favorable effects on limitation of bacterial peritonitis peritoneal damage in animal model [28].

Another therapeutic approach in peritoneal dialysis patients is to restrict extracellular matrix protein synthesis by penthoxifilin dipiridamol, troglitazone, diltiazem and statins use. Statins activate local fibrinolysis system via t-PA stimulation and PAI-1 inhibition [29].

There are some attempts to apply angiogenesis and fibrosis inhibitors (VEGF, TGF-β1 AGE and RAGE antibodies) and gene therapy – genetic modifications of peritoneal cells with its resistance to damage and inhibition of expression of many proinflammatory, profibrotic and proangiogenesis cytokines. Mesothelial cells culture derived from peritoneal membrane is useful in peritoneal membrane restoration in long term dialysis patients. Specifically, there were some attempts to apply these cells in prevention and treatment of peritoneal adhesions after abdominal surgery [30].

Contemporary methods of peritoneal membrane protection that are recommended by International Society of Peritoneal Dialysis - peritoneal fluids, medicines that interact with RAS and experimental methods are summarized in table II.

Table II. *Contemporary methods of peritoneal membrane protection*

Selected therapeutic options in peritoneal dialysis patients
1. Dialysis solutions – avoidance of glucose exposure.
-icodextrin, amino acids solutions, low GDPs solutions
2. AGEs inhibitors. (aminoguanidine)
3. Inhibition of NO synthesis – inhibition of L-arginin conjunction with eNOS.
-N-nitro-L-arginine metyl ester (L-NAME)
4. Inhibition of RAS.
-ACE-I, AT II receptor blockers, aldosterone antagonists
5. Angiogenesis modulations.
-VEGF and TGF-β1 antibodies
-VEGF receptor antibodies
-AGE and RAGE antibodies
6. Antioxidants.
7. Avoidance of any intraperitoneal drug except antibiotics.
8. Prevention, early diagnosis and treatment of peritonitis.
9. Intraperitoneal glycosaminoglycans administration.
10. Gene therapy.

In summary, success of peritoneal dialysis treatment is related to new peritoneal fluids application (glucose sparing regimen) and supportive pharmacotherapy. These methods guarantee maintaining of peritoneal membrane integrity and its function as dialysis membrane.

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REVIEW / PRACA POGLĄDOWA

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DIETARY FRUCTOSE – PREVALENCE AND EFFECTS ON METABOLISM, POTENTIAL RISK OF INCREASED METABOLIC SYNDROME COMPLICATIONS

WYSTĘPOWANIE I WPŁYW SPOŻYCIA FRUKTOZY NA PRZEMIANY METABOLICZNE ORAZ POTENCJALNE RYZYKO WZROSTU POWIKŁAŃ CHORÓB METABOLICZNYCH

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Summary

Fructose consumption, especially from mass – produced food products has increased considerably in the last few decades. Fructose metabolism is mainly hepatic and does not require insulin. Potentially adverse effects of fructose consumption are thought to be: increase in cardiac complications, kidney disease, obesity, hypertension, metabolic syndrome, and diabetes. Many studies have shown the ability of fructose to elevate serum uric acid levels in humans and animals. High fructose consumption among Afroamericans may explain their high rates of obesity, hypertension, diabetes, heart and kidney diseases. It is recommended that fructose consumption should be limited to that from natural sources, like fruit, vegetables, and honey, and not from highly processed – food products. This paper presents today's knowledge about prevalence and effects of dietary fructose on metabolism, and its highly probable effect on many metabolic diseases. It is certain that effects of dietary fructose require much more research.

Streszczenie

Spożycie fruktozy, zwłaszcza dodawanej do wielu produktów spożywczych produkowanych na masową skalę, zwiększyło się znacznie w ciągu ostatnich kilku dekad. Metabolizm fruktozy przebiega głównie w wątrobie i nie wymaga do tego insuliny. Za potencjalnie szkodliwe skutki spożywania fruktozy uważa się: wzrost chorób serca, nerek, otyłości, nadciśnienia tętniczego, zespołu metabolicznego i cukrzycy. Wiele badań wskazuje na zdolność fruktozy, jako jedynego cukru, do podnoszenia kwasu moczowego u ludzi i zwierząt. Wysokie spożycie fruktozy wśród Afroamerykanów może wyjaśniać ich większą zachorowalność na otyłość, nadciśnienie tętnicze, cukrzycę, choroby serca i nerek. Zachęca się do spożywania fruktozy pochodzącej z produktów naturalnych, jak owoce, warzywa, miód. Podkreśla się zbyt dużą zawartość fruktozy w artykułach przetworzonych w przemyśle spożywczym. Poniższy artykuł przedstawia obecny stan wiedzy na temat występowania fruktozy i jej wpływu na metabolizm oraz prawdopodobnego wpływu na zwiększenie liczby chorób metabolicznych. Jest oczywiste, że wpływ spożycia fruktozy wymaga jeszcze wielu badań.

Key words: fructose, sugar, fructosuria, metabolic syndrome, endothelial dysfunction, hypertension, obesity, chronic kidney disease, uric acid

Słowa kluczowe: fruktoza, cukier, fruktozuria, zespół metaboliczny, dysfunkcja śródbłonka, nadciśnienie, otyłość, przewlekłe choroby nerek, kwas moczowy

INTRODUCTION

Carbohydrates are a common naturally occuring group of polyhydroxide aldehydes and ketones, and their derivatives. They occur as monosaccharides and their polymers: oligosaccharydes and polysaccharides. A classification of monosaccharides is based on the number of carbon atoms in a molecule. All monosaccharides participate in carbohydrate metabolism, however, pentoses and hexoses play a special role, often as derivatives – alcohols, acids and aminosaccharides.

There are four hexoses which take part in metabolic processes in human body: fructose, galactose, glucose and mannose. Only fructose and glucose occur naturally in free from [1]. Fructose can be supplied to the body in free from (it is the main saccharide in fruit and honey) and as a compound, in sucrose. Sucrose is broken into glucose and fructose in the intestine by enzymatic hydrolysis [2]. Relatively small amounts of fructose occur in natural foods such as fruit, vegetables, or honey, when compared with the products containing high fructose corn syrup (High Fructose Corn Syrup - HFCS) [3]. In the second part of the 20th century the diet of people from Western Countries became loaded with a new ingredient: derived from corn high fructose syrup (mixture of fructose and glucose). In the USA it is widely used in sweet drinks, cakes, sweets, fruit preservatives, jams, gellies and dairy products [4]. Because it is inexpensive to produce and mixes well with many products, food producers choose HFCS over sucrose. Fructose content in various products differs greatly. Animal derived products in natural form do not contain fructose. However, processed fish or cold meats often contain fructose called 'sweeteners'. The fruit group with high fructose content comprises apples, pears, cherries, kiwi, grapes, and dried fruit (eg. dates, figs, raisins). Also fruit, and fruit and vegetable juices are rich in fructose. Examples of sweet beverages with fructose are lemonades, colas, and 'light' drinks containing, apart from fructose, artificial sweetener, sorbit. All kinds of sweets, fruit preserves, chocolate, ice cream, cakes and pastries contain high amounts of fructose. Natural dairy products do not contain fructose, however, products such as yogurts or cottage cheese containing fruit or fruit flavouring become a source of fructose. Many kinds of sauces, salad dressings, ketchups, mayonnaise and mustard are all processed foods with high fructose content. Fructose is also present as a supplement or carrier, in many medicines, eg. cough syrup. Many food products for diabetics and products with sugar substitutes marked as 'no sugar added' contain sorbit. In natural form it occurs in some fruits, such as plums, cherries and peaches. Some kinds of beer and wine, chewing gum and sweets can also contain sorbit. Sorbit blocks GLUT – 5 transport system responsible for absorption of fruit sugar in the small intestine [5]. Excessive consumption of refined sugar of any type constituting so called 'empty calories' is undesirable. It lowers the intake of minerals, vitamins, aminoacids and other necessary nutrients, and can lead to obesity. Intake of fructose from fruit and vegetables amounts to about 15g /day. The peak concentration of fructose occurs about 30 – 60 minutes after its consumption [6]. Serum fructose concentration on empty stomach in a healthy person is 1 mg / dl or less [7].

ABSORPTION AND METABOLISM OF FRUCTOSE

When looking into the effects of fructose consumption on metabolic processess in humans we have to consider their genetic conditioning.

So far, three types of genetically conditioned fructose metabolism disorders have been recognised. Two of them are caused by deficiency of enzymes responsible for fructose metabolism: idiopathic fructosuria (deficiency of fructokinase) and congenital fructose intolerance (deficiency of fructose – 1 – phosphatic aldolase).

The third disorder caused by deficiency of hepatic fructose – 1.6 – biophosphatase, even though it does not involve a fructose metabolism enzyme, is classified as a disorder of fructose metabolism [8] Out of these three disorders only idiopathic fructosuria is clinically neutral (indifferent). All three disorders are called fructosemias , however, only the congenital fructose intolerance caused by deficiency of fructose – 1 – phosphatic aldolase should be described by that term [9]. All these disorders are inherited in autosomal recessive way [10].

Idiopathic fructosuria occurs very rarely. Due to its lack of symptoms many cases go unrecognised. Fructosuria and increased serum fructose levels are recognised only during laboratory tests [10].

In congenital fructose intolerance, the first symptoms of fructosemia occur after an introduction of food containing fructose. The disorder reveals itself

particalarly in infants artificially fed with sweetened sucrose food. In breastfed babies the revealing trigger is an introduction of fruit juices [11]. The main symptoms of fructosemia are severe hypoglycaemia and vomiting soon after fructose ingestion. Further fructose ingestion leads to hepatomegaly, jaundice, haemorrhaging, renal Proxima tubuli damage, apathy, sleepiness and convulsions. The most common symptom in infants and small children is lack of appetite and physical growth retardation. Fructose is discovered in urine; urinary tract infection with bakteriemia is often diagnosed. Failure to exclude fructose from the diet can lead to recurring episodes of hypoglicaemia and liver and kidney disease, which can lead to death. It should be noted that in some babies the course of the disease is oligosymptomatic and mild [8, 9, 10]. Sometimes fructosemia is diagnosed in preschoolers and school – age children [8].

Fructose intolerance is a disorder of fructose transport in the intestine. It is caused by the damage to the GLUT – 5 protein responsible for fructose transport. The symptoms can occur even after a few minutes, or a day or two after the ingestion of fructose – rich food. Fruit sugar molecules stay in the digestive system and in an unaltered form leave the small intestine moving to the large intestine, where they undergo decomposition and fermentation. Hydrogen, carbon dioxide, methane and short – chain fatty acids are created. High amount of gases is created in a short time, leading to flatulence. Gasses creation can lead to dizziness, fullness and nausea. Fructose binds water molecules and excess water from food, and instead of reaching tissues, it is retained in the large intestine leading to osmotic diarrhoea accompanied by painful cramps and borborygmus (rumbling in the intestine). Fructose intolerance seldom occurs in isolation. Digestive disorders and intestinal symptoms also occur in lactose or histamine intolerance or in irritable bowel syndrome [5]. Fructose absorbed into the digestive system in the small intestine is transported to the liver by portal vein [12].

There are two fructose metabolism process tracts. One tract is formed in muscles and fatty tissue, the other in the liver (the main tract). In muscles and fatty tissue fructose may undergo phosphorylation to fructose – 1 – phosphate, under the action of hexokinase [13].

Most of fructose is included in glycolysis by fructose – 1 – phosphate. Phosphorylation of fructose to fructose – 1 – phosphate takes place under the action of fructosekinase - liver enzyme which does not phosphorylates glucose [14]. Subsequently it is broken down to glycemic aldehyde and phosphodihydroxyactone by fructose – 1 – phosphate aldolase. Gliceric aldehyde undergoes phosphorylation to 3 – phosphoglyceric aldehyde under the action of triokinase, and then is included in glycolysis.

The second tract concerns most of the fructose metabolised in the liver [13].

POTENTIALLY HARMFUL EFFECTS OF FRUCTOSE CONSUMPTION

Fructose is the cause of the rapid increase of uric acid production in humans. As a result of fructose phosphorylation ATP becomes AMP, which in turn becomes converted to uric acid under the action of AMP deaminase. Uric acid causes dysfunction of vascular endothelium [15].

Experimentally induced hyperuricaemia causes diabetes and arterial hypertension in laboratory animals. Lowering hyperuricaemia by limiting fructose ingestion in rats causes lowering of arterial blood pressure, insulin resistance, obesity, and hypertrigliceridaemia. Experimental and clinical test results seem to suggest a role played by excessive fructose ingestion in pathogenesis of metabolic syndrome and positive influence of xantyne oxidase inhibitor – allopurinol – in suppressing the syndrome [16].

Fructose, as opposed to glucose, is sometimes thought to be relatively safe. Glucose level regulates insulin secretion in the body and restricted consumption of fructose does not negatively influence serum glucose levels, at least over a short period of its ingestion [6].

Potentially harmful effects of fructose consumption are expressed by the incidence of e.g. obesity, insulin resistance, nephropathy, retinopathy, neuropathy, non – alcoholic liver steatosis, hypertriglyceridaemia, hyperuricaemia [3].

Hyperlipaemic fructose effect can be suppressed by physical exercise. A test where 8 patients with hypertriglyceridaemia have been given fructose dose of 80 g/day over 7 days has been conduced twice. All 8 patients showed increased triglyceride serum levels. In the second test, apart from fructose ingestion physical exertion was included. It constituted 45 minute exercise every day and a 15-minute swim 3 times a week. Triglyceride serum levels were only slightly

increased, but 3 patients showed significant decrease of triglyceride serum levels. It is likely to be the effect of increased activity of lipoprotein lipase after exercise [17].

SUSCEPTIBILITY OF AFROAMERICANS TO CARDIOVASCULAR DISEASES

Incidence of obesity, arterial hypertension, diabetes and cardiac and kidney diseases among Afro-Americans is much higher than among indigenous Americans [18]. One can speculate that Afro-Americans who had earlier worked on sugar cane plantations consumed high amounts of molasses, which caused high incidence of hypertension among them. It was confirmed by the studies conducted in the Caribbean and in Louisiana [19]. Latest studies also show that Afro-Americans consume more sugar than Caucasians [20]. Afro-Americans have higher levels of serum uric acid (av 8.3 mg/dl), reported in studies on humans with hypertension and kidney diseases [21].

Afro-American newborns show high incidence of low body mass at birth [22]. It has been proven that the low number of nephrones in this group of newborns is related to subsequent development of hypertension and excessive body mass [23], early hyperuricaemia and dysfunction of endothelium [24]. There is more and more evidence that uric acid can play a negative role in these disorders [25]. Afro-American diet is high in sodium and low in potassium [26], which encourages arterial hypertension and higher risk of cardiovascular disorders. This increased incidence is in sharp contrast with nearly complete lack of incidence of hypertension and obesity in studies on indigenous Africans conducted at the beginning of the XX century [27].

CONCLUSION

There is evidence that excessive consumption of fructose and high sugar food products can lead to higher incidence of modern day diseases. It seems prudent to limit any sugar consumption to a minimum. If the hypothesis that fructose plays a significant role in the development of cardiac and kidney diseases, and metabolic syndrome posed by researches proves to be true, it can greatly influence the therapies in particular disorders , and also force food manufactures to change their attitudes.

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REVIEW / PRACA POGLĄDOWA

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NEW BIOMARKERS IN LABORATORY DIAGNOSIS OF KIDNEY DISEASES

NOWE BIOMARKERY W DIAGNOSTYCE LABORATORYJNEJ CHORÓB NEREK

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S u m m a r y

With regard to insufficient sensitivity of diagnostic markers used so far for the evaluation of kidney function, the search for new markers of renal failure, which may enable the early recognition of renal disease and commencement of a proper therapy, is evolving. Neutrophile gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) are newly discovered biomarkers which, besides of traditional indices, seem to be useful in the diagnosis of acute and chronic renal failure. Their determination in biological materials such as serum and urine, which are routinely used in laboratory diagnostics, may be useful in the clinical practice.

S t r e s z c z e n i e

Ze względu na niewystarczającą czułość dotychczas stosowanych do oceny czynności nerek parametrów diagnostycznych wciąż poszukuje się nowych markerów uszkodzenia nerek, które umożliwiałyby wczesne rozpoznanie choroby i włączenie odpowiedniego leczenia. Lipokalina związana z żelatynazą neutrofili (NGAL) oraz cząsteczka-1 uszkodzenia nerek (KIM-1) to nowo odkryte wczesne biomarkery, które, obok tradycyjnych wskaźników, mogą znaleźć zastosowanie w diagnostyce ostrej i przewlekłej niewydolności nerek, a ich oznaczanie w surowicy lub moczu, które są materiałami biologicznymi stosowanymi w rutynowej diagnostyce laboratoryjnej, może okazać się przydatne w praktyce klinicznej.

Key words: neutrophile gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), kidney diseases
Słowa kluczowe: lipokalina związana z żelatynazą neutrofilii (NGAL), cząsteczka-1 uszkodzenia nerek (KIM-1), choroby nerek

Taking into account serum creatinine and the results of radiological and histological evaluation of the kidneys, the diagnosis of kidney dysfunction is often insufficient due to the low accuracy or high invasiveness. The determination of serum creatinine used so far is not a sensitive indicator of impaired renal function and depends on the factors such as muscle mass, sex, age and diet. Taking into account the limited diagnostic value of the concentration of creatinine in the early stages of acute renal failure and difficulties in the proper daily urine collection to assess creatinine clearance, there is a need to search for new markers of kidney damage, the concentration of which in the blood or urine changes already in preclinical phase of the disease, and which would allow the early diagnosis and inclusion of appropriate treatment. New markers with higher sensitivity and specificity, such as neutrophile gelatinase-associated lipocalin (NGAL), kidney injury molecule-1, acting as a KIM-1 and interleukin 18 (IL-18) are defined as "renal troponins" [1, 2, 3].

Neutrophile gelatinase-associated lipocalin NGAL appearing in the literature also under the name of human neutrophiles lipocalin (HNL) is a secreted protein belonging to the family of lipocalins present in neutrophile granules [1, 4]. In addition, this glycoprotein is known in mice as lipocalin 2, oncogene protein 24p33 or uterocalin, and in rats as neu-related-

protein or 25 kDa α2-microglobulin-related protein [4]. It was demonstrated that the human form of NGAL consists of a single disulphide-bridged polypeptide chain containing 178 amino-acid residues with a molecular mass of 22 kDa, which, due to glycosylation of proteins, increases to 25 kDa. Isoelectric point of this glycoprotein is at pH= 8,4 [1].

Activation of neutrophiles leads to releasing of NGAL from the granules, which may be secreted as a monomer, and in low concentration as a dimer, or may occur in complex with type IV collagenase, called gelatinase B or matrix metalloproteinase-9 to form heterodimer with a mass of 125 kDa (Fig. 1) [4]. Like other lipocalins, NGAL has a β-barrel structure with a hydrophobic calyx. The common feature of these protein families, due to their structure, is the ability to bind and transport small lipophilic molecules, such as free fatty acids, retinoids, arachidonic acid and steroids [5]. It has been shown that many lipocalins have specific surface receptors, but NGAL receptor has not been identified yet [4]. However, the ligand for NGAL was discovered by Goetz et al [6]. It has been shown that the main ligands for NGAL are siderophores – small iron-binding molecules, which are synthesized in bacteria [5].

PHYSIOLOGICAL FUNCTION OF NGAL

Although NGAL was identified over a decade ago, the physiological function of this protein is still poorly understood. Human NGAL was originally isolated from the supernatant of the activated neutrophiles. Initially it was considered a marker of infection, since the elevated levels of this protein were observed in inflammatory and acute bacterial infections. It has been demonstrated that NGAL gives a bacteriostatic effect by destroying siderophores, and therefore may be involved in inflammatory processes in the cells [5]. Moreover, regardless the infectious process, increased concentrations of NGAL are found in asthma, chronic obstructive pulmonary disease, as well as adenomas, adenocarcinomas of the breast and urothelial carcinomas [4, 7]. Moreover, this protein is present in the trachea, stomach, colon, and proximal renal tubules [7]. The expression of NGAL has been demonstrated in many human tissues at very low levels only, which , however, significantly increase in the damaged epithelial cells, including kidney, affecting (through the transcription factors, i.e. NF-κB) the cells survival and proliferation [2, 5]. NGAL activates the formation of nephrons in the early stage of kidney development, which has a protective effect [8]. Due to the low molecular mass and resistance to degradation NGAL can be easily secreted by the cells of the thick section of ascending arm of the Henle Loop and collecting tubules and excreted in the urine, both free and complex form with MMP-9. LCN2 appears to be upregulated in cells under the “stress” (e.g. from infection, inflammation, in tissues undergoing involution to ischemia or neoplastic transformation). he concentration of this protein in the urine correlates well with the concentration in the blood, hence the NGAL may be a useful marker in the diagnosis of kidney diseases [1].

NGAL IN EARLY DIAGNOSIS OF ACUTE KIDNEY INJURY

Acute kidney injury (AKI), a new term appearing in the literature to replace “acute renal failure”, is a common complication after cardiac surgery, kidney transplantation, cardiogenic and septic shock, after the administration of X-ray contrast or chemotherapeutic agents such as certain antibiotics or NSAIDs. Studies have shown that AKI develops in approximately 7% of the hospitalized patients and up to 30-50% of patients after heart surgery [9]. In 5.7% of patients requiring dialysis mortality rate is as high as 60-80%, which demonstrates that acute renal failure is an independent risk factor for death [9, 10]. Despite the well documented importance of the modified RIFLE classification for AKI, including changes in serum creatinine, there is still a need for a marker of higher sensitivity, which would be helpful in early diagnosis of acute kidney injury [2].

The preclinical studies demonstrated that NGAL is a protein easily detectable in the blood or urine after nephrotoxic factor activation, and therefore suggested its use as a non-invasive marker in the diagnosis of AKI [5, 9, 11]. Study of Bennett et al. [12] showed that NGAL determined in urine is an early indicator of acute renal damage in patients after cardiac surgery. In 51% of the 196 children who developed acute renal failure after cardiac surgery, after 2 hours a 15-fold increase of NGAL concentrations in the urine was observed and up to 25-fold after 4 and 6 hours [12]. For comparison, serum creatinine increased only after 2-3 days following the cardiopulmonary bypass [12]. This was also confirmed by studies of Mishra et al [13]. Other prospective studies conducted in children

after cardiac surgery showed a significant increase in the concentration of NGAL in the urine and plasma after 2-6 hours after surgery, while the increase in serum creatinine was observed only after several days [14], suggesting that urine NGAL can serve as an early marker for ischemic renal damage in children after the operation [4].

Also, in adult patients who developed acute renal failure after heart surgery, defined as an increase in serum creatinine, an increase of NGAL in urine was found after 1-3 hours after surgery [14].

In addition, many studies indicate a correlation between early postoperative NGAL concentrations determined in the urine or plasma and the severity of acute renal failure, the duration of patient's hospitalization, the need for dialysis and increased mortality [14]. Therefore, the concentration of NGAL in the urine after 2 hours correlates with the severity and duration of acute renal failure [12.13].

NGAL is considered to be an independent factor in the development of acute renal failure [14]. Many studies demonstrated that the concentration of NGAL closely correlated with the acute state, and therefore a significant increase of its concentration was observed in patients with ischemic renal injury often leading to acute renal failure, acute tubular necrosis, or acute tubulo-interstitial nephropathy [4]. It is worth to emphasize that patients with ischemic heart disease often exhibit different degrees of renal dysfunction due to the associated diseases such as diabetes, hypertension and congestive heart failure, despite normal serum creatinine [15]. Due to the increase of NGAL concentration prior to an increase in serum creatinine, and the possibility of its determination in the urine with commonly available analyzers, it seems to be a very helpful new biomarker in the early diagnosis of patients with acute renal injury [14].

In addition, the concentration of NGAL also increases in patients undergoing coronary angiography due to coronary artery disease [15]. It has been shown that NGAL increases in the serum after 2-4 hours after coronarography and in the urine after 4-8 hours. A significant correlation between NGAL and other markers of kidney function: cystatin C, GFR and serum creatinine has also been observed, which suggests that NGAL can become a useful marker of acute renal failure in patients after coronary angiography [15]. A similar correlation between the time after surgery and the NGAL concentration was observed by Mishra et al [13]. Studies of Devarajan [9] also showed that serum creatinine significantly correlated with NGAL determined in serum or urine. It is interesting that the increase of serum NGAL concentrations was observed after 2 hours after coronary angiography, and NGAL in the urine increased after 4 hours after surgery and lasted for up to 8 hours [15]. Study of Mishra et al [13] indicates a similar relationship, which proves that NGAL is released into the systemic circulation by inflammatory activation of neutrophiles initiated during the coronary angiography [15]. Moreover, NGAL has been demonstrated in atherosclerotic plaques, which suggests that it is secreted into the circulation [15].

It is emphasized that many other disorders of renal function are also associated with increased concentrations of NGAL found in plasma or urine [4]. It is suggested that NGAL may also be an early biomarker of contrast induced nephropathy (CIN) development, which is often exposed to several patients with chronic kidney disease, diabetes, impaired fasting glucose or heart failure [16]. In addition, increased serum creatinine as a symptom of CIN, is observed in patients after 24-48 hours and sometimes only within 3-5 days which significantly impedes the early diagnosis of renal damage [16]. In the group of children administrated to X-rays with contrast, after 2-4 hours a significant increase in the concentration of NGAL was observed in the urine and plasma [17], which indicates that NGAL can predict radiographic contrast-induced nephropathy and is potentially a useful marker in clinical practice [14].

Furthermore, the increase of serum and urine NGAL concentration was also observed in patients after renal transplantation. Multicenter studies conducted in adults and children after kidney transplantation showed an increase in NGAL concentration in the urine and plasma one day after kidney transplantation in those who have lost kidney function [14].

NGAL IN CHRONIC RENAL FAILURE

Chronic kidney disease (CKD) represents a serious risk of cardiovascular complications and death. It is estimated that the incidence of CKD in the U.S. is as high as 16%, while in Poland the incidence of CKD in the general population is determined as more than 10% [3].

It has been demonstrated that the determination of NGAL in the urine may not only be a marker of acute kidney injury (AKI), but also an indicator of chronic kidney disease (CKD) [2]. Bolignano et al [18] proposed the use of NGAL in clinical practice as a predictive factor of worsening renal function in patients with chronic renal failure. In patients with CKD several times higher levels of NGAL in the urine have been observed which correlated with serum creatinine, GFR and proteinuria [9]. Furthermore, Mitsnefes et al [19] found a significant correlation between serum NGAL and cystatin C ($r = 0.74$). Due to the fact that NGAL is a sensitive marker of renal damage [20], its determination in chronic kidney disease with proteinuria allows to specify the degree of kidney damage, so there is a possibility of using this protein for an early identification and monitoring of patients with chronic kidney disease. In addition, it was demonstrated that even a single determination of NGAL in the urine, but not serum creatinine, may be helpful in differentiating patients with acute renal failure and chronic kidney disease [21]. Studies have shown that NGAL is also involved in the pathological process leading to the polycystic kidney disease or glomerulonephritis.

It seems that NGAL may also be an early predictive marker of disease activity in patients with lupus nephritis [3, 22]. Suzuki et al [22] observed a significant NGAL increase in plasma and urine of children with SLE when compared with juvenile idiopathic arthritis and a group of healthy children. Also Rubinstein et al [23] indicated a significant relationship between NGAL concentration in the urine and disease activity in the course of lupus nephritis. Table I.

KIDNEY INJURY MOLECULE-1, KIM-1

Kidney injury molecule-1 determined as Kim-1 in rodents and KIM-1 in humans is a transmembrane tubular protein of previously unrecognized function [24]. KIM-1 also known as TIM-1 (T-cell immunoglobulin and mucin-containing molecule) and hepatitis A virus cellular receptor-1 (HAVCR-1) were discovered in the studies of molecules involved in the pathogenesis of acute kidney injury, i.e. AKI [24, 25, 26]. It has been demonstrated that KIM-1 is a type I cell membrane glycoprotein containing a novel six-cysteine Ig-like domain and a threonine/serine and proline-rich domain characteristic of mucin-like O-gllycosylated proteins [26, 27, 28]. Kidney injury molecule-1 belongs to a family of proteins KIM / TIM consistsing of 8 members in mice, 6 in rats, and 3 in humans [24, 25].

Although the protein is undetectable in physiological kidneys, it is highly expressed in proliferating and dedifferentiating epithelial cells of proximal renal tubules after 48 hours from ischemic renal damage [25]. Therefore, numerous studies indicated that the KIM-1 is produced in the proximal renal tubules as a result of toxic or pathological damage of the dedifferentiated epithelium. It has been proved that dedifferentiation is a very early manifestation of epithelial cells response to the damage [25]. It has been shown that KIM-1 mRNA damage leads to the increased release of protein and its accumulation at high concentration at the top of the membrane of proximal tubule, and therefore the presence of KIM-1 is mainly in the S3 segment of proximal tubule [24]. The production of this protein is probably regulated by the MAP kinase which can be activated under stress conditions [25, 26].

KIM-1 expression has been demonstrated in vitro and in vivo in the urine of rodents and humans in the proximal renal tubular damage, as well as in patients with renal cell carcinoma (RCC) [24, 26, 29]. The first study conducted in humans, published in 2002, indicated a significant increase in KIM-1 expression in renal biopsies from patients diagnosed with acute tubular necrosis, as well as high concentrations of KIM-1 in the urine (uKIM-1) in patients with clinical symptoms of acute kidney injury [30]. However, the relationship between expression of KIM-1 in renal biopsy and the KIM-1 levels in urine is not yet completely understood. It has been shown that uKIM-1 may reflect the tissue KIM-, because it was demonstrated that irrespective of kidney disease KIM-1 correlated positively with renal injury. The increase in KIM-1 levels is observed in renal diseases associated with kidney fibrosis or inflammation. A similar relationship was demonstrated between uKIM-1 and inflammation and renal function, suggesting that

KIM-1 may be used as a non-invasive biomarker for diagnosis of kidney diseases [24, 30].

ROLE OF KIM-1

Although the role of this protein has not been fully clarified, it is known that KIM-1 epithelial cells confer the ability to identify phagocytosis of dead cells, which may be present in the kidneys as a result of ischemic damage and lead to blockage of the light of tubule, which is characteristic of acute kidney injury (AKI). In addition, it was demonstrated that except the fact that KIM-1 is a phosphatidylserine receptor that recognizes apoptotic cells and directs them to the lysosomes, it is also a receptor for oxidized lipoproteins (ox LDL). It is suggested that KIM-1 can also detach an important role in reducing autoimmune response to damage [24].

Recently published studies have shown that KIM-1 is a highly specific marker of renal tubular damage. This protein appears to be a very sensitive indicator of toxic acute renal failure, which concentration increases before the rollers appear in the urine. Therefore, it is suggested that KIM-1 determined in the urine may be a non-invasive marker of kidney damage, useful in the diagnosis of kidney diseases [24,30]. It was indicated that KIM-1 may also be a sensitive marker of renal damage in children after cardiac surgery. In addition, this protein was also detectable in biopsies (100%) of patients with worsening renal function and histological changes indicating the damage of renal tubules. KIM-1 levels also correlated with serum creatinine and blood urea nitrogen [24,30]. Results of Han et al [29] have shown that KIM-1 may also be a useful biomarker of an early detection of RCC, because of its presence in a very high concentration in the urine of patients with renal cell carcinoma. RCC, as well as renal tubular damage, is associated with dedifferentiation of the proximal tubular cells [24].

Due to insufficient sensitivity of previously used parameters assessing renal function, the search for new diagnostic markers of kidney damage is currently a subject of numerous studies. Neutrophile gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1, KIM-1 are newly discovered early biomarkers of renal damage which, in addition to traditional indicators, may be used in the diagnosis of acute and chronic renal failure. Their determination in the urine or serum may be very useful in clinical practice. Table II.

Table I. *NGAL as a biomarker of acute kidney injury*

Significance of NGAL
specific marker of acute kidney injury (AKI)
differentiation of AKI types (pre-renal nitrogenemia and intrinsic AKI)
sensitive marker in early and established diagnosis
increases proportionally to damage or kidney function loss
detected for development of kidney failure
available as automated and standardized assay

Table. II. *Clinical application of NGAL and KIM-1 determination*

Usefulness of NGAL in early AKI detection
Cardiopulmonary bypass operations in adults and children
Percutaneous coronary interventions (PCI)
Patients presenting at the emergency department or in the intensive care unit (heart failure, sepsis, multi-organ failure)
Renal transplantation
Patients with chronic kidney disease
Potential clinical application of KIM-1
Kidney damage in children after cardiac surgery
Patients with worsening renal function
Patients with renal cell carcinoma (RCC)

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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DIAGNOSTIC USEFULNESS OF NON-HDL CHOLESTEROL CONCENTRATION AS A PROGNOSTIC FACTOR FOR CORONARY HEART DISEASE

OCENA PRZYDATNOŚCI DIAGNOSTYCZNEJ STĘŻENIA CHOLESTEROLU NIE-HDL JAKO WSKAŹNIKA PROGNOSTYCZNEGO CHOROBY NIEDOKRWIENNEJ SERCA

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Summary

Introduction Non-HDL cholesterol (non-HDL-C), reflecting cholesterol concentration in all proatherogenic serum lipoproteins, can be considered as a risk factor for atherosclerosis and cardiovascular disease.

The aim of this study was an evaluation whether increased non-HDL-C concentration in young, dyslipidemic subjects may have a diagnostic value in forecasting of coronary heart disease in the future.

Material and methods: Glucose, TC (total cholesterol), HDL-C, TG (triglycerides), C-reactive protein (hsCRP), apolipoproteins AI (apoAI) and B_{100} (apoB) were measured; LDL-C, non-HDL-C, TC:HDL-C, TG:HDL-C and apoB:apoAI levels were calculated in 69 healthy, non-smoking subjects aged 25-40 years, 44 - with moderate hyperlipidemia (24 women, 20 men) and 25 - with lipid values within norms (control group: 10 women, 15 men).

Results: Among individuals with dyslipidemia 55% had non-HDL-C>160 mg/dL and this was observed more frequently in men. Non-HDL-C correlated better with traditional and new CHD risk factors like: TC (R=0.85; p<0.001), LDL-C (R=0.92; p<0.001), apoB (R=0.53; p<0.001) and apoB:apoAI (R=0.76; p<0.001) than LDL-C. Non-HDL-C increased with raising apoB and apoB:apoAI and in the highest concentration of non-HDL-C (169-221 mg/dL) these parameters were also in the highest tertile.

Conclusions: Non-HDL-C concentration seems to be more useful than LDL-C in evaluating the risk of cardiovascular disease in the future.

Streszczenie

Wstęp: Cholesterol nie-HDL (nie-HDL-C), obrazujący stężenie cholesterolu we wszystkich proaterogennych frakcjach lipoproteinowych osocza, może być zastosowany jako wskaźnik oceny zagrożenia miażdżycą i chorobami sercowo-naczyniowymi.

Celem pracy była ocena występowania podwyższonego stężenia nie-HDL-C u młodych osób z dyslipidemią oraz określenie przydatności tego wskaźnika dla oceny ryzyka wystąpienia ChNS w przyszłości.

Materiał i metody: Badaniem objęto 69 zdrowych klinicznie, niepalących osób w wieku 25-40 lat. Grupę badaną stanowiły 44 osoby (24 kobiety, 20 mężczyzn) z hiperlipidemią, natomiast do grupy kontrolnej włączono 25 osób (10 kobiet, 15 mężczyzn) z normolipidemią. U wszystkich osób oznaczono na czczo w surowicy stężenie cholesterolu całkowitego (TC), cholesterolu frakcji HDL (HDL-C), triglicerydów (TG), apolipoproteiny B100 (apoB) i AI (apoAI), CRP i glukozy. Obliczono także wartość stężenia cholesterolu frakcji LDL (LDL-C) i nie-HDL-C oraz wartości wskaźników aterogenności TC:HDL-C, TG:HDL-C i apoB:apoAI.

Wyniki: W grupie młodych osób z dyslipidemią u 55% stwierdzono podwyższone stężenie nie-HDL-C (>160 mg/dl). Podwyższona wartość tego parametru występowała częściej u mężczyzn. Cholesterol nie-HDL wykazywał silne korelacje, wyższe niż w przypadku LDL-C, z tradycyjnymi i nowymi lipidowymi czynnikami ryzyka: TC (R=0,85; p<0,001), LDL-C (R=0,92; p<0,001), apoB (R=0,53; p<0,001) i apoB:apoAI (R=0,76; p<0,001). Stwierdzono, że wraz ze wzrostem stężenia nie-HDL-C wzrastały wartości

proaterogennych wskaźników: apoB i apoB:apoAI, a w grupie badanych z najwyższymi stężeniami nie-HDL-C (169-221 mg/dl) dominowały osoby, u których wartości apoB i wskaźnika apoB:apoAI odpowiadały ich najwyższemu tercylowi.

W n i o s k i : Stężenie nie-HDL-C u młodych osób z dyslipidemią wydaje się być bardziej przydatne niż LDL-C dla oceny ryzyka wystąpienia chorób sercowo-naczyniowych w przyszłości.

Key words: non-HDL-cholesterol, lipid profile, apolipoproteins, atherosclerosis, coronary heart disease risk factors
Słowa kluczowe: cholesterol nie-HDL, profil lipidowy, apolipoproteiny, miażdżyca, czynniki ryzyka choroby niedokrwiennej serca

INTRODUCTION

Laboratory diagnosis of coronary heart disease (CHD) is based on low density lipoprotein cholesterol (LDL-C) measurement. LDL-C was recognised as the best marker of cardiovascular risk. However, numerous studies have shown that heterogeneity of LDL fraction, containing highly proatherogenic small dense LDL (sd-LDL), and also overpassing other lipoproteins contribution to pathogenesis of atherosclerosis makes LDL-C estimation more or less precise. From the clinical point of view evaluation of apoliporotein B (apoB), a component of all proatherogenic lipoproteins: VLDL, IDL, LDL, Lp(a), seems to be more valuable but it is not widely used in practice [1].

An alternative for apoB assaying could be an evaluation of non-HDL cholesterol (non-HDL-C), which is a sum of cholesterol in all proatherogenic serum lipoproteins, except for HDL [2]. Non-HDL-C concentration can be simply calculated in routine lipid profile examination: non-HDL-C (mg/dL) = TC – HDL-C.

The diagnostic usefulness of non-HDL-C can be confirmed in different ways. First of all, this parameter includes cholesterol of every proatherogenic lipoprotein and changes of non-atherogenic HDL cholesterol levels. Non-HDL-C estimation can be used in subjects with hypertriglyceridemia as opposed to LDL-C calculated from Friedewald's equation [3]. Clinical data has shown that non-HDL-C correlates with hypetriglyceridemia and sd-LDL concentration [4]. This property brings a great importance in evaluating a CHD risk in patients with diabetes and obesity in whom elevated levels of triglycerides and sd-LDL and reduced HDL-C concentration are observed [5]. Several reports emphasized better correlation between apoB and non-HDL-C than LDL-C, therefore non-HDL-C seems to be a better predictor of cardiovascular disease because of strong association with a number of atherogenic particles [4].

In accordance with NCEP ATPIII recommendations, the goals for non-HDL cholesterol are 30 mg/dL higher than those for LDL-C. Non-HDL-C should be calculated in routine lipid profile and chosen as a preferred secondary target of CHD therapy, especially in patients with elevated triglycerides [6].

SUBJECTS, MATERIAL AND METHODS

The study included 69 non-smoking subjects aged 25-40 years, respectively 44 subjects with moderate hyperlipidemia (24 women, 20 men) and 25 subjects with lipid profile values recommended by NCEP ATPIII [6] (control group; 10 women, 15 men). Subjects with hyperglycemia or diabetes, cardiovascular disease, hypertension, infection or inflammatory diseases and familial atherosclerosis or cardiovascular disease were excluded.

The written informed consent was obtained from each participant of the study which was approved by the Bioethics Committee of Nicolaus Copernicus University in Toruń Collegium Medicum in Bydgoszcz.

Fasting blood samples were collected and serum glucose (Glu), lipid profile (TC, HDL-C, TG), apolipoproteins B_{100} and AI and C–reactive protein (hsCRP) concentrations were measured. LDL-C, non-HDL-C and atherogenicity ratios (TC:HDL-C, apoB:apoAI, TG:HDL-C) values were calculated. The following cut-points were used: TC<200 mg/dL, LDL-C<130 mg/dL, HDL-C≥40 mg/dL (men) and ≥50 mg/dL (women), TG<150 mg/dL, CRP<3 mg/L, apoAI 101-223 mg/dL (women) and 95-186 mg/dL (men) - according to the manufacturer data, TC:HDL-C<3.4 (women) and <3.3 (men), apoB:apoAI<0.6 (women) and <0.7 (men), TG:HDL-C<0.9 [7]. As optimal apoB values <100 mg/dL and non-HDL-C values <160 mg/dL were accepted [1].

Lipid profile, glucose and apolipoproteins concentrations were measured by Abbott Architect

ci8200 analyzer and hsCRP was assayed by using Siemens BN II analyzer.

Statistical methods

The statistical analysis was performed using Statistica 9.0 (StatSoft Inc., 2009). Data was presented as mean±standard deviation (normal distribution) or median, 25th and 75th percentiles (non-Gaussian distribution). Student t-test and Mann-Whitney U test were used and Pearson's test for assessment of correlation between the variables after log10 transformation was used as well.

RESULTS

Non-HDL-C concentration in all subjects ranged within the limit of 44-221 mg/dL, whereas in those with moderate hyperlipidemia it was 83-221 mg/dL. Table I shows the differences in the measured variables between normo- and dyslipidemic subjects, depending on sex. Dyslipidemic women had higher TC, LDL-C, non-HDL-C, apoB, TC:HDL-C and TG:HDL-C values than normolipidemic women. Similar differences were observed in men, furthermore dyslipidemic men had higher TG and apoB:apoAI values. Relevant distinction between dyslipidemic men and women were noticed. Women had lower TG (85 vs 151 mg/dL; p=0.005), TC:HDL-C (3.3 vs 4.3; p<0.001), apoB:apoAI (0.6 vs 0.8; p=0.005), TG:HDL-C (1.3 vs 3.21; p<0.001) and higher HDL-C (68 vs 53 mg/dL; p<0.001) and apoAI (143 vs 109 mg/dL; p<0.001) values than men. The median non-HDL-C concentration was higher in men than in women (145 vs 170; p=0.1).

Among individuals with dyslipidemia non-HDL-C was strongly or moderately positively correlated with lipid profile parameters (TC, LDL-C), TC:HDL-C and apoB:apoAI ratios and negatively correlated with HDL-C. Correlation factors (R) were higher in these cases for non-HDL-C than LDL-C (Table II).

Elevated non-HDL-C (≥160 mg/dL) was observed in 55% of dyslipidemic subjects. All these subjects had TC>200 mg/dL while 96% had LDL-C>130 mg/dL. Hypetriglyceridemia was noticed in about 1/3 of this group, only in men.

Non-HDL-C concentration ≥160 mg/dL, related to higher risk of CHD, was slightly more frequent in men (54%). In subjects with elevated non-HDL-C significantly higher values of TC, LDL-C, TG, apoAI and apoB:apoAI ratio were found, compared to those with normal levels (<160 mg/dL). High non-HDL-C concentration (≥160 mg/dl) was neither related to increased apoB, although the median value was much higher than in the group with non-HDL-C <160 mg/dl or with elevated CRP (Table III). In the group with elevated non-HDL-C significant correlations between non-HDL-C and TC (R=0.85; p<0.001) and LDL-C (R=0.86; p<0.001) were found.

Table I. *Comparison of measured variables in normolipidemic and dyslipidemic subjects, depending on sex*
Tabela I. *Porównanie wartości oznaczanych parametrów i obliczonych wskaźników w grupie badanej i kontrolnej, w zależności od płci*

	All/Wszyscy (n=69)		Women /Kobiety (n=34)		Men/ Mężczyźni (n=35)	
Variable /Zmienna	**Dyslipidemia (n=44)**	**Normolipidemia (n=25)**	**Dyslipidemia(n=24)**	**Normolipidemia (n=10)**	**Dyslipidemia(n=20)**	**Normolipidemia(n=15)**
Age//Wiek (years/lata)	30 ± 4.6	29 ± 4.9	30 ± 5	30 ± 5.6	30 ± 4	29 ± 4.6
Glu (mg/dL)	87 ± 7.9	86 ± 7.4	84 ± 6.9	85 ± 6.4	90 ± 7.7	87 ± 8.2
TC (mg/dL)	**219 (204-236)**	**161 (134-186)****	**217 (206-233)**	**147 (129-186)****	**219 (202-238)**	**167 (143-186)****
HDL-C (mg/dL)	58 (53-70)	57 (52-71)	68 (64-83)	73 (65-93)	53.00 (46-54)	53 (45-60)
LDL-C (mg/dL)	**133 (116-152)**	**85 (67-107)****	**131 (116-151)**	**71 (51-91)****	**141 (115-161)**	**101 (72-116)****
non-HDL-C (mg/dL)	**160 (132-179)**	**97 (80-121)****	**146 (131-167)**	**81 (57-96)****	**170 (149-181)**	**115 (88-132)****
TG (mg/dL)	**97 (71-159)**	**76 (57-95)***	85 (62-129)	61 (51-76)	**151 (94-188)**	**80 (68-104)***
CRP (mg/L)	0.9 (0.4-1.9)	0.4 (0.2-0.9)	1 (0.2-3.5)	0.3 (0.2-0.6)	0.9 (0.4-1.5)	0.5 (0.2-1.6)
TC:HDL-C	**3.5 (3.1-4.3)**	**2.7 (2.1-3.3)***	**3.3 (2.8-3.5)**	**2.1 (1.8-2.4)***	**4.3 (3.7-4.7)**	**3.2 (2.7-3.5)***
TG:HDL-C	**1.7 (1.2-3)**	**1.1 (0.9-1.7)***	1.3 (0.9-1.8)	0.9 (0.6-1.1)*	**3.2 (1.6-3.7)**	**1.5 (1-2.4)***
ApoAI (mg/dL)	127 (99-144)	117 (89-142)	143 (120-156)	136 (92-170)	109 (94-127)	101 (87-132)
ApoB (mg/dL)	**87 (70-100)**	**55 (42-68)****	**89 (66-103)**	**51 (35-62)***	**85 (71-96)**	**55 (43-69)***
ApoB:ApoAI	**0.7 (0.6-0.8)**	**0.5 (0.3-0.6)****	0.6 (0.5-0.7)	0.3 (0.2-0.5)	**0.8 (0.6-0.9)**	**0.5 (0.4-0.7)***

*p<0.05; **p<0.001

Table II. *Correlation factor R values between selected biochemical parameters in dyslipidemic group*
Tabela II. *Wartości współczynników korelacji R między wybranymi parametrami biochemicznymi w grupie badanej*

Variable/Zmienna	TC	LDL-C	non-HDL-C
TC	-	0.83**	0.85**
LDL-C	0.83**	-	0.92**
HDL-C	ns	ns	-0.31*
TC:HDL-C	0.37*	0.61**	0.79**
TG:HDL-C	-0.33*	ns	ns
apoB	0.36*	0.45*	0.53**
apoB:apoAI	0.41*	0.67**	0.76**

*p<0.05; **p<0.001; ns: p>0.05

Table III. *Values of measured parameters in two groups of relative CHD risk, specified by non-HDL-C concentration*
Tabela III. *Wartości oznaczonych parametrów w dwóch grupach relatywnego ryzyka ChNS, określonego stężeniem nie-HDL-C*

non-HDL-C (mg/dL)	**<160 n=20**	**≥160 n=24**	**p**
CHD Risk /Ryzyko ChNS	**Low/Niskie**	**Elevated/Podwyższone**	-
Glu (mg/dl)	86 ± 9.2	87 ± 6.7	ns
TC (mg/dl)	**204 (183-211)**	**234 (223-249)**	**<0.001**
HDL-C (mg/dl)	67 (53-82)	56 (53-64)	ns
LDL-C (mg/dl)	**112 (81-127)**	**152 (143-168)**	**<0.001**
TG (mg/dl)	**96 (64-164)**	**110 (76-154)**	**<0.001**
CRP (mg/l)	1.1 (0.5-3.5)	0.8 (0.3-1.5)	ns
TC:HDL-C	3.1 (2.6-3.4)	4.2 (3.6-4.6)	ns
TG:HDL-C	1.76 (0.91-2.87)	1.69 (1.23-3.06)	ns
ApoAI (mg/dl)	**135 (103-149)**	**122 (98-142)**	**<0.001**
ApoB (mg/dl)	72 (57-90)	92 (84-104)	ns
ApoB:ApoAI	**0.58 (0.49-0.65)**	**0.79 (0.67-0.88)**	**0.003**

ns: p>0.05

ApoB concentration above the accepted value was observed in 25% of subjects with hyperlipidemia and elevated TC:HDL-C and apoB:apoAI ratios in 61% and 77%, respectively. Relationship between tertiles of non-HDL-C and apoB (Figure 1) or apoB:apoAI ratio was analyzed. In the highest concentrations of non-HDL-C (169-221 mg/dL) properly 18% and 23% of dyslipidemic individuals had apoB and apoB:apoAI in their 3rd tertiles (apoB 93-193 mg/dL, apoB:apoAI 0.79-1.05).

TG:HDL-C ratio, which is related to sd-LDL appearance [7], was also analyzed. Its elevated values coexisted with apoB>100 mg/dL in 27% of dyslipidemic group. No significant difference in two groups of cardiovascular risk and no association between TG:HDL-C ratio and non-HDL-C were found, except for the weak correlation with TC (R=-0.33; p=0.027), TC:HDL (R=0.5; p=0.001) and apoB:apoAI (R=0.3; p=0.045). It is worth emphasizing that elevated values of TG:HDL-C ratio (≥0.9) were observed also in the control group.

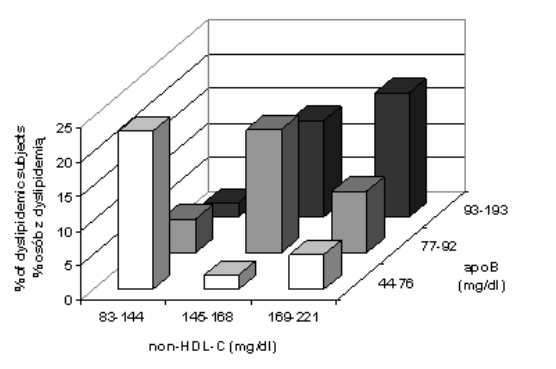

Fig. 1. *Frequency of elevated apoB values in non-HDL-C tertiles in dyslipidemic subjects*
Ryc. 1. *Częstość występowania podwyższonych wartości apoB w tercylach stężenia nie-HDL-C w grupie z dyslipidemią*

DISCUSSION

Lipid profile is a routine examination recommended by general practitioners for evaluation of atherosclerosis and cardiovascular risk. However total cholesterol, HDL-C and LDL-C concentration do not fully assess the risk of CVD. Numerous reports confirm diagnostic usefulness of non-HDL-C in primary and secondary CVD prevention, both in hyperlipidemic and normolipidemic patients, because of the fact that this parameter estimates a risk caused by all potential proatherogenic lipoproteins. The Ibaraki Perfectural Health Study [8] showed non-HDL-C >120 mg/dL in 77% and >140 mg/dL in 56% subjects in a population of over 91 thousand healthy Japanese aged 40-79 years. Elevated non-HDL-C occurred twice as often in women. Increasing non-HDL-C concentration with age in women and inverse relationship in men were observed. Individuals with higher non-HDL-C levels had higher TC, LDL-C and lower HDL-C values.

On the contrary, lower non-HDL-C levels in women and higher frequency of non-HDL-C>160 mg/dL in men were noticed in our study.

Liu et al. compared diagnostic value of non-HDL-C as a prognostic factor for myocardial infraction in healthy subjects and diabetics [9]. Patients were divided into three groups of relative CHD risk, specified by non-HDL-C levels: low (<130 mg/dL), moderate (130-159 mg/dL) and high (≥160 mg/dl). In

diabetics elevated non-HDL-C levels and higher relative risk of acute coronary syndromes were observed. Both groups had higher CHD risk with increasing non-HDL-C levels. However, in each interval it was 1.5-2 times greater in diabetics. Increased cardiovascular risk with simultaneous elevation of non-HDL-C and LDL-C (>100 mg/dL) was found in healthy population. The authors emphasized that each increase by 1 mg/dL of non-HDL cholesterol was associated with a 5% increased risk for CHD death and it seems that non-HDL-C is a more useful risk factor than other lipid profile components.

Better CHD risk estimation by non-HDL-C than by traditional lipid markers in healthy, non-diabetic Saudi population was shown by Al-Daghri et al. [10]. Data from the SAFARI Study and the Copenhagen City Heart Study [11, 12] indicated a better relationship between non-HDL-C and apoB or CHD risk than LDL-C in healthy subjects and among patients treated with statins. Sniderman et al. observed a strong association of non-HDL-C with apoB and their close diagnostic value in evaluating cardiovascular risk in general and normolipidemic population [13]. Also, the Health Professionals Follow-up Study proved a relation between apoB and non-HDL-C in estimating fatal myocardial infraction during 6 years of follow-up among American men aged 40-79 years [14].

Significant relationship between non-HDL-C and apoB was noticed in our study. Non-HDL-C was related to other serum lipid components much stronger than LDL-C. Furthermore, elevated apoB appeared with increasing non-HDL-C. Subjects with greater relative CVD risk (>160 mg/dL) had relevant higher apoB:apoAI values.

TG:HDL-C ratio associated with sd-LDL occurrence can be used as a risk factor for coronary heart disease. In our data no significant relation between this parameter and non-HDL-C was observed and its elevated values were noticed also in normolipidemic subjects. Maruyama et al. [7] noticed the presence of highly atherogenic LDL particles with diameter below 25.5 nm in young, non-diabetic subjects without prominent hyperlipidemia. On the basis of the data, TG:HDL-C could be approved as a helpful predictor of atherosclerosis in the healthy population. However, the relationship between non-HDL-C and TG:HDL-C is not fully recognized yet. TG:HDL-C ratio can be used as an independent risk factor which correlates with apoB and sd-LDL concentrations better than other lipid components [15]. On the other hand, many researchers agree that both non-HDL-C and TG:HDL-C ratio had close and strong predictive value for CHD and they may be of significant importance especially in hypertriglyceridemia associated with diabetes, obesity and metabolic syndrome [16, 17].

Despite many advantages of assessing the concentration of non-HDL cholesterol as a risk factor for coronary heart disease, greater diagnostic value of apoB and apoB:apoAI ratio is still emphasized [18]. Although Ridker et al. observed close correlation between non-HDL-C, TC:HDL-C and a risk of CHD, superior to apoB, the importance of measurement of apolipoproteins and CRP as risk factors should not be rejected [19]. Calculation of non-HDL-C combined with measurement of apoB and small dense LDL-C concentration or LDL particles number may actually become the most optimal solution in estimation of cardiovascular risk [20].

CONCLUSIONS

Elevated non-HDL-C concentration can be considered as a clinically useful risk factor for cardiovascular disease in young dyslipidemic subjects. We are aware of the limitation of this study that includes a relatively small group of young, apparently healthy women and men. However, we believe that the received significant relationship between non-HDL-C, apoB and apoB:apoAI values could have several important clinical implications. We conclude that the use of a simple non-HDL-C calculation in routine lipid profile allows an accurate risk assessment of atherosclerosis and coronary heart disease.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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STEREOLOGICAL ANALYSIS OF THE EFFECTS OF SWIMMING ON HEART MUSCLE IN RATS

STEREOLOGICZNA ANALIZA EFEKTU PŁYWANIA NA MIĘSIEŃ SERCOWY U SZCZURÓW

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S u m m a r y

The capillary network and myocytes in normal and in rats exercised by swimming were studied using morphometric techniques to determine the effect of exercise. The capillary contents were significantly increased. Nuclear profile density(Na) increased but a number of nuclei per unit volume of the cardiac myocytes (Nv) was found to be unchanged. As a result of exercise highly significant quantitative change in the chromatin patterns of nuclei was found. Mitochondrial contents distribution in their cardiac myocytes were studied and highly significant alterations in the volume fractions distribution were found.

S t r e s z c z e n i e

Badania zostały przeprowadzone na 12 szczurach, w tym 6 poddano progresywnemu wysiłkowi (pływaniu) przez 35 dni. Siatka kapilarna i miocyty u obu grup szczurów zostały opracowane morfometryczną (stereometryczną) techniką.

Gęstość kapilarnej siatki powiększyła się istotnie u szczurów poddawanych codziennemu pływaniu. Gęstość profilowa jąder (Na) była powiększona, a liczba jąder na jednostkę objętości sercowych miocytów (Nv) pozostała niezmieniona. W efekcie treningu zanotowano wysoko statystycznie istotne zmiany w materiale chromatynowym jąder. Zanotowano również istotne zmiany w liczbie i objętości mitochondriów.

Key words: heart muscle, exercise, stereology, rats
Słowa kluczowe: mięsień sercowy, wysiłek fizyczny, stereologia, szczury

INTRODUCTION

Several quantitative morphometric studies have been carried out on cardiac muscle to determine the magnitude of structural alterations which occur in that tissue as a response to exercise and variously with or without partial aortic ligation or renal hypertension. The results of such studies are often contradictory and a considerable amount of work is required to establish whether there are any general tendencies in the pattern of alteration of the cells and organelles in cardiac muscle following exercise or other types of adaptation. The disappearances between the varying results of different investigations are primarily due to four main causes. Firstly, species varying from small rodents such as mice [1] to large pigs [2] and humans [3]. Scaling effects [4] due to metabolic rates being unevenly related to body size are important and may be reflected in differences in quantitative measurements made on micrographs from different species. Secondly, different sampling techniques and different regimes of the heart have been used together with morphometric

techniques which do not allow for the natural anisotropy of cardiac tissue. Thirdly, the techniques of statistical analysis have not usually been comparable and no study has used either the more appropriate multivariate techniques nor allowed for the effects of multiple comparisons on statistical significance levels. Fourthly, various methods have been used to induce cardiac hypertrophy e.g. treadmill running or varying periods of time [5] swimming with or without additional body weighting and also partial aortic ligation [6]. Some studies have also been carried out in spontaneously hypertensive animals [7]. Some investigations have used combinations of cardiac inducers of hypertrophy, for example, pigs have been run for prolonged periods on treadmills following recovery from surgical operations in which partial aortic legation has been carried out [2]. It is possible and probably likely that cardiac muscle may respond differently to the various methods of inducing hypertrophy compared with the isotonic and isokinetic hypertrophies (or adaptation). Experimental studies should, therefore, attempt to study the effects of single variable factors on cardiac adaptation.

In the present study, swimming was actually selected as a simple, convenient, clearly reliable, widely used method using a normal rodent activity to induce cardiac adaptation in adult male rats. The morphometric techniques and theories used were developed specifically for anisotropic tissues [8, 9, 10] and the appropriate statistical analyses were carried out on the data obtained.

MATERIAL AND METHODS

Twelve adult male rats were used in the present study. Six animals were selected to be controls using a random number selection techniques. Animals were maintained in individual cages 40 cm x 18 cm each having a smooth plastic floor. The were allowed food and water ad libitum.

The 6 exercised rats underwent a swimming program consisting of gradually increasing periods of communal swimming in a tank 1.25 m x 1.00 m filled with warm water in the range 30°C to 33°C to a depth of 50 cm. The rats swam twice a day for a 30 minutes in each period of exercise for 35 consecutive days. During the first twelve days the duration of exercise was gradually increased from 5 to a maximum of 30 minutes. The exercise periods were separated by rest intervals of a least 6 hours. After the last exercise period on the 35th day of exercise the rats were allowed a rest period until the next day prior to being sacrificed. Under ether anesthesia the apex of the left ventricle was removed from each animal.

The tissue preparation methods previously used in other investigations [11, 12] were used in the present study.

The number of capillaries per unit area in semi thin transverse $(Na)_I$ and longitudinal $(Na)_{II}$ sections were counted and used to estimate the mean capillary length density per unit volume of heart (Lv or Jv) using the relations

$$Lv = (Na)_I + (Na)_{II} \quad [9, 10]$$

$$Jv = 2/3\ [(Na)_I + 2(Na)_{II} \quad [13]$$

Indices for the relative degree of orientation of the capillaries Ω1,3 and the concentration parameter, K were calculated using the relations

$$1.3\ \Omega = (Na)_I - (Na)_{II} /\ (Na)_I + (Na)_{II} \quad [9]$$

and

$$K = 2 \text{ left } (1 - \{(Na)_I / (Na)_{II}\} \text{ right}) \quad [10]$$

The numerical density of myonuclei, i.e. the number of nuclei per unit volume of heart, Nv, was estimated using the relation

$$Nv = Na / \bar{I} \quad [13, 14]$$

where Na is the number of nuclear profiles seen per unit area of a muscle fiber cross section and $\bar{I}$ the mean length of nuclei measured in thick longitudinal sections.

The mean volume of nuclei, v, was obtained by relation

$$v = Vv / Nv \quad [14]$$

where Vv is the nuclear volume fraction.

The volume fractions of heterochromatin within the nuclei of both the cardiac myocytes and the capillaries were analyzed using a previously detailed technique [11] which is a modification of a well known method for analyzing mosaic patterns morphometric [15, 16]. The method consists of placing a series of transparent test screens bearing randomly distributed and oriented test lines variously 1cm, 2cm and 3cm in length on random sections of nuclei and recording whether the

ends of each test line are "hits" on either heterochromatin or euchromatin.

Two specific morphometric measures of size of mitochondria were analyzed, mitochondrial volume fractions (Vv) and their external membrane surface density (Sv) per unit volume of cardiac myocyte. Surface densities were estimated using the relation

$$Sv = 2/3\ \{(I_I) + 2\ (I_{II}) \quad [13]$$

where (I_I and I_{II}) are the intersection counts between test grid line and the profile boundary of mitochondria. Differences between the control and experimental data were analyzed using procedures for multiple comparisons. Differences between means were tested using Student's t-test but allowances for the effect of multiple comparisons were made [17]. Values of Student's t critical value were taken from Sidak's or Bonferroni's tables for multiple comparison [18]. Homogeneity of empirical and control variances was tested using the variance ratio (F-test) test. Preliminary testing for normality was carried out using the Shapiro and Wilk "W" test [19].

RESULTS

The main results are summarized in Table I-III inclusive.

Table I. *Summary statistics (mean ± SD) for capillary in heart of control and experimental rats*

Tabela I. *Sumaryczne, statystyczne dane (x ± SD) kapilar w mięśniu sercowym u kontrolnych i eksperymentalnych szczurów*

Control	Experimental	Significance levels (t-test)
$(Na)_I$ (mm^{-2})	$(Na)_I$ (mm^{-2})	
1490.30±271.94	1807.26±170.46	.05
$(Na)_{II}$ (mm^{-2})	$(Na)_{II}$ (mm^{-2})	
1050.34±61.46	1234.91±69.86	.002
Lv ($mm\ mm^{-3}$)	Lv ($mm\ mm^{-3}$)	
2540.64±248.24	3042.18±193.61	.002
Jv ($mm\ mm^{-3}$)	Jv ($mm\ mm^{-3}$)	
2393.94±158.95	2851.38±157.31	.002
1.3 Ω (°)	1.3 Ω (°)	
.166±.1o4	.186±.053	n.s.
K (°)	K (°)	
.852±.620	.928±.306	n.s.

Footnote: $(Na)_I$ and $(Na)_{II}$ - number of capillaries per unit area in transverse and longitudinal sections; Lv and Jv – mean capillary length density per unit volume of heart; 1.3 Ω – relative degree of orientation of the capillary and K – concentration parameter of the capillary.

The difference in capillary density count (Na) between control and swum animals in transverse sections was significant only at the 6.4% level whilst the capillary density seen in longitudinal sections was significantly increase. Similarly, all the mean total capillary lengths per unit volume (Jv and Lv) were found to by highly significantly altered. The index of orientation, 1.3 Ω, and concentration parameter (K) were not found to be significantly altered.

Table II. *Summary statistics (mean± SD) for heart myonuclei in control and experimental rats*

Tabela II. *Sumaryczne, statystyczne dane (x ± SD) komórek mięśnia sercowego u kontrolnych i eksperymentalnych szczurów*

Control	Experimental	Significance levels (t-test)
Na (mm^2)	Na (mm^2)	
284.96±60.79	375.00±45.50	.05
$\bar{l}$ (μm)	$\bar{l}$ (μm)	
12.01±.63	10.52±.84	.05
Nv ($mm^{-3} \times 10^4$)	Nv ($mm^{-3} \times 10^4$)	
2.70±.50	3.12±.28	n.s.
v (μm^3)	v (μm^3)	
333.4±198.3	343.4 ±136.2	n.s.
Aa (%)	Aa (%)	
60.4	43.5	.001

Footnote: Na – nuclear profile densities per unit area; $\bar{l}$ – mean length of nuclei (in longitudinal sections); Nv – number of nuclei per unit volume of heart muscle; v – mean volume of nuclei and Aa – mean volume fraction of whole nuclei occurred by heterochromatin.

The nuclear profile density per unit area, Na, of cardiac tissue were found to be significantly increase whilst mean number length ($\bar{l}$) appeared to by significantly decreased. However, the nuclear volume density (Nv) was not altered as a consequence of swimming. Similarly, mean nuclear volume (v) was not altered. The chromatin patterns of the cardiac myocytes (Aa) indicate a significant reduction in the heterochromatin content of experimental nuclei. The size of the heterochromatin patches was slightly reduced whilst those of intervening euchromatin region increased.

Table III. *Summary statistics (mean ±SD) of mitochondrial volume fractions (Vv) and their surface densities (Sv) per unit volume of cardiac myocyte*

Tabela III. *Sumaryczne, statystyczne dane (x ± SD) frakcyjnej objętości mitochondriów (Vv) i ich powierzchniowej gęstości (Sv) na jednostkę objętości komórki mięśnia sercowego*

Control	Experimental	Significance levels (t-test)
Vv (%)	Vv (%)	
37.7	48.0	.001
Sv (μm^2 μm^{-3})	Sv (μm^2 μm^{-3})	
2.84±.19	3.47±30	.001

Table III indicates the alterations in mitochondrial structure which occur as a result of swimming. Significant increase in the external surface area per unit volume of cardiac myocytes, Sv , and in the volume fraction of the cardiac fibers were observed

DISCUSSION

The finding of an increased capillary length density (Lv, Jv) in the myocardium as a result of exercise is entirely consistent with the large number of studies [20, 21, 22] previously expecting and receiving increased capillary profile number density seen only in transverse sections [2, 23]. More important than simple capillary profile count in transverse sections are the estimates Lv, Jv since they depend on capillary profile counts in longitudinal sections, allow for the anisotropy of the capillary network and produce more information than simple profile count. For example, in the present investigation, capillary profile density in the longitudinal plane are more significantly increased than those in the classical transverse plane.

The use of estimation formula for Jv as applied to cardiac tissue has previously been demonstrated [24, 25] though some difference between capillary counts and estimates of measures of size are to be noted. The finding of highly significant alterations in values for Na suggest these raw results may well be more useful than the less sensitive 1.3 Ω and K estimates.

Studies on the myocardial nuclei can yield much data on the mechanisms of cardiac adaptation or hypertrophy [26, 27, 28, 29, 30] and are a potential source of information for objective pathological diagnosis [31]. In the present exercise study myocardial nuclear volumes (v) and nuclear volume densities (Nv) were not significantly altered. Clearly, the adaptive responses of the myocyte nuclei indicate a lack of overall cytoplasmatic hypertrophy. It is possible that the exrcise regime in the present study was not enought to induce hypertrophy. More fundamental nuclear changes indicating greatly increased activity (i.e. significant reduction in overal heterochromatin content and larger mean size of euchromatin) have occurred indicates that more nuclear volume and shape measures are themselves insufficient indication of altered nuclear activity. Clearly, the modified spatial pattern method for analysing eu- and heterochromatin content of nuclei is of as much value in studying cardiac myocyte nuclei as has previously been found in skeletal muscle fibre myonuclei [11, 16].

The alteration in mitochondrial content whilst expected in cardiac myocytes as a response to exercise were nevertheless substantial. Average mitochondrial volume fraction, (Vv) and their external membrane surface density (Sv) were significantly and substantially increased thus agreeing with the findings of some authors [5, 24, 32, 33]. In both normal and post exercised heart muscle evidence of a substantial gradient of mitochondria, highes at the capillary surface regions and lowest at the central axis of the cell as often seen in skeletal muscle fibers [12] was not convincing as in some fibres of that tissue [24].

CONCLUSIONS

As a result of exercise significantly increased number of capillaries in transverse and longitudinal sections, mean capillary length density, nuclear profile densities, mean length od nuclei, mean volume fraction of euchromatin, mitochondrial volume fractions and their surface densities of the cardiac myocytes in rats.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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COMPARISON OF EFFECTIVENESS OF SEGMENTARY AND CLASSICAL MASSAGE IN THE TREATMENT OF LOW BACK PAIN SYNDROME

PORÓWNANIE SKUTECZNOŚCI MASAŻU SEGMENTARNEGO I KLASYCZNEGO W ZESPOLE BÓLOWYM DOLNEGO ODCINKA KRĘGOSŁUPA

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Summary

Introduction: Low back pain syndrome is a social ailment, one of ten causing limited activity among the young and middle-aged. It is estimated that around 30-60% of population suffer from back disorders, and among them nearly 80% of adults experience the pain symptoms in lower part of the spine. Massage is one of the therapy methods.

Aim: The aim of the paper is to compare the effectiveness of segmentary and classical massage in the treatment of low back pain syndrome.

Material and methods: The research involved twenty people with low back pain syndrome. The studied group included 10 people treated with segmentary massage, whereas the other group composed the control group, treated with classical massage. Each group was subject to a series of 10 procedures. The study was based on measurement of range of mobility in the lower part of the spine, pain assessment using a test in VAS scale before and after the therapy, as well as a questionnaire completed after finishing the series of procedures. The evaluation of the survey was conducted according to a statistical method – student's T distribution.

Results: Based on evaluation of the increase of mobility in the lower part of the spine, a standard deviation assigned to segmentary massage was calculated, which shows a greater dispersion of examined values, whereas for the classical massage it takes comparable values. In majority of kinds of movements segmentary massage caused a considerable change in mobility to a much higher degree than classical massage. Moreover, segmentary massage reduced suffering significantly.

Conclusions: After completion of a series of procedures of both segmentary and classical massage mobility in the lower part of the spine improved and pain was reduced. Moreover, segmentary massage proved to be more effective, causing greater mobility improvement and pain reduction. Also, according to the aforementioned questionnaire segmentary massage appeared to be more efficient.

Streszczenie

Wstęp: Zespół bólowy dolnego odcinka kręgosłupa jest to choroba społeczna, która powoduje najczęściej ograniczenie aktywności u ludzi młodych i w średnim wieku. Szacuje się, że dolegliwości bólowe kręgosłupa odczuwa 30-60% populacji, natomiast u 80% dorosłych objawy dotyczą dolnego odcinka kręgosłupa. Jedną z metod leczenia jest masaż.

Cel pracy: Celem pracy jest porównanie skuteczności masażu segmentarnego i klasycznego w leczeniu zespołu bólowego dolnego odcinka kręgosłupa.

Materiał i metody: Badaniem objęto 20 osób z zespołem bólowym dolnego odcinka kręgosłupa. Grupa badana stanowiła 10 osób leczonych masażem segmentarnym, natomiast pozostała część stanowiła grupę kontrolną leczoną masażem klasycznym. W każdej grupie

chorych stosowano zabiegi w serii 10. Badanie polegało na pomiarze zakresu ruchomości w dolnym odcinku kręgosłupa, ocenie bólu testem w skali VAS przed i po terapii oraz ankiecie po zakończeniu serii zabiegowej. Do oceny badania posłużono się metoda statystyczną – rozkładem T student.

W y n i k i : Na podstawie oceny przyrostów ruchomości w dolnym odcinku kręgosłupa zostało obliczone odchylenie standardowe wyznaczone dla masażu segmentarnego, które wykazuje większy rozrzut badanych wartości, natomiast dla masażu klasycznego przyjmuje zbliżone wartości. W zdecydowanej większości rodzajów ruchów masaż segmentarny powodował istotną zmianę ruchomości w stopniu wyższym niż klasyczny. Również dolegliwości bólowe znacznie się zmniejszyły po masażu segmentarnym.

W n i o s k i : Po zakończeniu serii zabiegowej masażu segmentarnego i klasycznego ruchomość w dolnym odcinku kręgosłupa zwiększyła się i zmniejszył się ból. Jednak skuteczniejszy okazał się masaż segmentarny, który spowodował znaczniejszą poprawę ruchomości i odczuwalne zmniejszenie bólu. Według przeprowadzonej ankiety również masaż segmentarny okazał się skuteczniejszy.

Key words: segmentary massage, classical massage, pain syndrome, low part of the spine
Słowa kluczowe: masaż segmentarny, masaż klasyczny, zespół bólowy, dolny odcinek kręgosłupa

INTRODUCTION

The human spinal column is a type of biomechanical complex, composed of stiff segments (vertebrae) separated by flexible segments (intervertebral discs and ligaments). Owing to that it maintains its inner stability and it is additionally strengthened by muscles and trunk, thorax and abdomen. It is subjected to both physiologic and pathologic tensions, loads and injuries. It also concerns lumbar spine among others. The functional unit of the spine is composed of two neighbouring vertebrae joined by an intervertebral disc. They have great importance for efficiency and competence of lumbar spine. [1] Most probably it is the intrevertebral disc which most often causes backbone pains and it may constitute even above 85% cases [2].

Low back pain syndrome is an ailment often referred to as sacralgia. It is a predominant of muscular-skeletal system disorders in the industrialized societies. Pain is located mostly in lumbar, lumbar-sacral and sacroiliac regions. There are numerous causes of that disorder. Among them the most often are : degenerative changes (of intervertebral disc, vertebral body, intervertebral joints), inflammation of vertebral joints, inborn anomalies, overloading and injuries, hyperalgesia, ailments located beyond spinal column [3, 4].

Contemporary medicine offers various methods of management of low back pain syndrome. They include both conservative and operational treatment. The medical treatment requires considering the span of suffering: acute or chronic. The treatment is based on using physiotherapy, kinesitherapy, pharmacotherapy and massage [5, 6]. The central objective of massage is to stimulate nervous system thanks to coordinating activity of cerebral cortex and using reflexes, which influence all organs and systems of our bodies.

In the treatment of the lower back pain syndrome there were two types of massage used: segmentary and classical, each in separate group. The mentioned kinds of massage differ significantly in many ways, e.g. different techniques (much deeper in segmentary massage), the direction of movements, the first one does not use lubricants. Low back is treated using the following techniques of segmentary massage: patting and segmentary rubbing (spiral), as well as grips: right-side and left-side screwing, on spinous processes, sawing, pushing, pulling; vibrating and pelvis shaking. The direction of the movements in the massage could be towards the head, centripetal and paravertebral. Classical massage includes the following techniques: patting, rubbing, stroking, vibration, pelvis shaking. The direction of the movements could be towards the head, centrifugal and paravertebral [7, 8, 9, 10]. The other method of treatment is an operation. If conservative treatment does not give the expected results (1-10%), surgical intervention is necessary [5].

AIM

The aim of the paper is to compare the effectiveness of segmentary and classical massage in the treatment of the lower back pain syndrome.

MATERIAL AND METHODS

The research was conducted on a group of 20 patients treated in the NZOZ "REH-MED" [Rheumatology and Rehabilitation Clinic] in Bydgoszcz between September 2007 and May 2008. The studied group included 10 people treated with segmentary massage, whereas the other 10 patients formed a control group given the therapy of classical massage. Patients in both groups were subject to a

series of 10 procedures. All the people participating in the research were above 18 years old and of both sexes. In both groups women constituted majority; 60% in the first one and 80% in the other group.

The examination consisted of the measurement of mobility range in the lower part of the vertebral column (flexion and extension of lumbar spine, sideward flexion and rotation in thoracic spine) using centimeter tape as well as pain assessment using VAS scale before the first massage and after a series of 10 procedures [11].

VAS scale (Visual Analogue Score) is a tool used for the assessment of the intensity of pain. It is made of a straight 10 cm line on which we mark two opposite ends and separate segments. According to that scale '0' means no pain at all, whereas '10' stands for the strongest pain a patient can imagine. It is a comprehensible, easy to use scale requiring no complicated terminology [12, 13].

After the therapy a questionnaire was developed to assess the effectiveness of the performed type of massage.

The evaluation of the research was made using the statistical t-Student method. It allowed to compare the received results of the treatment.

RESULTS

Table I presents statistical assessment of the treatment results considering the increase of mobility in lumbar and thoracic spine, whereas Fig. 1 and 2 show the results of statistical analysis from Table I.

Table I. *Statistical assessment of results based on the increase of mobility in lumbar and thoracic spine after segmentary and classical massage*

Tabela I. *Statystyczna ocena wyników na podstawie przyrostów ruchomości w odcinku lędźwiowym oraz piersiowo-lędźwowym kręgosłupa po masażu segmentarnym i klasycznym*

		Zgięcie/ Flexion L [mm]	Wyprost/ Extension L [mm]	Zgięcie boczne w prawo/ Flexion to the right Th-L [mm]	Zgięcie boczne w lewo/ Flexion to the left Th-L [mm]	Rotacja w prawo/ Rotation to the right Th-L [mm]	Rotacja w prawo/ Rotation to the left Th-L [mm]
Masaż Segmentarny/ segmentary massage	**średnia/ mean**	**9.5**	**19.5**	**13.5**	**16.5**	**12**	**11**
	odchylenie standardowe/ standard deviation	5.50	13.01	7.84	13.13	4.83	5.68
	p	0.012	0.015	0.080	0.052	0.008	0.003
Masaż klasyczny/ classical massage	**Średnia/ mean**	**11.5**	**11.5**	**13.5**	**11.0**	**11.0**	**8.5**
	odchylenie standardowe/ standard deviation	5.30	5.80	6.69	5.68	6.15	6.26
	p	0.056	0.032	0.087	0.118	0.002	0.007

Standard deviation reached after segmentary massage shows a greater dispersion of the examined values, whereas for the classical massage it takes comparable values.

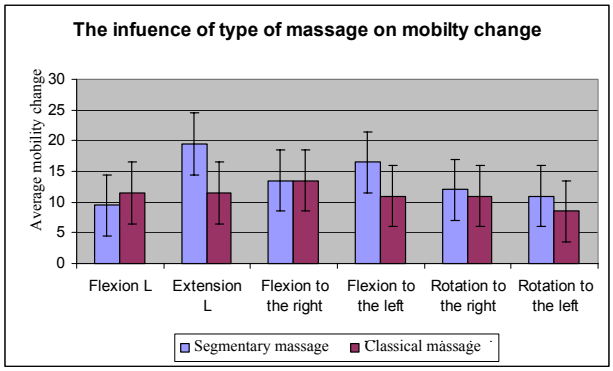

Fig. 1. *The influence of type of massage on mobility change considering average mobility increase*

Ryc. 1. *Wpływ rodzaju masażu na zmianę ruchomości z uwzględnieniem średniego przyrostu ruchomości*

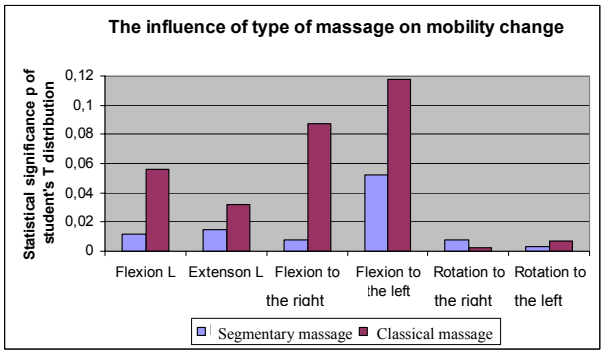

Fig. 2. *The influence of type of massage on mobility change considering statistical significance*

Ryc. 2. *Wpływ rodzaju masażu na zmianę ruchomości z uwzględnieniem poziomu istotności*

From the analysis of Fig. 2, it can be assumed that for the vast majority of the examined movements segmentary massage proved to induce a greater change in mobility than classical massage.

Table II presents relative mobility change using the means from Table I.

Relative mobility change (u)

$$u = \frac{u_{SEG} - u_{CLAS}}{u_{CLAS}} * 100\%$$

Table II. *The relative mobility change of segmentary massage in relation to classical massage and statistical significance*

Tabela II. *Względna zmiana ruchomości masażu segmentarnego względem masażu klasycznego oraz poziom istotności*

	Zgięcie/ Flexion L	Wyprost/ Extension L	Zgięcie boczne w prawo/ Flexion to the right Th-L	Zgięcie boczne w lewo/ Flexion to the left Th-L	Rotacja w prawo/ Rotation to the right Th-L	Rotacja w prawo/ Rotation to the left Th-L
u [%]	-17.39	69.57	0.00	50.00	9.09	29.41
p	0.056	0.032	0.087	0.118	0.002	0.007

The analysis of the above table shows that segmentary massage proved to be more effective than classical massage, increasing the extension of lumbar spine of about 70%, flexion of thoracic spine to the left of 50%, rotation of thoracic spine to the right of about 9% and to the left of about 29%. Nonetheless, in flexion of lumbar spine segmentary massage turned out to be worse than the classical one of about 17%. Sideward flexion of thoracic spine to the right showed no changes.

Fig. 3 shows the effectiveness of classical and segmentary massage. Statistically, the most important differences occur in the extension of lumbar spine. There are no differences for the flexion of thoracic spine to the right side.

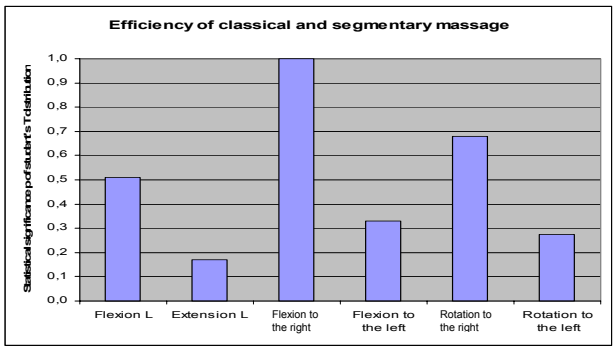

Fig. 3. *Efficiency of classical and segmentary massage considering statistical significance*

Ryc. 3. *Skuteczność masażu klasycznego i segmentarnego z uwzględnieniem poziomu istotności*

Fig. 4, 5 and table III show VAS scale pain assessment before and after segmentary and classical massage therapy and statistical analysis.

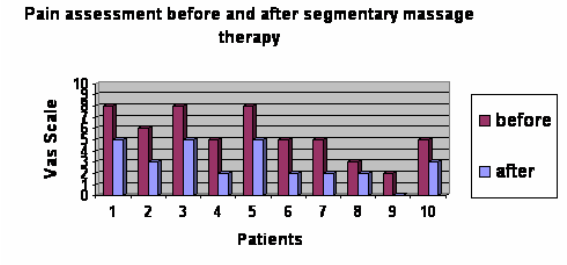

Fig. 4. *VAS scale pain assessment before and after segmentary massage therapy*

Ryc. 4. *Ocena bólu w skali Vas przed i po terapii masażem segmentarnym*

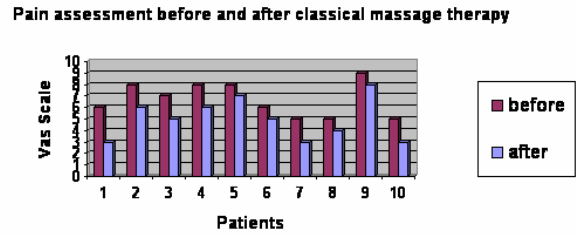

Fig. 5. *VAS scale pain assessment before and after classical massage therapy*

Ryc. 5. *Ocena bólu w skali Vas przed i po terapii masażem klasycznym*

Table III. *Algesia variation and statistical assessment*

Tabela III. *Zmiana odczuwania bólu oraz ocena statystyczna*

	Klasyczny/ Classical	Segmentarny/ Segmentary
średnia/mean	-1.7	-2.6
odchylenie standardowe/ standard deviation	0.67	0.70
p	0.031	0.006

The questionnaire concerning the efficiency of the type of massage applied consisted of three questions.

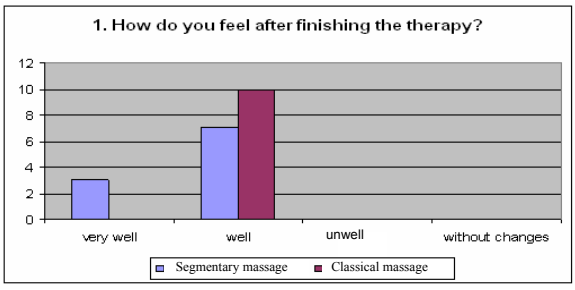

Fig. 6. *First question*

Ryc. 6. *Pytanie pierwsze*

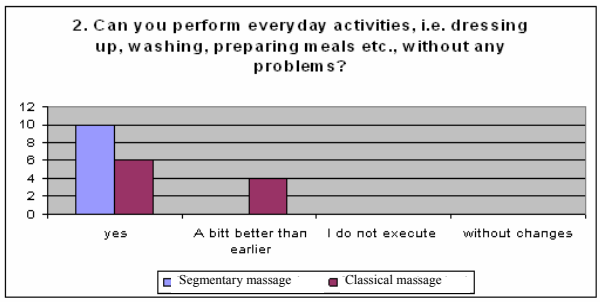

Fig. 7. *Second question*
Ryc. 7. *Pytanie drugie*

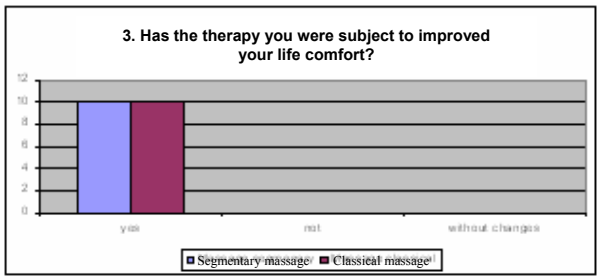

Fig. 8. *Third question*
Ryc. 8. *Pytanie trzecie*

DISCUSSION

After conducting the examination and data analysis it was assumed that both segmentary and classical massage help improving mobility of the lower part of the spine and reducing the pain. The comparison of the mentioned procedures shows that majority of the tested movements of segmentary massage resulted in a greater mobility change than classical massage. Also pain was reduced more significantly after segmentary massage. The questionnaire on the effectiveness of implemented therapy also assigns a priority to the mentioned technique. The third question, which both groups of patients answered positively to, (the therapy improved their life comfort), is important.

The researches of: Chołodecki D., Kassolik K., Zosuł G., Krawiecka-Jaworska E. prove medical massage to be an effective and non-invasive method of treatment and to prevent suffering in the discussed ailment [14].

Moreover, Andrzejewski W., Witkowski T., Kassolik K. believe that medical massage can improve mobility in the chronic lower back pain disorders and alleviate suffering [15].

According to the recommendations of the Polish Rehabilitation Society on the management of the lower back pain syndrome in both acute and chronic cases, the treatment includes: pharmacotherapy, physiotherapy, massage, kinesitherapy and psychotherapy [5, 6].

CONCLUSIONS

1. Increase in mobility of the lower part of the spine after both segmentary and classical massage.
2. Pain reduction subsequent to both types of massage.
3. To majority of the tested movements segmentary massage resulted in a greater mobility change than classical massage.
4. Segmentary massage helped to reduce the pain more significantly than classical massage.
5. According to the developed questionnaire segmentary massage emerged to be more effective.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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THE DIFFERENCES IN THE MORPHOLOGICAL BUILD OF FULL-TIME AND PART-TIME PHYSICAL EDUCATION STUDENTS OF KAZIMIERZ WIELKI UNIVERSITY IN BYDGOSZCZ

RÓŻNICE W BUDOWIE MORFOLOGICZNEJ STUDENTÓW STUDIÓW STACJONARNYCH I NIESTACJONARNYCH Z KIERUNKU WYCHOWANIA FIZYCZNEGO UNIWERSYTETU KAZIMIERZA WIELKIEGO W BYDGOSZCZY

Institute of Physical Education, Kazimierz Wielki University in Bydgoszcz
Head: dr Mariusz Zasada

Summary

The objective of this work was to indicate the differences of the chosen somatic parameters of the first year full-time and part-time students.

The research was conducted between December 2008 and August 2009 in the Kazimierz Wielki University (UKW) in Bydgoszcz. 114 (75 male and 39 female) physical education students aged 19 to 26 took part in the research. The anthropometric measurements were made in order to determine the following values: body height, body mass, forearm length, arm length, upper limb length, lower limb length, foot length, shoulder width, hip width, pelvis width, hand width, palm width and foot width. Apart from the above, circumference measurements of chest – on full inhale and full exhale, waist, hips, arm – both tensed and loosened, as well as thigh and calfwere made. Apart from the above, measurements of the thickness of three dermato-adipose folds (mm) placed over the triceps muscle of arm, below the inferior angle of shoulder blade and over iliac crest were made. Based on the above measurements, Arm Muscle Circumference (AMC), Waist to Hip Ratio (WHR), Body Mass Index (BMI), Fat Mass (FM), proportional Fat Mass (%FM), Fat Free Mass (FFM) in kilograms and proportional Fat Free Mass (%FFM) values were calculated. The measurements were made using the portable medical scale (model: TANITA BF 662 and the anthropometric tool-kit (anthropometer, anthropometric tape, foldometer) produced by a Swiss firm Siber Hegner & Co. Ltd.

Average values and standard deviations were calculated from the taken measurements and statistical inference was conducted by comparing the examined populations. The t-student test and the Mollison index were used in this case.

From the calculations made it results that in the majority of cases the values of examined morphological features do not differ. The differences, which are statistically relevant, appear in the measurements of the features which influence the amount of fat in the body, which gives evidence of higher body fat in the bodies of part-time students comparing to full-time students.

Streszczenie

Celem pracy było wskazanie wielkości różnic wybranych parametrów somatycznych u studentów pierwszego roku studiów stacjonarnych i studiów niestacjonarnych.

Badania prowadzono na Uniwersytecie Kazimierza Wielkiego (UKW) w Bydgoszczy od grudnia 2008 roku do sierpnia 2009 roku. Objęto nimi 114 studentów (75 mężczyzn i 39 kobiet) w wieku 19-26 lat studiujących na kierunku wychowania fizycznego. Przeprowadzono badania antropometryczne, wyznaczając następujące wielkości: wysokość ciała, masa ciała, długość przedramienia, długość ramienia, długość kończyny górnej, długość kończyny dolnej, długość stopy, szerokość barków, szerokość bioder, miednicy, ręki, dłoni i stopy. Poza tym zmierzono obwód klatki piersiowej przy pełnym wdechu i wydechu, obwód talii, bioder, obwód napiętego i rozluźnionego ramienia, oraz uda i łydki. Dodatkowo zmierzono grubość trzech fałdów skórno-tłuszczowych położonych: nad mięśniem trójgłowym ramienia, pod dolnym katem łopatek, nad grzebieniem kości

biodrowej. W oparciu o powyższe pomiary wyliczono: wskaźnik obwodu mięśni ramienia (AMC), wskaźnik talia/biodra (WHR) wskaźnik wagowo-wzrostowy (BMI), masę tłuszczu w ciele (FM) (kg), procentową zawartość tłuszczu w ciele (%FM) (%), oraz beztłuszczowa masę ciała w kilogramach i procentach (FFM) (kg), (FFM) (%). Pomiary wykonano używając przenośnej wagi lekarskiej TANITA BF 662M oraz zestawu narzędzi antropometrycznych (antropometr, taśma, fałdomierz) szwajcarskiej firmy Siber Hegner & Co. Ltd.

Z przeprowadzonych pomiarów wyliczono wartości średnie, odchylenia standardowe, oraz przeprowadzono wnioskowanie statystyczne, porównując ze sobą badane populacje. W tym celu wykorzystano test t-Studenta oraz wskaźnik Mollisona.

Z wykonanych obliczeń wynika, że w większości przypadków wielkość badanych cech morfologicznych nie różni się. Różnice istotne statystycznie pojawiają się przy pomiarach cech mających wpływ na rozmiar masy tłuszczu w ciele, co świadczy o większej zawartości tkanki tłuszczowej u studentów studiujących w trybie niestacjonarnym w stosunku do studentów stacjonarnych.

Key words: morphological features, physical education, students
Słowa kluczowe: cechy morfologiczne, wychowanie fizyczne, studenci

INTRODUCTION

The size of human body has been undergoing continuous changes throughout centuries. The process of phylogenetic formation of a man led to today's form of *homo sapiens*. Human body shows a significant elasticity in adjusting to the surrounding conditions, both in the area of inner-body processes e.g. the speed of puberty, and in the sphere of the morphological build. The ontogenetic changes lead to shaping the individuals, which are able to take part in the procreation process. The human individual's development (ontogenesis) is a cohesive process, which takes into account genetic conditions and the environmental conditions [1]. These are certainly the most important reasons of formation of individual differences in the population presenting the most diverse living conditions. A clear trend of tall growth observed among persons living in the developing countries may be an example. It is supposed that the reason of emerging those development characteristics is the degree of realization of the genetic records in the area of progressive somatic development, which results from the increasing standard of living of the consecutive generations. The confirmation of correctness of the conclusions of the presented thesis is the lack of the phenomenon of growing tall in the societies, which reached a high level of socio-economic development for a number of generations. The trend was stopped when the consecutive generations realized that their genetic potential was related to the somatic development in almost one hundred percent [2]. The knowledge of the course of the generation development processes allows to make such a selection of educational and pedagogical methods that will encourage optimal development of the next generations.

The objective of this work was to determine the differences in the morphological build of the full-time and part-time Kazimierz Wielki University in Bydgoszcz physical education students (male and female).

RESEARCH MATERIAL AND METHODOLOGY

The research was conducted among 114 physical education students (75 male and 39 female) of Kazimierz Wielki University in Bydgoszcz aged 19 to 26 between December 2008 and August 2009. The group was divided into two subgroups – one consisted of full-time students (32 male, 18 female) and the other consisted of part-time students (43 male, 21 female). Male and female part-time students were, on average, four years older than their colleagues from the full-time studies. The following anthropometric measurements were taken from all examined students (cm): body height (V – B), arm length (a – r), forearm length (r – sty), upper limb length (a – da III), lower limb length (tro – B), foot length (ap – pte), shoulder width (a – a), hip width (ic – ic), pelvis width (is – is), hand width (mm – mu), palm width (mr – mu) and foot width (mtt – mtf). Apart from the above, chest circumference – on full inhale and full exhale, waist, hip circumference as well as arm – both tensed and loosened, thigh and calf circumference measurements were taken (cm). Apart from the above, body mass was assigned (kg). Apart from the above, measurements of the thickness of three dermato-adipose folds (mm) placed over the triceps muscle of arm (TSF, triceps skinfold), vertical skinfold, below the inferior angle of

shoulder blade (SCSF, subscapular skinfold), horizontal skinfold, and over iliac crest (SISF, suprailiac skinfold), diagonal skinfold were taken. Based on the above measurements, Body Mass Index (BMI, kg/m^2), Arm Muscle Circumference (AMC), Waist to Hip Ratio (WHR), Fat Mass (FM, kg), proportional Fat Mass (%FM), Fat Free Mass in kilograms (FFM, kg) and proportional Fat Free Mass (%FFM) values were calculated [1].

In order to explain the calculated indexes, the values below were presupposed:

- BMI values for men and women: below 19.0 – body mass deficit, 19.0 – 25.0 - proper body mass, 25.1 – 29.9 - overweight, 30.0+ - obesity,
- the border value of WHR index, which enables to note obesity, is over 0.95 for men and over 0.85 for women,
- AMC evaluation criteria: the degree of aluminous nutrition was calculated according to the following formula: arm circumference – (3.14 x the thickness of the dermato-adipose fold over the triceps), the following values were presupposed: proper aluminous nutrition: men – over 22.8. women – over 20.9; slight malnutrition: men - 22.7-20.2. women - 20.8-18.6; moderate malnutrition: men – 20.1-17.7. women – 18.5-16.2; heavy malnutrition: men - below 17.7. women - below 16.2 [3].

The measurements were made using a portable medical scale – model: TANITA BF 662M and the anthropometric tool-kit (anthropometer, anthropometric tape, foldometer) produced by a Swiss company Siber Hegner & Co. Ltd.

Average values and standard deviations were calculated from the taken measurements and statistical inference was conducted by comparing the examined parameters between the groups. The t-student test and the Mollison index were used in this case.

THE ANALYSIS OF THE RESEARCH RESULTS

The majority of the results of the comparison of the morphological features between the groups of full-time and part-time students proved to be similar.

It appears that in the group of men, the part-time students, in comparison with the full-time students have, on average, their skinfolds placed below the inferior angle of shoulder blade (the statistically relevant difference on 1% level) and the one over the triceps muscle of arm (statistically relevant difference on 5% level) thicker. The length measurements prove that the full-time students, in comparison with the part-time students, have higher forearms (statistical relevance – 1%) and longer feet (statistical relevance 5%). Part-time students have higher waist and thigh circumferences (relevance at 1% level) as well as calf circumferences (5%) and, on average, higher WHR index (the statistically relevant difference at 1% level) (table I).

The largest differences between the examined features determined by the Mollison index were discovered in the sizes of the skinfolds under the shoulder blade (3.38) and in the thigh circumference (0.86) (figure 1). The detailed numeral characteristics are presented in the table I.

The following was noted with regard to the part-time female students (table II) in comparison with the full-time students: averagely larger proportional in-body amount of fat (%FM) and averagely larger fat free body mass (%FFM) (both differences are statistically relevant at 1%level), skinfolds measured over the triceps muscle of arm and over the iliac crest and the sum of the skinfolds (in all three cases the differences are statistically relevant at 5% level). Apart from the above, there was averagely higher waist circumference and higher WHR index (both cases had their statistical relevance at 1% level) in comparison with their colleagues from the full-time studies. However, in the same group, in comparison with the female full-time students, it appeared that the arm and the forearm were, on average, shorter (statistical relevance at 1% level for both measurements), the hips, on average, were narrower (the difference statistically relevant at 5% level) as well as the AMC index was lower on average (statistical relevance at 1% level).

Between the compared groups of female full-time and part-time students the largest differences measured using the Mollison index are indicated by the WHR index (1.40), waist circumference (1.09), proportional in-body fat amount (%FM) (1.08) and proportional fat free mass (1.08) (figure 2). The numeral characteristics of the compared groups of women are presented in the table II.

Tabela I. *Charakterystyki liczbowe badanych cech somatycznych mężczyzn*
Table I. *Numerical characteristics of the tested somatic features of men*

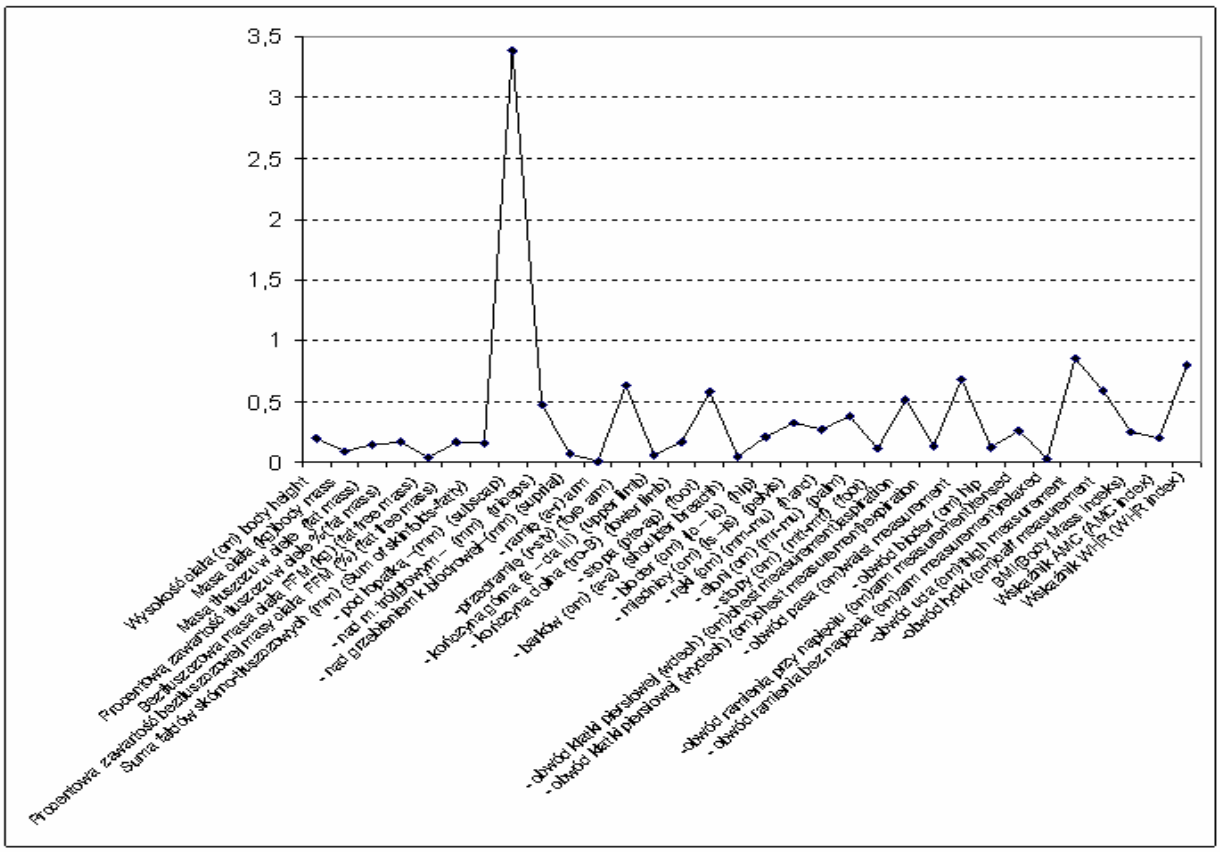
Badana cecha Tested feature	Mężczyźni stacjonarni Men (N-32)		Mężczyźni niestacjonarni (N – 43)		d	u	Wskaźnik Mollisona Mollison's rate
	$\overline{x}_1$	σ_1	$\overline{x}_1$	σ_1			
Wysokość ciała (body height) (cm) (B – V)	181.0	8.01	180.0	5.0	1.0	0.62	0.20
Masa ciała (kg) (body mass)	76.7	9.7	77.6	9.6	0.9	0.4	0.09
Masa tłuszczu w ciele FM (kg) (fat mass) (kg) Procentowa zawartość tłuszczu w ciele FM (%) (fat mass) (%)	12.23 15.73	3.62 3.14	12.86 16.26	4.09 3.13	0.63 1.16	0.71 0.72	0.15 0.17
Beztłuszczowa masa ciała FFM (kg) (fat free mass) (kg) Procentowa zawartość beztłuszczowej masy ciała FFM (%) (fat free mass) (%)	64.48 84.27	7.17 3.14	64.73 83.74	6.05 3.13	0.25 0.53	0.16 0.72	0.04 0.17
Suma fałdów skórno-tłuszczowych (mm) (Sum of dermato-adipose folds) (mm)	30.3	6.70	31.5	7.6	1.2	0.72	0.16
- pod łopatką – (mm) (shoulder blade)	10.8	2.6	19.6	2.6	8.8	14.5**	3.38
- nad m. trójgłowym – (mm) (triceps)	9.0	2.0	10.3	2.7	1.3	2.4*	0.48
- nad grzebieniem k. biodrowej – (mm) (over iliac crest)	10.5	3.3	10.7	2.9	0.2	0.27	0.07
Pomiary długościowe (cm):(length measurements)	32.51	2.51	32.40	7.6	0.11	0.09	0.01
- ramię (a-r) arm	27.43	1.86	25.86	2.49	1.57	3.13**	0.63
- przedramię (r-sty) (fore arm)	79.45	3.95	79.17	4.72	0.28	0.28	0.06
- kończyna górna (a – da III) (upper limb)	91.88	3.89	91.08	4.73	0.80	0.8	0.17
- kończyna dolna (tro-B) (lower limb)	27.15	1.51	26.46	1.18	0.69	2.14*	0.58
- stopa (pte-ap) (foot)							
Pomiary szerok. (cm): (width measurements)							
- barków (cm) (a-a) (shoulder breadth)	43.07	1.89	43.19	2.48	0.12	0.24	0.05
- bioder (cm) (ic – ic) (hip)	30.59	2.19	31.08	2.32	0.49	0.93	0.21
- miednicy (cm) (is –is) (pelvis)	24.23	2.29	24.88	1.96	0.65	1.29	0.33
- ręki (cm) (mm-mu) (hand)	10.93	0.64	10.75	0.64	0.18	1.2	0.28
- dłoni (cm) (mr-mu) (palm)	8.78	0.64	8.57	0.55	0.21	1.49	0.38
- stopy (cm) (mtt-mtf) (foot)	10.14	0.67	10.43	2.50	0.29	0.73	0.12
Pomiary obwod. (cm): (circuit measurements)	97.31	91.82	100.61	6.31	3.30	0.2	0.52
- klatki piersiowej (wdech) (cm) (chest measurement) (inhale)	91.82	5.53	93.62	13.03	1.80	0.81	0.14
- klatki piersiowej (wydech) (cm) (chest measurement) (exhale)	78.02	5.32	82.69	6.78	4.67	3.34**	0.69
- pasa (cm) (waist measurement)	95.53	6.15	96.14	4.79	0.61	0.47	0.13
- bioder (cm) hip	32.88	2.28	33.74	3.28	0.86	1.34	0.26
- ramienia przy napięciu (cm) (arm measurement) (tensed)	29.93	2.67	29.85	2.83	0.08	0.13	0.03
- ramienia bez napięcia (cm) (arm measurement) (loosen)	52.93	3.77	56.21	3.83	3.28	3.7**	0.86
- uda (cm) (thigh measurement)	36.78	2.82	38.22	2.45	1.44	2.31*	0.59
- łydki (cm) (calf measurement)							
BMI (Body Mass Index)	23.4	1.70	24.0	2.4	0.6	1.27	0.25
Wskaźnik AMC (AMC index)	27.11	2.42	26.61	2.54	0.5	0.87	0.20
Wskaźnik WHR (WHR index)	0.82	0.04	0.86	0.05	0.04	3.85**	0.80

N – numbers, $\overline{X}$ – average value, σ - standard variation, d – difference of statistical averages, u - statistic importance of differences
* $p<0.05$;** $p<0.01$; $t_{\alpha=0.05} = 1.98$; $t_{\alpha=0.01} = 2.56$. W_M - Mollison indicator

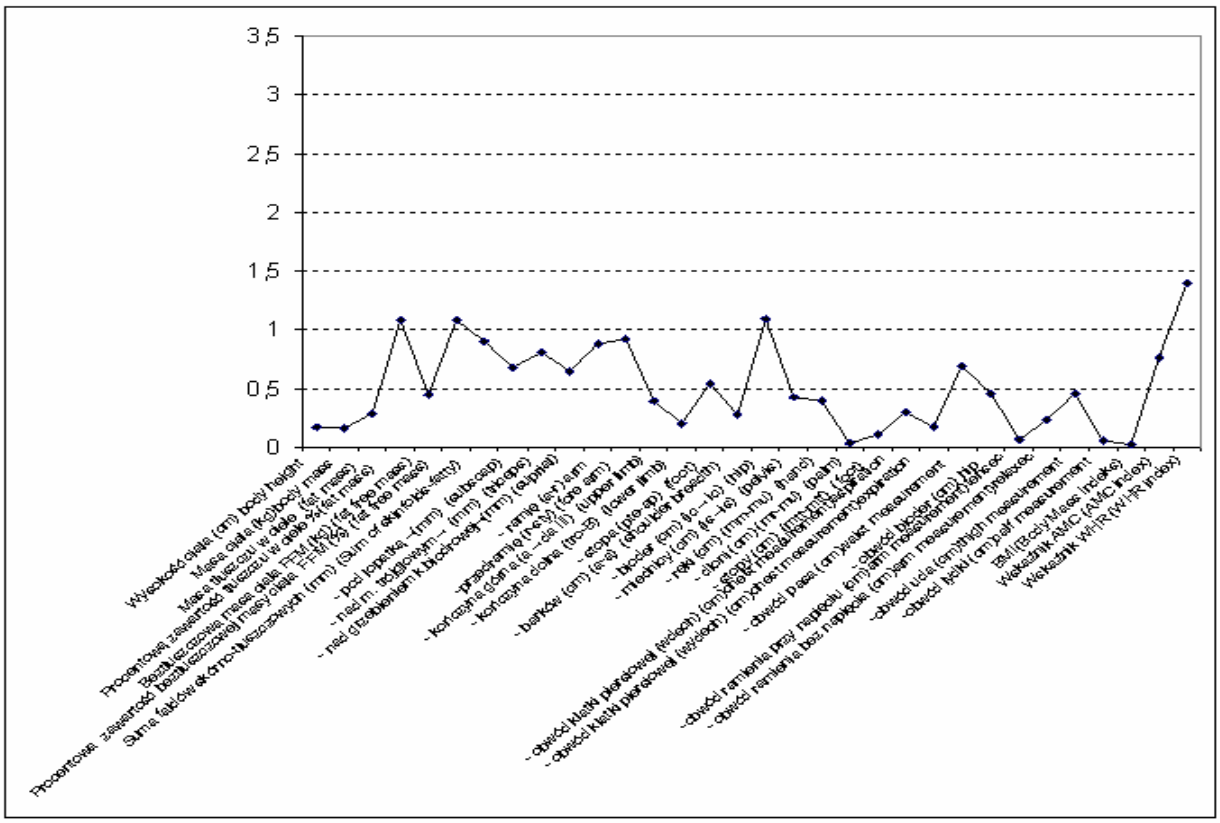
Tabela II. *Charakterystyki liczbowe badanych cech somatycznych kobiet*
Table II. *Numerical characteristics of the tested somatic features of women*

Badana cecha Tested feature	Kobiety stacjonarni Women (N – 18)		Kobiety niestacjonarni (N – 21)		d	u	Wskaźnik Mollisona
	$\overline{x}_2$	σ_2	$\overline{x}_1$	σ_1			
Wysokość ciała (body height) (cm) (B – V)	170.01	7.02	169.0	6.0	1.01	0.48	0.17
Masa ciała (kg) (body mass)	64.3	6.36	63.0	7.97	1.3	0.57	0.16
Masa tłuszczu w ciele FM (kg) (fat mass)	18.34	3.25	19.30	3.27	0.96	0.92	0.29
Procentowa zawartość tłuszczu w ciele FM (%) (fat mass)	28.38%	2.80	30.49%	1.96	2.11	2.68**	1.08
Beztłuszczowa masa ciała FFM (kg) (fat free mass) (kg)	45.93	3.66	43.73	5.00	2.2	1.58	0.44
Procentowa zawartość beztłuszczowej masy ciała FFM (%) (fat free mass) (%)	71.62%	2.80	69.51	1.96	2.11	2.68**	1.08
Suma fałdów skórno-tłuszczowych (mm) (Sum of dermato-adipose folds)	34.2	8.0	39.7	6.1	5.5	2.38*	0.90
- pod łopatką – (mm) (shoulder blade)	11.1	2.88	12.6	2.20	1.5	1.8	0.68
- nad m. trójgłowym – (mm) (triceps)	12.9	3.72	15.1	2.76	2.2	2.07*	0.80
- nad grzebieniem k. biodrowej – (mm) (over iliac crest)	10.2	2.70	12.0	2.82	1.8	2.03*	0.64
Pomiary długościowe (cm):(length measurements)							
- ramię (a-r) arm	29.04	1.65	32.09	3.48	3.05	3.57**	0.88
- przedramię (r-sty) (fore arm)	25.65	1.78	23.81	1.99	1.84	3.05**	0.92
- kończyna górna (a – da III) (upper limb)	72.48	3.64	74.13	4.24	1.65	1.31	0.39
- kończyna dolna (tro-B) (lower limb)	86.85	6.23	85.85	4.94	1	0.55	0.20
- stopa (pte-ap) (foot)	25.19	1.30	24.45	1.37	0.74	1.73	0.54
Pomiary szerok. (cm): (width measurements)							
- barków (cm) (a-a) (shoulder breadth)	39.61	1.56	38.86	2.65	0.75	1.09	0.28
- bioder (cm) (ic – ic) (hip)	32.62	3.32	30.50	1.95	2.12	2.38*	1.09
- miednicy (cm) (is –is) (pelvis)	23.97	2.36	24.77	1.92	0.8	1.15	0.42
- ręki (cm) (mm-mu) (hand)	9.59	0.35	9.78	0.49	0.19	1.41	0.39
- dłoni (cm) (mr-mu) (palm)	7.79	0.28	7.80	0.38	0.01	0.09	0.03
- stopy (cm) (mtt-mtf) (foot)	9.35	0.52	9.30	0.45	0.05	0.32	0.11
Pomiary obwodów (cm): (circuit measurements)							
- klatki piersiowej (wdech) (cm) (chest measurement) (inhale)	91.81	5.22	93.38	5.17	1.57	0.94	0.30
- klatki piersiowej (wydech) (cm) (chest measurement) (exhale)	87.66	4.61	88.65	5.78	0.99	0.59	0.17
- pasa (cm) (waist measurement)	71.57	3.45	76.03	6.51	4.46	2.72**	0.69
- bioder (cm) hip	97.11	5.43	94.47	5.82	2.64	1.46	0.45
- ramienia przy napięciu (cm) (arm measurement) (tensed)	28.35	1.71	28.49	2.30	0.14	0.22	0.06
- ramienia bez napięcia (cm) (arm measurement) (loosen)	26.76	1.69	26.20	2.39	0.56	0.85	0.23
- uda (cm) (thigh measurement)	54.70	3.04	53.05	3.66	1.65	1.54	0.45
- łydki (cm) (calf measurement)	36.68	1.72	36.83	2.73	0.15	0.21	0.05
BMI (AMC index)	22.3	1.7	22.2	5.4	0.1	0.08	0.02
Wskaźnik (AMC index)	22.81	1.30	21.45	1.79	1.36	2.74**	0.76
Wskaźnik WHR (WHR index)	0.74	0.03	0.81	0.05	0.07	5.38**	1.40

N – numbers, $\overline{X}$ – average value, σ - standard variation, d – difference of statistical averages, u - statistic importance of differences
* $p<0.05$;** $p<0.01$; $t_{\alpha=0.05} = 1.98$; $t_{\alpha=0.01} = 2.56$. W_M - Mollison indicator



Ryc. 1. *Różnice wielkości badanych cech w grupach mężczyzn*
Fig. 1. *Differences in the sizes studied the characteristics of groups of men*



Ryc. 2. *Różnice wielkości badanych cech w grupach kobiet*
Fig. 2. *Differences in the sizes studied the characteristics of groups of women*

DISCUSSION

All around the world, especially in the developed countries, disproportions are observed in the relations between the impetuous progress of science, technology in life, its fast increasing pace and the time dedicated to improving or maintaining the proper composition of the body and the physical efficiency [4.5]. The biological human development is realized in specific environmental, social and biological conditions. The statement, that the standard and the quality of life of different social groups significantly influence the effects of that development and at the same time describes somatic differences and efficiency differences of the human development is obvious.

The changes of civilization habits, apart from the positive effects, bring some negative phenomena for the human body, especially for the children and the young (e.g. limitation of physical activity, exercise, bad nutrition habits, attractiveness of various forms of television message, computer addiction, etc.) [6]. It is known that the physical activity is a biological human need related to the idea of health and physical education promotion. Thus the role of health education and the operations aiming at improving the physical efficiency of children and the young, and in the future - of the grown-up man, still increase. These operations have to become a permanent process embracing all the levels of the process of education [7].

It is important to constantly monitor the morphological features, which undoubtedly influence human physical activity. In the times of today's civilization, the rapid development of science and the lifestyle seized in determined frames of actions cause unfavorable relations with regard to time assigned for the physical activity and developing suitable human effort abilities needed in everyday life. The lifestyle changes, apart from their negative aspects, have also positive ones, related, first of all, to a dynamic development of the scientific thought in various life's fields.

Development is an inherent characteristics of each single life form. It undergoes constant progressive or regressive transformations and the pace depends on the lifestyle, the environmental conditions and the individual genetic features. The ontogenesis, the transformations related to the organism's development, which occur since egg cell's fertilization until the end of life, aims at achieving the perfection of the organism in order to obtain an optimal self-dependence and the ability to procreate. These transformations, which terms like physical development, somatic development or motoric development are related to, are constantly observed and are subject to analysis. As a result of the analysis of the human development, it may be stated that the surrounding environment has a large influence, but not all of its elements and not at every stage of the development form the organism with the same force. The moment, when the organism adjusts to given conditions, a given factor may play the supporting and not the stimulating role. For this reason it is so important to recognize and to clearly determine those factors, which in a particular way (the most important way) influence the somatic and motoric development of a young man. Those factors, in different periods of life, influence the organism with a different force, causing in some periods accumulation of quality changes, which decide about the organism's autonomy in the period and provide the differences with regard to the previous and the following periods. It is difficult to set close limits between them, nevertheless, it needs to be underlined that proceeding to the next development level is possible after reaching an adequate stage of the previous level. It receives a particular meaning on the background of the accelerated development (acceleration) phenomenon commonly observed in the consecutive generations. Many years of research allowed to determine specific development indexes, which enable determining the factual state of the child's physical maturity, so-called development age (biological age) [7].

There are strong traditions of research in the Kuyavia and Pomerania region. It is worth adding that the Kuyavia and Pomerania Province, from the sociological point of view, belongs to the regions, placed in the middle ranges of any Polish sociological parameters, which may certify that the researched phenomena are rather typical [8].

The conducted research and its results may become a valuable documentation material, which will help to better know the biological worth of the young generation from the Kuyavia and Pomerania region, for the examined candidates to become future teachers come from the Kuyavia and Pomerania region.

The research related to the state of the morphological features are normally directed to searching relations between those features and the health state, the danger of suffering from the civilization diseases and the influence of those features to the elements of motoric efficiency. It may be

admitted that the amount of fat in the body increased over a particular extent favors the increased danger of suffering from circulation diseases and it does affect the quality and the length of life. The tendency to increase the body fat increases along with the age with a simultaneous decrease of the physical activity. The phenomenon is observed, regardless of sex, in different environments [9]. A clear tendency appearing in this range may be seen in this work (increased amount of the body fat in the group of the part-time female students and a higher WHR index, both in the group of male and female part-time students in comparison with the full-time students). Extensive Body Fat and the Lean Body Mass reflect the values of motoric features. The relatively strongest links occur between the level of static strenght and the amount of Lean Body Mass. The LBM as the only of the body components describes quite adequately the muscle mass, which is considered the main predisposition of strength abilities. From the other features, height and body mass [10] are in a more feeble relation with static strength. It is worth to underline the meaning of the individual motoric abilities, which enable a systematic physical activity, preventing from many civilization diseases and supporting the process of maintaining a good state of health for long years.

CONCLUSIONS

The research results enable to reach the following conclusions:

1. The majority of the results of the compared morphological features between the groups of full-time and part-time students is similar.
2. Part-time male and female students in comparison with the full-time students have, on average, larger body fat amount in similar general body mass, which is indicated by: average higher proportional fat mass (%FM), average thicker skinfolds and average longer circumferences of waist and higher WHR indexes.
3. The higher amount of part-time students body fat in comparison with their colleagues from full-time studies may result from less physical activity of the first group. The part-time students in determined majority are committed to their professional work and to studies and they are left with little time for physical activity, whereas the full-time students very often combine the studies with sport practiced at the professional or recreation level.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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THE INFLUENCE OF COTININE ON THE CELL LINE DERIVED FROM THE URINARY BLADDER TRANSITIONAL EPITHELIUM (UROTHELIUM)

WPŁYW KOTYNINY NA KOMÓRKI WYPROWADZONE Z NABŁONKA PRZEJŚCIOWEGO PĘCHERZA MOCZOWEGO (UROTELIUM)

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Summary

Introduction: Cotinine, a major metabolite of nicotine, is one of the most important biomarkers of tobacco smoke exposure. In the present study some doses of cotinine were selected in order to approach urine cotinine concentrations similar to those typical of active smokers (two groups: <20 cigarettes per day, >20 cigarette per day), passive smokers, non-smokers.

The objective of the study: The aim of this study was to determine the influence of cotinine on the urothelium *in vitro* and create an experimental model for further investigations.

Material and methods: The study material was a cell line derived from urinary bladder transitional epithelium. The cultured cells were treated with six different doses of cotinine concentrations (6; 9.2; 18; 36; 3600; 7200 ng/ml) for 24 hours. Cell viability was determined by the trypan blue dye exclusion method. Morphological changes were observed in a light microscope. The fluorescent method was used to evaluate F-actin organization.

Results: The cotinine's action resulted in alteration of cell shape and in the number of viable cells. Significant changes appeared in the organization of F-actin cytoskeleton. An increase in the concentration of cotinine caused the shapes of the cell to gradually become more spindle-like, with elongated nuclei.

Conclusions: The results observed in these *in vitro* studies probably suggest that cotinine as a major degradation product of nicotine can induce some alterations in human urinary bladder transitional epithelium cells, especially in concentrations similar to those typical for smokers (COT doses: 3600 ng/ml and 7200 ng/ml).

Streszczenie

Wstęp: Kotynina, pochodna nikotyny, jest jednym z najlepszych biomarkerów ekspozycji organizmu na dym papierosowy. W prezentowanych badaniach zastosowano stężenia kotyniny, które były analogiczne do fizjologicznie występujących stężeń kotyniny w moczu u osób palących papierosy (dwie grupy: <20 papierosów dziennie, >20 papierosów dziennie), biernych palaczy oraz osób niepalących.

Cel badań: Założeniem badań było określenie wpływu, kotyniny na komórki urotelialne *in vitro* jak również stworzenie modelu doświadczalnego wykorzystywanego w dalszych, bardziej szczegółowych eksperymentach.

Materiał i metody: Materiał do badań stanowiła linia komórkowa wyprowadzona z nabłonka przejściowego pęcherza moczowego. Hodowane komórki traktowane były

sześcioma różnymi dawkami kotyniny (6; 9,2; 18; 36; 3600; 7200 ng/ml) przez 24 godziny. Ocena żywotności komórek została dokonana przy pomocy barwienia błękitem trypanu. Zmiany morfologiczne obserwowano przy pomocy mikroskopu świetlnego. Fluorescencję wykorzystano w celu oceny organizacji F-aktyny.

W y n i k i : W wyniku działania kotyniny zaobserwowano zmiany w kształcie i ilości żywych komórek. Znaczące różnice pojawiły się w organizacji cytoszkieletu aktynowego. Wraz ze wzrastającą dawką kotyniny komórki przybierały coraz bardziej wrzecionowaty kształt, a jądra ulegały wydłużeniu.

W n i o s k i : Wyniki zaobserwowane w badaniach *in vitro* sugerują, że kotynina, główny metabolit nikotyny, może indukować zmiany komórek wyprowadzonych z nabłonka przejściowego pęcherza moczowego, przede wszystkim w dawkach analogicznych do tych występujących w moczu osób palących papierosy (stężenie COT: 3600ng/ml i 7200ng/ml).

Key words: cotinine, urothelium, F-actin
Słowa kluczowe: kotynina, urotelium, F-aktyna

INTRODUCTION

Cotinine, the major degradation product of nicotine in a human body, has been widely used as a biomarker of environmental tobacco smoke exposure [1, 2, 3]. It has been established that approximately 70-80% of nicotine which enters an organism undergoes C-oxidation to cotinine in a two-stage NADPH-dependent process that occurs in liver and involves cytochrome P450 and cytosolic aldehyde oxidase. Cotinine is widely spread in the human body (serum, saliva, urinary) and approximately 10-15% of circulating cotinine is excreted with the urine in unchanged form. The elimination half-time of cotinine is about 17 hours, which is much longer when compared to nicotine (only 6 hours) [1, 3, 4, 5, 6]. It has been established that urine cotinine concentration depends on the nicotine intake with tobacco smoke [2, 7]. Data collected by Wall et al. shows the significant difference cotinine between urine concentration in smokers and non-smokers. People who smoke cigarettes have concentration of urine cotinine increased by about 100 times in comparison to non-smokers [2]. Tobacco smoke is one of the major risk factors for bladder cancer in humans. Cigarette smoking is the primary environmental risk factor for urinary bladder cancer in humans, causing a twofold to threefold increase in the risk [8, 9, 10]. According to the U.S. National Cancer Institute, the annual incidence rate of bladder cancer was 21.2 per 100,000 men and women. These rates are based on cases diagnosed in 2001-2005 [11]. The major causative agents of bladder cancer in cigarette smoke are believed to be aromatic amines, but apart from that more than 3000 substances identified as carcinogens have been found [4, 8, 12]. Cigarette smoking is also responsible for hyperplasia of bladder epithelium. [8, 13]. Changes in the bladder epithelium (neoplasia, hyperplasia) are strongly related to tobacco smoke exposure [8, 13].

It is possible that the interrelation of cigarette smoking and cotinine concentration in urine, as well as the influence of cotinine on bladder epithelial (urothelium) cells allow us to better understand the mechanisms connected with changes caused by cigarette smoke.

MATERIALS AND METHODS

Cell culture. The study material was a cell line derived from the human urinary bladder transitional epithelium (urothelium). The primary culture was prepared from the bladder tissue fragment (explant) collected by dr Wroński at the Department of Urology of Dr Jan Biziel Hospital in Bydgoszcz during a routine operation in a patient with a urinary bladder carcinoma. An explant obtained from the patient was immediately inserted into a sterile container with a physiological saline and transported in aseptic conditions. Next, material was rinsed with PBS (3x) and the transitional epithelium was separated from an explant and rubbed through a metal sieve. An over-night incubation with 0.25 % trypsin was performed at 4°C. After inactivation of trypsin (addition of serum), material was centrifuged (2000 r.p.m., 10 min.) and harvested into a petri dish. Culture medium used in this study consists of a 1:4 mixture of Dulbecco's Modified Eagle's medium (DMEM) and Ham's F-12, supplemented with 10% foetal bovine serum, 10 µg/ml insulin, 5 µg/ml glucose, 100 mg/ml hydrocortisone and 5 ng/ml EGF, antibiotics (penicillin, streptomycin) and amphotericin B. Cells were incubated at 37 °C in an atmosphere of 5% CO_2. The passages were made when cell culture obtained minimum 70% of confluence (culture medium was changed every 2 days). The cells were incubated with cotinine for 24h in six concentrations: 6; 9.2; 18; 36; 3600; 7200 ng/ml. As a control we used cells that grow in the same

condition but without addition of cotinine. For all experiments we used passages from 8 to 12. Cell culture and preparation for further experiments were made in the Regional Bank of Tissues of Dr Jan Biziel Hospital in Bydgoszcz.

Cell viability. Suspension of cells derived from urinary bladder transitional epithelium (5-10 µl) in culture medium was mixed with solution of 2 % trypan blue diluted in 0.9 % NaCl (1:1 mixture) on a microscope slide and covered with a coverslip. The number of dying blue-stained cells was counted in a Neubauer counting chamber after 3 minutes.

HE staining. Cells were fixed with 4% paraformaldehyde in HBSS (15 min, RT) and rinsed with PBS (3x 5 min, RT). Then, cells were embedded in 0,1 M glycine solution (5 min, RT). Following double rinsing in PBS, cells were stained in Meyer's hematoxylin (5 min, RT). Then, cells were washed thoroughly under running water for 20 minutes. After that, cells underwent dehydratation in series of alcohols and xylenes. Optimally fixed and prepared material was examined using Eclipse E800 microscope (Nikon, Tokyo, Japan) with computer imaging system analysis – NIS-Elements software version 4.20 (Nikon Instruments).

F-actin staining. Cells were fixed with 4% paraformaldehyde in HBSS (15 min, RT) and rinsed with PBS (3x 5 min, RT). Then cells were incubated with 0.1 M glycine (5 min, RT) and afterwards rinsed in PBS (3x 5 min, RT). Staining of F-actin was performed using phalloidin conjugated with derivative of rodamin (phalloidin/TRITC, Sigma). Cells were incubated in darkness with 10µM of stock (phalloidin/TRITC) diluted 1:5 in 20% methanol (20 min, RT). Counterstaining of nuclei was done with DAPI (Sigma) at a dilution of 1:25000 (10 min, RT). Finally, the material was embedded in Gelvatol (Monsato). Serum derived from unimmunized mice (phalloidin/TRITC) was used instead of the first antibody in order to confirm reaction specificity. F-actin was estimated in Nikon C1 confocal laser-scanning microscope (Nikon, Tokyo, Japan). Computer analysis of fluorescent imaging was done with the use of Nikon EZ-C1 FreeViewer software version 3.70.843 (Nikon).

Statistical analysis. Results were subjected to statistical analysis. The number of total dead cells was analyzed after 24 hours of incubation with cotinine (control – without cotinine addition). The Mann-Whitney *U* test was carried out to indicate statistically significant differences. A diagram and statistical analysis was done in a Graph Pad Prism software version 4.0.

RESULTS

Cell line viability after cotinine treatment. Statistically significant differences ($p<0,05$) in the average percentage of surviving cells appeared after 24 hours cotinine treatment at concentrations of 18; 36; 3600; 7200 ng/ml. The data gained by counting dead cells indicated that after incubation with cotinine the viability of cells decreased. In the control cells we observed the highest viability – 91.8%. In doses of 6ng/ml and 9.2ng/ml viability decreased to 84.2% and 82.4%, respectively. At cotinine concentrations of 18 ng/ml and 36 ng/ml only 44.4% and 64.3% of cells survived these conditions. The lowest viability (23.1%, 27.8%) was observed at the highest doses of COT (3600 ng/ml, 7200 ng/ml). All data was shown in Figure 1.

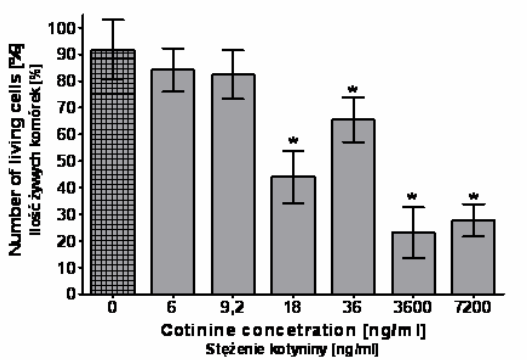

Fig. 1. *The influence of cotinine on the average percentage of surviving cells derived from urinary bladder transitional epithelium*

Ryc. 1. *Wpływ dawek kotyniny na średni procent przeżywających komórek wyprowadzonych z nabłonka przejściowego pęcherza moczowego*

Cell morphology examination in the light microscope. Light microscopic studies after hematoxylin staining revealed changes in the shape of cells treated with cotinine as compared with the control cells (Figure 2). However, significant changes were not observed at the dose of COT of 6ng/ml, (non-smokers) (Figure 2B). On the other hand, at 9.2 ng/ml cotinine (passive-smokers) some changes including more elongated morphology of part of the cells appeared (Figure 2C). According to the increase in concentration of cotinine (18; 36; 3600; 7200 ng/ml), the shape of the cell gradually became more spindle-like, with elongated nuclei (Fig 2 C, D, E, F, G).

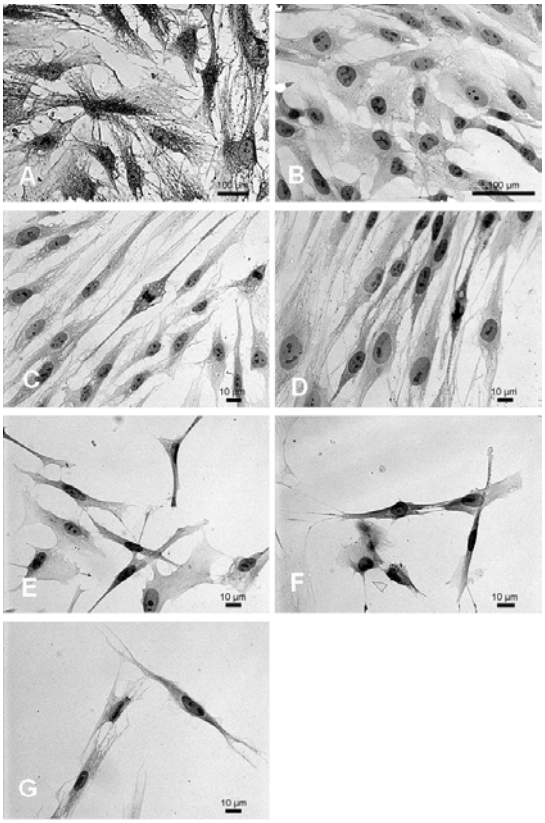

Fig. 2. *Light microscopy: (A) control, (B) cotinine dose of 6 ng/ml – non-smokers (B-no significant changes), (C) cotinine dose of 9.2 ng/ml – passive smokers, (D) cotinine dose of 18 ng/ml, (E) cotinine dose of 36 ng/ml, (F) cotinine dose of 3600 ng/ml – active smokers <20 cigarettes per 24h, (G) cotinine dose of 7200ng/ml – active smokers >20 cigarette per 24h. (C, D, E, F, G) shape of the cell gradually became more spindle-like, with elongated nuclei*

Ryc. 2. *Mikroskop świetlny: (A) kontrola, (B) dawka kotyniny 6ng/ml – niepalący (B-brak znaczących zmian), (C) dawka kotyniny 9,2 ng/ml – bierni palacze, (D) dawka kotyniny 18 ng/ml, (E) dawka kotyniny 36 ng/ml, (F) dawka kotyniny 3600 ng/ml – aktywni palacze <20 papierosów na dobę, (G) dawka kotyniny 7200 ng/ml – aktywni palacza >20 papierosów na dobę. (C, D, E, F, G) komórki stają się coraz bardziej wrzecionowate, jądra ulegają wydłużeniu*

Organization of F-actin – fluorescence confocal laser-scanning microscopic study. Significant changes were found in the organization of F-actin cytoskeleton (Fig. 3). However, at the dose of 6 ng/ml cotinine (non-smokers) such changes were not observed. At the concentration of 9.2 ng/ml (typical of passive-smokers) some changes, including depolymerized actin in the nucleus area as well as slightly-folded stress fibers appeared.

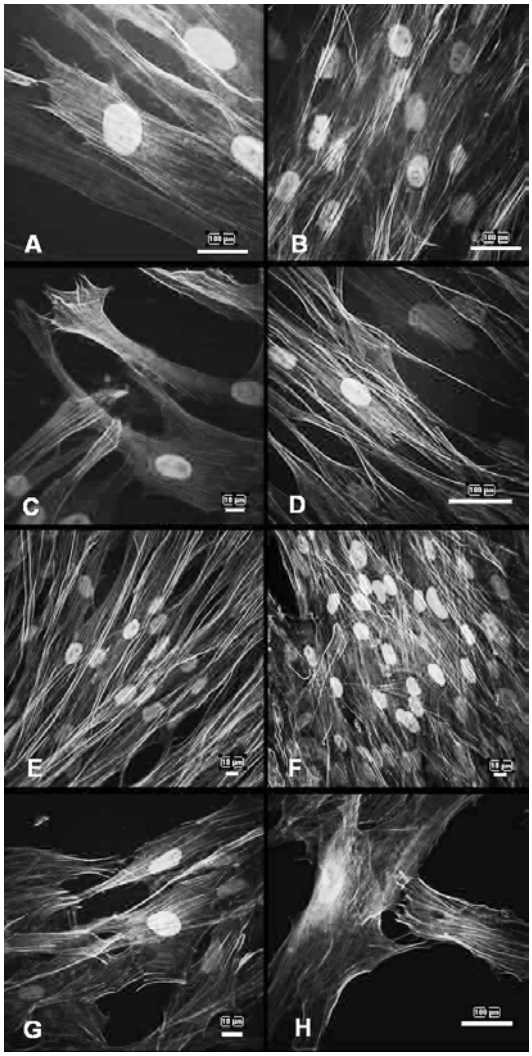

Fig. 3. *Fluorescence confocal microscopy (TRITC – F-actin, DAPI – nuclei) (A) control, (B) cotinine dose of 6ng/ml – non-smokers, (C) cotinine dose of 9.2 ng/ml – passive smokers, (D) cotinine dose 18ng/ml, (E) cotinine dose of 36ng/ml, (F, G) cotinine dose of 3600ng/ml – active smokers <20 cigarettes per 24 h, (H) cotinine dose of 7200 ng/ml – active smokers >20 cigarette per 24 h. A,B – no significant changes in stress fiber formation. C – slightly-folded stress fibers. D – long folded stress fibers. E – thick and long F-actin fibers concentrated at the cell periphery under the plasma membrane. F,G – thicker and more folded F-actin fibers. H- the thickest and most folded F-actin fibers*

Ryc. 3. *Mikroskop fluoroscencyjny konfokalny (TRITC – F-aktyna, DAPI – jądra) (A) kontrola, (B) dawka kotyniny 6ng/ml – niepalący, (C) dawka kotyniny 9,2 ng/ml – bierni palacze, (D) dawka kotyniny 18 ng/ml, (E) dawka kotyniny 36 ng/ml, (F, G) dawka kotyniny 3600 ng/ml – aktywni palacze <20 papierosów na dobę, (H) dawka kotyniny 7200ng/ml – aktywni palacza >20 papierosów na dobę. A,B – brak znaczących zmian w formowaniu włókien naprężeniowych. C – lekko pofałdowane włókna stresowe. D – długie, pofałdowane włókna stresowe. E – grube i pofałdowane włókna aktynowe skoncentrowane w części peryferyjnej komórki pod błoną komórkową. F, G – grubsze i bardziej pofałdowane włókna aktynowe. H – najgrubsze i najmocniej pofałdowane włókna aktynowe*

At the higher dose (18 ng/ml cotinine) long and folded stress fibers were particularly visible in some cells penetrating the nucleus. Thick and long F-actin fibers were concentrated at the cell periphery under the plasma membrane at the dose of 36 ng/ml. In the individuals smoking less than 20 cigarettes daily (3600 ng/ml), stress fibers were thicker and even more folded when compared to the previous dose. At the highest concentration (7200 ng/ml cotinine), that occurs in the urine of the individuals smoking more than 20 cigarettes daily, the thickest and most folded F-actin fibers among all groups analyzed in this experiment were noted.

DISCUSSION

The goal of this study was to investigate the influence of cotinine on the cell line derived from the human urinary bladder transitional epithelium (urothelium). Urothelium is a transitional epithelium consisting of 3-7 layers. The most differentiated cells are on the luminal surface (large, multinucleated, specialized cells called umbrella cells) and the least differentiated cells line in the basal layer. Normal urothelial cells are long-lived epithelial cells that show little mitotic activity [14]. Alterations in the urothelium are evidenced by histological changes (increased cell number, loss of tissue polarity) associated with cytoskeletal alterations, changes in adhesion molecules and cell surface markers or growth factor receptors [14]. All these changes connected with cell shape, structure and motility are one of the most important factors that are involved in cancer development and metastasis. Two main components of the cytoskeleton have been previously investigated in bladder cancer: actin and cytokeratins. These proteins are used as differentiation markers of the urothelial cells in cancer prognosis [14, 15, 16]. In urothelial cancer cells the amount of F-actin in comparison with G-actin is rather low, but in normal differentiated urothelium this relation is inverted, which is a useful hallmark in monitoring chemopreventive effects and tumor recurrence. [14, 15, 17]. Cytoplasmatic actin depolymerization, as measured by a decreased F/G-actin ratio, is a marker of cellular differentiation and early transformation and therefore it has been suggested that it could also be used as a marker for early detection in cancer [17]. One of the actin binding proteins, called gelsolin is very useful in bladder cancer research. It was established that expression of this protein is reduced or even absent in bladder cancer cells [14,15]. Other important part of the cytoskeleton – cytokeratins (CK) - plays an important role in bladder cancer. In the normal urothelium an expression of cytokeratins CK8, CK13, CK18, CK19 was observed. Loss of CK13 expression is a marker of tumor stage [14, 15].

Our observations of cotinine influence on the cell line delivered from human urothelium suggest that this alkaloid could play an important role in alterations of actin cytoskeleton, but further investigations should be conducted to evaluate if cotinine, major metabolite of nicotine, is involved in changes that could be related to cancer development.

Cucima et al. observed that nicotine enhanced release of PDGF, which in turn caused an alteration in actin cytoskeleton organization in the smooth muscle cells (SMCs) [18]. Carty et al. demonstrated that not only nicotine but also cotinine are potent regulators of basic fibroblast growth factor (bFGF) and matrix metalloproteinases (MMPs) synthesis in human SMCs, that are critical in cell migration [19]. Studies performed by Cucima and Carty suggested that cotinine plays an important role in alteration of actin cytoskeleton in the smooth muscle cells. Our results provided evidence that cotinine affects not only SMCs but also urothelial cells.

We used concentrations of cotinine similar to these occuring in urine of non-smokers, passive smokers and individuals who smoke less and more than 20 cigarettes per day. It is widely known that cigarette smoking is strongly connected with cotinine level in urine, as well as the fact that cigarette smoking is one of the major risk factors for the urinary bladder cancer [2, 20, 21]. Results of meta-analysis in epidemiologic studies done by Zeegers et al. indicate an approximately threefold higher risk of urinary tract cancer in the individuals smoking cigarettes [21]. Alberg et al. conducted a community-based prospective cohort study, the results of which give a confirmation of an important role of active cigarette smoking in the etiology of bladder cancer. But in the same cohort study data involving the secondhand smoke exposure and bladder cancer risk were mixed. [9]. The Zeegers's workgroup made a systematic literature review of the association between smoking, beverage consumption, diet and a bladder cancer. In conclusions, they pointed that the most important risk for bladder cancer is cigarette smoke. Prevention of cigarette smoking would result in a decrease of 50% of

male bladder cancer cases and 23% of female bladder cancer cases [22].

Our studies of viability after cotinine treatment performed *in vitro* on the urothelial cell line showed statistical differences after treatment of cotinine as comparable to the control cells. COT concentration of COT 18 ng/ml and 36 ng/ml gave also statistical differences. The number of living cells decreased after 24h cotinine treatment when we used following doses of cotinine: 18, 36, 3600 and 7200 ng/ml. We suggest that cotinine, an important biomarker of cigarette smoking, can have an influence on the increased risk of bladder cancer.

In agreement with our study, Ohnishi et al. detected in their study that mice exposed to cigarette smoke develop increased bladder urothelial and endothelial cell proliferation, which is due to citotoxicity and consequent regeneration [8].

Studies made by Auerbach et al. suggested a strong connection between cigarette smoking and changes in the bladder epithelium. This workgroup observed that the individuals who smoke cigarettes had dose - dependent histological alterations including increase of cell rows and increased number of cells with atypical nuclei [13]. We also observed histological changes in cells treated with cotinine (changes in cell shape – increasing concentration caused that the cell become more spindle-like with elongated nuclei). Our findings done in *in vitro* conditions suggest that metabolite of nicotine, one of the components of tobacco smoke, could be responsible for alterations related to smoking.

CONCLUSIONS

The present study shows the potential effect of cotinine on the urothelial cells in a cell culture model. Our data provides, for the first time, information that cotinine alters the morphology, the number of cells and organization of actin cytoskeleton in the cell line derived from human bladder transitional epithelium at the concentrations of COT, which are similar to the cotinine levels in urine of smokers. Further investigations need to be done to confirm the potential influence of cotinine on the urothelial cells and relation to bladder cancer occurrence as well as to understand better the mechanism of cotinine influence on the urothelium *in vivo.*

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