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## CONTENTS



## CUPRINS

# REVIEW
# HSP72 AND THE IMMUNE RESPONSE

**CARMEN TATU[1], V CIOCOTISAN[2], GABRIELA TANASIE[1], DANIELA PUSCASIU[1], RF TATU[1], CARMEN BUNU[1]**

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## ABSTRACT

Heat shock proteins (HSP) are the most phylogenetically conserved proteins present in all prokaryotes and eukaryotes. Intracellular HSP found within cells serve a cytoprotective role by chaperoning naive, misfolded and/or denatured proteins in response to stressful stimuli by a process known as the stress response. However, stressful stimuli also induce the release of intracellular heat shock proteins into the extracellular milieu and circulation. The extracellular heat shock protein proteins serve a cytostimulatory role by initiating immune responses designed to fend off microbial infection and destroy neoplastic transformed cells. This review will briefly cover recent advances into elucidating the mechanism by which stress induces the release of heat shock proteins into the circulation, how it initiates immune responses and suggest the possible biological significance of circulating HSP to the host.

**Key words:** heat shock proteins, HSP72, immune response, antigen presentation

### INTRODUCTION

Heat shock proteins (HSP) are highly conserved proteins found in all prokaryotes and eukaryotes. Heat shock proteins were originally described for their role as chaperones induced by temperature shock as well as various other kinds of stress including environmental (U.V. radiation, heat shock, heavy metals and amino acids), pathological (bacterial, parasitic infections or fever, inflammation, malignancy or autoimmunity) or physiological stresses (growth factors, cell differentiation, hormonal stimulation or tissue development), that induced a marked increase in intracellular HSP synthesis known as the stress response (2, 20). The stress response is designed to enhance the ability of the cell to cope with increasing concentrations of unfolded or denatured proteins. Of all heat shock proteins, the HSP70 family constitutes the most conserved and best-studied class. This family consists of the constitutively expressed HSP70 (Hsc70; 73 kDa), the stress inducible HSP70 (HSP70; 72 kDa), the mitochondrial HSP70 (HSP75; 75 kDa), and the endoplasmic reticulum HSP70 (Grp78; 78 kDa) (2, 3). The primary function ascribed to HSP72 was as an intracellular molecular chaperone of naive, aberrantly folded, or mutated proteins as well as in cytoprotection following the kinds of stressful stimuli including environmental (UV radiation, heat shock, heavy metals and amino acids), pathological (viral, bacterial, parasitic infections or fever, inflammation, malignancy or autoimmunity) or physiological stimuli (growth factors, cell differentiation, hormonal stimulation or tissue development), induces a marked increase in intracellular HSP72 synthesis (20, 21), known as the stress response. HSP72 display a distribution pattern closely paralleling the tissue solute concentration characteristic of the concentrating kidney (5). Consitent with this distribution pattern, in one study immunoreactivity to HSP72 was detected in the cortex in individual collecting duct cells only (22). In the outer medula, all tubules were stained weakly, whereas in the papilla intense staining of collecting ducts and the epithelium lining the papilla was noted (25). In another study, however, in wich a different antibody was used, staining was apparent in all cortical and medullary tubules after intravenous injection of vasopressin but not in the saline -injected control. With few exceptions, changes in medullary solute concentrations are associated with corresponding changes in inner medullary HSP72 expression (27). Another study show that accumulation of HSP90 mRNA in response to osmotic stress is unrelated to cellular protein denaturation and that synthesis of HSP90 may be regulated at both the level of transcription and translation (23). Because, in murine inner medullary collecting duct cells, maximal HSP72 mRNA expression is already reached at 500 mosmol/kg $H_2O$ and because the various stressors (high NaCl and urea concentrations, low pH) may act in concert to induce HSP72, the variability in these results may be explained by differences in the duration and/or extent of changes in medullary solute concentrations in the various studies. Experiments in cultured renal epithelial cells demonstrate that HSP72 is significantly induced only when medium osmolality is increased by solutes for which the cell membrane is poorly permeable (e.g., NaCl, mannitol) but not by readily permeating solutes (urea, glycerol) (10). This suggest that, in the renal inner medulla, the high extracellular NaCl concentration is a major determinant of the high HSP72 content. The idea that, under specific circumstances, HSP70 may

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act as a cytoprotectant has prompted a series of investigations examining the role and the putative protective effects of HSP72 in acute renal failure (10).

**HSP72 INDUCTION AND RELEASE**

The gene for HSP72 contains at least two regulatory elements that interact with heat shock transcription factors (HSFs). Specifically, the induction of HSP72 protein requires HSF1 binding to the heat shock element in the promoter region of the HSP70 gene (18). Many factors induce transcription and translation of intracellular HSP72 protein, and these vary depending on the tissue examined. The mechanism of release was studied by Gallucci and co-workers, who demonstrated that dendritic cells (DC) are stimulated by endogenous signals received from stressed, virally infected or necrosis-induced cells, but not by healthy cells or cells undergoing apoptosis (14). Additional studies for other laboratories demonstrated that necrotic but not apoptotic cell death leads to release of HSP's including gp96, calreticulin, HSP90 and HSP70 (8). These authors showed that exposure of DC to necrotic but not apoptotic cells resulted in maturation of DC. A condition in which necrotic cell death clearly contributes to the release of HSP72 from cells is after severe trauma. In a study by Pittet and colleagues a significant upregulation in circulating serum HSP72 can be measured in severely traumatized patients as early as 30 minutes after injury (24). Increased circulating serum HSP72 has also been measured in patients after coronary artery bypass grafting (2). Importantly, circulating serum HSP72 has been suggested as a marker of myocardial damage, and reported to have a role in the inflammatory response after acute myocardial infarction (AMI) (12). Other conditions in which elevated levels of circulating serum HSP72 has been demonstrated is in renal disease, hypertension, atherosclerosis, aging, and sickle cell disease (2). However, in these conditions although necrosis is proposed as the mechanism of release, conclusive experimental data is still lacking. Walsh and coworkers demonstrate that physical exercise results in the appearance of HSP70 in the circulation prior to any increase in gene or protein expression in contracting skeletal muscle (26). Additional studies have confirmed these findings and recently it was demonstrated that both intensity and duration of exercise influences the concentration of released eHSP72 in the plasma (3). Psychological stress induced by exposure of Sprague Dawley rats to cats results in the release of HSP72 into the circulation (13). The human brain is able to release HSP72 into the circulation in response to exercise (3). In an in *vitro* study, Guzhova and colleagues demonstrated that HSP70 is released by glia cells in the absence of necrotic cell death (3, 16). More recently, HSP70 has been shown to be released by B cells (11) and peripheral blood mononuclear cells and also under non necrotic conditions. Using conditions will not induce significant cell death, we showed that IFN-γ and IL-10 induce the active release of constitutive seventy-kilo-Dalton heat shock protein, HSC70 from tumors (3). Using conditions that will not induce significant cell death, Barreto and coworkers showed that IFN-γ and IL-10 induce the active release of constitutively expressed HSP70 also designed, Hsc70 or HSP73 from tumors (7). Recently Bausero and coworkers have begun to elucidate the mechanism of active release of iHSP72 from viable cells (9). It was demonstrated that certain pro-inflammatory cytokines normally found in high concentrations within inflammatory foci including IFN-γ, IL-10 but not the anti-inflammatory cytokine TGF-β1, mediate the active release of HSP72. It was showed that whereas some eHSP72 could be found as free HSP72, a proportion of eHSP72 was released within exosomes (9). Exosomes are internal vesicles of multivesicular bodies (MVB) released into the extracellular milieu upon fusion of multivesicular bodies MVB with the cell surface. In addition to containing HSP72 (9), exosomes are highly packed with immunostimulatory mediators including MHC class I and II and costimulatory molecules (2). Studies by Lancaster & Febbraio recently demonstrate that exosomes provide the major pathway for secretory vesicular release of HSP72 (19). However, using methyl-β-cyclodextrin (the cholesterol depleting agent) to disrupt lipid raft function, these authors were unable to confirm a role for lipid rafts in stress-induced HSP72 release from human peripheral blood mononuclear cells PBMC. In order to address the cellular location of HSP72 after stress, a recent study demonstrated that newly synthesized HSP72 protein localizes within the Golgi region of HeLa cells and also concentrates on the surface of the plasma membrane and in the ruffled zone of migrating cells (2). Taken together, these studies suggest that the active release hypothesis is an important mechanism by which HSP72 is released into the circulation. However, studies remain to be performed that conclusively demonstrate that T cell responses are primed in response to active release of HSP72 especially in the case of psychological stress or exercise.

**BIOLOGICAL SIGNIFICANCE OF RELEASED HSP72**

Irrespective of whether HSP72 enters the circulation via an active or passive release mechanism, what is the role of eHSP72 in circulation? The danger theory postulates that immune activation involves danger/non-danger molecular recognition schemas and suggests that innate immune cells are activated by danger signals that are derived from stressed or damaged self-proteins (1). It is now widely accepted that eHSP72 fit this criteria. The hypothesis is further reinforced by studies howing that circulating eHSP72 is increased and upregulated in diseased conditions including renal disease, hypertension, atherosclerosis and sickle cell disease. However, intriguing questions still remain about the role of increased circulating eHSP72 during psychological stress. Is it possible that in these situations the circulating eHSP72 is priming the immune system to real or perceived danger? These are important questions, the answers to which will provide a keen insight into numerous psychological and pathophysiological conditions. However, extensive studies and concerted efforts that bring together HSP researchers from such disparate fields as immunology, molecular biology, cell biology, neurophysiology and psychology utilizing new breakthrough technologies including the recently deciphered human genome and proteomics are required before conclusive answers can be given (1).

## THE CHAPEROKINE ACTIVITY OF HSP72, INITIATION OF IMMUNE RESPONSES

The term chaperokine describe the unique function of extracellular HSP72 (eHSP72) as both chaperone and cytokine (3). After admixing eHSP70 to APCs, specific signal transduction pathways are activated that result in the stimulation of an immune response. eHSP72 induces a plethora of immune responses and the list continues to grow. Asea and coworkers demonstrated that as early as 2-4 hours post exposure of APC to exogenous eHSP70, there is significant release of cytokines including TNF-α, IL-1β, IL-6 and IL-12 (10) and GM-CSF; nitric oxide, a potent apotogenic mediator; chemokines including MIP-1, MCP-1 and RANTES (3). It was demonstrated that both peptide-bearing and non peptide-bearing eHSP70 is capable of inducing pro-inflammatory cytokine production by APCs (3, 4). eHSP72 induces the DC maturation by augmenting the surface expression of CD40, CD83, CD86 and MHC class II molecules on DC and migration of DC and NK cells (15).

## HSP 72 AND ANTIGEN PRESENTATION

Once eHSP72 is bound to its specific surface receptor, two interrelated events occur. First, signal transduction cascades are activated. Second, the eHSP72 is endocytosed and the chaperoned peptides are presented to the antigen presentation pathway. Following receptor-mediated endocytosis heat shock proteins carrying their peptide load is transported *via* clathrin-coated pits to endosomes where it colocalizes with both MHCI and MHCII (2). The exact mechanism by which cross-presentation occurs has not been completely elucidated. However, eHSP72 may traffic through various intracellular compartments, which results in peptide release into the cytoplasm and re-presentation on the cell surface associated with MHC proteins (2). eHSP72 has been shown to deliver peptides to MHC class I molecules through the cross-presentation pathway (6). Following internalization of eHSP72, chaperoned peptides maybe released into the cytoplasm and processed by the classical antigen representation pathways. However, cross-presentation may proceed through a recently described alternative route which involves a specialized MHC class I structure known as the ER/phagosome fusion compartment, which is highly sufficient in inducing antigen cross-presentation (17).

## CONCLUSION

eHSP72 is released via necrotic as would be expected during non-physiological conditions including trauma. eHSP72 can also be released into the extracellular milieu within exosomes by a non classical protein transport mechanism, requiring intact lipid rafts. eHSP72 is found in the circulation of healthy individuals, however elevated levels can be found in various disease conditions and in response to moderate exercise and acute psychological stress. eHSP72 binds with high affinity and avidity to specific cells of the immune system and activates specific signal transduction cascades depending on which receptor it bind. Finally, eHSP72 stimulates the hosts immune response in an attempt to rid the host of infection or tumors.

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## HSP72 SI RASPUNSUL IMUN

## REZUMAT

Proteinele de stres (HSP) reprezinta proteinele cele mai bine conservate din punct de vedere filogenetic, prezente la toate celulele procariote si eucariote. Proteinele de stress intracelulare au rol protector asupra proteinelor denaturate sau incorect plicaturate ca reactie de raspuns la diversi stresori. Aceast raspuns este cunoscut sub numele de raspunsul la stres. Stimulii stresori induc de asemenea eliberarea proteinelor de stres in mediul extracelular. Aici HSP contribuie in calitate de citostimulatori, la initierea raspunsului imun in diverse situatii: aparare antimicrobiana, distrugerea celulelor neoplazice. Acest articol incearca sa elucideze cele mai noi mecanisme, propuse de diversi autori, prin care stresul induce eliberarea de HSP in circulatie, despre cum se initiaza raspunsul imun si posibile semnificatii biologice ale ale HSP.
**Cuvinte cheie:** proteinele de stres, HSP72, raspuns imun, prezentarea antigenului

# ANTHROPOMETRIC CHARACTERISTICS AND PHYSICAL PERFORMANCE IN YOUNG ATHLETES IN HUNGARY

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## ABSTRACT

The aim of this study was to investigate the age and sport specific features of anthropometric characteristics and their relationship with the training and physical performance parameters in football, water polo and handball players, and kayak-canoeists in Hungary.

Men of 10 to 25 years of age (n = 213) were involved in this cross-sectional study, performed in 2006. The anthropometric measurements included body mass, body height, body fat percent, upper arm girth and shoulder width. Training volume was characterized by the weekly average time, and physical performance was determined by two simple sport tests (Cooper's 12 minute run and Burpee tests).

An age dependent increase was seen in anthropometric parameters and weekly training times. The results of Cooper's test improved with age in all sport groups, and the Burpee test's results were rather variable. Correlation between anthropometric data and training volume was seen only in the older age groups. The results indicated differences between the anthropometric data and physical performance among male kayak-canoeists, football, water polo and handball players.

The results indicated typical differences in the anthropometric data and physical performance of male kayak-canoeists, football, water polo and handball players.

**Key words**: body mass index, body fat, sport, training

### INTRODUCTION

In the complex biological process called growth, the alteration of body composition is an important feature (9). Numerous studies were accomplished about effects of usual physical activity on fitness and anthropometric parameters in subjects of various age, physical activity, nutritional state etc. (3,4,5). The relationship between physical activity and changes in body composition in children was described by Stevens et al. (13). In a German study, made on persons aged between 17 and 26, body weight increased and fitness decreased in non-obese young adults (6). Rowlands et al. have found a positive relationship between activity and fitness, and a negative relationship between fatness and activity (12).

Anthropometric studies on athletes engaged in various sports indicated a relationship between body composition and the type of sport (1,7,8,10,14,15,16). Body composition has a significant effect on athletic performance and, conversely, exercise has the potential to alter body composition (11), so that the evaluation of body composition in connection with exercise and sport has emerged as an important field of interest.

In the present study, the age and sport specific features of anthropometric characteristics and their relationship with the training and physical performance was studied in football (soccer), water polo and handball players, and kayak-canoeists in Hungary. Football, water polo, kayak-canoeist and handball are very different sports. They are similar in comprising both aerobic and anaerobic components, but the proportions of the two vary. Our working hypothesis was that the athletes' anthropometric characteristics and level of physical performance are related to their age, the kind of sport, and the training volume.

### MATERIALS AND METHODS

Men of 10 to 25 years of age (n = 213) were involved in this cross-sectional study, performed in 2006. Of these, 65 (30.5%) were 10-13 years old, 73 (34.3%) were 14-17, and 75 (35.2%) were 18-25 years old. All subjects have had trainings since their childhood. According to the types of sport, 55 (25.8%) were football players, 52 (24.4%) were water poloists, 53 (24.9%) were kayak-canoeists, and another 53 (24.9%) were handball players. The athletes were selected from the most promising registered players of the given age group in sport clubs of Szeged, and included several age-group representative players, as well as European/World championship and Olympic Game winners among the adults.

The anthropometric parameters and physical fitness were measured under standardized conditions in the fitness gym in Szeged, always on Sunday afternoon, at least 2 hours after the last meal. The anthropometric measurements included body

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mass, body height, body fat percent (BF%), upper arm girth and shoulder width. Body mass was measured to the nearest 0.5 kg, and body height to the nearest 0.5 cm; with the subjects wearing gym shorts and T-shirts and no shoes. Body mass index (BMI) was calculated from body mass and body height, and expressed in kg/m$^2$. Body fat percent was determined by bioimpedance analyser (Beurer BF40). Upper arm girth was measured at the mid-point of the arm; and shoulder width, between the two acromia.

Training volume was characterized by the weekly average time (frequency and length of training sessions). Physical performance was determined by two simple sport tests: Cooper's 12 minute run test (run length in meters) was used for aerobic capacity, while anaerobic capacity was measured by the Burpee test performed in two minutes. The Burpee test involves the following practices: standing erect, arms by the side; placing the hands on the floor in front of the feet (squat position); thrusting the legs back to assume a push up position; squat position; returning to the starting position.

The data were processed by the Statistical Package for Social Sciences (SPSS), version 13.0 for Windows. For each age group and sport, mean and standard deviation of the anthropometric and performance characteristics was calculated. Comparison of the means by age-groups and sports was done by non-parametric Kruskal-Wallis test, because the Levene's test was significant. Spearman's rho correlations were used to determine the degree of association among selected variables. In all tests, $P<0.05$ was considered as statistically significant.

The study protocol (No. 11/2006) was approved by the Human Investigation Review Board of the University of Szeged. Informed written consent was obtained from the participants.

## RESULTS

Anthropometric and Physical Performance Characteristics

The results of measurements by age-groups and sport are shown in Table I. Significant age-group related differences were seen in anthropometric and physical performance parameters except the results of the Burpee test in football players and water poloists.

**Table I** – Anthropometric and physical performance parameters in highly trained young athletes

| Characteristics | Football players | | Handball players | | Water poloists | | Kayak-canoeists | |
|---|---|---|---|---|---|---|---|---|
| 10-13 years old | (n=16) | | (n=16) | | (n=16) | | (n=17) | |
| | mean | SD | mean | SD | mean | SD | mean | SD |
| Body mass index (kg/m$^2$)* | 17.06 | 2.07# | 20.69 | 2.12# | 21.51 | 2.69# | 19.23 | 2.79# |
| Body fat (%)* | 5.31 | 1.27# | 7.13 | 3.70# | 9.43 | 5.80# | 7.21 | 3.63# |
| Upper arm girth (cm)* | 21.87 | 1.99# | 25.13 | 2.33# | 23.80 | 3.01# | 24.20 | 2.73# |
| Shoulder width (cm)* | 42.09 | 2.05# | 47.75 | 1.88# | 47.80 | 2.48# | 46.53 | 3.18# |
| Weekly training time (min)* | 439.69 | 69.01# | 378.75 | 78.13# | 326.25 | 121.76# | 340.29 | 111.25# |
| Cooper's run test (m)* | 2106.51 | 176.89# | 2090.23 | 222.27# | 1862.50 | 257.88# | 2017.65 | 312.72# |
| Burpee test (number)* | 58.44 | 9.93 | 58.13 | 11.50# | 36.13 | 8.15 | 44.06 | 9.49# |
| 14-17 years old | (n=19) | | (n=18) | | (n=15) | | (n=21) | |
| | mean | SD | mean | SD | mean | SD | mean | SD |
| Body mass index (kg/m$^2$)* | 19.82 | 1.68 | 23.12 | 3.37 | 23.99 | 2.41 | 23.60 | 2.32 |
| Body fat (%)* | 8.20 | 3.77 | 13.93 | 6.25 | 16.90 | 4.07 | 13.69 | 5.77 |
| Upper arm girth (cm)* | 27.00 | 2.50 | 29.75 | 3.23 | 30.63 | 1.56 | 30.67 | 2.23 |
| Shoulder width (cm)* | 47.31 | 4.76 | 57.87 | 3.18 | 55.40 | 2.56 | 58.67 | 1.63 |
| Weekly training time (min)* | 706.84 | 357.89 | 465.28 | 88.12 | 451.67 | 68.34 | 638.57 | 219.44 |
| Cooper's run test (m)* | 2723.68 | 409.73 | 2655.56 | 178.96 | 2126.67 | 345.31 | 2666.67 | 03.86 |
| Burpee test (number)* | 52.37 | 11.75 | 42.05 | 5.22 | 44.00 | 15.75 | 49.00 | 6.79 |
| 18-25 years old | (n=20) | | (n=19) | | (n=21) | | (n=15) | |
| | mean | SD | mean | SD | mean | SD | mean | SD |
| Body mass index (kg/m$^2$)* | 22.11 | 2.07 | 24.14 | 2.21 | 24.03 | 1.55 | 24.58 | 1.40 |
| Body fat (%)* | 15.40 | 4.09 | 16.39 | 2.21 | 19.42 | 3.58 | 15.18 | 3.58 |
| Upper arm girth (cm)* | 29.07 | 2.68 | 32.63 | 2.22 | 32.86 | 2.61 | 33.60 | 2.03 |
| Shoulder width (cm)* | 56.30 | 2.81 | 61.26 | 2.64 | 59.86 | 5.59 | 62.67 | 8.03 |
| Weekly training time (min)* | 1074.00 | 395.48 | 610.79 | 183.83 | 803.09 | 377.51 | 780.00 | 247.38 |
| Cooper's run test (m)* | 3030.00 | 389.47 | 2963.16 | 208.73 | 2466.67 | 300.56 | 3157.33 | 253.08 |
| Burpee test (number)* | 58.50 | 11.70 | 52.21 | 11.87 | 42.62 | 8.65 | 60.60 | 8.09 |

*Significant differences between sports (Kruskall-Wallis test)

#Significant differences between age-groups within sports (Kruskall-Wallis test)

Anthropometric data of each age-group were significantly different by sports with lowest figures among football players, and highest among water poloists or kayak-canoeists.

Weekly training volume increased with age. Within a given age group, the longest overall training times were seen among football players, and the shortest, among water poloists and adult handball players.

The results of Cooper's test improved with age in all sport groups, with best performance among 18-25 years old kayak-canoeists and football players, and worst among water poloists. The results of the Burpee test were rather variable. Only the kayak-canoeists' results increased with age, water poloists again had the worst results.

**Analysis of Correlations**

There were correlation between BMI and other anthropometric parameters (Table II). BMI and BF% was in correlation except in 10-13 years old football players and 18-25 years old kayak-canoeists. BMI was in strong relationship with upper arm girth except 10-13 years old football players and 14-17 years old water poloists. A strong correlation between BMI and shoulder width values was in 10-13 years old water poloists, kayak-canoeists and 14-17 years old handball players (Table II).

Correlation between anthropometric data and training volume was seen only in the age groups above 13 years. In the 14-17 years old football players' group, training volume was in strong relationship with BF% ($r$=0.869; $P$<0.001) and with shoulder width ($r$=0.490; $P$=0.033). In the 18-25 years old groups, the kayak-canoeists' upper arm girth ($r$=0.581; $P$=0.023), as well as the handball players' BF% ($r$=0.482; $P$=0.037) and upper arm girth ($r$=0.473; $P$=0.041) was in connection with training volume, while no correlation was found in the water poloists' group.

**Table II** – Spearman's correlation coefficients between body mass index and other anthropometric parameters

| Age-groups and kinds of sport | Body fat (%) | Upper arm girth (cm) | Shoulder width (cm) |
|---|---|---|---|
| 10-13 years old | | | |
| Football players | 0.420 | 0.494 | 0.163 |
| Handball players | 0.692** | 0.745** | 0.201 |
| Water poloists | 0.829*** | 0.726** | 0.772*** |
| Kayak-canoeists | 0.849*** | 0.931*** | 0.776*** |
| 14-17 years old | | | |
| Football players | 0.869*** | 0.829*** | 0.647** |
| Handball players | 0.937*** | 0.857*** | 0.864*** |
| Water poloists | 0.913*** | 0.397 | 0.288 |
| Kayak-canoeists | 0.798*** | 0.638** | 0.506* |
| 18-25 years old | | | |
| Football players | 0.986*** | 0.826*** | 0.101 |
| Handball players | 0.887*** | 0.711** | 0.659** |
| Water poloists | 0.905*** | 0.502* | 0.390 |
| Kayak-canoeists | 0.151 | 0.755** | 0.589* |

* P<0.05, **P<0.01, ***P<0.001

The performance in Cooper's test was not influenced by the training volume, while the Burpee test, among the 14-17 old football players ($r$=0.584; $P$=0.009) and the 18-25 years old kayak-canoeists ($r$=0.531; $P$=0.042) had a correlation with the training volume.

When testing the relationship between physical performance and anthropometric parameters it was found that results of the Cooper's test were correlated to BMI and BF%, beside Burpee test outcome with BMI, BF% and body measures. In the 10-13 years old group, there was no correlation between anthropometric data and physical performance.

Among the 18-25 years old water poloists, negative correlation was found between Cooper's test results and BMI ($r$=–0.475; $P$=0.030), and between Burpee test performance and shoulder width ($r$=–0.579; $P$=0.006).

Among the 14-17 years old football players, the results of the Burpee test were in connection with BMI ($r$=0.478; $P$=0.039), BF% ($r$=0.467; $P$=0.044) and shoulder width ($r$=0.633; $P$=0.004). In the 18-25 year old kayak-canoeists' group, the Burpee test results were correlated with BMI ($r$=0.535; $P$=0.040), upper arm girth ($r$=0.852; $P$<0.001) and shoulder width ($r$=0.632; $P$=0.012).

## DISCUSSION

The observed age-dependent increase of anthropometric parameters might be, in the 10-13 and 14-17 years group, equally due to growth and training. In the subjects above 18, the major factors were likely the training volume and the type of sport. The dissimilar effect of intensive training on the anthropometric characteristics in different sports has already been described by other authors (1,8,16).

The choice of sport can, however, itself be influenced by body constitution: those of thin figure have a preference to football while the overweighed children's choice is probably water polo.

The high BF% of the water poloists is a sport specific feature, where fat has probably a thermal insulator role in the water. Water polo requires much of stamina. Beyond being a heat insulator, fat tissues actively produce hormones influencing blood level of sugar and triglycerides, the latter being utilised by the muscles for energy production (15).

Kayak-canoeists had, determined by the characteristics of this sport, the largest muscle mass; but this was nearly free of fat (high BMI together with low BF%), which was due partly to the high physical load and partly to that any overweight would be unwanted ballast in the boat.

Handball players had a likely good physical state, but in this sport direct body-to-body fight is frequent so that body mass, represented to a lesser extent by fat and not muscles, is more crucial. In several surveys, the data of BMI and BF% were only weakly correlated (14). Body fat measurement by bioimpedance seemed to be better in describing nutritional state than the standard BMI calculation from body height and weight (3,4).

Compared to the above sports, football players had lower BMI and BF% data and weaker upper body musculature. Beyond the influence of physical constitution on the choice of sport, this might be due to the higher energy consumption during training work by feet, compared to upper body training, but also to the low

level of physical training of football players in Hungary. Coerver's method (2), athletic training of football players, has less weight in Hungary compared to countries with more successful football. In those, technical development is prioritized in the 5-13 years old, whereas condition development and physical training is done mostly in the age groups of 13-14 to 18-20. Beyond increasing aerobic and anaerobic capacity, this includes the development of upper body musculature.

In all sports investigated in the present study, the 10-13 years olds' anthropometric data were sport specific without correlation to training intensity or physical performance (kind of sport chosen by physical constitution).

Among the older, kayak-canoeists had the best, and water poloists the worst, condition. The 18-25 years old kayak-canoeists' very good performance in the 4-beat pushup test was in correlation with their upper body composition, probably because this test requires short-term (2-4 min) high anaerobic capacity, a characteristic of this sport. In the same age group, the results of the water poloists' Cooper's test were in negative correlation with BMI: more corpulent players had lower aerobic performance.

The results described above indicated typical differences in the anthropometric data and physical performance of male kayak-canoeists, football, water polo and handball players. The differences arose partly from the influence of physical constitution on the choice of sport, but are later influenced by the physiological demands of the kind of sport and its specific training methods. In practice, the anthropometric examinations and ongoing performance measurements enable or help the suggestion and choice of a kind of sport in childhood.

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## CARACTERISTICILE ANTROPOMETRICE SI PERFORMANTA FIZICA LA SPORTIVI TINERI IN UNGARIA

## REZUMAT


Scopul acestui studiu a fost de a investiga varsta si caracteristicile antropometrice specifice sportului si relatia lor cu parametrii de antrenaj si performanta fizica la jucatorii fotbal, polo pe apa, handbal si caiac-canoe in Ungaria.

In studiul crossectional, executat in 2006, au fost involvati barbati de varsta intre 10 si 25 ani (n = 213). Ca parametrii antropometrici, indexul Masa-Corp, inaltimea, procentajul de grasime, circumferinta bratului si a umerilor au fost considerate. Antrenajul a fost caracterizat prin durata medie de timp al antrenajului saptaminal, performanta fizica a fost determinata prin doua testuri simple: Cooper de 12 minute si testul Burpee.

Parametrii antropometrici si durata saptaminala de antrenaj au aratat o crestere dependenta de varsta. Rezultatele testului Cooper au aratat o perfectionare cu varsta in toate felurile de sport, pe cand rezultatele testului Burpee au fost variabile. Corelatia dintre datele antropometrice si volumul de antrenaj a fost demonstrate doar la cei mai varstnici. Rezultatele au indicat diferinte intre datele antropometrice si perfomanta fizica intre barbati caiac-canoisti, jucatorii fotbal, polo pe apa si handbal.

Rezultatele au indicat diferinte tipice intre datele antropometrice si performanta fizica la barbatii barbati caiac-canoisti, jucatorii fotbal, polo pe apa si handbal.



**Cuvinte cheie:** indexul Masa-Corp, procentaj de grasime, antrenaj

# OLIVE LEAF EXTRACT ATTENUATES ETHANOL-INDUCED GASTRIC LESIONS IN RATS

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## ABSTRACT

Olive leaf extract (OLE) possesses, among other, antioxidative properties, but whether it influences the gastric defense mechanism and gastroprotection against ethanol-induced gastric lesions remains unknown. Previous studies demonstrated that the damaging action of absolute ethanol could be attributed to the enhancement in the reactive oxygen species (ROS) and the ROS-dependent increase in lipid peroxidation and inhibition of antioxidative enzyme activity. In this study we investigated the protective effect of OLE, a natural antioxidant, on gastric mucosal damage induced by absolute ethanol in rats. We applied three different doses of OLE intragastrically (i.g.) 30 minutes prior to absolute ethanol administration. One hour after i.g. applied ethanol, areas of gastric lesions were measured by planimetry using ImageJ computer programme. Ulcer index (UI) and percent of inhibition of UI in relation to the control ethanol group were calculated. Furthermore, we compared the effects of applied OLE on gastric mucosal lesions with effects of i.g. pretreatment of $H_2$ receptor antagonist, ranitidine. Histological evidence of gastric mucosal lesions was also obtained. Absolute ethanol treatment caused severe gastric submucosal haemorrhage, and this finding was confirmed histologicaly. Pretreatment with OLE (40, 80 and 120 mg/kg), as well as with ranitidine (50 mg/kg), significantly ($p < 0.001$) attenuated gastric lesions induced by absolute ethanol. Percents of inhibition in UI were 62%, 82% and 71%, respectively. The protective effect of OLE was similar to that obtained with ranitidine which inhibited UI for 76%. Our results suggest that OLE exerts a potent gastroprotective activity against ethanol-induced gastric lesions in rats.

**Keywords**: olive leaf, gastroprotection, rats

### INTRODUCTION

Gastric mucosa plays a role of a barrier that limits exposure of the gastric mucosal cells to numerous injurious luminal agents and irritants of exogenous and endogenous origin. Pretreatment with different substances could effectively prevent gastric mucosa from the development of erosions and ulceration. This action, called gastro- or cyto-protection is not related to the inhibition of gastric acid secretion and is known to account for gastroprotection by various irritants.

Interest in the olive leaf and its chemical constituents has recently been increasing. Its benefits, however, have been known for centuries, and it has been traditionally used to prevent and treat diseases. Olive leaf is used to enhance the immune system, as an antimicrobial and in heart disease. Folk medicine uses also include hypertonia, arteriosclerosis, rheumatism, gout, diabetes mellitus, and fever (19). Recently, experimental animal studies have demonstrated hypoglycemic (2,10), hypotensive (13), antiarrhythmic (25), and vasodilator effects (29), as well as spasmolytic effect on the intestinal smooth muscle (8). Antimicrobial (5, 9, 16,21), antiviral (15,18), anti-tumor (1,11) and antiinflammatory activity (22) were also reported. The beneficial properties of olive leaf are further enhanced by the bioavailability of its polyphenolic constituents, which are readily absorbed through the gastrointestinal tract, resulting in significant levels in the circulation (27,28).

The main constituent of the olive leaves is oleuropeine, one of iridoide monoterpenes, which is thought to be responsible for pharmacological effects. Furthermore, the olive leaves contain triterpenes including oleanolic and maslinic acid, flavonoides (luteolin, apigenine, rutin), and chalcones (olivin, olivin-diglucoside) (19,17,21). Its chemical content makes olive leaf one of the most potent natural antioxidant. Oleuropein has high antioxidant activity *in vitro*, comparable to a hydrosoluble analog of tocopherol (26) and exibits strong antioxidant protection in oxidative stress during ishemia-reperfusion i an *in vivo* experimental model (3).

Moreover, other constituents of olive leaf also exert antioxidative properties, which is experimentaly confirmed (4,7).

A variety of botanical products rich in flavonoids have been reported to possess anti-ulcer activity (especially from ethnopharmacological studies), and the documented literature has centered primarily on their pharmacological action in experimental animals (6, 20,30).

Whether olive leaf extract (OLE) influences the gastric defense mechanism and exhibits gastroprotection against

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experimentaly-induced gastric lesions remains unknown. Previous studies demonstrated that the damaging action of absolute ethanol could be attributed to the enhancement in the reactive oxygen species (ROS) and the ROS-dependent increase in lipid peroxidation and inhibition of antioxidative enzyme activity (14). Therefore, treatment with potent antioxidant as OLE, could decrease ethanol-induced gastric mucosal damage.

## METHODS

*Materials*

We used standardized dry olive leaf extract (*Olea europaea* L.) purchased from Frutarom Industry Ltd. (Switzerland) which contains 16.8 % of oleuropein, previosly determined by HPLC analysis. For the extrapolation of the dosage from humans to rats, we used the metabolic body size or food intake rather than body weight as a criterion (23, 24). Hence, 40 mg/kg of olive leaf extract was administered. A higher doses of 80 and 120 mg/kg was also given to test for a dose response. Ranitidine tablets were obtained from Galenika a.d., Serbia. Both OLE and ranitidine was suspended in distilled water before administration.

Gastric lesions induction and evaluation

This study was approved by the Ethical Committee, Medical School, University of Belgrade, and run in accordance to the statements of European Union regarding handling of experimental animals. Wistar male rats, weighting between 200 and 220 g were randomly divided into 5 groups (6 rats in each group). The animals were placed in individual metabolic cages. Before the experiment, they were fasted overnight, but had free access to water.

The first, control group received distilled water intragastricaly (i.g.) 30 minutes prior to 1 ml absolute ethanol administration. We applied three different doses of OLE on the next three groups, and finaly, the last group (positive control) received 50 mg/kg of ranitidine, $H_2$ receptor antagonist. One hour after i.g. applied ethanol, animals were sacrificed, abdomen was opened by the midline incision, the stomach was removed, opened along the greater curvature, rinsed gently with water and pinned open for macroscopic examination and for photodocumentation. Areas of gastric lesions were measured by planimetry using NIH ImageJ computer programme (12).

Ulcer index (UI) and % of inhibition in UI in relation to the control ethanol group were estimated from formulas:

$$UI = [\text{ulcerated area } (mm^2)/ \text{ total stomach area } (mm^2)] \times 100$$

$$\% \text{ inhibition} = [1-(UI \text{ treatment}/UI \text{ control})] \times 100$$

*Histopathological evaluation*

Histological evidence of gastric mucosal lesions in all experimental groups was also obtained. Gastric tissue samples were fixed in 10% buffered formalin and processed for routine histology. Paraffin sections of 5 μm thickness were cut by rotatory microtome, stained with haematoxylin and eosin, and examined microscopically for pathomorphological changes.

*Statistical analysis*

Results are expressed as means ± SD. Statistical analysis was done using t-test. Diferences with $p<0.01$ were considered as significant.

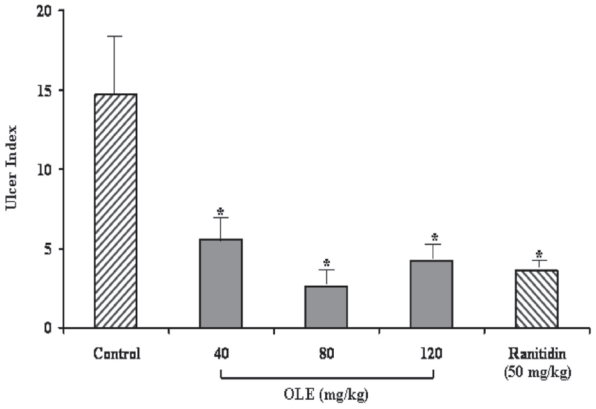
## RESULTS AND DISCUSSION

Effect of OLE suspension on gastric lesions induced by absolute ethanol

Administration of absolute ethanol to fasted rats resulted in severe gastric damage visible from the outside of the stomach as thick reddish-black lines. After opening, the gastric lesions were found in the mucosa and consisted of elongated bands, 1–10 mm long, usually parallel to the long axis of the stomach. They were located mostly in the corpus, the portion of the stomach secreting acid and pepsin. No visible lesions developed in the nonsecretory part of the stomach.

The effect of absolute ethanol and pretreatment with OLE applied i.g. in graded concentrations, as well as ranitidine on the ulcer index is shown on Figure 1. Ethanol caused typical widespread gastric lesions on 14.7 % ± 5.5 of total stomach area. Pretreatment with all three doses of OLE significantly ($p < 0.001$) reduced gastric lesions induced by absolute ethanol. The best inhibition of ulcer index was obtained in group pretreated with 80 mg/kg of OLE, were ethanol caused gastric lesions in only 2.6 % ± 1.4 of total stomach area. The gastroprotective effect of OLE was similar to that achieved by the pretreatment with $H_2$ receptor antagonist, ranitidine, where UI was 3.6% ± 0.8.

**Fig. 1.** Effect of intragastric pretreatment with OLE applied in graded doses ranging from 40 mg/kg up to120 mg/kg and ranitidine (50 mg/kg) on the ulcer index induced by absolute ethanol. Asterisk indicates statistical significance of inhibition ($P< 0.001$), as compared to the control value.




Percents of inhibition of UI are shown in Table I. All applied doses of OLE (40, 80 and 120 mg/kg) significantly inhibited ulcer index (62%, 82% and 71%, respectively). The protective effect of OLE was similar to that obtained with ranitidine which inhibited UI for 76%. Hence, we confirmed the useful role of gastric antisecretory medication in the prevention of ethanol-related gastric mucosal damage. The results obtained suggest that both antioxidants and antisecretory drugs may be beneficial in preventing gastric mucosal cell damage induced by ethanol.

**Table I.** Inhibition of ulcer index induced by absolute ethanol after pretreatment with different doses of OLE and ranitidine. Asterisk indicates statistical significance of inhibition ($P < 0.001$)

| Pretreatment | Dose (mg/kg) | % of inhibition in UE |
|---|---|---|
| OLE | 40 | 62* |
| OLE | 80 | 82* |
| OLE | 120 | 71* |
| Ranitidine | 50 | 76* |

*Effect of OLE suspension on histopathological changes*

Histological examination of gastric mucosa showed various histopathological changes including congestion, haemorrhage, edema, necrosis, inflammatory changes, erosions and ulcers in ethanol-treated rats, which were in significantly lesser extent seen in rats pretreated with both OLE and ranitidine.

**CONCLUSION**

In this study we investigated the protective effect of OLE, a natural antioxidant, on gastric mucosal damage induced by topical ulcerogen - absolute ethanol.

The role of oxygen derived free radicals in the generation of gastric injury is well known. The results obtained indicate that OLE posseses gastroprotective effect possibly related to its antioxidative properties. In order to further elucidate the OLE mechanism of gastroprotective effect, our further investigation will be focused on determination of gastric mucosal antioxidative enzyme activity.

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# EXTRACTUL DIN FRUNZE DE MASLIN ATENUEAZA LEZIUNILE GASTRICE INDUSE DE ALCOOL LA SOBOLANI


## REZUMAT

Extractul din frunze de maslin (OLE) poseda, printre altele, proprietati antioxidante, dar nu se stie inca daca influenteaza mecanismele de aparare gastrica si protectia gastrica in cazul leziunilor produse de alcool la acest nivel. Studii anterioare au demonstrat ca actiunea distructiva a etanolului pur s-ar putea datora cresterii speciilor reactive ale oxigenului (ROS) si cresterii ROS-dependenta a peroxidarii lipidice si inhibitiei activitatii enzimatice antioxidative. In acest studio am investigat rolul protector al OLE, un antioxidant natural, asupra injuriei mucoasei gastrice induse de etanol la sobolani. Am aplicat intragastric (i.g.) trei doze diferite de OLE cu 30 de minute inainte de administrarea etanolului pur. La o ora dupa administrarea i.g. de etanol, s-au masurat ariile leziunilor gastrice folosind planimetria, cu ajutorul programului ImageJ. Am fost calculate indexul ulcerativ (UI) si expresia procentuala a inhibitiei UI in relatie cu grupul de control caruia i s-a administrat doar etanol. In continuare, am comparat efectele OLE asupra leziunilor mucoasei gastrice cu efectele pre-tratamentului i.g. cu un antagonist al receptorilor $H_2$, ranitidina. Au fost obtinute de asemenea si dovezile histologice ale leziunilor mucoasei gastrice. Tratamentu cu etanol absolut a indus hemoragii gastrice submucoase severe, acest aspect fiind confirmat histologic. Pre-tratamentul cu OLE (40, 80 and 120 mg/kg), precum si cu ranitidina (50 mg/kg), a atenuat semnificativ ($p < 0.001$) leziunile gastrice induse de etanol. Procentual, inhibitia UI a fost 62%, 82% si respectiv 71%. Efectele protectoare ale OLE au fost similare cu cele obtinute folosind pre-tratament cu ranitidina, care a inhibat UI in proportie de 76%. Rezultatele noastre sugereaza ca OLE exercita o activitate gastroprotectoare puternica impotriva aparitiei leziunilor gastrice induse de etanol la sobolani.



**Cuvinte cheie**: frunze de maslin, protectie gastrica, sobolani

# EFFECTS OF DIFFERENT EXERCISE PROGRAM ON BLOOD MARKERS OF OXIDATIVE STRESS IN YOUNG WOMEN

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## ABSTRACT

**Introduction**: Increased aerobic metabolism during exercise is a potential source of oxidative stress. Only a few studies have investigated and compared different aerobic exercise programs and their relations to pro-oxidant and antioxidant activities.
**Aim**: The aim of this study was to compare biomarkers of oxidative stress: lipid peroxidation, protein oxidation and total antioxidants in blood, as well as functional variables, before and after 12 weeks of two different exercise programs.
**Methods**: Blood samples were collected from seven young women who performed pilates training as low-intensity aerobic exercise and from seven young women who performed tae-bo training as high-intensity aerobic exercise. Samples were analyzed for lipid peroxidation byproduct malondialdehyde, enzyme catalase, reactive carbonyl derivates, total sulfhydryl groups and serum antioxidant status. The results were statistically evaluated by the by the Wilcoxon Signed Ranks Test and Mann-Whitney U Test.
**Results**: There were no significant differences in any functional or biomarkers parameters between groups before training program at beginning of study. After 12 weeks CAT increased in both group but increase was significantly higher with pilates training, whereas TAS increased significantly higher with tae-bo training. Both training programs increased values of MDA, protein carbonyls and sulphydryls, but there were no significant differences between groups.
**Conclusion**: There was evidence of oxidative stress after both pilates and tae-bo training program. Biomarkers of oxidative stress in blood increased after both 12 weeks training program. Due to different metabolic demands of pilates and tae-bo training, we can conclude that both training types induced oxidative stress and action effects of O2 is not only mechanism for exercise-induced oxidative stress.
**Key words**: oxidative stress, pilates, tae-bo, exercise

### INTRODUCTION

It is widely assumed that oxidative stress is detrimental to exercise performance, but there is little experimental evidence to support this. The lack of such an effect suggests that exercise-induced oxidative stress has only minor effects on performance in the short term; long-term effects on health should not be so readily dismissed however, given the range of diseases that are associated with enhanced free radical production. On the other hand, the increase in oxidative stress during exercise may signal an increase in antioxidant defenses that protects against a wide range of oxidative stresses (5). It is usually stated that one of the most important source of reactive oxygen species (ROS) during exercise is mitochondrial superoxide production, via side-reactions of flavin or ubisemiquinoneradicals with oxygen (3,9,14). An alternative mechanism by which exercise may promote free radical production involves ischemia-reperfusion (13). Muscle damage subsequent to exercise (e.g. in delayed onset muscle soreness) can cause inflammation and release of superoxide from the neutrophil NADPH oxidase (11,12).

Increased aerobic metabolism during exercise is a potential source of oxidative stress. Only few studies have investigated and compared different aerobic exercise programs and their relations to pro-oxidant and antioxidant activities. Only high intensity, or long duration, exercise appears to lead to a large enough increase in free radical production to overwhelm the antioxidant defenses (11).

The aim of this study was to compare biomarkers of oxidative stress: lipid peroxidation, protein oxidation and total antioxidants in blood, as well as functional variables, before and after 12 weeks of two different exercise programs.

### METHODS

A total number of fourteen young women participated in investigation. None of the participants had suffered from any illness for at least two weeks before the study. The purpose of the study was explained to the subjects and all gave their informed consent. In a preliminary visit body height and weight were measured using standard techniques (4). Subjects from both groups were instructed not to participate in any physical activity during the day before the study.

Blood samples were collected from seven women (mean+/-SD: age 24.7+/-3.7 years, body height 1.61+/-5.1 m, body weight

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54.9 +/-8.3 kg, body fat 11.1+/-5.2%) who performed pilates training as low-intensity aerobic exercise and from seven young women (mean+/-SD: age 26.8+/-4.3 years, body height 1.65+/-6.7 m, body weight 58.4 +/-9.8 kg, body fat 15.1+/-6.4%) who performed tae-bo training as high-intensity aerobic exercise.

Low intensity experimental program consisted of pilates exercises. After one training of familiarization, subjects exercised under the author's supervision for 3 days per week for a total of 10 weeks. During this period total of 30 training sessions were performed. Each training lasted 55 min, and consisted from three parts: worm up, main part of the training and cooling down. The main part of the training lasted between 35-40 min, and included between 12-15 pilates exercises using standing, sitting and lying down positions. The supervisor cues subjects through the various stretches and movements, offering visualizations and motivating talks to help them make good postural alignment, proper breathing, balance, and functional muscle strengthening. The movements during pilates are performed in the rhythm of the slow music (50 bpm) along with telemetric monitoring of heart function (Polar, Finland). Heart rate frequency was held under the 60% of estimated maximal heart rate for age.

High intensity experimental program consisted of tae-bo exercise. The experimental program was consisted from three training sessions every week. This made total of 30 training sessions. Each training session lasted one hour. After the warm-up to properly prepare the muscles and joints for the challenge of the workout, the main part of the training was consisted from the combination of tae-bo exercises. The choreographies were made from next tae-bo basic moves: jab, hook, cross, upper-cut, kick (front, side and back). Cross train with other forms of exercise such as walking, jogging, knee up, leg curls, step touch, etc. This tae-bo exercises program involves boxing gloves and punching bags. The majority of these classes are driven by fast music (135-155 bpm), although the music in many instances is more for motivation because the cardio tae-bo choreography is not always performed to a specific tempo, but intensity in the main part of training is adjusting to held hart rate frequency in range between 60-85% of heart rate. If heart rate frequency reaches above 85% of estimated maximal heart rate for age subject was instructed to slow down the intensity of exercising.

Samples were analyzed for lipid peroxidation byproduct malondialdehyde, enzyme catalase, reactive carbonyl derivates, total sulfhydryl groups and serum antioxidant status. Blood markers of oxidative stress were determined by standardized spectrophotometry technics (6,7,10).

In order to process the results of the study, the SPSS statistical program for Windows (Release 10.0, Chicago, IL, USA) was used. The results were statistically evaluated by the by the Wilcoxon Signed Ranks Test and Mann-Whitney U Test.

## RESULTS

The results of study are presented in Tables I and II.

**Table I.** Blood markers of oxidative stress before different exercise programs

| Parameters | Exercise program | Min-max | **Me** (25-75. percentile) |
|---|---|---|---|
| **Malondialdehyde activity - MDA (μM/L)** | **pilates** | 9.96-28.11 | 13.22 (10.39-20.77) |
| | **tae-bo** | 7.72-37.41 | 13.60 (12.21-19.24) |
| **Catalase - CAT (IU/L)** | **pilates** | 1.05-41.47 | 13.41 (3.56-18.22) |
| | **tae-bo** | 3.77-50.48 | 25.14 (7.33-48.60) |
| **Total sulfhydryl groups - TSHG (mM/L)** | **pilates** | 180.36-423.81 | 281.46 (228.08-326.76) |
| | **tae-bo** | 177.13-420.58 | 229.70 (203.16-333.63) |
| **Reactive carbonyl derivates (μM/g protein)** | **pilates** | 0.51-1.54 | 1.20 (0.58-1.28) |
| | **tae-bo** | 0.41-2.14 | 1.31 (0.57-1.82) |
| **Serum antioxidant activity – AOA (%)** | **pilates** | 25.00-92.70 | 69.10 (30.90-86.80) |
| | **tae-bo** | 39.70-98.50 | 78.20 (60.90-89.60) |

**Table II.** Blood markers of oxidative stress after different exercise programs

| Parameters | Exercise program | Min-max | **Me (25-75. percentile)** |
|---|---|---|---|
| **Malondialdehyde activity - MDA (μM/L)** | **pilates** | 9.29-64.27 | 14.42 (10.82-50.72) |
| | **tae-bo** | 8.57-58.75 | 13.63 (10.39-22.38) |
| **Catalase - CAT (IU/L)** | **pilates** | 16.55-55.51 | 34.56 (28.49-51.74)* |
| | **tae-bo** | 1.89-29.95 | 13.82 (9.64-25.87) |
| **Total sulfhydryl groups - TSHG (mM/L)** | **pilates** | 205.44-884.83 | 242.64 (221.61-258.82) |
| | **tae-bo** | 200.58-283.89 | 219.18 (208.27-248.30) |
| **Reactive carbonyl derivates (μM/g protein)** | **pilates** | 0.87-2.39 | 1.31 (0.99-2.08) |
| | **tae-bo** | 0.35-2.79 | 0.91 (0.61-1.85) |
| **Serum antioxidant activity – AOA (%)** | **pilates** | 17.70-95.60 | 64.70 (22.10-83.80) |
| | **tae-bo** | 69.10-97.10 | 82.40 (71.35-92.65)* |

* Mann-Whitney U Test: **p < 0.05**

## DISCUSSION

Exercise intensity refers to how fast an action is performed, the power or strength required to achieve an activity, or the effort put forth by the participant during the activity. Measurement of heart rate is precise and most often used method to evaluate intensity in everyday life or to set the level of exercise in physical training.

There is a common acceptance that pilates and other forms of low intensity exercises done slowly and with the breath, will do far more to improve health status than a vigorous cardiovascular or strength workout (1). They won't overly raise heart rates and breathing rates, and won't overly disturb digestion (parasympathetic exercise) but will reduce stress levels as much as possible. Strenuous exercise is characterized by an increased oxygen consumption and disturbance of intracellular prooxidant-antioxidant homeostasis (2). Some conditions associated with intense exercise, such as local tissue hypoxia or elevated tissue temperatures, could also contribute to reactive oxygen production (8). Since the antioxidant reserve capacity in most tissues is rather marginal, strenuous physical exercise characterized by a remarkable increase in oxygen consumption with concomitant production of ROS presents a challenge to the antioxidant systems. There is sufficient credible evidence to suggest that exercise is accompanied by an increased generation of free radicals, resulting in a measurable degree of oxidative modifications to various molecules (15).

In conducted study there were no significant differences in any functional or biomarkers parameters between groups before training program at beginning of study. After 12 weeks CAT increased in both group but increase was significantly higher with pilates training, whereas TAS increased significantly higher with tae-bo training. Both training programs increased values of MDA, protein carbonyls and sulphydryls, but there were no significant differences between groups. However, t is clear that ROS play important roles in numerous physiological processes at rest; however, the detailed physiological functions of ROS in exercise remain to be elucidated.

## CONCLUSION

There was evidence of oxidative stress after both pilates and tae-bo training program. Biomarkers of oxidative stress in blood increased after both 12 weeks training program. Due to different metabolic demands of pilates and tae-bo training, we can conclude that both training types induced oxidative stress and action effects of $O_2$ is not only mechanism for exercise-induced oxidative stress.

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# EFECTELE DIFERITELOR PROGRAME DE EXERCITIU FIZIC ASUPRA MARKERILOR SANGUINI AI STRESSULUI OXIDATIV LA FEMEILE TINERE


## REZUMAT

**Introducere**: Cresterea metabolismului aerob in timpul efortului fizic este o sursa potentiala de stres ovidativ. Doar putine studii au investigat si comparat diferite programe de exercitii fizice aerobe si relatia acestora cu activitatile pro-oxidante si anti-oxidante.
**Scop**: Scopul acestui studiu a fost compararea biomarkerilor stresului oxidativ: peroxidarea lipidelor, peroxidarea proteinelor si concetratia sanguina totala a antioxidantilor, precum si variabilele functionale, inainte si dupa 12 saptamani de efectuare a 2 programe diferite de exercitiu fizic.
**Metode**: Au fost recoltate probe de sange de la sapte persoane de sex feminin, tinere, care desfasurau antrenament pilates ca exercitiu fizic aerobic de intensitate scazuta si sapte femei tinere care desfasurau activitati tae-bo ca exercitiu aerobic de intensitate crescuta. Analiza probelor a presupus determinarea produsilor metabolici ai peroxidarii lipidice, cum ar fi malondialdehida, catalaza, derivatii reactivi de carbonil, grupurile sulfhidril totale si statusul seric antioxidant. Rezultatele au fost evaluate statistic prin Wilcoxon Signed Ranks Test si Mann-Whitney U Test.
**Rezultate**: Nu au existat diferente semnificative intre parametrii functionali si biomarkeri la grupurile studiate, inainte de inceperea programenlor de antrenament, la inceputul studiului. Dupa 12 saptamani, CAT a crescut in ambele grupuri, dar cresterea a fost semnificativ mai crescuta in grupul care a efectuat pilates, in timp ce TAS a crescut semnificativ in cursul antrenamentelor tae-bo. Ambele grupuri de antrenament fizic au prezentat valori crescute ale MDA, protein carbonil si sulfidril, dar nu au existat diferente semnificative intre grupuri.
**Concluzii**: Nu exista dovezi ale stresului oxidativ dupa efectuarea programelor pilates si tae-bo. Biomarkerii sanguini ai stresului oxidativ au crescut dupa 12 saptamani de antrenament. Datorita diferitelor nevoi metabolice in pilates si tae-bo, am putut concluziona ca ambele tipuri de antrenament fizic induc stresul oxidativ, iar efectele actiunii O2 nu sunt singurele mecanisme implicate in stresul oxidativ indus de efort.


**Cuvinte cheie**: stres oxidativ, pilates, tae-bo, efort fizic

# SHORT AND LONG TERM HYPOBARIC HYPOXIA INDUCES OXIDATIVE STRESS IN RATS: THE PROTECTIVE EFFECTS OF N-ACETYLCYSTEINE

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## ABSTRACT

**Purpose:** The aim of this study was to investigate whether acute and severe hypobaric hypoxia induces the changes of the reactive oxygen species (ROS) and the antioxidant systems. Knowing the efficiency of N-acetylcysteine supplementation in preventing tissue lesions induced by hypoxic stress, we have also studied if NAC could ameliorate the disfunctionalities generated by the free radicals caused by hypoxia. **Methods:** The animals were exposed to hypobaric hypoxia in the barochamber (simulated high altitude was the equivalent of 5500 meters), for 2 and 14 days. We have followed up 6 groups of Wistar rats: 1st group – control group, which received a vehicle solution (placebo) ; 2nd group – control group, N-acetylcysteine (NAC, 20 mg/kg); 3rd group – rats exposed to hypobaric hypoxia for 2 days-placebo; 4th group – rats exposed to hypobaric hypoxia for 14 days-placebo; 5th group – rats exposed to hypobaric hypoxia for 2 days and treated with NAC (20 mg/kg), prior to each exposure; 6th group – rats exposed to hypobaric hypoxia for 14 days and treated with NAC (20 mg/kg), prior to each exposure. After 14 days, the oxidative parameters in the blood were determined. **Results:** The results indicate an increase in the oxidative stress as seen by the increase in free radical production, malondialdehyde (MDA) and carbonylated proteins levels, after the exposure to hypobaric hypoxia. The antioxidant defense systems such as reduced superoxide dismutase (SOD) and catalase (CAT) levels were significantly decreased after "acute" and long term hypobaric hypoxia but they significantly increase after the administration of NAC. **Conclusions:** This study suggests that the administration of NAC can be beneficial in attenuating the oxidative stress associated with exposed to high altitude.
**Key words:** hypobaric hypoxia, oxidative stress, N-acetylcysteine

### INTRODUCTION

N-Acetylcystein(NAC), also called Acetylcysteine, is well-known by its effectiveness in the treatment of acute and chronic bronchitis and chronic obstructive bronchopneumapthy. Recent research have brought numerous arguments regarding its role in different diseases related to oxidative stress.

Nowadays, the implication of reactive species of oxygen (ROS) in a high number of physiological and pathological processes is well-known, some of them having an importance in the clinical practice, as a causal factor, leading, accompanying or even aggravating primary lesions.

Acute and chronic exposure to altitude constitutes an oxidative stress, the ROS sources being: mitochondrial respiratory chain alterations (1), membrane and cytosol alterations, endothelial cell alterations and lipid and protein perturbations. They are caused by hypobaric hypoxia, ultraviolet radiations and, possibly, by the temperature variations, dehydration and nutritive deficient. The oxidative metabolism is altered, and the anaerobic one is activated. The mitochondria can become targets of the oxidation, leading to membrane lipid peroxidation (5), protein oxidation, DNA clivation and, subsequently, deficitary production of ATP (6, 7, 8). The purpose of this study was to evaluate whether acute and severe hypobaric hypoxia induces the changes of the ROS and the antioxidant systems and also to assess the efficiency of N-acetylcysteine (NAC) supplementation in preventing tissue lesions induced by hypoxic stress.

### MATERIALS AND METHODS

We have studied 6 groups of 10 Wistar white male rats each, with the body mass between 150-170g.

The animals were exposed to hypobaric hypoxia in the barochamber (the simulated high altitude was the equivalent of 5500 meters), for 2 days and 14 days.

All studies were conducted in accordance with the National

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Institute of Health "Guide for the Care and Use of Laboratory Animals" and the experiments were carried out with the permission of the Institutional Ethics Committee.

The animals were divided into 6 groups:

- 1st group - Control group, which received vehicle solution (physiological serum-placebo)
- 2nd group- Control group, which received N-acetylcysteine (NAC, 20 mg/kg);
- 3rd group- rats exposed to hypobaric hypoxia for 2 days-placebo;
- 4th group- rats exposed to hypobaric hypoxia for 14 days-placebo;
- 5th group - rats exposed to hypobaric hypoxia for 2 days and treated with NAC (20 mg/kg), prior to each exposure;
- 6th group - rats exposed to hypobaric hypoxia for 14 days and treated with NAC (20 mg/kg), prior to each exposure.

At the end of the experiment, the rats were fasted overnight and sacrificed by cervical decapitation. Venous blood samples were collected from the rats' retroorbital sinus. The serum was separated for the estimation of the free radical production: malondialdehyde (MDA) (by Satoh method) (11) and carbonylated proteins levels (by Reznick method) (9); erythrocytes were separated for the estimation of superoxide dismutase (SOD, by Kakkar method) (4), catalase (CAT, by Sinha method) (12).

## RESULTS

Tables I and II show the levels of free radicals: MDA and carbonylated proteins levels in the serum of control and experimental animals. A significant increased oxidative stress was observed after the exposure to hypobaric hypoxia. Supplementation with NAC (20 mg/kg body weight) in the hypoxia-exposed group for 2 and 14 days respective shows significant decreased oxidative stress.

**Table I.** Effect of N-acetylcysteine (NAC) on MDA, carbonylated proteins levels in the serum and SOD and CAT in the RBC lysate of normal and experimental rats after 2 days the exposure to hypobaric hypoxia (***p<0.001; **p=0.005; *p<0.05)

| Parameters | MDA (nmol/ml) | Carbonylated proteins (nmol/mg) | SOD (U/mg Hg) | CAT (U/mg Hg) |
|---|---|---|---|---|
| 1st Group | 2.01± 0.26 | 0.61± 0.06 | 3.17±0.11 | 2.81±0.09 |
| 2nd Group | 2.03±0.21 | 0.63± 0.15 | 3.08±0.18 | 2.76±0.09 |
| 3rd Group | 4.57±1.1*** | 1.65±0.12 *** | 2.67±0.5** | 2.41±0.01* |
| 5th Group | 3.55±1.4** | 0.96±0.20 *** | 2.96±0.36* | 2.73±0.33* |

Tables I and II show the activities of enzymatic antioxidants: SOD, CAT of control and experimental animals. Activities of these enzymes significantly decreased after "acute" and long term hypobaric hypoxia exposure but they significantly increased after supplementation with NAC (20 mg/kg body weight) for 2 and 14 days, respectively, of hypoxia exposure.

**Table II.** Effect of N-acetylcysteine (NAC) on MDA, carbonylated proteins levels in the serum and SOD and CAT in the RBC lysate of normal and experimental rats after 14 days the exposure to hypobaric hypoxia (***p<0.001; **p=0.005; *p<0.05)

| Parameters | MDA (nmol/ml) | Carbonyla ted proteins (nmol/mg) | SOD (U/mg Hg) | CAT (U/mg Hg) |
|---|---|---|---|---|
| 1st Group | 2.01± 0.26 | 0.61± 0.06 | 3.17±0.11 | 2.81±0.09 |
| 2nd Group | 2.03±0.21 | 0.63± 0.15 | 3.08±0.18 | 2.76±0.09 |
| 4rd Group | 6.97±0.5*** | 2.02±0.3*** | 1.72±0.3*** | 1.79±0.1*** |
| 6th Group | 4.67±1.85** | 1.30±0.1*** | 2.97±0.3*** | 2.63±0.3*** |

## DISCUSSION

NAC actions by itself extracelularly, and intracelularly it actions as a precursor of L-cysteine and reduced glutathione (GSH) through a variety of mechanisms, ROS reduction being one of them (2). These properties are extremely relevant for the skin carcinogenesis induced by UV, due to ROS involvement in these processes, in conditions in which cutaneous antioxidants are depleted in the cells exposed to UV (10). NAC is efficient in decreasing their incidence, multiplication and prevents the appearance of scuamous cell carcinoma. In a high number of studies there was showed that that NAC acts as an inhibitor of apoptosis, due to its ability to attenuate the damages launched by these processes over DNA (2, 3).

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O2•-), which damages the membrane and biological structures.

Catalase has been shown to be responsible for the detoxification of significant amounts of $H_2O_2$.

SOD and catalase are the two major scavenging enzymes that remove the toxic free radicals in vivo. Reduced activities of SOD and catalase in erythrocytes have been observed after the exposure to hypobaric hypoxia and this may result in a number of deterious effects due to the accumulation of superoxide radicals (O2•-) and hydrogen peroxide.

## CONCLUSIONS

The data we obtained showed that hypoxic aggression induces an increased production of lipid peroxides and protein oxidation. Moreover, our study suggests that NAC is able to soften the impact of hypobaric hypoxia, based on the fact that the markers of ROS production and oxidative damage in rats were substantially lower in the hypoxic animals treated with NAC than in the hypoxic animals not treated with NAC.

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## HIPOXIA HIPOBARICA PE TERMEN SCURT SI LUNG INDUCE APRITIA STRESULUI OXIDATIV LA SOBOLANI: EFECTELE PROTECTOARE ALE N-ACETILCISTEINEI

## REZUMAT

**Scopul** acestui studiu a fost sa evidentieze daca hipoxia hipobara acuta si severa induce modificari ale speciilor reactive ale oxigenului (SRO) si ale sistemelor antioxidante. Cunoscand eficienta suplimentului de N-acetilcisteina in in prevenirea leziunilor tisulare induse de stresul hipoxic, am studiat de asemenea daca NAC poate ameliora disfunctionalitatile generate de radicalii liberi ce apar in urma hipoxiei. **Metode:** Animalele au fost expuse la hipoxie hipobara in barocamera (altitudinea simulate a fost echivalentul a 5500 de metri) timp de 2, respectiv 14 zile. Am urmarit 6 grupuri de sobolani rasa Wistar: grupul 1-grup control, care a primit solutie vehicul (placebo); grupul 2- grup control- a primit NAC, 20 mg/kg; grupul 3-sobolani expusi la hipoxie hipobara timp de doua zile- placebo; grupul 4- sobolani expusi la hipoxie hipobara timp de 14 zile-placebo; grupul 5-sobolani expusi la hipoxie hipobara timp de 2 zile si tratati cu NAC (20 mg/kg), inainte de fiecare expunere; grupul 6- sobolani expusi la hipoxie hipobara timp de 14 zile si tratati cu NAC (20 mg/kg) inainte de fiecare expunere. Dupa 14 zile, parametrii stresului oxidativ au fost dozati din sange. **Rezultate:** indica o crestere a stresului oxidativ, marcata de cresterea productiei de radicali liberi, malondialdehida (MDA) si proteine carbonilate, dupa expunerea la hipoxie hipobara. Sistemele de aparare antioxidanta, cum ar fi superoxid-dismutaza (SOD) redusa si catalaza (CAT), au avut niveluri semnificativ scazute dupa hipoxia acuta si de durata, dar nivelurile acestor parametri au fost crescute dupa adminitrarea NAC. **Concluzii:** Acest studiu sugereaza ca administrarea de NAC poate fi benefica pentru atenuarea stresului oxidativ asociat cu expunerea la altitudine.
**Cuvinte cheie**: hipoxie hipobara, stres oxidativ, N-acetilcisteina

# REST AND POSTURAL TREMOR IN THE POWER SPECTRAL STRUCTURE IN PATIENTS WITH PARKINSON'S DISEASE

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## ABSTRACT

It has recently been suggested that frequency harmonics found in power spectra of pathological tremors may contain information on dynamical properties of tremors, with each finite frequency band (mode) corresponding to a fluctuation period of a single independent source of oscillation. It was thus shown earlier, in the case of Parkinson's disease (PD) tremor, that the first two modes of oscillation display different patterns of dynamic behaviour. The aim of the present study was to investigate possible difference between the dynamic oscillatory behaviour of the rest and the postural tremor in PD.

We have studied the time series of hand tremor movements PD by applying the classical Fourier analysis and the detrended fluctuation analysis (DFA) method, to distinguish between their power spectra frequency bands. The analysis of these tremor series revealed the type of dynamic behaviour that can help differentiate between different modes. The application of DFA analysis helped us also distinguish between neural mechanisms active in rest and postural tremor in PD patients, as well as discerning difference of tremors in PD, when compared to hand oscillations in healthy subjects. Our findings suggest that pathological tremors in movement disorders may entail multiple oscillatory circuits that underlie the complexity of their initiation and development.

**Keywords**: Parkinson's disease, PD, tremor, power spectra, Fourier analysis

### INTRODUCTION

Tremors are typically defined as complex movements, manifested through seemingly rhythmic, oscillatory limb contractions, or contractions of muscle groups, producing oscillatory movements at one or more joints (6). While it is believed that most of pathological tremors have their origins in the central nervous system, the pathophysiology of tremor-generating mechanisms is still an open key question. The power spectra (PwS) of most tremors usually appear as semi-periodic wave forms, with more than one typical frequency (see Fig.1b). The recent studies support the assumption that, particularly in tremors of Parkinson's disease (PD), multiple sets of neuronal oscillators, rather then a single one, generate the tremulous activity (1,5,7,9,11).

It has been proposed recently (11) that the presence of multiple oscillatory drives of tremor could be assessed by probing the difference in dynamic behaviour that is, in values of functions characterizing the dynamic behaviour, of different modes, extracted from different frequency domains of the power spectra of recorded tremor data series. It was in this way suggested that the rest tremor in PD most likely originates from multiple superimposed biological oscillators (11).

The aim of our study was to confirm previous results in the case of PD tremor (11). Having in mind that the typical tremor (6) in PD patients is the rest tremor, while the other types of pathological tremors manifest in the existence of postural tremor, we have investigated both the rest and postural tremor in PD, and have compared our findings with the normal (physiological) oscillations of the same kind (rest and postural) in healthy subjects.

*The details of DFA method*

In order to elucidate the dynamic behaviour of analyzed tremor data, we applied the detrended fluctuation analysis (DFA), technique that stems from the field of statistical physics, on a PtP (point-to-point) series of tremor data (10). The DFA method requires three consecutive steps. In the first step, for each sequence of T(k), one should calculate the partial sum $y(\eta) = \sum_{k=1}^{\eta} T(k) - T_{ave}$, where $T_{ave}$ is the average of PtP durations (in all cases studied the total number of PtP data for a given series was of the order of $10^3$). In the next step, the entire series of y(η) is divided into a set of overlapping segments of the length n and evaluate the local trend for each segment, which is the polynomial fit for the segment data (4). The order of the polynomial fit k specifies the order of the DFA (denoted DFAk). We used a sliding segment, of size n, which slides over the entire y(η) series, producing thereby a new series of segments $y_{n,i}(\eta)$. For the new series, one has to define the so-called detrended walk $y^{d}_{n,i}(\eta)$ that is, for a given segment, the difference between the original series (represented by the partial sums y(η) and the local trend. Finally, one has to calculate the variance about the local trend for each segment and determine the average of these variances over all segments, which brings about the *detrended*

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*fluctuation function:*

$$F(n) = \sqrt{1/(N-n+1)n \sum^{N-n+1} \sum^{n} (y^{d}_{n,i})^2}$$

It has been proven that the function F(n) increases with increasing the segment length n. A linear relationship on a log-log plot indicates the presence of power-law type scaling behaviour of the analyzed data. Under such conditions, the fluctuations can be characterized by the scaling exponent η, a slope of the line relating log F(n) to log n. The scaling exponent η characterizes the dynamics of the analyzed series, for it is connected to scaling exponent η of the related PwS, and the exponent η of the related autocorrelation function, through the scaling relation $a = 1 - g/2 = (1+b)/2$ (12). It may happen, as was shown in our previous work, that in two neighbouring intervals of n the function F(n) displays two different power-law behaviours (2,3). The so-called crossover region is then defined by the values of n on which the function F(n) changes its behaviour.

## MATERIALS AND METHODS

Altogether, 20 patients participated in the study (mean age 62.1 years, range 47-76, mean UPDRS 25.3; see Table 1). The findings in this patient group were compared with corresponding data recorded in 20 healthy subjects (mean age 46 years, range 30-72), who had no history of neurological diseases. Patients were pre-selected from the records of the Institute of Neurology, Clinical Centre of Serbia and Neurological Service of the Military Medical Academy in Belgrade. All patients underwent routine neurological examination that excluded other neurological abnormalities. All subjects gave informed, written consent, acknowledging that the employed methods had been clearly explained, and that they understood them prior to the study. All procedures were approved by the Local Ethics Committee, and all the precautions were taken to comply with the Helsinki Declaration of the World Medical Associations (with all current revisions and amendments).

Tremor movements were recorded by monoaxial accelerometers (EGAX-5, Etran Devices Inc., USA), placed on subject's middle finger. All the subjects (patients and healthy controls) were free of drugs known to influence the tremor for at least 24 hours before the examination. In all the subjects, the tremor was recorded while comfortably seated in reclined position, with hands at rest (rest condition) and with arms and hands fully extended (postural condition). Subjects were instructed to keep their eyes closed during the recordings. Three successive 2-minutes epochs were recorded in all the studied cases. In order to avoid fatigue, each 2-minute recording session was followed by a 5-minute break.

Fourier analysis of signals was performed off-line, and the peak frequencies of the power spectra and the total power of the records were calculated, in the range between 1 and 25 Hz. We have analyzed, using the extended DFA method, the first three PwS modes, in order to access their possible independence, based on comparison of their dynamic behaviour (8). The total number of the analyzed data (PtP) of a single mode has been of the order of $10^3$ data.

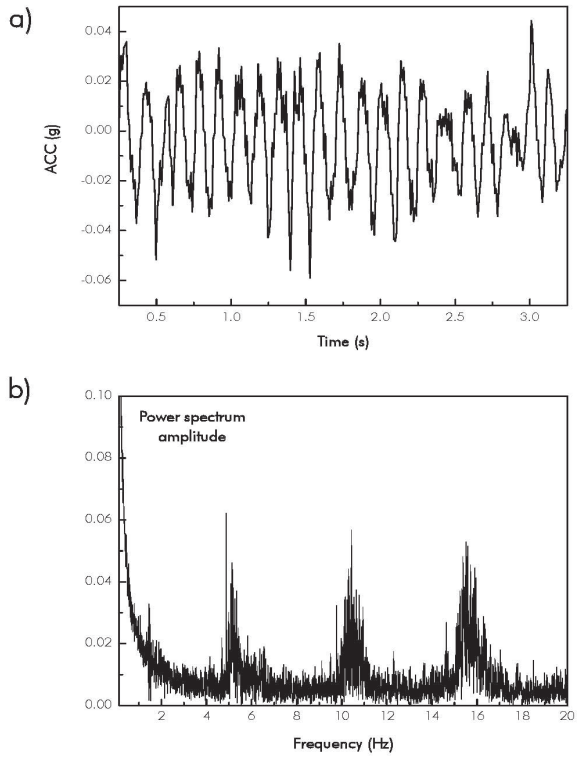



**Fig.1.** a) Typical example of recorded hand tremor from a patient with Parkinson's disease; b) The corresponding power spectrum of the recorded tremor, shown in a), displays three distinct frequency modes, used for filtering the original data

## RESULTS

For all PD patients studied, functions F(n) of the second order DFA (denoted DFA2) have been calculated, for the PtP time series extracted from the regions around three main PwS modes, while in the healthy controls, since it was impossible to discriminate different PwS modes, DFA2 curves were calculated for the area covering law frequencies (from 1Hz to 25Hz) (8).

**Table I:** Characteristics of the 20 patients with PD, and 20 healthy controls.

| | **PATIENTS No. (%)** | **HEALTY SUBJECTS No. (%)** |
|---|---|---|
| **• Sex** | | |
| Male | 11 (55) | 12 (60) |
| Female | 9 (45) | 8 (40) |
| **Mean age, range** | 61.47-76 | 46.30-72 |
| **Handedness** | | |
| Right | 19 (95) | 19 (90) |
| Left | 1 (5) | 2 (10) |
| **• Family history** | | |
| Yes | 12 (60) | 4(20) |
| No | 8 (40) | 16(80) |
| **• Mean disease duration, range** | 8.2-14 | |
| **• UPDRS** | 25.3 | |
| **• H&Y** | 2.5 | |

In Fig.2, the results obtained for a patient with Parkinsonian (PD) rest tremor are shown. We present DFA functions on a $\log_{10}$-$\log_{10}$ graphs, to measure ηby a linear fit, in the small n regions (within two decades), which was the range for estimating scaling exponent η. Although there was a notable difference between the first and the second PwS mode (see Fig.2), the difference in corresponding values of scaling exponents was, dissimilar to findings of Sapir and colleagues (11), not statistically significant. However, it is visible from the Fig.2 that the third PD mode displays markedly different dynamics, in comparison to the first two modes (with $p<0.05$ for differences of scaling exponents of the first and the third region, and second and the third region, respectively). This was the case in all PD patients studied.

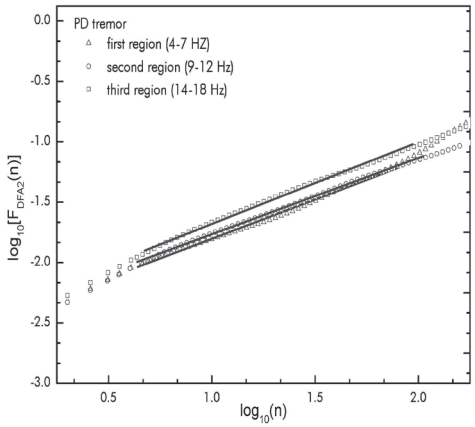
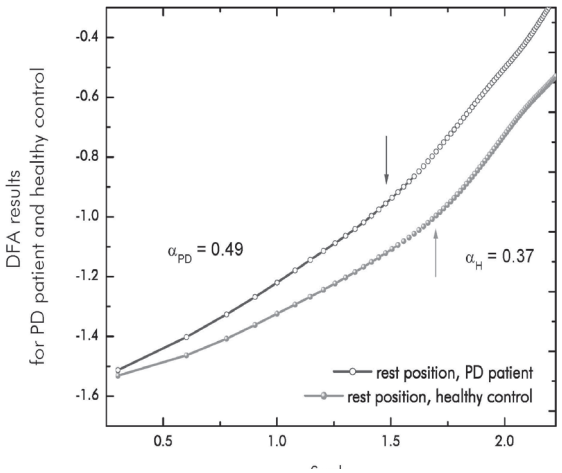



**Fig.2.** The DFA functions $F_{DFA2}(n)$ of second order, for the series of peak-to-peak (PtP) intervals, extracted around the first three modes of the power spectra of hand tremor from patient with Parkinson's disease (PD). Straight lines represent the linear least-squares fits to DFA functions. One can observe markedly different dynamics of the third harmonic mode, in comparison to the first two PwS modes, in the small n region. This is a typical result obtained for PD patients

The results obtained for the low frequency regions of power spectra of healthy controls group, depicted in Fig. 3, show distinct difference between the values of the scaling exponent α of the recorded tremor when compared to the corresponding tremors in PD. All the results obtained from healthy controls in the rest condition show existence of scaling in the DFA2 curves, with the scaling exponent $\alpha=0.34\pm0.01$, and with a visible crossover on higher scales (more than two orders of magnitude; see Fig. 3), while the corresponding results for the first (main) mode in PD case equals to $\alpha_{PD}=0.45\pm0.04$, with the visible crossover on lower scales. In the case of the recorded postural tremor, the results obtained from healthy controls show existence of scaling in the DFA2 curves, with the scaling exponent $\alpha=0.32\pm0.04$, and with a crossover on high scales, while the corresponding results for the first (main) mode in PD case equals to $\alpha_{PD}=0.48\pm0.01$, with the visible crossover on lower scales. The DFA analysis thus differentiated between the dynamical behaviour of the rest and postural tremor in PD patients and healthy controls.

**Fig.3.** Comparison of the DFA functions $F_{DFA2}(n)$ for the first (main) modes for a patient with PD and a healthy subject, in recorded rest tremor condition. Arrows point to the positions of crossovers. The calculated values of DFA exponents α are given, for PD case and a healthy control case




**DISCUSSION**

We have studied the time series of hand tremor movements PD by applying the classical Fourier analysis and the detrended fluctuation analysis (DFA) method, to distinguish between their power spectra frequency bands. The analysis of these tremor series revealed the type of dynamic behaviour that can help differentiate between different modes.

The application of DFA analysis helped us also distinguish between neural mechanisms active in rest and postural tremor in PD patients, as well as discerning difference of tremors in PD, when compared to hand oscillations in healthy subjects. Namely, the rest and the postural tremor recorded in PD patients display the existence of higher degree of correlations and the existence of crossover in scaling behaviour on lower scales than the corresponding (rest and postural) oscillations in healthy subjects that show lesser degree of correlations and have crossovers on a much higher scales.

Our findings suggest that pathological tremors in movement disorders may entail multiple oscillatory circuits that underlie the complexity of their initiation and development. Our findings also suggest that the existence of pathology in PD tremor may be connected to higher degree of correlations in corresponding tremor data. In the PD case, the positions of crossovers in behavior were shifted toward lower scales, in comparison to the data obtained in healthy subjects, in both rest and postural condition. Generally, the most likely explanation for the appearance of crossover (2) is that it is the sign of effects of various global and/or local processes (with longer characteristic time scales) within the organism. The appearance of these processes on lower scales in PD case could thus be interpreted as another sign of existent pathology.

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# TREMORUL DE REPAUS SI POSTURAL IN STRUCTURA SPECTRALA DE PUTERE LA PACIENTII CU BOALA PARKINSON


## REZUMAT

Recent a fost sugerat ca frecventa armoniilor din spectrele de putere ale tremorului patologic ar putea contine informatii cu privire la proprietatile dinamice ale tremorului, fiecare banda de frecventa finita (mod) corespunzand unei perioade de fluctuatie a unei singure surse independente de oscilatie. A fost aratat anterior, in cazul tremorului din boala Parkinson (PD), ca primele doua moduri de oscilatii prezinta patternuri diferite de comportament dinamic. Scopul acestui studiu a fost investigarea posibilei diferente existente intre comportamentul oscilator dinamic in repaus si tremorul postural in PD.

Am studiat in evolutie miscarile de tremor ale mainii persoanelor cu PD prin aplicarea analizei Fourier clasica si metoda analizei fluctuatiilor (DFA), pentru a diferentia spectrul de putere in benzile de frecventa. Analiza seriata a relevat tipul comportamentului dinamic care poate ajuta la diferentierea diferitelor moduri. Aplicarea analizei DFA a ajutat la diferentierea mecanismelor neurale active in repaus si tremorul postural la pacientii cu PD, precum si la diferentierea diferitelor tipuri de tremor in PD, atunci cand au fost comparate cu oscilatiile prezente la nivelul mainii unei persoane sanatoase. Rezultatele noastre sugereaza ca tremorul patologic in afectiunile de miscare pot avea diferite circuite oscilatorii care stau la baza complexitatii initierii si dezvoltarii acestora



**Cuvinte cheie**: boala Parkinson, PD, tremor, spectru de putere, analiza Fourier

# THE CORRELATION BETWEEN CARDIOVASCULAR RISK FACTORS, ARTERIAL STIFFNESS AND LEFT VENTRICULAR DIASTOLIC FUNCTION IN THE PATIENTS WITH HYPERTENSION

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## ABSTRACT

**Background and aim:** Hypertension accelerates atherosclerosis, collagen synthesis, and arterial smooth muscle hyperplasia and hypertrophy, thereby increasing arterial stiffness. It is well known that left ventricular diastolic function declines in the elderly, especially in patients with hypertension. The study aims determine the relationship between cardiovascular risk factors, arterial stiffness and left ventricular diastolic dysfunction in patients with hypertension with preserved left ventricular ejection fraction using newly developed ultrasonic imaging.

**Methods:** We performed a cross-sectional study including 105 (52 men and 53 women) patients with hypertension, mean age 58.5 (12.4). Left ventricular systolic and diastolic function was evaluated by 2D, M-mode and measuring transmitral flow velocity, mitral annular motion velocity using conventional and tissue Doppler ultrasonic system. Subclinical atherosclerosis also was determined by measuring the intimae – media thickness and stiffness of the common carotid arteries using 2D-, M-mode and tissue Doppler by ultrasonography. The dosages of lipids were measured by enzymatic method and the dosages of apolipoproteins were measured by immunoturbidimetric methods.

**Results:** The mitral annular motion velocity is correlated with apolipoprotein B ($r = 0.64$, $p<0.05$), and LDL-cholesterol ($r = 0.54$, $p = 0.05$) plasma levels, and apoB/apoA-I ratio ($r = 0.58$, $p<0.05$). The diastolic transmitral flow velocity is correlated with hypertension, without associated with lipids and apolipoproteins. The stiffness of carotid arteries correlated with diastolic mitral annular motion velocity ($r = 0.65$, $p < 0.05$) and with apoB/apoA-I ratio level ($r = 0.62$, $p < 0.05$). No significant correlation was obtained between the intimae – media thickness and echocardiographical parameters that define systolic and diastolic function of left ventricle.

**Conclusions:** These results suggest that the cardiovascular risk factors interact with arterial stiffness and left ventricular relaxation in patients with hypertension. The modern non-invasive imagistic exploration describes a useful method for early tracking of subclinical atherosclerosis.

### INTRODUCTION

Hypertension accelerates atherosclerosis, arterial smooth muscle hyperplasia and hypertrophy, and collagen synthesis, thereby increasing arterial stiffness. Arterial stiffening is found in patients with hypertension, but it is not clear whether the arterial disease precedes or is a consequence of sustained elevated blood pressure (29). Increased stiffness of large arteries commonly occurs with advancing age, thereby leading to the development of atherosclerosis regardless of the presence of coexisting diseases (22). In addition, increased arterial stiffness is associated with hypertension (26), elevated levels of triglycerides (2), diabetes (36), obesity (25), and may be an early marker for the development of cardiovascular disease.

As asymptomatic patient with hypertensive left ventricular hypertrophy and an echocardiography showing a normal ejection fraction and abnormal left ventricle filling can be said to have diastolic dysfunction (1,7). Diastolic dysfunction refers to an abnormality of diastolic distensibility, filling, or relaxation of the left ventricle, regardless of whether the ejection fraction is normal or abnormal and whether the patient is symptomatic or asymptomatic (7).

Arterial stiffness of hypertension patients has received growing attention during the last decade because of its association with cardiovascular diseases (3). Interestingly, recent results demonstrate that arterial stiffness is an independent predictor of progression to hypertension in normotensive subjects suggesting that lower arterial elasticity is related to the development of hypertension (5).

The measurement of pulse wave velocity is generally accepted as the most simple, non-invasive, and reproducible method indirect to determine arterial stiffness (13). However, the progresses made in last year in noninvasive ultrasonography concur to assessment of subclinical atherosclerosis by using tissue Doppler ultrasonography as direct method of simultane-

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ous assessment of left ventricular diastolic function as well as carotid distensibility (8).

The study aims determine the relationship between cardiovascular risk factors, arterial stiffness and left ventricular diastolic dysfunction in patients with hypertension with preserved left ventricular ejection fraction using newly developed ultrasonic imaging.

## METHODS

### Patients and study design

We performed a cross-sectional study with 105 patients with arterial hypertension (52 men and 53 women), hospitalized in the Department of Internal Medicine, Coulommiers, France between May 2005 and December 2005. Patients were informed about the study protocol and written consent was obtained from each patient. All the patients were evaluated with the same structural quiz, the same clinical and paraclinical protocol: medical history, physical examination, 12-lead electrocardiography, 2D, M-mode and Doppler echocardiography, 2D, M-mode and Doppler carotid echography, biochemical analysis.

Essential arterial hypertension was defined in patients as a systolic blood pressure (SBP) >140 mm Hg and/or diastolic blood pressure (DBP) >90 mm Hg or being treated with blood pressure lowering drugs (14). Only patients with normal resting left ventricular systolic function (ejection fraction >50% and left ventricular restrictive filling pattern) and without significant valve dysfunction were enrolled (12). The patients were divided in two groups according to the presence or absence of left ventricular diastolic dysfunction: group 1 – patients without left ventricular diastolic dysfunction and group 2 – patients with left ventricular diastolic dysfunction.

Exclusion criteria comprised: 1) any causes of secondary hypertension; 2) history of cardiovascular disease, defined as myocardial infarction, coronary heart disease, stroke (including transient ischemic attack), congestive heart failure, and peripheral vascular disease; 3) current use of oral hypoglycemic, antihypertensive, or lipid-lowering drugs; 4) renal failure; and 5) ejection fraction < 50%.

### Blood sampling and biochemical analysis

Venous blood samples were obtained after 12 h of fasting, and samples for lipids, glucose and apolipoproteins, were drawn without stasis into evacuated glass tubes containing 1/100 volume of 0.5 mmol of ethylenediaminetetraacetic acid/L. Plasma was obtained by centrifugation at 1500 g for 15 minutes was measured in fresh samples.

All patients had lipids: cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglycerides (TG) from plasma, analyzed by enzymatic tests performed by a Roche-Hitachi 911 analyzer (9). The plasma concentration of apo A-I and B and Lp(a) were measured by immunoturbidimetry with the help of the Roche/Hitachi Modular analyzer, using reagents and standard controls traceable to the International Federation of Clinical Chemistry primary standards (15,16).

### Clinical variables

We recorded the presence of several risk factors such as: age, sex, family background of coronary heart disease, smoking, hypertension, diabetes mellitus, obesity, dyslipidemia – all defined in accordance to international rules. Family history of coronary heart disease (CHD) was defined by a history of premature coronary artery disease in first-degree relatives (having occurred in those relatives at age < 55 years for men and < 65 years for women). Active smoking was defined as the smoke of at least one cigarette per day within the previous two months.

The presence of diabetes at baseline was defined as fasting plasma glucose >110 mg/dL (6.1 mmol/L) or use of oral hypoglycaemia agents or insulin (33). A surrogate marker for obesity content is the body mass index (BMI), which is determined by weight (kilograms) divided by height squared (square meters). In clinical terms, a BMI of 25–29 kg/m² is called overweight; higher BMI (30 kg/m²) are called obesity. The waist circumference was measured on admission, midway between the last rib and iliac crest, and the average of 2 measures was recorded (27).

### Echocardiography

Patients were studied by conventional and tissue Doppler echocardiography with HP, Sonos 5500, Philips ultrasound system, using a 2.5 MHz transducer. Recordings were made with a simultaneous superimposed electrocardiography.

*Conventional echocardiography 2D,* M-mode and Doppler was used for each patients, tracings from the parasternal long axis view for measuring septal thickness, left ventricular diameter at end-diastole and end-systole, and posterior wall thickness. Ejection fraction was derived from Simpson's modified single plane method using the apical 4-chamber view (12).

Comprehensive assessment of left ventricular diastolic function included transmitral pulsed wave Doppler from an apical 4-chamber view. From the transmitral flow, the peck early (E) and late atrial (A) diastolic velocities, E-deceleration time (DT), and isovolumetric relaxation time (IVRT) were successfully recorded for all patients. Diastolic function was classified as normal, impaired relaxation, pseudonormal, or restrictive according to standard diagnostic criteria. Normal diastolic function was defined by E/A of 0.75–1.5 and a deceleration time of 160–230 ms. Impaired LV relaxation was defined by the combination of E/A< 0.75 and E wave deceleration time >230 ms (20). No patients had a restrictive filling pattern.

*Tissue Doppler echocardiography* was used to left ventricular longitudinal myocardial wall motion from the apical 4-chamber view (31). Peck systolic myocardial velocity during ejection (Sm), early (Em) and late (Am) diastolic velocities were measured at the septal and lateral mitral annulus with pulsed wave tissue Doppler imaging in the apical 4-chamber view. The transducer was positioned to align the ultrasound beam with longitudinal left ventricular motion. A sample volume of 2 mm was used with the frame rate exceeding 100 m/s. The ratio of E to lateral Em was used to estimate left ventricular filling pressures. Normal diastolic function was defined by E/Em of <8, and the impaired left ventricular filling pressure was defined by E/Em of >8 (19).

### Carotid Ultrasonography

Ultrasound images were acquired using a 7.5 MHz linear array transducer and HP, Sonos 5500, Philips ultrasound sys-

tem. Common carotid artery intima – media thickness (IMT) was determined by high-resolution 2D-mode ultrasound (29). The transducer was manipulated so that the near and far walls of the common carotid arteries were parallel to the transducer foot print, and the lumen diameter was maximized in the longitudinal plane. The reference point for measurement of the carotid IMT was the carotid bulb. On that image, carotid IMT was measured as the leading edges corresponding to the transition zones between lumen – intima and media – adventitia over a length of 1 cm proximal to the reference point. Maximum IMT were defined as the greatest measured from 3 contiguous sites at 1-cm intervals.

Posterior wall velocity of common carotid artery in systole (DTI-Vmax m/s) was measured in tissue Doppler ultrasonography for a velocity of 100 mm/s. Sample of 1-2 mm was placed perpendicular to the posterior wall of common carotid artery.

Stiffness of the common carotid artery was evaluated by M-mode ultrasonography were used to calculate 2 estimates the arterial stiffness index β=ln(SBP/DBP)/[(Ds – Dd)/Dd], and the elastic modulus (EM)=[(SBP-DBP)/(Ds – Dd)]x Dd, where Ds and Dd are the end-systolic and end-diastolic diameters of the common carotid artery, respectively (24). The pulse pressure was evaluated as difference between SBP and DBP.

**Statistical analysis**

A commercially available statistical program, Statistical Package of Social Sciences (SPSS 6.0, Chicago, Ill) was used. All date are presented as the mean and standard deviation (SD). For univariate analyses were used Chi square or Fisher tests, according to standard application criteria, to test differences between qualitative data in the two groups according to the presence or absence of left ventricular diastolic dysfunction, respectively. Continuous data were analyzed using Student test for independent samples.

Pearson's and when relevant (n<20) additional Spearman's correlation was applied and linear regression plot was used to show relationships. A receiver operating characteristic (ROC) curve was used to discriminate patients with cardiac diastolic dysfunction (E/A ratio of <0.75 and E/Em>8) by arterial compliance. The results were considered statistically significant for p-value < 0.05.

## RESULTS

**Characteristics of patients**

In our study, the diastolic filling pattern was normal in 72 (68.6%) of patients with hypertension and the dysfunction pattern (relaxation or pseudonormal) was in 33 (31.4%) patients with hypertension. There were no significant differences in the clinical characteristics at baseline between the groups (Table I). The mean (SD) age did not differ significantly between the 2 groups. The cardiovascular risk factors – diabetes, obesity, smoking, dyslipidaemia, familial history of CHD – there were not significant differences between the 2 groups.

**Table I.** Characteristics of patient population

| Parameters | Group 1 | Group 2 | p-value |
|---|---|---|---|
| *Number patients, n* | 72 (68.6) | 33 (31.4) | <0.05 |
| *Mean age, years* | 57.6 (12.1) | 59.2 (11.4) | NS |
| *Women/Men, n* | 38/34 | 18/15 | NS |
| *Systolic blood pressure, mmHg* | 146.8 (10.7) | 148.9 (11.5) | NS |
| *Diastolic blood pressure, mmHg* | 96.3 (8.5) | 102.5 (6.9) | <0.05 |
| *Pulse pressure, mmHg* | 55.3 (9.3) | 67.5 (8.4) | <0.05 |
| *Body mass index, kg/m$^2$* | 28.4 (5.8) | 28.9 (5.5) | NS |
| *Family history of CHD, n (%)* | 14 (19.4) | 7 (21.2) | NS |
| *Obesity, n (%)* | 15 (20.5) | 8 (24.2) | NS |
| *Diabetes mellitus, n (%)* | 13 (18.1) | 7 (21.2) | NS |
| *Smoking status, n (%)* | 21 (29.1) | 12 (36.3) | NS |
| *Total Cholesterol level, mg/dL* | 180.2 (49.7) | 190.6 (42.3) | NS |
| *LDL-Cholesterol level, mg/dL* | 113.4 (46.6) | 115.6 (41.2) | NS |
| *HDL-Cholesterol level, mg/dL* | 54.5 (23.1) | 45.8 (12.4) | <0.05 |
| *Triglycerides level, mg/dL* | 134.3 (48.2) | 148.4 (42.7) | NS |
| *Apolipoproteins A-I, g/L* | 1.3 (0.3) | 1.2 (0.2) | NS |
| *Apolipoproteins B, g/L* | 0.8 (0.2) | 0.9 (0.3) | <0.05 |
| *ApoB/ApoA-I ratio* | 0.6 (0.2) | 0.7 (0.3) | <0.05 |

Continuous numerical data were expressed as mean (std. dev.); NS, not significant

**Echocardiography**

There were no significant differences in the M-mode and 2-D echocardiography between the 2 groups, regarding systolic function estimated by ejection fraction and diastolic function estimated by the transmitral flow velocity variables. Left ventricular parameters by echocardiography in the 2 groups are shown in Table II. Regarding the diastolic tissue Doppler parameters, where demonstrated significant differences between the 2 groups.

**Table II.** Comparisons of left ventricular parameters between the 2 groups

| Parameters | Group 1 | Group 2 | p-value |
|---|---|---|---|
| *End-diastolic ventricular septal thickness (mm)* | 11.2 (1.7) | 11.7 (1.9) | NS |
| *End-diastolic LV posterior wall thickness (cm)* | 11.5 (1.6) | 11.9 (2.1) | NS |
| *End-diastolic LV diameter (cm)* | 46.3 (4.5) | 47.4 (5.4) | NS |
| *End-systolic LV diameter (cm)* | 30.1 (2.3) | 31.5 (2.7) | NS |
| *LV ejection fraction (%)* | 68.2 (9.1) | 67.7 (8.9) | NS |
| *Transmitral flow velocity* | | | |
| – *Peak E velocity (m/s)* | 0.67 (0.2) | 0.72 (0.1) | NS |
| – *Peak A velocity (m/s)* | 0.71 (0.1) | 0.68 (0.2) | NS |
| – *E-DT (ms)* | 224 (44) | 212 (32) | NS |
| – *E/A* | 0.9 (0.3) | 1.18 (0.3) | <0.05 |
| *Mitral annular motion velocity* | | | |
| – *Peak Sm velocity (m/s)* | 7.8 (1.7) | 8.9 (1.4) | <0.05 |
| – *Peak Em velocity (m/s)* | 9.2 (2.9) | 11.4 (3.1) | <0.05 |
| – *Peak Am velocity (m/s)* | 9.6 (3.8) | 10.2 (3.9) | <0.05 |
| – *E/Em* | 8.6 (3.3) | 6.6 (1.9) | <0.05 |

Continuous numerical data were expressed as mean (std. dev.); NS, not significant

Peak E velocity, peak early diastolic velocity of transmitral flow; peak A velocity, peak atrial systolic velocity of transmitral flow; E-DT, deceleration time from peak to baseline of the early diastolic transmitral flow velocity; Em, peak early diastolic mitral annular motion velocity; Am, peak atrial systolic mitral annular motion velocity; Sm, peak systolic mitral annular motion velocity; E/Em, the ratio of E to Em.

*Carotid IMT and arterial stiffness*

There were no significant differences in the carotid IMT between the 2 groups. Carotid stiffness parameters and posterior wall velocity of common carotid artery was significantly lower in the group 1, whereas there was no significant change in the carotid diameter between the 2 groups.

**Table III.** Comparison of carotid ultrasonographic parameters between the 2 groups

| Parameters | Group 1 | Group 2 | p-value |
|---|---|---|---|
| *Intima-media thickness, mm* | 0.8 (0.3) | 0.9 (0.3) | NS |
| *End-diastolic carotid diameter, mm* | 6.5 (1.1) | 6.8 (1.3) | NS |
| *End-systolic carotid diameter, mm* | 7.4 (1.4) | 7.8 (1.2) | NS |
| *Stiffness parameters* | | | |
| – *Carotid stiffness index β* | 10.3 (3.7) | 11.8 (4.5) | <0.05 |
| – *Elastic modulus, kPa* | 124 (32.4) | 152 (42.5) | <0.05 |
| *Posterior wall velocity of common carotid artery, m/s* | 0.3 (0.2) | 0.4 (0.4) | <0.05 |

Correlation between left ventricular diastolic dysfunction parameters and cardiovascular risk factors

Table IV presents univariate relations assessed with simple linear regression analysis of left ventricular diastolic dysfunction parameters and cardiovascular risk factors. The E/A ratio correlated well the apoB/apoA-I ratio and the E/Em ratio correlated well the LDL-Cholesterol, apoB and apoB/apoA-I ratio.

**Table IV.** Correlation coefficients of left ventricular diastolic dysfunction parameters and cardiovascular risk factors

| Parameters | *E/A<0.75* | | *E/Em>8* | |
|---|---|---|---|---|
| | *R* | *p* | *R* | *p* |
| *Total Cholesterol* | **0.199** | **0.042** | **0.125** | **0.205** |
| *LDL-Cholesterol* | **0.176** | **0.073** | **0.542** | **0.032** |
| *HDL-Cholesterol* | **0.104** | **0.291** | **0.014** | **0.887** |
| *Triglycerides* | **0.027** | **0.787** | **0.129** | **0.189** |
| *Apolipoprotein A-I* | **0.077** | **0.435** | **0.010** | **0.917** |
| *Apolipoprotein B* | **0.104** | **0.291** | **0.641** | **0.008** |
| *ApoB/ApoA-I* | **0.490** | **0.000** | **0.589** | **0.029** |
| *Intima-media thickness* | **0.474** | **0.000** | **0.435** | **0.047** |

Correlation between DTI-Vmax, pulse pressure, arterial stiffness and diastolic dysfunction parameters

Significant correlations were found between posterior wall velocity of common carotid artery in systole and pulse pressure ($r = 0.47$, $p < 0.05$), carotid elastic modulus ($r = 0.71$, $p < 0.05$), and left ventricular diastolic dysfunction parameters – peck early (E) diastolic velocities measured by transmitral flow ($r = 0.52$, $p < 0.05$), and peck early (Em) diastolic velocities measured by the mitral annulus motion ($r = 0.67$, $p < 0.05$).

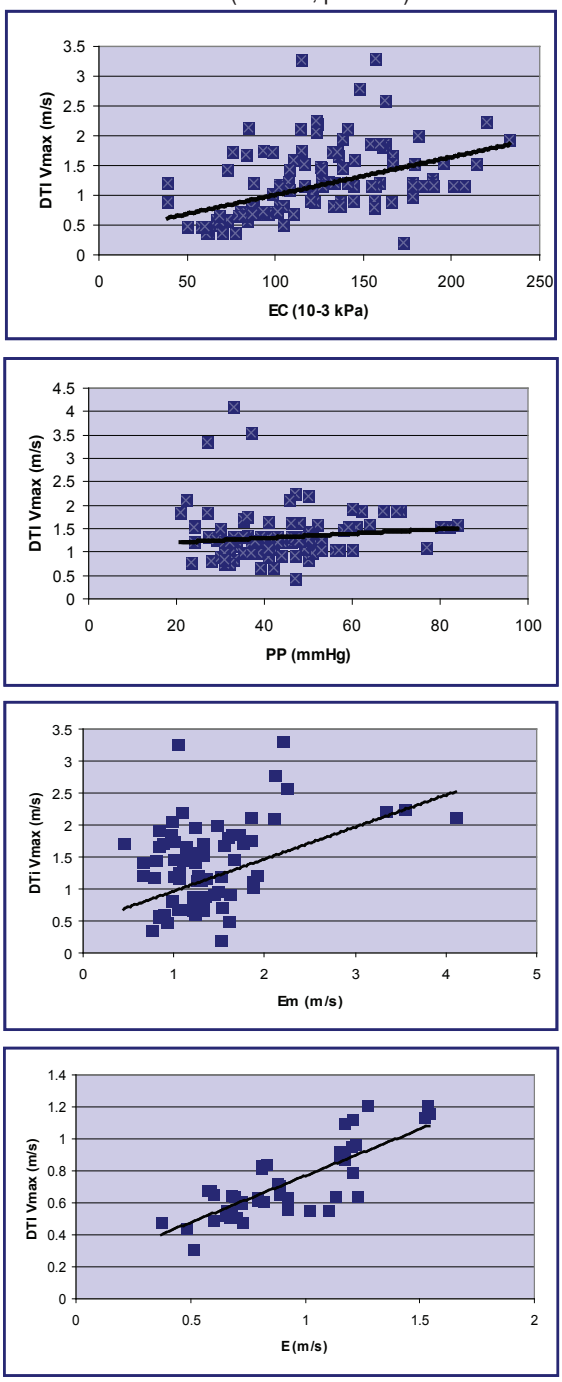


**Fig. 1.** Correlation between carotid DTI-Vmax and pulse pressure, arterial stiffness, left ventricular diastolic dysfunction parameters

Correlation between carotid stiffness and the presence/absence of left ventricular diastolic dysfunction

In the ROC curve for discriminating patients with hypertension and cardiac diastolic dysfunction by carotid stiffness, the area under the curve was 0.71, and the highest discriminating sensitivity and specificity were 70% and 95% as is shown in Fig. 2.

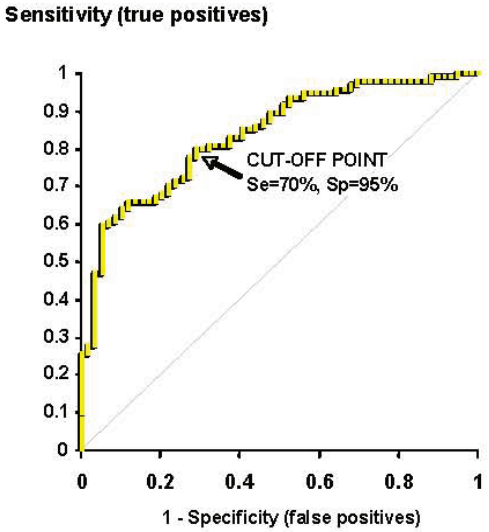


**Fig. 2.** Receiver operating characteristic curve between carotid stiffness and the presence/absence of diastolic dysfunction

## DISCUSSIONS

In the present study we assessed conventional echocardiography and tissue Doppler parameters regarding diastolic function and explored the relationship between arterial stiffness and cardiovascular risk factors. A method for reliably detecting the onset of left ventricular systolic and diastolic dysfunction and subclinical atherosclerosis of carotid arterial in patients with essential hypertension before transition to irreversible damage of the myocardium would be of crucial importance.

Cardiovascular risk factors, including hypertension, abdominal obesity, dyslipidemia, and glucose intolerance (4) contribute to vascular endothelial dysfunction, determining the development of atherosclerosis. The atherosclerosis should be evaluated according to the following 2 aspects: atherosis, which reflects structural changes in the intima and media of vascular walls, and sclerosis, which reflects changes in vascular stiffness or distensibility. The carotid IMT used in the present study is an index of atherosis, and the stiffness index is an index of sclerosis.

Decreased distensibility of the arterial wall increases SBP and decreases DBP, leading to increased left ventricular afterload and impairment of myocardial blood flow because of decreased coronary perfusion pressure. These abnormalities are reflected in the decreased left ventricular diastolic function preceding left ventricular systolic dysfunction (34). The present study - as well as previous ones - shows that arterial stiffness increases with left ventricular diastolic dysfunction in hypertensive patients.

The relationship between structural and functional changes in the carotid arteries and left ventricular myocardial function in patients with cardiovascular risk factors was investigated and found that left ventricular relaxation is significantly associated with carotid stiffness (17,18). In our study, the most significant correlations were obtained between left ventricular diastolic dysfunction and apoB/apoA-I ratio.

With pulsed Doppler echocardiography, left ventricular diastolic function can now be evaluated noninvasively by recording transmitral flow and mitral annular or left ventricular wall motion velocities (19). Many studies have indicated that left ventricular diastolic dysfunction occurs before left ventricular systolic dysfunction in patients with hypertension (21) even in the absence of apparently cardiovascular disease. Recently, previous studies (6,11) have demonstrated significant univariate relation between arterial distensibility, mitral inflow propagation velocity, and mitral E/A in patients with newly diagnosed hypertension. Our study showed that hypertension increase arterial stiffness and there is an association between left ventricular diastolic dysfunction measured by mitral E/A.

The relationship between blood flow derived velocities and regional myocardial wall motion derived velocities, measured by tissue Doppler echocardiography, and expressed as the ratio of peak early diastolic velocities E/Em, has been shown in several studies (23,32). In addition, E/Em ratio measured both at the lateral and medial parts of the mitral annulus, has limitations in discriminating patients with mildly to moderately elevate left ventricular filing pressures such as hypertension patients (32). Increased fibrosis in the left ventricular is known to be a part of the pathological process in hypertension patients (35). In the present study, we demonstrated that hypertension increase arterial stiffness and there is an association between left ventricular diastolic dysfunction measured by mitral E/Em ratio.

Redfield et al. (28) proposed the criteria of mild cardiac diastolic dysfunction by Doppler ultrasound examination in the general population. According to their criteria, Yambe et al. (26) showed than the ROC curve that has a brachial-ankle PWV over 1,600 cm/s is a marker of abnormal cardiac diastolic function (E/A ratio of 0.75) (sensitivity = 78% and specificity = 58%). The present study demonstrated that increased arterial stiffness is correlated with the parameters reflecting atherosclerosis but also to those reflecting cardiac diastolic dysfunction.

**In conclusion** the tissue Doppler echography is a simple method for evaluation of arterial stiffness rising and left ventricular relaxation alteration in patients with hypertension recently tracked. The reliability of the method is similarly or exceeds the more recently validated markers in diagnosis of studied pathology, e.g. apoB/apoA-I ratio. The physiological and physiopathological parameters – posterior wall velocity of common carotid artery in systole, E/Em ratio – have good performance in characterizing of arterial stiffness and left ventricular diastolic function and can be used for diagnosis and monitoring.

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## CORELATIILE DINTRE FACTORII DE RISC CARDIOVASCULAR, SCLEROZA ARTERIALA SI FUNCTIA DIASTOLICA A VENTRICULULUI STANG LA PACIENTII HIPERTENSIVI

## REZUMAT

**Obiective:** Hipertensiunea accelereaza ateroscleroza, sinteza de colagen, precum si hiperplazia si hipertrofia musculara neteda arteriala, crescand astfel rigiditatea arteriala. Este bine cunoscut faptul ca functia diastolica ventriculara scade la persoanele in varsta, mai ales la pacientii cu hipertensiune. Acest studiu isi propune sa determine relatia dintre factorii de risc cardiovascular, rigiditatea arteriala si disfunctia diastolica la pacientii cu hipertensiune au fractia de ejectie pastrata, folosind imagistica ultrasonografica recent descoperita.

**Metode:** Am efectuat un studiu incrucisat care a inclus 105 pacienti (52 barbati si 53 femei) cu hipertensiune, cu varsta medie de 58,5 (12,4). Functiile sistolica si diastolica ale ventriculului stang au fost evaluate 2D, M-mode si prin masurarea vitezei fluxului transmitral, vitezei de miscare a inelului mitral folosind sistemul ultrasonic Doppler conventional si tisular. Ateroscleroza subclinica a fost determinata de asemenea prin masurarea grosimii si rigiditatii intima-medie la nivelulu arterei caroide comune, folosind metode ultrasonografie 2D-, M-mode si Doppler tisular. Concentratia de lipide a fost masurata prin metode enzimatice, in timp ce concentratiile apolipoproteinelor au fost determinate prin metode imunoturbidimetrice.

**Rezultate:** Viteza de miscarea a inelului mitral este corelata cu apolipoproteina B ($r = 0,64$, $p<0,05$) si cu nivelurile plasmatice ale LDL-colesterol ($r = 0,54$, $p = 0,05$), precum si cu raportul apoB/apoA-I ($r = 0,58$, $p<0,05$). Viteza fluxului diastolic transmitral este corelata cu hipertensiune, fara asociere cu lipidele si apolipoproteinele. Rigiditatea si ingrosarea arterelor coronare s-a corelat cu viteza de miscarea a inelului mitral in timpul diastolei ($r = 0,65$, $p < 0,05$) si cu raportul apoB/apoA-I ($r = 0,62$, $p < 0,05$). Nu au fost observate corelatii semnificative intre grosimea intima-medie si parametrii ecocardiografici care definesc functia sistolica si diastolica a ventriculului stang.

**Concluzii:** Aceste rezultate sugereaza ca factorii de risc cardiovasculari interactioneaza cu rigiditatea vasculara si relaxarea ventriculara la pacientii cu hipertensiune. Explorarea prin metodele imagistice non-invazive moderne este descrisa ca o metoda utila pentru depistarea precoce a aterosclerozei subclinice.

# SDS-PAGE ELECTROPHORESIS OF URINARY PROTEINS: DIAGNOSTIC AND PROGNOSTIC VALUE

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## ABSTRACT

Sodium-dodecyl-sulphate polyacrylamide gel electrophoresis of urinary protein (SDS-PAGE) is a non-invasive method for the study of proteinuria. Besides its diagnostic usefulness it has recently been shown to have prognostic value in patients with glomerular diseases. The *aim* of this study was to evaluate diagnostic and prognostic value of SDS-PAGE in our center. *Material and methods*: In 288 patients, in whom SDS-PAGE was performed between 1995 and 2005, we retrospectively studied the link between the pattern of proteinuria (PP) by SDS-PAGE and the diagnosis established by other methods. We then identified patients with glomerular diseases who were followed-up for at least 6 months and studied the prognostic value of certain SDS-PAGE patterns in this group. *Results*: The PP by SDS-PAGE (glomerular-7%, glomerulo-tubular-65%, tubular 21%, physiologic-7%) correlates with the diagnosis (glomerular diseases-42%, tubulo-interstitial diseases-23%, multiple myeloma-2%, other-33%; p = 0.0001, $\chi 2$ 57.71). PP also correlates with quantitative proteinuria (p = 0.0001). In 31 patients with glomerular diseases, at a follow-up of 26 ± 20 months, we analysed the prognostic value of urinary high molecular weight (HMW ≈150 kda) proteins, very low molecular weight (VLMW <25kda) proteins and polymers of albumin (PA), which are potential risk factors for progression of renal failure. As compared to their negative counterparts, the HMW positive patients displayed a faster decline in creatinine clearance (CrCl) (1.49±0.21 ml/min/month versus 0.14 ± 0.02 ml/min/month, p = 0.01). The same applied to the VLMW positive vs. VLMW negative patients (1.42 ± 0.25 versus 1.15 ± 0.18ml/min/month; p = 0.08), as well as for the PA positive vs. the PA negative patients (1.79 ± 0.24 vs. 0.89 ± 0.17ml/min/month; p = 0.04). There was no statistically significant difference between decline in CrCl of patients with or without low molecular weight (LMW tubular proteins 25-45 kda) proteins in SDS-PAGE. In *conclusion* SDS-PAGE confirms its value as a diagnostic tool; also its prognostic use in patients with glomerular diseases is to be encouraged, as certain PP are associated with a worse course of disease.

**Key words**: SDS-PAGE, urinary proteins, very low molecular weight, prognostic, progression – chronic renal failure

### BACKGROUND

Proteinuria is an important progression factor of chronic renal failure as it correlates not only with glomerulosclerosis but also with tubulo-interstitial damage, which is an important determinant in the prognosis of chronic renal failure (11,18). In predicting the outcome of glomerular diseases, composition of proteinuria is essential, since excretion of certain urinary proteins carry prognostic significance (19, 1-6, 8). This justifies the qualitative study of proteinuria in glomerular patients.

First described by Laemmli (16), sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of urinary proteins is a non-invasive method for the qualitative evaluation of proteinuria. SDS-PAGE has been in use in our center since 1980 (9). There are some reports about the prognostic value of some patterns of urinary SDS-PAGE (11, 17, 18).

The aim of our study was to establish the diagnostic efficiency of SDS-PAGE on a large cohort of patients in our clinical practice and to assess the prognostic value of specific components of proteinuria in patients with glomerular diseases.

### PATIENTS AND METHODS

We reviewed the records of patients admitted in our unit between January 1995 and December 2005 in whom SDS-PAGE had been performed. Patients whose results were reported prior to hospital discharge (so that discharge diagnosis could have been influenced by SDS-PAGE findings) were excluded. The study cohort consisted of 288 patients; chart records were noted. Creatinine clearance was estimated according to the Cockroft formula.

SDS-PAGE was performed following the method initially described by Laemmli (16). 24 hour urine samples were dialysed, concentrated and then electrophoresed on 7.5% polyacrylamide gel slabs (170/120/0.5 mm with 10 protein bands/ slab) in discontinuous buffer. Each sample contained 12 µg proteins. After migration bands were colored with Coomassie Brilliant Blue R 250. Classification was made by visual inspection in comparison to the migration band of known molecular weight (Amersham International).

The following types of urinary proteins were identified:

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proteins ≈ 150 kda (mainly immunoglobulins) – termed high molecular weight (HMW) proteins; proteins with a molecular weight of

60-72 kda (albumin, transferrin) – termed middle molecular weight (MMW) proteins, proteins with molecular weight between 25 and 45 kda termed low molecular weight (LMW) proteins and proteins with molecular weight under 25 kda termed very low molecular weight (VLMW) proteins. These types of proteins were sometimes associated, creating various patterns. According to data in the literature (3, 4) we classified patients into four patterns of proteinuria: pure glomerular proteinuria (presence of HMW and MMW proteins), glomerulo-tubular pattern (presence of MMW and/or HMW proteins accompanied by LMW and/or VLMW proteins), physiologic proteinuria – albumin alone and tubular proteinuria (LMW and/or VLMW proteins). Presence of PA was also recorded.

We then selected patients with glomerular diseases who had a follow-up of at least 6 months. Patients with improving renal function (acute renal insufficiency superimposed) or rapidly deteriorating renal function (decrease in CrCl of more than 50% in 3 months) were excluded. Cockroft CrCl was calculated on the first and last visit and the decline in CrCl was divided by the number of months of follow-up. Correlations between monthly decline in renal function and various proteinuric patterns were then studied.

The statistical methods used were $\chi^2$ test, t-test, simple and multiple regression analyses. Data are presented as mean ± SEM.

## RESULTS

*Diagnostic value of SDS-PAGE*

Our group consisted of 288 patients (55% male) with a mean age of 40.60±15.83 years. They had moderately altered renal function, significant proteinuria and relatively well controlled blood pressure (Table I).

**Table I** – Characteristics of the patients

| Parameter | Mean ± SEM |
|---|---|
| Age (years) | 48±1 |
| Serum Creatinine (mg/dl) | 3.36±0.19 |
| Initial creatinine clearance (ml/min) | 38±2 |
| Blood Urea (mg/dl) | 99±4 |
| Hemoglobin (g/dl) | 11.6±0.13 |
| CRP (mg/dl) | 1.61±0.17 |
| Total serum proteins (g/dl) | 6.70±0.05 |
| Serum albumin (%) | 49±1 |
| Serum cholesterol (mg/dl) | 219±4 |
| Proteinuria (g/24 h) | 3.01±0.13 |
| Systolic BP (mmHg) | 135±1 |
| Diastolic BP (mmHg) | 84±1 |

Legend: CRP – C reactive protein, SBP – systolic blood pressure, DBP – diastolic blood pressure

120 patients (42%) had glomerular diseases, 69 patients (23%) tubulo-interstitial diseases, 5 patients (2%) had multiple myeloma and 94 patients (33%) other renal diseases. This last group consisted mainly of patients presenting with relatively advanced chronic renal insufficiency in which diagnostic certitude could not be obtained.

SDS-PAGE patterns were as follows: 23 (8%) pure glomerular pattern, 182 (63%) glomerulo-tubular pattern, 60 (21%) tubular pattern and 23 (8%) physiologic pattern. Among patients with glomerulo-tubular pattern, 81 (44.5%) had VLMW proteinuria and 101 (55.5%) had only LMW proteins in their urine.

The diagnosis established by other methods correlated significantly with SDS-PAGE pattern (p=0. 0001 $\chi^2$=57. 71) – Figure 1.

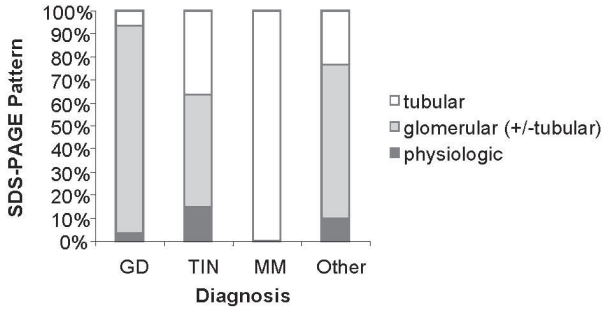


**Fig. 1**. Correlation between SDS-PAGE pattern and diagnosis
Legend: GD – glomerular diseases; TIN – tubulointerstitial nephritis; MM – multiple myeloma; other – other nephropathies

As expected, SDS-PAGE pattern also significantly correlated with proteinuria (p = 0.0001) - Figure 2.

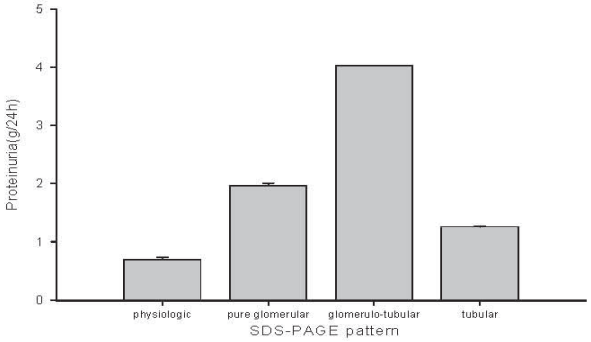


**Fig. 2** - Relation between proteinuria and SDS-PAGE pattern

*Prognostic value of SDS-PAGE*

We recorded the follow-up of 31 patients (77.4% male) with glomerular renal disease who were followed for at least 6 months (mean 26 ± 10 months). Patients were divided according to their SDS-PAGE pattern.

First we divided patients according to presence/absence of HMW proteins in their urine and compared mean monthly decline in renal function. Patients with HMW proteinuria exhibited a significant, more rapid deterioration in renal function than

HMW negative ones (1.49 ± 0.21ml/min/month versus 0.14 ± 0.02 ml/min/month, p = 0.01). We compared other factors known to influence progression of renal failure between the two above-mentioned groups: patients with HMW proteinuria had a significantly greater proteinuria. Diastolic BP and CRP were more elevated in the HMW negative group (Table II).

Patients divided according to presence or absence of LMW proteins in their urine had a similar decline in renal function (1.16 ± 0.18 ml/min/month versus 1.38 ± 0.25 ml/min/month, NS).

Patients with VLMW proteinuria had a more rapid decline in renal function than patients without VLMW proteinuria (1.42 ± 0.25ml/min/month versus 1.15 ± 0.18ml/min/month, p = 0.08). No other parameters known to influence progression of renal disease were different between subgroups except for slightly different initial renal function (Table II).

We then divided these patients according to the presence or absence of PA in the SDS-PAGE and again the decline in renal function was compared. Patients with PA in the urine had a more rapid progression of renal insufficiency as compared to patients without PA (1.79 ± 0.24 ml/min/month versus 0.89 ± 0.17 ml/min/month, p = 0.04). However they also had significantly more proteinuria and more severe hyperlipidemia (Table II).

Results were similar if only patients with nephrotic syndrome were analyzed. However, this group was very small (n =13): HMW positive patients had a more rapid decline in renal function than HMW negative patients (1.8 ± 0.4 ml/min/month versus 0.16 ± 0.05 ml/min/month, p = 0.08), as did VLMW positive patients versus VLMW negative patients (3.57 ± 0.44ml/min/month versus 0.84 ± 0.33 ml/min/month, p = 0.01) and PA positive patients versus PA negative patients (2.23 ± 0.44 ml/min/month versus 0.40 ± 0.27ml/min/month, p = 0.02). Again there were no significant differences when we divided patients according to LMW proteinuria.

## DISCUSSION

The main finding in our study was that SDS-PAGE analysis of proteinuria carries prognostic significance, as certain SDS-PAGE patterns (HMW, VLMW proteins and PA) are associated with more rapid progression of renal failure.

As already stated, in glomerular diseases, the qualitative study of proteinuria by SDS-PAGE also has prognostic implications. Presence of HMW proteins excreted in the urine is a direct marker of the severity of glomerular lesions. In fact it has been shown that increased urinary excretion of IgG correlates better

**Table II** – Comparison of patients with glomerular diseases divided according to presence or absence of urinary HMW, VLMW, PA proteins

| **Glomerular disease n=31** | **HMW +** | **HMW -** | **p** | **VLMW +** | **VLMW -** | **p** | **PA +** | **PA -** | **p** |
|---|---|---|---|---|---|---|---|---|---|
| Decline in cretinine clearance(ml/min/month) | **1.49± 0.21** | **0.14±0.02** | 0.01 | **1.42± 0.25** | **1.15±0.18** | 0.08 | **1.79±0.24** | **0.89±0.17** | 0.04 |
| Time (months) | 18±1 | 60±1 | 0.002 | 22±4 | 27±5 | NS | 10±1 | 32±5 | 0.01 |
| Age (years) | 49±1 | 42±0.5 | NS | 50±4 | 43±2 | NS | 56±3 | 41±3 | 0.01 |
| Initial creatinine clearance (ml/min) | 54±1 | 46±1 | NS | 42±6 | 63±6 | 0.05 | 50±3 | 53±6 | NS |
| ESR 1 (mm/h) | 74±1 | 66±1 | NS | 43±8 | 60±7 | NS | 72±7 | 57±8 | NS |
| ESR 2 (mm/2h) | 95±2 | 71±1 | NS | 102±9 | 75±8 | NS | 97±7 | 69±7 | NS |
| CRP (mg/dl) | 0.45±0.02 | 2.5±0.05 | 0.01 | 0.87±0.14 | 0.8±0.33 | NS | 0.60±0.15 | 1.01±0.35 | NS |
| Hemoglobin (g/dl) | 11.34±10.32 | 12.5±0.64 | NS | 11.21±1.79 | 11.9±3.59 | NS | 11.19±3.59 | 11.70±3.59 | NS |
| Serum albumin (%) | 48±1 | 50±1 | NS | 46±5 | 49.00±4 | NS | 49±1 | 47±1 | NS |
| Serum cholesterol (mg/dl) | 233±2 | 236±1 | NS | 206±9 | 265±1 | NS | 272±12 | 215±12 | 0.02 |
| Proteinuria (g/24h) | 4.21±0.08 | 1.68±0.11 | 0.05 | 3.78±0.48 | 3.80±0.66 | NS | 5.07±0.61 | 3.05±0.50 | 0.04 |
| SBP (mmHg) | 139±1 | 148±1 | NS | 136±4 | 146±4 | NS | 141±4 | 148±4 | NS |
| DBP (mmHg) | 85±2 | 98±0.5 | 0.02 | 82±2 | 92±2 | NS | 89±3 | 86±2 | NS |

Legend: HMW – high molecular weight, VLMW – very low molecular weight, PA – polymers of albumin, ESR 1– erythrocyte sedimentation rate at 1 h, ESR 2– erythrocyte sedimentation rate at 2h, CRP – C reactive protein, SBP – systolic blood pressure, DBP – diastolic blood pressure

than 24 hour proteinuria with progression to chronic renal failure (19) with reduced remission and histological changes in patients with membranous glomerulopathy (2,8) and focal segmental glomerulosclerosis (6). The same kind of information results from studies of the predictive value of selectivity of proteinuria in various glomerular diseases: low selectivity proteinuria correlates with more prominent tubulo-interstitial changes and accelerated progression to chronic renal failure, reduced clinical remission or response to therapy (5, 1).

As information about excretion of different molecules (IgG, β2 microglobulin, α1 microglobulin) are not routine in our center, we turned to SDS-PAGE for the characterization of proteinuria. Available for decades, SDS-PAGE was used for hardly more than diagnostic purposes, and even in research it tends to be neglected in favour of direct measurements of various proteins. It is, however, a reliable method and relatively easy to perform, so we considered it worthwhile to explore its prognostic value. Although our initial population was large enough, since SDS-PAGE was needed in most cases for diagnostic purposes, the group of patients with confirmed glomerular diseases and having had SDS-PAGE is relatively small because diagnosis of glomerular diseases was in most cases obtained by other methods. HMW proteins in the urine of glomerular patients should account for unselective proteinuria and therefore more severe disease. As expected, presence of urinary HMW proteins identified a group of patients with more severe disease, greater proteinuria and more rapid decline in renal function. Other factors of progression (hypertension and inflammation) were worse in the HMW negative group.

In proteinuric patients with glomerular diseases, filtered proteins will stress the reabsorptive mechanism of the proximal tubule, eventually inducing tubular lesions and activation of cytokine and growth factors that produce tubulo-interstitial damage (10). Presence of abnormal quantities of albumin and HMW proteins in the urine also results in an impaired reabsorption and increase in the urinary excretion of LMW "tubular" proteins, turning them into a valuable marker for the severity and outcome of glomerular diseases. In fact β2 microglobulin has been shown to correlate with the progression to chronic renal failure in patients with membranous glomerulopathy (19, 8). Also fractional excretion of α1 microglobulin was significantly associated with severity of tubulo-interstitial changes, functional outcome and response to therapy in membranous glomerulopathy (2). It also correlates with tubulo-interstitial damage in patients with focal segmental glomerulosclerosis (6). It is true that other studies contested the role of excretion of LMW proteins in predicting the response to therapy (12).

Using SDS-PAGE for the identification of tubular proteins, several studies have already demonstrated the role of LMW and VLMW proteins in predicting the renal outcome in glomerular diseases (17, 21, 3, 15, 13). Bazzi et al. (3) studied 145 patients with biopsy-proven primary glomerular diseases and showed a correlation between presence of VLMW proteinuria and tubulo-interstitial damage, development of CRF and response to therapy.

In our study we obtained a significant relationship between the presence of VLMW proteins in SDS-PAGE and rapid progression of renal failure in patients with glomerular diseases. This confirms Bazzi et al.'s findings of VLMW proteins as a marker of worse outcome. Other factors that potentially influence progression of chronic renal failure were compared between patients with and without VLMW proteins in SDS-PAGE and their values were similar between the two groups, excluding thereby other causes of worse outcome in the VLMW positive patients.

Presence of PA in the urine of patients with glomerular diseases, first described by Boesken (7), has been suggested to be a negative prognostic factor for the development of chronic renal failure (14). Polymerisation of albumin requires certain conditions to occur, one of which is the presence of a LMW ultrafiltrable factor (14), so significance of presence of PA and VLMW in urine could be interconnected. Our study also finds PA to be a negative marker of accelerated progression of renal insufficiency in patients with glomerular diseases. However, patients presenting with PA also had other possible confounding progression factors as they had more severe proteinuria and higher cholesterol levels, and were also older. This can reflect a more severe underlying disease. Of course, again the relatively small number of patients and the retrospective nature of the study prompt to prudence in interpreting the results.

Although the majority of our patients were biopsied, we were not able to study the link between different SDS-PAGE patterns and histological findings since re-examination of biopsy samples was not feasible.

Proteinuria is the cornerstone of many renal diseases and therefore an essential diagnostic tool. In the present study we evaluated the value of SDS-PAGE as a non-invasive tool in the diagnosis of different nephropathies. An unequivocal statistically significant relationship was obtained between SDS-PAGE pattern and different diagnostic categories (diagnosis being established by other methods, including renal biopsy). As expected SDS-PAGE patterns also correlated with proteinuria. These strong correlations confirm its diagnostic value and suggest that we should probably prescribe it more frequently with diagnostic purpose, at least in our local experience.

**CONCLUSION**

Our study reiterates the clinical usefulness of SDS-PAGE in establishing the nephrological diagnosis. But most importantly it confirms the role of certain SDS-PAGE patterns, in particular presence of HMW and VLMW proteins as well as PA, in predicting accelerated progression of chronic renal failure in patients with glomerular diseases. In our opinion, given the non-invasive character of SDS-PAGE and the fact that the method can be performed without the need of costly material, it should be performed especially in centers where imunonephelometric dosage of urinary proteins is not available. Larger prospective studies are warranted to confirm the prognostic value of the method; also its use as a diagnostic tool should be encouraged.

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# VALOAREA DIAGNOSTICA SI PROGNOSTICA A ELECTROFOREZEI PROTEINELOR URINARE IN GEL DE POLIACRILAMIDA CU TAMPON DODECILSULFAT DE SODIU (SDS-PAGE)


## REZUMAT

Electroforeza proteinelor urinare in gel de policarilamida cu tampon dodecilsulfat de sodiu (SDS-PAGE) este o metoda non-invaziva de evaluare a proteinuriei. Pe langa valentele sale diagnostice s-a demonstrat recent ca detine si o semnificatie prognostica in glomerulopatii. *Scopul* acestui studiu a fost aprecierea valorii diagnostice si prognostice a metodei in centrul nostru. *Pacienti si metoda*: Pe un lot de 288 de pacienti la care s-a efectuat SDS-PAGE intre 1995 si 2005 au fost studiate corelatiile intre tipul proteinuriei si diagnosticul stabilit prin alte metode. Au fost de asemenea selectati pacienti cu nefropatii glomerulare cu urmarire de minimum 6 luni si a fost evaluata valoarea prognostica a tipurilor proteinuriei la acest grup de pacienti. *Rezultate*: Tipul proteinuriei conform SDS-PAGE (glomerulara-7%, glomerulo-tubulara-65%, tubulara-21%, fiziologica-7%) se coreleaza cu diagnosticul (glomerulopatii-42%, nefropatii tubulointerstitiale-23%, mielom multiplu-2%, altele-33%; p = 0,0001, $\chi 2$ 57,71) si cu proteinuria cantitativa (p = 0,0001). La 31 de pacienti cu glomerulopatii si o urmarire medie de 26 ± 20 luni, a fost analizata valoarea prognostica a proteinelor urinare cu greutate moleulara mare (high molecular weight -HMW ≈150 kda), greutate moleculara foarte mica (very low molecular weight -VLMW < 25kda) si polimerilor de albumina (PA), care sunt potential factori de risc ai progresiei insuficientei renale. Pacientii cu HMW in urina au avut o rata de declin a clearance-ului creatininic mai rapida in comparatie cu cei fara HMW (1,49 ± 0,21 ml/min/luna versus 0,14 ± 0,02 ml/min/luna, p = 0,01). Aceasta observatie este valabila si pentru pacientii cu VLMW fata de cei fara fara VLMW (1,42 ± 0,25 versus 1,15 ± 0,18 ml/min/luna; p = 0,08), si pentru pacientii cu PA fata de cei fara PA (1,79 ± 0,24 vs. 0,89 ± 0,17ml/min/luna; p = 0,04). Rapidiatatea declinului functiei renale a fost similara la pacienti cu, respectiv fara, proteinelor cu greutate moleculara mica (25-45 kda) in urina. In *concluzie* SDS-PAGE isi confirma valoarea ca mijloc diagnostic; utilizarea sa ca marker prognostic la pacientii cu nefropatii glomerulare ar trebui incurajata deoarece anumite tipuri proteinurice sunt asociate cu o evolutie nefavorabila.



**Cuvinte cheie**: SDS-PAGE, proteine urinare, greutate moleculara foarte joasa, prognostic, progresie-insuficienta renala cronica

Societatea Română de Ştiinţe Fiziologice

Colegiul Medicilor din România

Universitatea de Medicină şi Farmacie din Craiova

# A XXIII-a Conferinţă Naţională a Societăţii Române de Ştiinţe Fiziologice

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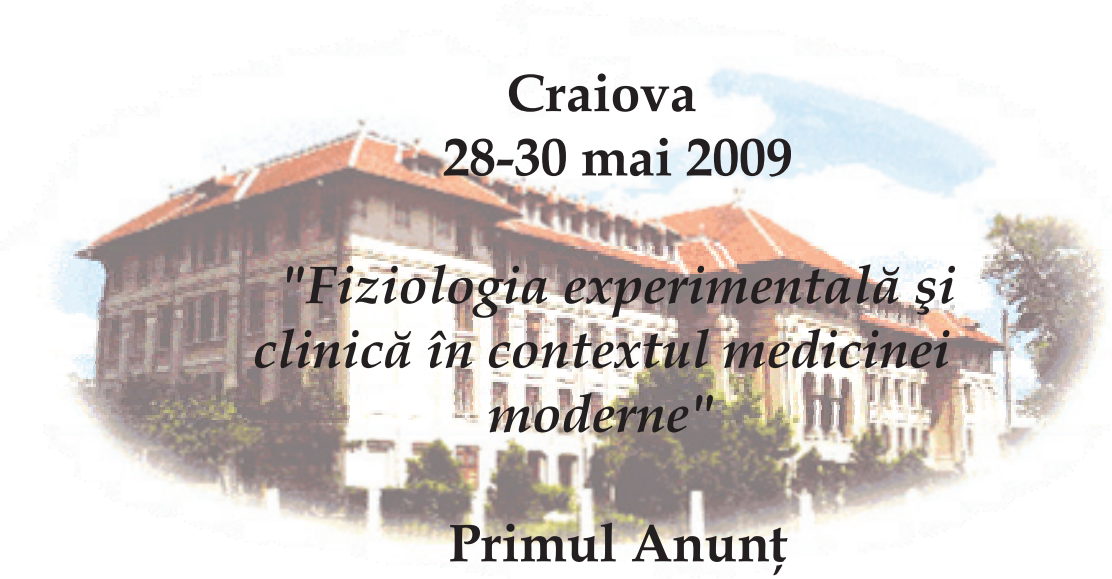
## 28-30 mai 2009



## *"Fiziologia experimentală şi clinică în contextul medicinei moderne"*

## Primul Anunţ

**Stimaţi colegi,**

**Societatea Română de Ştiinţe Fiziologice şi Catedra de Fiziologie a Universităţii de Medicină şi Farmacie din Craiova, vă invită să participaţi la *A XXIII-a Conferinţă Naţională a Societăţii Române de Ştiinţe Fiziologice,* intitulată *"Fiziologia experimentală şi clinică în contextul medicinei moderne"*, care se va desfăşura în Craiova, în perioada 28-30 mai 2009, fiind onorate de participarea dumneavoastră.**

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*Preşedinte SRSF*

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