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Secular Trends of Children from Birth to Age 3: Meta-Analysis of Data from Russia and the Neighboring Countries

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ABSTRACT

The goal of the study is to investigate secular changes in main anthropometric traits of the newborns, infants and children of early age, using a wide spectrum of the urban samples from modern Russia and the former USSR, through the period of several decades, from 1920th till now. The study summarizes the data of 338 samples of the newborns, 188 samples of 12-months old infants, 168 samples of 2-year old children, 256 samples of the 3-year old children. The secular dynamics of the body length describes the process of a significant increase of the skeletal development of children of both sexes in all growth periods. This trend is combined with the stability in body mass parameter of the newborns and infants, significant increase in body mass of 2-year-olds, and a slight increase in body dimension in 3-year-old children. The values of head circumference in newborns decrease from the 1950's to 2000's by 1.1–1.2 cm, which could be explained by the trend of a decrease in maternal pelvic width discussed in the literature. This trend completely stops by the end of the first year of life, and the 12-months old infants experience secular stability of this parameter. The head and chest circumferences of 2- and 3-year-olds keep stable through the time period under consideration.

Key-words: auxology, secular trends, physical development, newborns, infants, early age children, Russia

Introduction

Monitoring of children's growth is one of the important tasks for human biologists in every country. The latest literary sources present a wide spectrum of local (regional) studies, limited often by small historical time space of 10–30 years, rarely more^{1–11}. The whole sum of the world studies, describing secular dynamics of the main physical development indices in children and their somatic status, gives a very mixed picture, and points to a variety of possible particular (special) factors of secular changes and their effects: different geographical locations^{12–18}, different historical intervals, different ontogenetic periods^{19–20}, for rural and urban children, for the full-term and pre-term children^{21–24}, for parents and their offspring, for different body dimensions (body length, body mass, circumferences, skinfolds, etc.) and their combinations, resulting in somatotype variability^{25–26}. The diversity of these factors may be still generalized by the common concept – the level of anthropogenic pressure, which means the level of economic modernization of the society, the level of technogenic and informational stress, the level of the ecological, in a broad sense, comfort or discomfort.

Monitoring of anthropometric dimensions of children aged 0–3 years is very important for Russian anthropology, as the studies on the morphology of children at the beginning of postnatal ontogenesis are not numerous. There are several studies of the newborns from Moscow in the 20th century^{27–29}, infants of Moscow in the 20th century³⁰, the newborns of modern Belgorod^{31–33}, modern Moscow newborns, infants and early age children^{34–42}, and from the neighboring countries – infants of Lithuania in the 20th century^{43–45}.

The goal of this research is the analysis of secular changes of four main anthropometric traits in children from birth to three years: total dimensions (body length and mass) and circumferences of the head and chest as markers of body proportions.

Child's growth from birth to three years is a very specific period in the life history. Its biological meaning is the rapid morphofunctional development, intensive quantitative growth, compared only to the pubertal growth spurt, and the active search of the stable growth curve⁴⁶. The newborn's body dimensions mark the quality of the intrauterine growth, mediated significantly by the maternal conditions.

One-year old children's body dimensions mark the quality of the period of infancy or the period of compensatory growth. Two-year old children's body dimensions describe the status of the organism, almost completely independent from the circumstances of the intrauterine growth. Three-year-old's body dimensions may be considered, with some reservations, as the beginning of the stable ontogenetic trajectory and the conventional boundary between physiologically and behaviorally dependent organism and the relatively autonomic growing organism. Hence, our analyses include the secular somatic dynamics of the newborns, one-, two- and three-year old children.

Material and methods

The study is based on the data from Russia and the former USSR. The subject of the study, as already mentioned, are the newborns – 338 samples from the 1920's to 2010's with the ten-year interval; one-year old infants – 188 samples from the 1930's to 2010's with the ten-year interval; two-year old children – 116 samples from the 1950's till now and three-year old children – 256 samples from the 1950's till now, also with the ten-year intervals. Using the fixed age intervals allows, in addition, to minimize the effect of the age dynamics of the parameters of intragroup variations and correlations of the dimensions^{47–49}.

The main source of the data are collections of the materials on physical development of children and adolescents of Russia and the former USSR, regularly published by the Research Institute of Children and Adolescents Hygiene and Health Protection. The data were collected according to the common research protocol and were completely comparable^{50–54}. The additional source of the data include local standards^{55–58}, articles and some archive data of the authors of the present paper.

The number in each age/sex group was not less than 100 subjects, while the total number of the data array was over 90,000 individuals. The object of the research were

four main anthropometric body dimensions: body length as a marker of the skeletal development, body mass as an integrative marker of metabolism, head and chest circumferences as markers of the somatotype proportionality. The change of the body proportions in the direction of the leptosomic somatotype, e.g. a decrease of the circumferences as compared to the total body dimensions, usually coincides with the weakening of the physical conditions and the adaptive potential of the organism, which was proved by a number of auxological studies on elder children. Besides, a decrease of the ratio »circumferences/total body dimensions« in the newborns is associated with an increase of a number of the intrauterine growth disorders (»disembriogenesis stigma«), while an increase of this ratio together with a decrease of a number of stigma are related to the middle level of the heterozygosity and a better forecast for further development⁵⁹.

Statistics

To estimate the level and direction of the associations between anthropometric dimensions and temporal factor, the scattering diagrams were used as an effective instrument while dealing with the big set of sample means⁶⁰, when individual data are not available and hence the multivariate biometrical methods cannot be applied. The scattering diagrams were built for each dimension and each age/sex group with the obligatory calculation of the correlation coefficients and the estimation of the confidence level.

Results and discussion

The long-term dynamics of body length is uniform for the children of all age and sex groups and consists in a significant increase of the dimension or the acceleration of the skeletal development. The secular increase for the newborns (Fig. 1a) from the 1920's till 2010's is over 2 cm

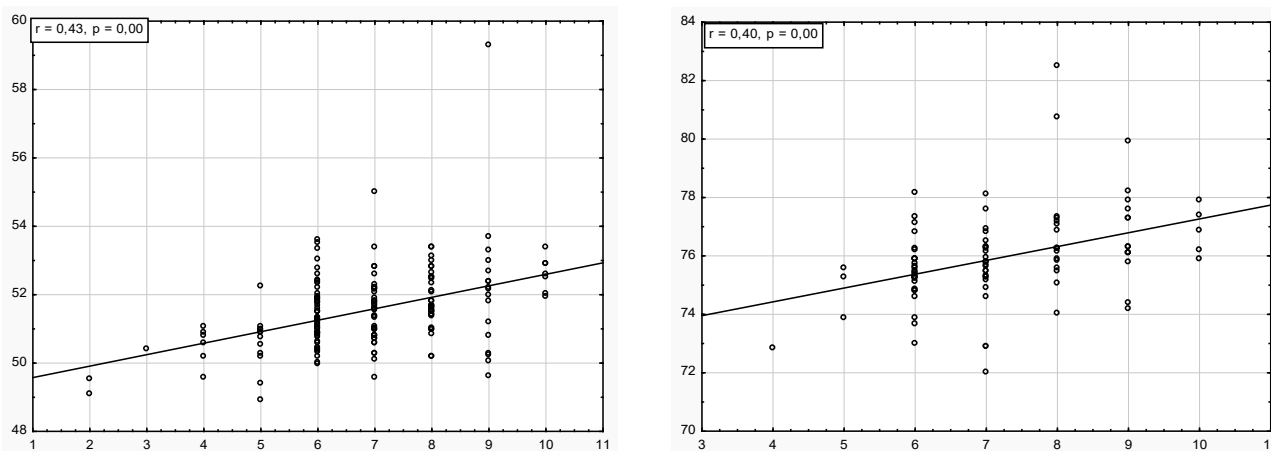


Fig. 1. Secular dynamics in body length of male newborns (a) and 1-year old infants (b) of the Russian cities. Axe X – time decade intervals: 3 – 1930's, 4 – 1940's 10 – 2000's, 11 – 2010's; Y – average values of body length, cm.

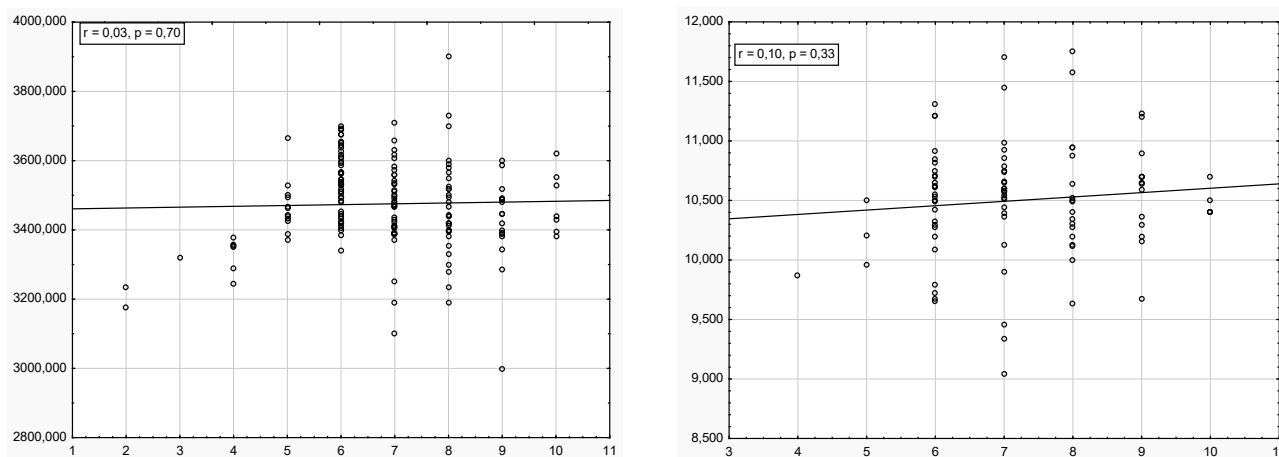


Fig. 2. Secular dynamics in body mass of male newborns (a) and 1-year old infants (b) of the Russian cities. Axe X – time decade intervals: 3 – 1930's, 4 – 1940's.... 10 – 2000's, 11 – 2010's; Y – average values of body mass, kg.

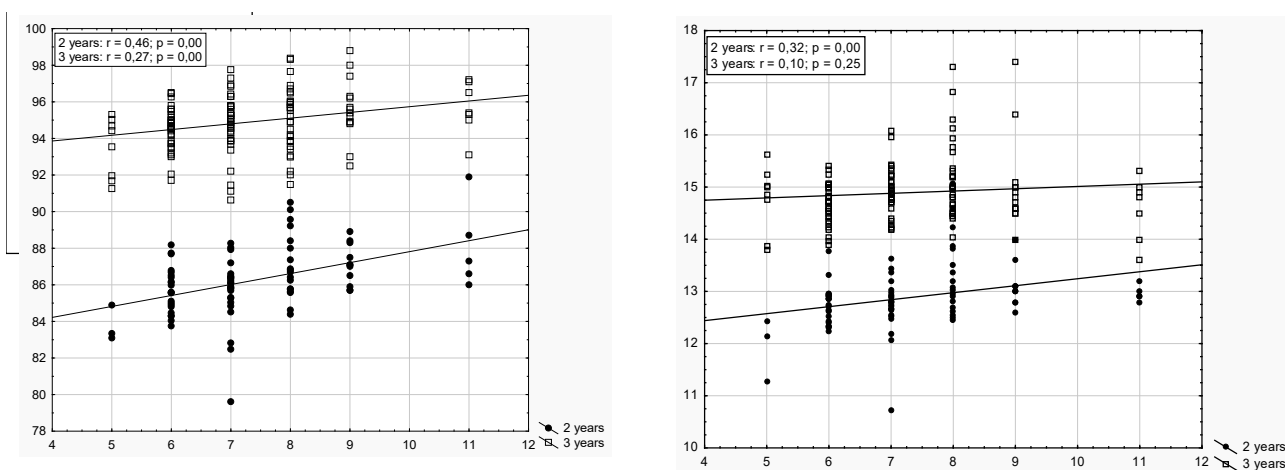


Fig. 3. Secular dynamics in body length (a) and body mass (b) of the 2-year old boys and 3-year old children of the Russian cities. Axe X – time decade intervals: 5 – 1950's, 6 – 1960's.... 11 – 2010's; Y – average values of body length, cm (a) and body mass, kg (b).

for both sexes, $r=0.27-0.37$, $p=0.00$. The increase for the 12-months old infants (Fig. 1b) for the 1930–2010 time period is 4.7 cm for boys and 3.8 cm for girls ($r=0.4$ for both sexes, $p=0.00$).

The value for the 2-year old children (Fig. 3a) from the 1950's till 2010's is about 3 cm ($r=0.46-0.47$ for boys and girls respectively, $p=0.00$).

The increase for the 3-year old children (Fig. 3a) through 1950–2010 interval is about 2 cm ($r=0.27$ for both sexes, $p=0.00$). A smaller secular increase in length for 3-year old children as compared to the 2-year-olds through the same time interval may reflect the well-known fact of the gradual (progressive) decrease in growth velocities of the discussed dimensions in children from 0 to 3 years, although their common level is still high enough⁶¹.

Such uniformity is not typical for the secular trends in body mass. Newborns and infants (Fig.2a, Fig.2b) experi-

ence the temporal stability of this dimension. A slight increase of the body mass in newborn girls (50g throughout 80 years) is not significant ($r=0.08$, $p=0.33$). It is worth mentioning that the newborns' body mass is the classic object of the stabilizing selection⁶², and the greatest contribution to its variability belongs to the »family« factor – i.e. body mass at birth of parents and sibs, the determination coefficient of which is about 0.16 as compared to 0.01–0.04 for all other possible factors³⁵.

Some increase in this dimension of 1-year old children through the period of 60 years (300–400 g) is not significant as well ($r=0.03-0.33$, $p=0.17-0.33$). Such combination of secular trends in two total body dimensions reflects the increase of leptosomy in newborns and infants.

Different secular changes of the body mass are typical for the children of an early age. Two-year old children (Fig. 3b) have the synchronous increase of both body length and

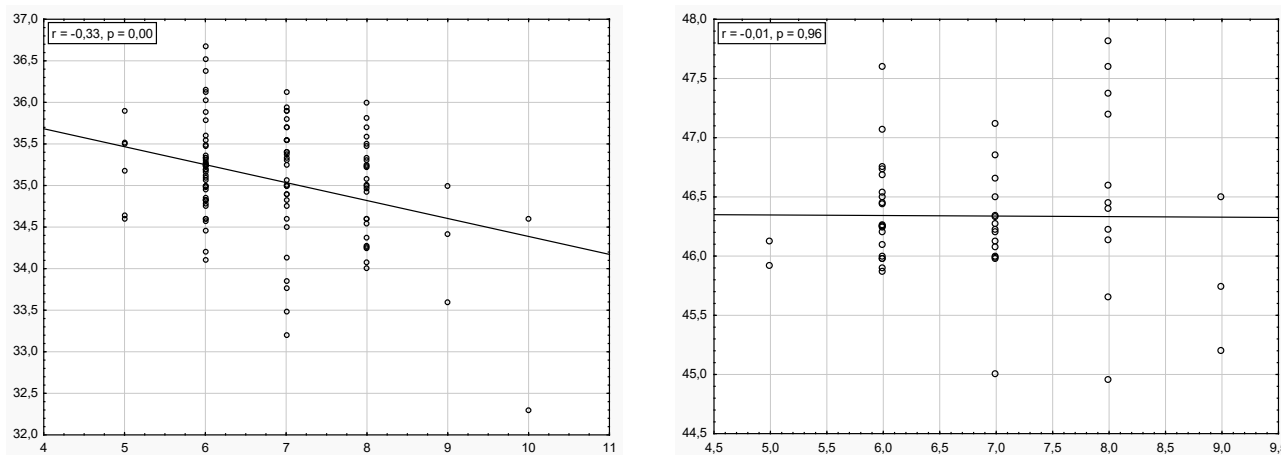


Fig. 4. Secular dynamics in head circumference of newborn girls (a) and 1-year old infants (b) of the Russian cities. Axe X – time decade intervals: 5 – 1950's, 6 – 1960's.... 9 – 1990's; Axe Y – average values of head circumference, cm.

body mass, the latter being about 700 g for the period of 1950–2010 ($r=0.32$, $p=0.00$ for boys and $r=0.27$, $p=0.01$ for girls). For 3-year-olds (Fig. 3b) this trend is close to the significant level. Thus, the secular trend to leptosomy is less expressed in 2- and 3-year old children as compared to the newborns and infants.

Secular dynamics of the chest circumference through different age periods is not uniform as well. For the newborns, secular changes in this dimension repeat the dynamics of body length, which allows to postulate the acceleration of the skeletal development in general, because body length can be considered as an «absolute» marker and the chest circumference as a reliable enough marker of skeletal development. The trend is significant for the newborn boys ($r=0.18$, $p=0.03$) and close to the significant level for the girls ($r=0.16$, $p=0.06$). The long-term dynamics of the chest circumference of the 1-year old children demonstrates a very slight secular decrease of this dimension, which means the change in infants' body proportions towards leptosomy as compared to the newborns.

This trend is not significant, but different vectors of secular changes in newborns and infants deserve attention. It means that secular increase in chest circumference as the result of intrauterine growth completely disappears by the age of 12 months as a result of the compensatory growth through the infancy period. For 2- and 3-year-olds no secular changes in this dimension were found. Combined with the permanent secular increase in body length this trend also confirms the phenomenon of a secular increase in leptosomy.

The secular dynamics of head circumference also has some specific traits in different age periods. For the newborn boys and girls (Fig. 4a) in the 1950–2010 time period, there is a significant decrease of this dimension by 1.2 and 1.1 cm correspondingly ($r=0.27$ – 0.37 , $p=0.00$). This trend corresponds to the secular decrease in maternal pelvic width, frequently discussed in the literature.

The 1-year old children (Fig. 4b) have stable values of this dimension through the same time interval, thus the secular trend of deceleration in the growth of head circumference in the newborns is completely compensated till the end of the infancy period. Equally, the secular stability of the dimension is characteristic for the 2- and 3-year old children.

Secular trend towards leptosomy embraces children of both sexes and all age groups under consideration, though in various degree, and reflects the heterochrony of the secular dynamics in body length, body mass and circumferences. The trend of the secular increase in leptosomic body build was noted for children and adolescents from 3 to 17 years of age in the 1970's–1990's, mostly from urban locations^{38,62,64–67}. Nowadays the situation is opposite – the tendency towards adiposity prevails^{2,68–71}.

Thus, the secular increase in leptosomy of the newborns and early age children in the 2000's, may correspond to the morphological peculiarities of their potential mothers – adolescents in the 1970's –1990's – with the pronounced leptosomic somatotype.

This similarity points to the existence of the inter-generational associations of the long-term microevolutionary somatic changes. The same parallelism in the secular dynamics of body dimensions of «parent and offsprings» (newborns and 18-year-old young females) is demonstrated for the population of the Polish cities, Poznan and Krakow⁷².

The long-term deceleration of the intrauterine growth of the head circumference may be probably regarded as an adaptation to some secular changes to more narrow width of maternal pelvis and an increase of mothers' leptosomic body build in general, regularly registered for different regions of Russia since the 1980's^{73–77}.

It is worth noting that the analysis of changes in anthropometric dimensions of Moscow newborns from 1880's to 1960's revealed the most intensive increase exactly for

the head circumference combined with more moderate increase in body length and mass and the decrease in abdomen circumference. Such variations in the dynamics of head circumference of the newborns during different historical periods confirm the thesis about fluctuations in secular changes²⁷.

Apparently, the most advanced approach towards monitoring of children's growth from birth to 3 years of age should consist of the analysis of the intra-group variability and dynamics of body dimensions of the offspring in close connection with the maternal morphological status. In this way, the physical status of the consecutive generations may be examined in their inevitable and logical succession and interdependence^{21,25,32,38,78–82}.

It is important to notice that the patterns of secular changes in different ethnic and territorial groups in Russia and the neighboring countries are not identical through different decades, and have some local peculiarities^{4–5}. But the vector of the secular trends is common for all regions — the increase in body length combined mostly with in-

significant or negligible changes in body mass. Local variations in growth patterns point to the necessity of the regional growth charts.

Conclusions

The results of this research reveal the heterochrony of secular changes in different characteristics of physical development of children at the beginning of their postnatal ontogenesis. This leads to the increase in leptosomic somatotypes of the newborns, infants and early age children. The absence of uniform patterns in temporal dynamics of the anthropometric traits of the newborns, 12-months-old infants, 2- and 3-year old children is shown as well. The secular dynamics of the body shape of children aged 0–3 years corresponds to the morphological status of their potential mothers, and points to the existence of the inter-generational associations in the long-term microevolutionary morphological changes.

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SEKULARNI TRENDVI U DJECE OD ROĐENJA DO TREĆE GODINE ŽIVOTA: META-ANALIZA PODATAKA IZ RUSIJE I SUSJEDNIH ZEMALJA

S A Ž E T A K

Cilj rada je istraživanje sekularnih promjena u glavnim antropometrijskim karakteristikama novorođenčadi, dojenčadi i djece u ranoj dobi, koristeći širok spektar urbanih uzoraka iz sadašnje Rusije i bivšeg SSSR-a i to kroz razdoblje od nekoliko desetljeća, od 1920. godine do danas. Studija sažima podatke o 338 novorođenčadi, 188 djece u dobi od 12 mjeseci, 168 djece stare dvije godine i 256 djece u dobi od tri godine. Sekularna dinamika duljine tijela opisuje proces značajnog povećanja skeletnog razvoja djece oba spola u svim razdobljima rasta. Taj se trend kombinira sa stabilnošću parametra tjelesne mase novorođenčadi i dojenčadi, značajnim porastom tjelesne mase u dobi od dvije godine i malim povećanjem dimenzije tijela kod trogodišnje djece. Vrijednosti opsega glave u novorođenčadi smanjuju se u razdoblju od 1950. do 2000. godine za 1,1–1,2 cm, što se može objasniti trendom smanjenja širine zdjelice u majki koji se spominje u literaturi. Taj se trend potpuno zaustavlja do kraja prve godine života, a u dobi od 12 mjeseci dolazi do sekularne stabilnosti ovog parametra. Opsezi glave i prsa u dvo- i trogodišnje djece stabilni su kroz promatrano vremensko razdoblje.

Impact of Pre-Pregnancy BMI on Blood Glucose Levels in Pregnancy and on the Anthropometry of Newborns – Preliminary Insights from The Croatian Islands' Birth Cohort Study (CRIBS)

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ABSTRACT

The aim of this study was to investigate the relationship between pre-pregnancy body mass index (BMI) and fasting blood glucose level in pregnancy on the anthropometry of newborns. The sample consisted of 171 healthy pregnant women and their newborns from the Croatian Islands' Birth Cohort Study. Peripheral blood of pregnant women was taken in the second trimester and fasting glucose values >5.1 mmol/l were considered elevated. Anthropometric variables (body weight, height and waist circumference) were measured according to the International Biological Program. Pre-pregnancy BMI and fasting glucose levels during pregnancy were significantly positively correlated ($p < 0.01$), as were pre-pregnancy BMI and birth weight ($p < 0.05$), length ($p < 0.01$), and head circumference ($p < 0.05$) of the newborns. Hyperglycaemic women gave birth to significantly heavier newborn girls than normal glycaemic women ($p < 0.05$). In conclusion, there was a positive correlation between pre-pregnancy BMI, fasting glucose during pregnancy and the anthropometry of newborns in this study.

Key words: Croatian Islands' Birth Cohort Study, pre-pregnancy BMI, fasting glucose in pregnancy, anthropometry, newborns

Introduction

It is well known that the biological, physical and social environment, to which a child is exposed early in life may lead to disease or disability in childhood and adulthood. Various risk factors acting during critical periods of development in pre- and early postnatal life play an important role in the etiology of these non-communicable diseases^{1,2}. It is now widely accepted that an adverse peri-conception (e.g. increased pregravid weight) and intrauterine environment (e.g. gestational diabetes mellitus, excessive weight gain during pregnancy) are associated with predisposition to chronic metabolic disorders later in life: a phenomenon

termed 'early life programming'²⁻⁷. Population-based pregnancy and birth cohort studies are particularly salient for studying early origins of health and disease that begin in fetal life and infancy^{8,9}.

Early identification of all biological, environmental and behavioural risk factors for poor pregnancy outcomes and MetS related disorders is important for the development of preventive and early intervention strategies¹⁰. Increased pre-pregnancy body mass index (BMI) and elevated glucose levels are known to independently influence fetal growth and to increase the risk of subsequent development of obesity. A previous systematic review and meta-analysis

of 66 studies suggested a promising strategy to lower overweight risk in the long term by adequately managing maternal overweight¹¹. The main goal of this study was to investigate the influence of body mass index (BMI) before pregnancy and glucose levels in the second trimester on birth weight, birth length, and head circumference of newborns and to determine the trend of measured anthropometric variables of pregnant women and derived indices during pregnancy.

The Study and Sample

The Croatian Islands' Birth Cohort Study (CRIBS) is the first birth cohort study in South-Eastern Europe with the aim of identifying the prevalence of modifiable risk factors for the development of the metabolic syndrome (MetS). The total sample of 500 healthy pregnant women and their offspring were recruited from the Adriatic islands of Hvar and Brač and the adjacent mainland City of Split and Dalmatian County. The sample for this analysis consisted of 171 healthy pregnant women and their newborns all delivered at the University Hospital Center Split (Split, Croatia).

The enrollment into the study of pregnant woman and all scheduled visits (follow-ups) were carried out at doctor's office where the pregnancy is routinely monitored and in the maternity unit in the University Hospital Center Split, which is the only maternity hospital in the Split-Dalmatia County. In this study we enrolled pregnant women (age 18 or older), from 12 weeks of gestation onwards. Women were examined three times during pregnancy. Inclusion criteria were: 12 weeks or more of single pregnancy, not assisted with reproductive technology. Exclusion criteria were: diagnosed acute or chronic medical conditions (cancer; renal, endocrinologic, psychiatric, neurological, infectious and cardiovascular diseases), multiple gestations, persistent second or third trimester bleeding, placenta praevia after the 26th week of gestation, poorly controlled hypertension, diabetes mellitus or thyroid gland disease, as well as incompetent cervix, history of recurrent miscarriages.

Methods

Pre-pregnancy BMI was calculated as the ratio of weight in kg and the square of height in meters based on pre-pregnancy weight and height both extracted from the projects' pregnancy booklet. Extensive medical and biochemical data (medical records, cord blood, serum and DNA samples – both from pregnant women and newborns) were collected during pregnancy and after birth. Comprehensive questionnaires (including questions on family data, genealogy, nutrition, health-related behaviour, psychosocial characteristics, etc.) were filled in during pregnancy and postnatally at particular child developmental milestones. Peripheral blood of pregnant women was taken between the 22nd and 26th week of gestation, fasting glucose in pregnant women was measured at the licensed

biochemical laboratory at the Dubrava University Hospital (Zagreb, Croatia). Fasting glucose values >5.1 mmol/L were considered elevated according to the reference values of Croatian Society of Medical Biochemistry and Laboratory Medicine (CSMBLM). The 75-g oral glucose tolerance test (oGTT) in pregnancy was analysed according to International Association for the Diabetes and Pregnancy Study Groups¹² (IADPSG 2010) and World Health Organization¹³ (WHO 2013). Anthropometric variables (body weight, height and waist circumference) were measured according to the International Biological Program. Anthropometry of newborns (body length, weight, head circumference) – conversion to Z-scores was done using WHO Anthro software¹⁴ (WHO 2005) Statistical analyses were performed by SPSS 10.0 for Windows.

Results

There was a significant positive correlation between pre-pregnancy BMI and fasting glucose levels between the 22nd and 26th week of gestation ($R^2=0.056$; $R=0.236$; $p<0.01$). (Fig. 1.a). Likewise, there was a significant positive correlation between pre-pregnancy BMI and birth weight ($R^2=0.066$; $R=0.257$; $p<0.05$), birth length ($R^2=0.038$; $R=0.195$; $p<0.01$) and birth head circumference ($R^2=0.039$; $R=0.198$; $p<0.05$) of the newborns (Fig. 1.b).

We also tested the correlation between newborn anthropometry (measures z-standardized according to WHO) between mothers with different levels of glucose in the second trimester (22nd and 26th week of gestation). The sample of mothers was defined in two groups according to the reference values of Croatian Society of Medical Biochemistry and Laboratory Medicine (CSMBLM). The first group included normal glycaemic mothers (GLUC1) and the second one included hyperglycaemic mothers (GLUC2). Although in general no significant differences have been observed, we however detected that hyperglycaemic women gave birth to significantly heavier newborn girls than normal glycaemic women ($p<0.05$). (Table 1).

Discussion

A limited number of studies has previously focused on variables of pre-pregnancy BMI, glucose level and newborn length, weight and head circumference. In studies where only pre-pregnancy BMI was brought into association with newborn birth weight, increased risk of LGA (large-for-gestational-age) newborns, macrosomia, and subsequent offspring overweight/obesity was observed in women with a high pre-pregnancy BMI (overweight or obese mothers)^{15–17}. Only several previous studies have focused on the investigation of fasting blood glucose as a predictor of birth weight among neonates of non-diabetic mothers^{18–20} and have detected a positive correlation between elevated glucose levels of mothers in pre-pregnancy and increased birth weight of the newborn.

In this study, participants were categorized into two groups; women with normal glucose (GLUC1) and those

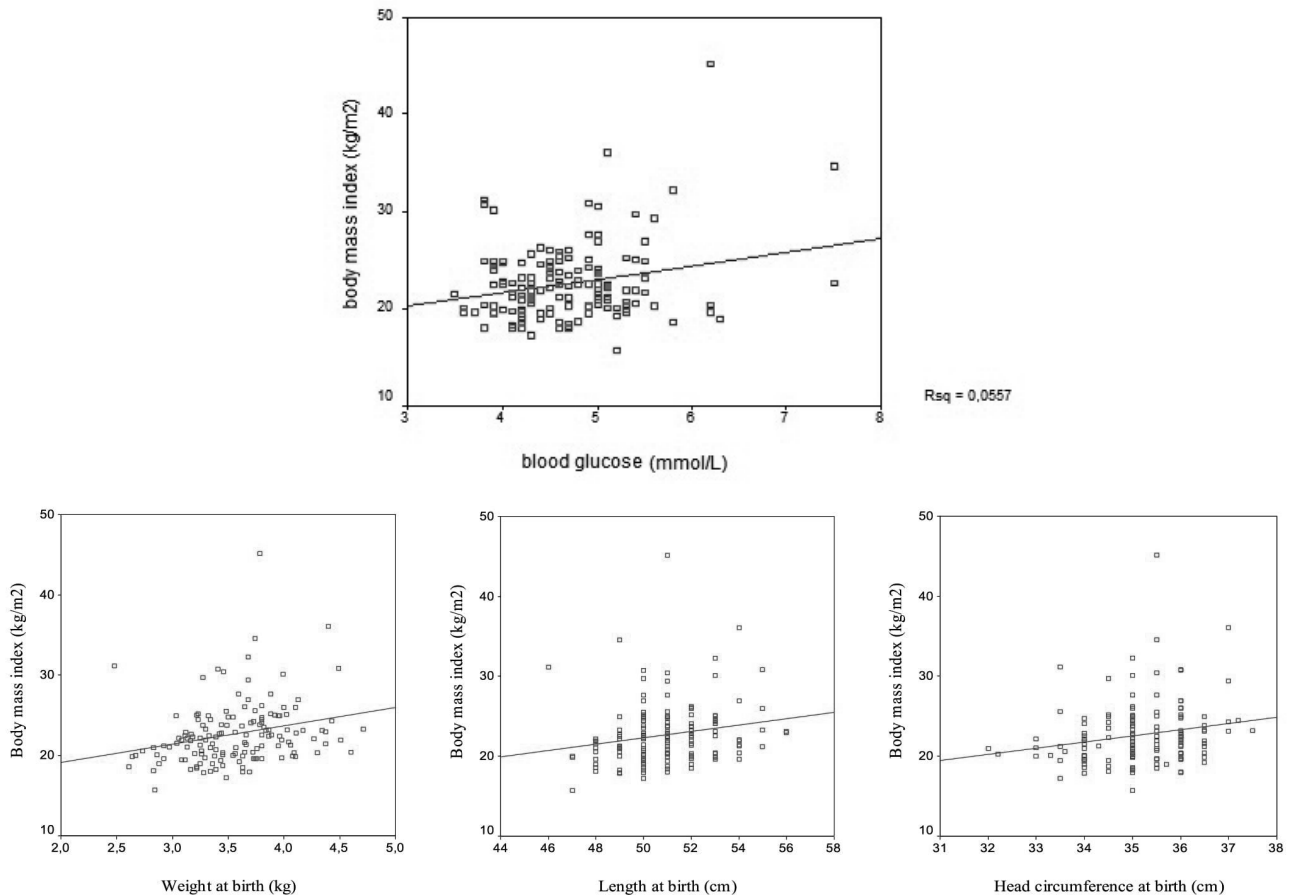


Fig. 1.a and Fig. 1.b. The correlation between pre-pregnancy BMI and glucose levels in the second trimester (22nd and 26th week of gestation) as well as between pre-pregnancy BMI and birth weight, length, and head circumference of the newborns.

TABLE 1
THE COMPARISON OF ANTHROPOMETRIC VARIABLES OF NEWBORNS BETWEEN MOTHERS WITH DIFFERENT LEVELS OF GLUCOSE IN THE SECOND TRIMESTER (22ND AND 26TH WEEK OF GESTATION).

Variable	N	GLUC1	N	GLUC2	p
weight-at-birth (kg)	139	3,55±0,43	32	3,68±0,48	ns
length-at-birth (cm)	139	50,94±1,91	32	50,78±1,81	ns
head circumference-at-birth (cm)	137	35,14±1,03	32	35,37±0,87	ns
Boys					
weight-at-birth (kg)	66	3,67±0,44	13	3,67±0,52	ns
length-at-birth (cm)	66	51,60±1,94	13	50,77±1,48	ns
head circumference-at-birth (cm)	65	35,45±0,97	13	35,62±0,65	ns
Girls					
weight-at-birth (kg)	73	3,45±0,38	19	3,68±0,47	<0,05
length-at-birth (cm)	73	50,34±1,67	19	50,79±2,04	ns
head circumference-at-birth (cm)	72	34,87±1,02	19	35,20±0,97	ns

hyperglycaemic (GLUC2) according to CSMBLM reference values. Only the difference in birth weight of newborn girls was statistically significant (Table 1). The re-

sults were expected due to a small sample, although the trend of increased birth weight, birth length and head circumference of newborns of hyperglycaemic mothers is

observed. The results of our study were consistent with a similar research involving mothers of increased BMI (excessive weight and obesity) during pregnancy with hyperglycaemia, which showed that there was a trend in the birth of children with increased birth weight, birth length and head circumference when compared to mothers with normal glucose in pregnancy²¹.

Subsequent research on the same sample of mother-child dyads and new cohort studies are necessary to further investigate 'early life programming' and importance of pre-pregnancy and pregnancy period for birth outcomes and health of children later in life.

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In conclusion, a growth trend has been observed between pre-pregnancy BMI, fasting glucose during pregnancy and the anthropometry (body length, weight and head circumference) of newborns in the CRIBS study. We can conclude based on our results that there is a minor influence of pre-pregnancy BMI on the anthropometry of newborns.

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UTJECAJ INDEKSA TJELESNE MASE (ITM) PRIJE TRUDNOĆE I RAZINE GLUKOZE U TRUDNOĆI NA ANTROPOMETRIJSKA OBILJEŽJA NOVOROĐENČADI – PRELIMINARNI REZULTATI STUDIJE CRIBS

SAŽETAK

U ovoj pilot studiji pod nazivom »Kohortna studija rođenih na istočnojadranskim otocima« (*CRIBS*) koja je još u tijeku, analizirani uzorak je obuhvatio 171 zdravu trudnicu te njihovu novorođenčad s područja Splitsko – Dalmatinske županije, točnije hrvatskih otoka Brača i Hvara te područja grada Splita i bliže okolice. Cilj ovog rada bio je istražiti utjecaj indeksa tjelesne mase (ITM) prije trudnoće i razine glukoze u trudnoći na antropometrijska obilježja novorođenčeta (porodajnu težinu, porodajnu duljinu i opseg glave). Antropometrijske varijable majke (tjelesna masa, tjelesna visina i opseg struka) mjerene su prema metodama opisanima u Praktikumu biološke antropometrije (1975.), izrađenima u skladu s protokolom International Biological Programme. U okviru biokemijskih analiza trudnicama su izuzeti uzorci periferne krvi u drugom trimesteru trudnoće. Izmjerene vrijednosti glukoze >5.1 mmol/L smatrane su povišenima. Statističke analize provedene su pomoću programa SPSS10.0 for Windows. Preliminarni rezultati pokazali su značajnu korelaciju porasta glukoze u krvi trudnica s povećanim indeksom tjelesne mase prije trudnoće ($p<0,01$) kao i značajnu korelaciju povećanog indeksa tjelesne mase prije trudnoće s većom porodajnom težinom ($p<0,05$) porodajnom duljinom ($p<0,01$) i opsegom glave ($p<0,05$) novorođenčadi. Utvrđena je i povezanost povišene vrijednosti glukoze s povećanom porodajnom težinom ženske novorođenčadi ($p<0.05$). Iz prikazanih rezultata vidljiv je trend rasta antropometrijskih osobina novorođenčeta s povećanim vrijednostima glukoze u krvi trudnica i indeksom tjelesne mase prije trudnoće. Temeljem dobivenih preliminarnih rezultata možemo zaključiti da postoji utjecaj tjelesnog i zdravstvenog statusa (prehrambenog) trudnica na antropometrijske značajke novorođenčadi.

Gender Differences in Relation to Knowledge and Risky Behavior among Students of Medical Colleges in Serbia: Study of Prevalence

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ABSTRACT

The aim of this study was to determine the level of knowledge about sexually transmitted diseases and the frequency of behavior presenting risk for acquiring those diseases among students of Medical colleges in relation to gender. The study is based on a cross-sectional survey conducted in two Medical colleges in Belgrade in December 2016. A total of 670 students (96%) completed an epidemiological questionnaire. Respondents were selected through random sampling, they took part in the study voluntarily and filled in the questionnaire anonymously and independently. The questionnaire included 18 questions on demographic characteristics, knowledge and risky behavior. Female students were significantly older than male students ($p=0.014$). Male students were more often single, while female students were mostly in the relationship or married ($p<0.001$). Female students have more knowledge about symptoms of sexually transmitted diseases ($p=0.008$) and more than males are familiar with the causality of HPV infection and cervical cancer ($p=0.002$). Males students earlier had the first sexual intercourse ($p<0.001$), had sex on the first date ($p<0.001$), changed sexual partners during their lives ($p<0.001$), and paid for sex ($p<0.001$). Male students have specific risk factors for unhealthy sexual behavior. It is necessary to upgrade the education programs and implement population-targeted prevention and control measures for sexually transmitted infections.

Keywords: Students, gender, knowledge, sexual behavior.

Introduction

Sexually transmitted infections (STIs) represent a significant public health problem for young people¹. People aged 15-24 make up 25% of the sexually active population, and at the same time, 50% of all new STIs in the world are registered in this population².

The World Health Organization (WHO) estimated that more than a million STIs per day are generated globally, and 131 million STIs are caused by *Chlamydia* annually, 78 million infections caused by *Neisseria gonorrhoeae*, 5.6 million by *Treponemapallidum* and 143 million by *Trichomonasvaginalis* infection. More than 500 million people live with genital herpes, and more than 290 million women with HPV infection (1). Genital chlamydiasis one of the most common STIs in Europe in young people 15-24 years of age³.

According to the Institute of Public Health of Serbia, the basic characteristic of STIs in the period 2010-2014 was a decrease in the number of registered cases of infections caused by human immunodeficiency virus (HIV) and genital chlamydiasis. At the same time, there is a trend of an increase in the number of new cases of syphilis and gonorrhea, with certain oscillations. Given the epidemiological characteristics of STIs, as well as the problems in laboratory diagnostics, there is a reasonable assumption that the reported cases do not show the real situation, due to the unknown extent of diagnosis, as well as due to the insufficient reporting of laboratory-confirmed cases from the private health sector⁴.

Numerous studies indicate that students are at high risk of acquiring STIs⁵⁻⁸. The most commonly described forms of risky behavior for obtaining STI in the student population are age⁸, early entry into sexual intercourse^{8,9},

TABLE 1
DEMOGRAPHIC CHARACTERISTICS OF STUDENT POPULATION

Variables	Male N=214	Female N=431	p value
Age (years)	MV ± SD	MV ± SD	
All ages	23.63 ± 5.72	25.45 ± 7.30	0.014**
<20	19.65 ± 0.48	19.78 ± 0.42	0.048**
21–30	23.10 ± 2.55	23.24 ± 2.81	0.865**
31–40	35.41 ± 2.24	35.40 ± 2.84	0.852**
>41	44.33 ± 3.45	44.95 ± 2.57	0.394**
Marital status	No (%)	N (%)	
Married	19 (8.9)	102 (23.7)	
In a relationship	95 (44.4)	203 (47.1)	
Divorced	4 (1.9)	9 (2.1)	0.001*
Widow/Widower	–	1 (0.2)	
Single	96 (44.9)	116 (26.9)	
Current smokers	73 (34.1)	157 (36.4)	0.624*
Current alcohol users	116 (54.2)	114 (26.5)	0.001*

*Chi-square test;

**Mann-Whitney U test;

No – number;

MV – mean value;

SD – standard deviation.

not using a condom^{6,8,9}, psychoactive substances abuse^{9,10} and the number of sexual partners⁸. As risk factors for acquiring STI in the student population, sex and the level of knowledge about STIs are described^{11,12}. A study conducted among students in the United States of America showed that psychological differences, attitudes and sexual behavior among male and female students are significant factors for the emergence of STIs¹¹. A study conducted on a Turkish student population has shown a less risky behavior, as well as a lower incidence of STIs among students with a higher level of knowledge than in those less educated about STIs¹².

The aim of this study was to determine the level of knowledge about STIs and the frequency of risky behavior for acquiring STIs among the students of medical colleges in Belgrade, as well as to determine differences according to gender.

Materials and Methods

The research was designed as a cross-sectional study and was carried out in December 2016. The respondents completed an epidemiological questionnaire based on the questionnaires used in the previous research^{5–10,12}. The questionnaire collected the answers to three sets of questions: about the demographic characteristics of the respondents (gender, age, marital status, smoking and alcohol consumption habits), their knowledge of STIs (the ways of acquiring knowledge, the knowledge of the symptoms, the existence of a vaccine against HPV infection, relationship between cervical cancer and STIs) and their behavior (age at first sexual intercourse, sexual orientation, number of

sexual partners during the past year and during the course of life, frequency of sexual intercourse, usage of condoms, sexual intercourse at first date, sexual intercourse under the influence of alcohol and/or other psychoactive substances, tattooing, testing on STIs, self-assessment of risky sexual behavior). The research covered students of all three years in two schools, High Medical College of Professional Studies »Milutin Milankovic« and High Medical College of Vocational Studies in Belgrade. Respondents took part in the study voluntarily and filled in the questionnaire anonymously and independently. The approval for conducting this research was obtained by the Ethics Committee for the Higher Education of the University of Belgrade, whose students participated in the research.

The complete statistical analysis of data was performed using the statistical software package, PASW Statistics 18® [SPSS (Hong Kong) Ltd., Hong Kong]. All variables were presented as the frequency of certain categories. Continuous variables were presented as means and standard deviations. Differences between categorical variables were tested by Chi-square test, while the significance of differences between continuous variables was tested by non-parametric Mann-Whitney U test. The normality of the data was assessed using the Kolmogorov-Smirnov test. The relationship between the variables was tested by Spearman's coefficient correlation. All the analyses were estimated at $p < 0.05$ level of statistical significance.

Results

Of 670 students who participated in the study, 645 (96%) completed the questionnaire, including 431 (66.8%) females and 214 (33.2%) males. Female students were sig-

TABLE 2
KNOWLEDGE AND BEHAVIOR OF STUDENTS ABOUT SEXUALLY TRANSMITTED INFECTIONS (STI)

Variables	Male No (%)	Female No (%)	p value
Source of information about STI			
From family	100 (46.7)	187 (43.4)	0.472*
From friends	84 (39.3)	170 (39.4)	1.000*
In school /faculty	173 (80.8)	382 (88.6)	0.010*
Through counseling	30 (14.0)	50 (11.6)	0.453*
In the health center	36 (16.8)	87 (20.2)	0.359*
Over the Internet	120 (56.1)	263 (61.0)	0.263*
Through the daily press	60 (28.0)	131 (30.4)	0.599*
Other	2 (0.9)	4 (0.9)	1.000*
Whether STI always give symptoms			
Yes	58 (27.1)	84 (19.5)	0.008*
No	116 (54.2)	288 (66.8)	
I don't know	40 (18.7)	59 (13.7)	
Is there an effective vaccine against a HPV			
Yes	53 (24.8)	107 (24.8)	0.707*
No	78 (36.4)	144 (33.4)	
I don't know	83 (38.8)	180 (41.8)	
Is there a link between HPV infection and cervical cancer			
Yes	82 (38.3)	229 (53.1)	0.002*
No	11 (5.1)	18 (4.2)	
I don't know	121 (56.5)	184 (42.7)	
Age at first sexual intercourse (years)	(MV ± SD) 17.01 ± 1.84	(MV ± SD) 18.49 ± 1.79	0.001**
Sexual status			
Heterosexual	208 (97.2)	430 (99.8)	0.012*
Homosexual	5 (2.3)	1 (0.2)	
Bisexual	1 (0.5)	-	
Number of sexual partners	(MV ± SD)	(MV ± SD)	
Total	5.86 ± 5.37	2.35 ± 2.74	0.001**
Last 12 months	1.85 ± 1.67	0.91 ± 0.74	0.001**
Frequency of sexual intercourse			
Every day	24 (11.2)	24 (5.6)	0.001*
2-3 per week	90 (42.1)	200 (46.4)	
1-3 per month	62 (29.0)	108 (25.1)	
1 in 3 month	20 (9.3)	15 (3.5)	
1 in 6 month	4 (1.9)	5 (1.2)	
1 in 12 month	5 (2.3)	10 (2.3)	
Virgin	9 (4.2)	69 (16.0)	
Usage of condom during sexual intercourse			
	78 (36.4)	162 (37.6)	0.845*
The reasons for not using condoms			
It is expensive	11 (8.1)	8 (3.0)	0.001*
Partner refuses to use it	10 (7.4)	7 (2.6)	
Use other contraceptives	13 (9.6)	58 (21.6)	
It is not necessary to use a condom	4 (2.9)	15 (5.6)	
I trust my partner	42 (30.8)	133 (49.4)	
Reduces the sensitivity of pleasure during intercourse	56 (41.2)	48 (17.8)	

Sexual intercourse on the first date?	74 (34.6)	21 (4.9)	0.001*
Sexual intercourse for money?	20 (9.3)	2 (0.5)	0.001*
Alcohol and other psychoactive substances?			
Always	10 (4.7)	1 (0.2)	
Rarely	96 (44.9)	82 (19.0)	0.001*
Never	108 (50.5)	348 (80.8)	
Tattooing?	21 (9.8)	35 (8.1)	0.568*

*Chi-square test;

**Mann-Whitney U test;

No – number;

MV – mean value;

SD – standard deviation;

STI – sexually transmitted infections.

nificantly older than male students (23.63 ± 5.72 vs 25.45 ± 7.30 , $p=0.014$). Male students were more often single (44.9% vs 26.9%; $p<0.001$), while female students were mostly in the relationship or married (70.8% vs 53.3%; $p<0.001$). Gender differences in smoking status were not found but were found in alcohol consumption. Statistically, there were significantly more men who consumed alcohol than women (54.2% vs 26.5%; $p<0.001$) (Table 1).

Mainly, there were no differences between male and female students in the way of acquiring knowledge about STIs, except in case of acquiring knowledge at school/faculty (80.8% vs 88.6%, $p=0.010$) (Table 2). Female students (66.8%) significantly more often than males (54.2%) knew that STIs do not always give symptoms. The majority of students of both sexes did not know that there is an effective vaccine for HPV infections (75.2% of both sexes). More than half of female students (53.1%) were informed about the association between HPV infections and cervical cancer, while only 38.3% of male students knew that ($p=0.002$).

Male students had first sexual intercourse significantly earlier than female students (17.01 ± 1.84 vs 18.49 ± 1.79) (Table 2). Males significantly more often changed sexual partners during their lives (5.86 ± 5.37 vs 2.35 ± 2.74), and also over the past 12 months (1.85 ± 1.67 vs 0.91 ± 0.74). As for the frequency of practicing sexual relations, the majority of males (71.1%) and females (71.5%) usually engage in sexual relations 2–3 times per week or 1–3 times in a month. However, there are significantly more females who had never practiced sexual intercourse and males who practice it daily.

More than 60% of all students do not use condoms, without significant differences between genders. As the reason why they do not use a condom, the majority of male students gave an answer that condom reduces pleasure during sexual intercourse (41.2%), while the majority of female students do not use a condom because they trust their partners (49.4%). Male students more significantly often than female students: engage in sexual intercourse on the first date (34.6% vs 4.9%), pay for sexual intercourse (9.3% vs 0.5%) and engage in sexual intercourse under the influence of alcohol and psychoactive substances (49.5% vs 19.2%). There is no significant difference between genders regarding tattooing (9.8% vs 8.1%) and testing on STIs any time

in their life. Male students had significantly more frequent sexual relations with persons of the same sex and both sexes than females. On the basis of self-critical thinking, male students were significantly at greater risk for getting STI than the females (24.8% vs 17.6%).

Discussion

A recent sociological research conducted in youth population in Serbia showed that they are patriarchal and that sexual relations and STIs are still a taboo in the society. These claims are supported by the fact that every tenth young person older than 15 is unaware of the importance of particular methods of contraception, not only in preventing unwanted pregnancies but also in preventing the transmission of STIs¹³.

Demographic characteristics of the respondents

As in similar studies carried out in the countries in our region and in Europe^{5,9}, there were significantly more female than male respondents in our study (66.8% vs 33.2%). Our respondents were older (24.85 years) in comparison to respondents from Brazil (20.16 years)⁸.

Male students were most often single (44.9%), while female students were most often in a relationship (47.1%). In our study, 32.6% of respondents were married, in contrast to Sao Paulo students where 97.3% were not in a marital union⁸.

In our respondents, there was no significant difference between genders regarding tobacco smoking habits, but male students were more likely to drink alcohol than females (54.2% vs 26.5%). Male students from Uganda significantly more often consumed alcohol than female students (54.3% vs 41.9%)¹⁴.

Knowledge

Male students who participated in our research, compared to women, as the most common source of knowledge about STIs cited: school/faculty (80.8% vs 88.6%), internet (56.1% vs 61.0%), family (46.7% vs 43.4%), and at the end the newspaper (28.0% vs 30.4%). In contrast, the study in

Ghana showed that the majority of respondents, 83% acquired knowledge of STIs through TV programs (84.0% males *vs* 82.0% females)¹⁵. Nearly 80% of respondents from Turkey acquired knowledge of STIs from the book-magazine-newspaper (76.5% males *vs* 82.4% females)¹⁶. It is interesting that, unlike our respondents, only 19.3% of respondents in a study from Turkey identified school/faculty as a source of knowledge about STIs¹⁶.

In our study, female respondents were significantly more familiar with the causality of HPV infection and cervical cancer (53.1% *vs* 38.3%) than males. A survey conducted on 2500 students in North India showed that only 15.0% were familiar with this causal relationship¹⁷. As within our study, in this research, knowledge of female students of the causality of HPV infection and cervical cancer was higher than in male students (68.5% *vs* 31.5%)¹⁷. A study conducted among medical students in Nigeria showed that 73.9% of respondents knew that HPV infection has a causal relationship with cervical cancer¹⁸. The limited knowledge of our respondents about this STI is in line with the fact that in 2002 Serbia had the highest incidence of cervical cancer in Europe¹⁹, while in 2012 it was fourth in terms of incidence, and third in terms of mortality from cervical cancer in Europe²⁰.

Risky behavior

Numerous studies indicate that there are gender differences in risky behavior for acquiring STIs^{5,8,21}. Our research indicated that men entered into the first sexual relationship earlier than women (17.01 *vs* 18.49 years old). Male students from Brazil entered into the first sexual intercourse earlier than female students (13.0 *vs* 17.5 years old)⁸. A study in a population of Portuguese students showed that majority of the respondents had first sexual intercourse when they were 16 or more years old (72% of males *vs* 82.5% females)⁹.

Male students from Brazil had more sexual partners during their lives than females (5.8 *vs* 3.0), which is in line with the results of our research (5.8 *vs* 2.3)⁸. Our male respondents were significantly more likely to have sexual intercourse under the influence of alcohol and other psychoactive substances than females (49.6% *vs* 19.2%), which is in line with the results of the authors from Portugal (53.1% *vs* 27.3%)⁹. Authors from Croatia point out that 19.2% of men had sexual relations under the influence of alcohol compared to 7.6% females²². It is similar among students in Uganda (16% *vs* 9%)²³. Bjekić et al. showed that respondents with STIs were significantly more likely to enter into sexual relations under the influence of alcohol compared to the control group of the respondents²⁴.

A survey conducted among young people in Denmark indicated that a third of respondents do not use a condom²¹, while about two-thirds of our respondents of both sexes had exhibited this type of risky behavior (63.6% males *vs* 62.4% females). Unlike students from Croatia whose main reason for not using condoms is taking other contraceptives²⁵, most of our male students said that they do not use a condom because it reduces the pleasure during sexual intercourse (41.2%), while the majority of female students do not use condoms because they trust their partners (49.4%).

Our male students were tested for STIs more often than female students (12.1% *vs* 9.7%) as opposed to young people in Denmark, where more young women were tested for STI (14.8% of males *vs* 18.5% females)²¹.

Limitations

This study, like other observational studies, had some limitations. Due to the cross-sectional design of the current study, causal inferences could not be drawn from the associations. Another limitation was that findings from this study may not be generalized to the whole population of the young people because the study involved only those young people who are in higher educational institutions.

Conclusion

A survey conducted in the population of students of medical colleges in Belgrade showed that male students, compared to female students, had a lower level of knowledge about STIs and that they were more likely to behave in a risky manner. The most common forms of risky behavior in both sexes are not using condoms and frequent changes of sexual partners, as well as sexual intercourse at the first date in male respondents. It is necessary to upgrade the STI education programs and implement population-targeted prevention and control measures for the STIs.

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RODNE RAZLIKE U ODNOSU NA ZNANJE I RIZIČNO PONAŠANJE MEĐU STUDENTIMA VISOKIH MEDICINSKIH ŠKOLA U SRBIJI: STUDIJA PREVALENCIJE

SAŽETAK

Cilj ovog istraživanja bio je odrediti razinu znanja o spolno prenosivim bolestima i učestalost ponašanja koja predstavlja rizik za stjecanje tih bolesti među studentima medicinskih fakulteta u odnosu na spol. Istraživanje se temelji na pregledu poprečnog presjeka provedenom na dva Medicinska fakulteta u Beogradu u prosincu 2016. godine. Ukupno 670 studenata (96%) završilo je epidemiološko ispitivanje. Ispitanici su birani slučajnim uzorkovanjem, dobrovoljno su sudjelovali u studiji te su anonimno i samostalno popunili upitnik. Upitnik je uključivao 18 pitanja o demografskim karakteristikama, znanju i rizičnom ponašanju. Studentice su bili znatno starije od muških ispitanika ($p = 0,014$). Studenti su bili češće bez partnera, dok su učenice većinom bile u vezi ili oženjene ($p < 0,001$). Ženske ispitanice imaju više znanja o simptomima spolno prenosivih bolesti ($p = 0,008$), a više od muškaraca su upoznati s uzročnicima infekcije HPV-om i rakom vrata maternice ($p = 0,002$). Muškarci su ranije imali prvi seksualni odnos ($p < 0,001$), imali su seks na prvom spoju ($p < 0,001$), mijenjali seksualne partnere tijekom svog života ($p < 0,001$) i plaćali za seks ($p < 0,001$). Studenti imaju specifične faktore rizika za nezdrav seksualno ponašanje. Potrebno je nadograditi obrazovne programe i provoditi mjere prevencije i kontrole usmjerene na stanovništvo za spolno prenosive infekcije.

Prevalence and Risk Factors of Vitamin D Deficiency among a Sample of Kuwaiti Population: A Sociocultural Study

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ABSTRACT

Despite the high amount of sunshine that Kuwaitis can receive from sunlight most of the year, there is a high prevalence of vitamin D deficiency among Kuwaiti individuals. This descriptive study aimed to determine the prevalence of vitamin D deficiency and related sociocultural and daily life behavior risk factors among 276 Kuwaiti adults based on their blood serum 25-hydroxyvitamin D 25(OH)D levels. Subjects were selected by using a convenient non-random opportunistic sample. A cross-sectional survey was conducted using a closed-ended questionnaire. The mean vitamin D 25(OH)D level of the participants was 23.44 nmol/L (SD=10.89). The social demographic factors that were significantly associated with vitamin D deficiency were {sex ($P<0.05$), age, monthly income, marital status, and occupational status ($P<0.01$)}. Participants' daily lifestyle behaviors risk factors that were significantly associated with vitamin D deficiency were {working environment, smoking habit, fast-food consumption, taking supplements ($P<0.05$), and clothing style ($P<0.001$)}. The present study demonstrates that vitamin D deficiency is common among Kuwaiti adults identifies many sociocultural risk factors. Continued effort to increase knowledge and awareness of vitamin D deficiency risk factors and health outcomes is needed through intensive educational programs.

Key words: Vitamin D deficiency prevalence, Sociocultural factors and health, Kuwait.

Introduction

Despite the ideal geographical region of Kuwait, located between the latitudes of 28.45 and 30.05 degrees north of the Equator and its sunny dry weather most of the year, there is a high prevalence of vitamin D deficiency among Kuwaiti individuals^{1,2}. Kuwaitis can obtain a sufficient amount of ultraviolet rays in the summer where the highest recorded temperature under shadow can sometimes reach 50 °C (122 °F), which is an optimal source of vitamin D one can absorb through the skin³. Even with the high amount of sunshine that Kuwaitis can receive from sunlight in the summer months, many risk factors play a significant role in vitamin D synthesis, which contributes to vitamin D deficiency among Kuwaitis. These factors include insufficient exposure to sunlight, genetic background and ethnicity, sedentary lifestyle, socio-demographic characteristics, anthropometric ratios, quality of food consumed, clothing style and smoking habits^{4–11}. Vitamin D deficiency outcomes have been investigated by several researchers. The findings reveal that

vitamin D deficiency has many health comorbidity and side effects^{12–15}.

Several clinical and public health studies have been performed in the Kuwaiti population to determine the prevalence of vitamin D inadequacy, risk factors, and health comorbidity^{2,16}. These studies include 17 El-Sonbaty (1996), 18 Molla et al. (2005), 1 Al-Yahya et al. (2014), and 19 Zhang et al. (2016) report a high prevalence of vitamin D deficiency among Kuwaiti individuals^{1,17–19}. A study examined the association between clothing style and vitamin D deficiency among veiled Kuwaiti women between 14 and 45 years old¹⁷. The finding reveals that serum 25-hydroxy vitamin D 25(OH)D level was significantly lower among veiled Kuwaiti women compared to non-veiled women. Another study about the relationship between lifestyle behavior and vitamin D deficiency among Kuwaiti¹ shows that the objective of the study was to consider the risk factors (anthropometric and bone measurements, exposure to sunshine, food intake, physical activity, and skin color) compared to serum 25(OH)D levels < 25 nmol/L among adolescent Kuwaiti females to determine the impact on the women's bone mass. The study

shows that most (98.7 %) of the Kuwaiti female adolescents had < 50 nmol/L of 25(OH)D serum levels. The following risk factors for their inadequate levels of vitamin D were veiling, not having a private room, and waist-to-hip ratio. The study states that the independent predictors of bone mineral content among adolescent Kuwaiti females were age at puberty, animal protein intake, physical activity, and anthropometric measurements.

Furthermore, a study among Kuwaiti athletes aimed to assess the relationship between vitamin D deficiency and its risk factors such as body composition, lifestyle behavior, and fasting during the month of Ramadan from sunrise to sunset¹⁶. The study finding reveals that 83% of Kuwaiti athletes had inadequate levels (< 75 nmol/L) of vitamin D; 23% of them were deficient (< 25 nmol/L) in vitamin D. The data also show an inverse relationship between fasting and an athlete's body fat and vitamin D inadequacy. The median serum of 25(OH)D was higher before Ramadan than after. The study also detected a positive significant association between serum 25(OH)D concentrations and both dietary habits and a high dose of vitamin D supplement.

The following research explores the prevalence of vitamin D deficiency among newborn infants and children in Kuwait. The study goal was to detect a correlation between the serum 25(OH)D concentration of the mothers in Kuwait and their newborn infants and the prevalence of rickets among these mothers and infants¹⁸. Their finding reveals that on the day of delivery, the incidence of vitamin D deficiency among the Kuwaiti mothers was 40% and among their infants it was 60%. The study also demonstrates a significant correlation between the mothers and their newborn infants' serum 25(OH)D levels. The values for the mean level of the mothers' serum 25(OH)D was 13.3 (6.5) ng/mL and the corresponding values for their babies was 8.2 (6.5) ng/mL.

Another study was conducted to examine the association between vitamin D and weight status among Kuwaiti children aged 5 to 19 years old². The data present that 45.2% of the children have vitamin D insufficiency (25(OH)D = 30–49.9 nmol/L) and 42.1% have vitamin D deficiency [25(OH)D < 30 nmol/L]. The study also detects the serum 25(OH)D concentration was significantly lower among girls than boys (30.9 vs. 36.0 nmol/L, $p < 0.01$). The children's family income and physical activity were positively associated with the serum 25(OH)D concentration, and negatively associated with the children's age.

In addition, there was a study that showed the association between serum levels of 25(OH)D and the prevalence of diabetes and pre-diabetes among 960 Kuwaiti adults¹⁹. The study finding illustrates that more than half (56%) of the Kuwaiti adults were diagnosed with vitamin D inadequacy [25(OH)D = 12–19.9 ng/mL], and 27% of them were diagnosed with vitamin D deficiency [25(OH)D < 12 ng/mL]. And there was a significant association between vitamin D inadequacy and pre-diabetes and diabetes health status of Kuwaiti adults.

Anthropological research on the relationship between sociocultural factors and daily life behavior risk factors of vitamin D deficiency is rare in Kuwait. To the best of the author's knowledge, no clinical research has been conducted in Kuwait to examine sociocultural factors associated with vitamin D deficiency based on participants' blood serum 25-hydroxyvitamin D 25(OH)D levels from an anthropological perspective.

Objective of the Study and Research Questions

The objective of this medical anthropological study is (1) to determine the prevalence of vitamin D deficiency among a sample of Kuwaiti adults, and (2) to detect sociocultural and daily life behavior risk factors of vitamin D deficiency such as clothing style (whether women cover their faces and bodies), amount of exposure to sun, smoking, consumption of fast foods, and physical activity among a sample of Kuwaiti adults based on their blood serum 25-hydroxyvitamin D 25(OH)D levels. The study was designed to answer the following research questions: 1) what is the prevalence of vitamin D deficiency among a sample of Kuwaiti adults? 2) Are there significant differences among the sociocultural characteristics of Kuwaiti adults and how they affect vitamin D serum 25-hydroxy vitamin D 25(OH)D levels? 3) Are there significant differences among Kuwaiti adults' cultural traditions and daily life behaviors (consumption of fast food, working environment, clothing style, exposure to sun, smoking habits, and physical activity) and their vitamin D serum 25-hydroxy vitamin D 25(OH)D levels?

Methodology

Study Design and Population

This is a cross-sectional descriptive study that was conducted among 276 Kuwaiti adults from March 2017 to January 2018. Participants were recruited using a convenient non-random opportunistic sample from the populations living in six Kuwaiti governorates. All of the participants had attended a medical laboratory to have blood drawn for health assessment and they all had 25-hydroxy vitamin D 25(OH)D blood test levels.

Participation in the study was voluntary. The participants were asked to assess their eligibility and willingness to participate in the study. A written informed consent was handed to all study participants after they were completely informed of the study objectives and procedures and of their right to withdraw from the study at any time. The data collection procedures follow the Kuwait University research method rules and regulations. All participants who had had a surgical intervention in the previous year were excluded. Representative subjects included both non-veiled and veiled women, and men who wore the traditional Kuwaiti long garment and Western clothes.

The questionnaire was translated into Arabic and went through a process of forward and backward translation by four faculty experts at Kuwait University in the fields of social science and anthropology to ensure its content validity. Prior to data collection, the translated questionnaire was checked to insure the accuracy and meaning of the questions. The questionnaire was pretested for content, design, clarity, and recognition in 25 subjects. The reliability of the study instrument showed high internal consistency (Cronbach's alpha was 0.83).

Study sample

As shown in Table 1, one hundred and sixteen (42.0%) of the participants' age was between 23 and 39 years, more than half ($n = 146$; 52.9%) of them were female, half of the participants' marital status was single ($n = 138$; 50.0%), and more than half ($n = 156$; 56.5%) of the participants was overweight. The majority ($n = 126$; 45.7%) of the participants' occupational status was students, and more than half ($n = 176$; 63.8%) of the participants' educational level was a university degree and above, and half of the participants reported a monthly income between 801–1110 KD ($n = 50$; 18.1%). About one-fourth of the participants lived in the Al-Jahra district ($n = 62$; 22.5%), the majority ($n = 245$; 88.8%) of the participants' religious affiliation was Sunni, and more than half ($n = 162$; 58.7%) of the participants' ethnic background was Bedouin (tribal).

Study Instruments and Data Collection

The daily behaviors assessment sections of the questionnaire for this study, such as consumption of fast food, intake of vitamin D supplements, smoking habits, and engaging in regular physical activity, were modified from a previously used questionnaire⁸. The extent of exposure to the sun, clothing style, and working environment sections of the questionnaire for this study were modified from a previously used questionnaire³. The self-administrative questionnaire composed of closed-ended questions to measure the identified study variables included the following five sections:

(1) *Socio-demographic characteristics*: These characteristics included age, sex, body mass index (BMI), marital status, educational level, working patterns, ethnic background, religious affiliation, monthly income, and governorate district.

(2) *Clothing style (conservative/modern)*: Conservative style of clothing for women refers to the traditional long and black dress that covers the body from the shoulders down to the feet, with a headscarf that covers the hair. The modern style of clothing for women refers to European-style clothing that does not completely cover the arms, legs or hair. Similarly for men, the conservative style of clothing refers to the traditional long and white garment that covers the body from the shoulders down to the feet, with a separate head-cover. The modern style of clothing for men includes pants, shorts, and a shirt which do not completely cover the arms, legs, or head. The questions asked, Do you wear the hijab (headscarf) (Yes = 1, No = 0); Do you wear traditional clothes (Yes = 1, No = 0).

(3) *Daily life behaviors assessment*: These characteristics included consumption of fast food, intake of vitamin D supplements (Yes = 1, No = 0); smoking habits (non-smoker = 0, previously a smoker = 1, smoking = 2), engaging in regular physical activity (Do you walk consistently? Yes = 1, No = 0), exercising indoors or outdoors, duration of exercising indoors and outdoors (How many hours did you exercise outdoors in the last seven days?)

(4) *Frequency of exposure to the sun seasonally*: We asked, Do you get exposure to the sun (for 3–4 days) during the summer months between 9:00 and 10:30 a.m. and between 2:00 and 3:00 p.m.? (Yes = 1, No = 0), Do you get exposure to the sun (for 3–4 days) during the winter months between 10:00 a.m. and 2:00 p.m.? (Yes = 1, No = 0).

(5) *Health profile*: We asked, Do you or a member of your family have vitamin D deficiency? (Yes = 1, No = 0), and Were you diagnosed with a chronic disease? (Yes = 1, No = 0), or, What is your serum 25(OH)D score?

The value of BMI was computed as the weight in kg divided by height in meters squared (kg/m^2). Based on the World Health Organization's measurement of obesity, the participants were classified as underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$), normal ($\text{BMI} 18.5 - 24.9 \text{ kg/m}^2$), overweight ($\text{BMI} 25 - 29.9 \text{ kg/m}^2$), and obese ($\text{BMI} \geq 29.9 \text{ kg/m}^2$).

Statistical Analysis

The data was entered and analyzed by using SPSS 25 (SPSS, Inc., USA). Categorical variables were expressed as frequencies and percentages, and continuous variables were presented as means $\pm$ standard deviation ($M \pm SD$). For all statistical analyses, a p-value of < 0.05 was considered as statistically significant. Chi-square was used to compare between participants with optimal vitamin D levels, insufficient levels, and deficiency of vitamin D (measuring total 25(OH)D concentrations) and the independent variables of socio-demographic characteristics, clothing style, daily life behaviors assessment, frequency of exposure to the sun seasonally, and health profile. The results of 25(OH)D were analyzed as a continuous variable and were also stratified with respect to the Institute of Medicine categories definition of vitamin D status that diagnosed vitamin D as sufficient for serum 25(OH)D levels $< 50 \text{ nmol/L}$, insufficient for $50 - 75 \text{ nmol/L}$ and sufficient for $75 - 250 \text{ nmol/L}$ ^{20,21}. Vitamin D deficiency in the current study was defined as serum 25(OH)D $< 50 \text{ nmol/L}$.

Results

Sociocultural Characteristic and Vitamin D serum 25(OH)D Levels

The mean age of the participants was 31.55 years ($SD = 13.52$); three-fourths ($n = 209$; 75.7%) of the participants reported deficiency in total serum 25(OH)D $< 50 \text{ nmol/L}$, and mean vitamin D 25(OH)D level was 23.44 nmol/L ($SD = 10.89$). Table 1 reveals that a majority of the female participants said that they regularly wore a headscarf (*Hijab*) ($n = 125$; 81.16%), more than half of the partici-

TABLE 1.
DISTRIBUTION OF PARTICIPANTS' SOCIODEMOGRAPHIC VARIABLES (N = 276).

Characteristics	N (%)	Characteristics	N (%)
Age category, years		Walking outdoor	
< 22	94 (34.1)	Yes	72 (26.1)
23–39	116 (42.0)	No	204 (73.9)
>40	63 (22.8)		
Sex		Season of walking	
Male	130 (47.1)	summer	59 (21.4)
Female	146 (52.9)	winter	164 (59.4)
		Hours of exercising outdoor	
BMI, kg/m²		< than half an hour	144 (52.2)
Normal	156 (56.5)	0.5 to 2	37 (13.4)
Overweight	126 (45.7)	2–4	31 (11.2)
		> 4	15 (5.4)
Occupational status		Smoking habits	
Student	74 (26.8)	Non smoker	184 (66.7)
Student & employed	24 (8.7)	Previous smoker	23 (8.3)
Employed	7 (2.5)	Smoker	56 (20.3)
Retired	12 (4.3)		
Private business	245 (88.8)	Frequency of eating fast food weakly	
Housewife	28 (10.1)	Non	58 (21.0)
Religious affiliation		Once	76 (27.5)
Sunni		2–6 times	120 (43.5)
Shiite		Everyday	18 (6.5)
Marital status		Exposing to sun (3–4 days) in summer from 9–10:30 and from 2–3 p.m	
Not married	138 (50.0)	Yes	77 (27.9)
Married	129 (46.7)	No	196 (71.0)
Roots		Exposing to sun (3–4 days) in winter from 10–2	
Urban	101 (36.6)	Yes	97 (35.1)
Bedouin	162 (58.7)	No	176 (63.8)
Kuwait governorates		My dress cover my whole body	
AlAsimah	44 (15.9)	Yes	186 (67.4)
Hawalli	37 (13.4)	No	87 (31.5)
Mubarak Al-kabeer	38 (13.8)		
ALFarwayniyah	56 (20.3)	Diagnosed with chronic disease	
ALJahra	62 (22.5)	Yes	35 (12.7)
ALAhmadi	35 (12.7)	No	239 (86.6)
Educational level		Taking vitamin D supplement	
< High school	20 (7.2)	Yes	90 (32.6)
High school & Diploma	78 (28.3)	No	182 (65.9)
>University	176 (63.8)		
Do you wear a scarf (hijab)		25 OHD category	
Yes	125 (81.16)	Deficient (<50 nmol/L)	209 (75.7)
No	29 (18.83)	Insufficient (50–75 nmol/L)	37 (13.4)
Working indoor		Sufficient (>75–125/L)	26 (9.4)
Yes	208 (75.4)	Income K.D	
No	44 (15.9)	< 500	27 (9.8)
Do you your family have Vit D deficiency ?		501–800	19 (6.9)
Yes	251 (90.9)	801–1110	50 (18.1)
No	20 (7.2)	1101–1400	46 (16.7)
Do you regularly go for walking		1401–1700	27 (9.8)
Yes	88 (31.9)	1701–2000	21 (7.6)
No	183 (66.3)	2001–2300	23 (8.3)
		2301–2600	8 (2.9)
		2601–2900	5 (1.8)
		>2901	26 (9.4)

TABLE 2.
PARTICIPANTS' SOCIODEMOGRAPHIC PROFILE, AND 25(OH)D LEVELS

	25 OHD level				X ²	Sig
	Deficient (<50 nmol/L) N (%)	Insufficient (50–75 nmol/L) N (%)	Sufficient (>75–125/L) N (%)	Total N (%)		
Age (years)						
< 22	81 (39.3)	2 (5.4)	9 (34.6)	92 (34.2)	25.892	0.000
23–39	89 (43.2)	20 (54.1)	6 (23.1)	115 (42.8)		
> 40	36 (17.5)	15 (40.5)	11 (42.3)	62 (23.0)		
Sex						
Male	106 (50.7)	15 (40.5)	7 (26.9)	128 (47.1)	5.985	0.050
Female	103 (49.3)	22 (59.5)	19 (73.1)	144 (52.9)		
Monthly income						
< 500	23 (12.0)	1 (3.0)	3 (12.0)	27 (10.8)	33.625	0.014
501–800	14 (7.3)	3 (9.1)	2 (8.0)	19 (7.6)		
801–1110	42 (22.0)	7 (21.2)	1 (4.0)	50 (20.1)		
1101–1400	35 (18.3)	4 (12.1)	6 (24.0)	45 (18.1)		
1401–1700	21 (11.0)	1 (3.0)	3 (12.0)	25 (10.0)		
1701–2000	11 (5.8)	8 (24.2)	2 (8.0)	21 (8.4)		
2001–2300	18 (9.4)	4 (12.1)	1 (4.0)	23 (9.2)		
2301–2600	4 (2.1)	1 (3.0)	3 (12.0)	8 (3.2)		
2601–2900	3 (1.6)	0 (0.0)	2 (8.0)	5 (2.0)		
>2901	20 (10.5)	4 (12.1)	2 (8.0)	26 (10.4)		
Marital status						
Unmarried	113 (55.9)	13 (37.1)	8 (30.8)	134 (51.0)	8.920	0.012
Married	89 (44.1)	22 (62.9)	18 (69.2)	129 (49.0)		
Occupational status						
Students	105 (50.5)	7 (18.9)	12 (46.2)	124 (45.8)	24.348	0.007
Students & employee	24 (11.5)	7 (18.9)	1 (3.8)	32 (11.8)		
Employee	56 (26.9)	11 (29.7)	7 (26.9)	74 (27.3)		
Retired	11 (5.3)	8 (21.6)	4 (15.4)	23 (8.5)		
Private business	5 (2.4)	1 (2.7)	0 (0.0)	6 (2.2)		
Housewives	7 (3.4)	3 (8.1)	2 (7.7)	12 (4.4)		

pants reported wearing long clothes that cover their whole body ($n = 186$; 67.4%), about three-fourths of the participants said their working environment is indoors ($n = 198$; 71.7%), and more than one-third of the participants reported that they eat fast food weekly from two to six times ($n = 120$; 43.5%). In addition, daily physical activity behavior data presents that three-fourths of the participants said they don't walk outdoors in fresh air ($n = 204$; 73.9%). Out of 223 participants who said that they do walk, more than half ($n = 164$; 59.4%) of the participants said that they walk in the winter season, and half ($n = 144$; 52.2%) of the participants reported that they usually exercise outdoors for less than half an hour.

The data in Table 1 shows hours of exposure to the sun and time of exposure to the sun according to season. The data illustrates that in answer to the question about their exposure to the sun in the summer season between 9:00 and 9:30 a.m. and between 2:00 and 3:00 p.m. on average three to four days a week, only one-fourth ($n = 77$; 27.9%) of the participants said yes they do get exposure to the

sun during these hours, compared to only one-third ($n = 97$; 35.1%) of the participants who said that they have exposure to the sun during the winter season from 10:00 a.m. to 2:00 p.m. on average three to four days a week. Furthermore, Table 1 shows that only one-third ($n = 90$; 32.6%) of the participants reported that they are taking a vitamin D supplement, the majority ($n = 239$; 86.6%) of the participants stated that they were not diagnosed with any chronic diseases, and the majority ($n = 251$; 90.9%) of the participants reported that a member of their family was diagnosed with vitamin D deficiency.

Table 2 displays the social demographic factors that were significantly associated with vitamin D deficiency after applying chi square analysis of the predicted associated factors. These include age ($P < 0.001$), sex ($P < 0.05$), monthly income ($P < 0.01$), marital status ($P < 0.01$), and occupational status ($P < 0.01$). The remainder of the associated factors listed under methods were not significantly associated with vitamin D deficiency. A statistical significance was found between participants' age and

serum levels of total 25(OH) ($X^2 = 25.892$, $p = 0.000$), 43.2% of participants whose ages are between 23 and 39 years old reported significantly lower total 25(OH)D than 39.3% of participants whose ages are 22 years old and younger and 17.5% of participants whose ages are 40 years old and older. Also a statistical significance was found between participants' sex and serum levels of total 25(OH) ($X^2 = 5.985$, $p = 0.050$), (50.7%) of Kuwaiti males reported significantly lower total 25(OH)D than Kuwaiti females. Moreover, the findings reveal that there were significant differences between participants' monthly income and serum levels of total 25(OH) ($X^2 = 33.625$, $p = 0.014$); 22.0% of the participants whose monthly income are between 801–1110 KD reported significantly lower serum levels of total 25(OH) compared to the other participants' monthly income.

The findings also revealed an statistically significant association ($X^2 = 19.231$, $p = 0.012$) between the participants' marital status and serum levels of total 25(OH); 55.9% of unmarried participants reported significantly lower serum levels of total 25(OH) than did married participants (44.1%). Table 2 also shows that participants' occupational status is significantly associated ($X^2 = 24.348$, $p = 0.007$) with serum levels of total 25(OH) D, half (50.5%) of the participants who are students reported significantly lower serum levels of total 25(OH) compared to 26.9% of employee participants, and 11.5% of students who are employee participants.

Vitamin D deficiency risk factors and serum 25(OH)D levels

Table 3 presents the vitamin D deficiency risk factors of participants' daily lifestyle behaviors {working environment ($P < 0.05$), smoking habit ($P < 0.05$), clothing style ($P < 0.001$), fast-food consumption ($P < 0.05$), and taking supplements ($P < 0.05$)} that were significantly associated with vitamin D deficiency after applying chi square analysis of the predicted associated factors. The remainder of the associated factors listed under Methods was not significantly associated with vitamin D deficiency. A statistical significance was found between participants' smoking habits and total 25(OH) serum levels ($X^2 = 10.296$, $p = 0.036$); more than half (65.3%) of the nonsmoking participants reported significantly lower total 25(OH)D levels than did the smoking participants (25.3%). And 48.1% of the participants who reported high prevalence (between 2 to 6 times per week) of fast-food consumption reported significantly lower total 25(OH)D levels compared to 25.7% of participants who reported they consumed fast food once a week ($X^2 = 12.682$, $p = 0.048$).

Clothing style also revealed a significant association with vitamin D deficiency: more than half (65.7%) of the participants who said they wear clothing that covers their whole body reported significantly lower total 25(OH)D levels compared to 34.3% of participants who answered they do not wear clothing that covers their whole body ($X^2 = 5.538$, $p = 0.05$). Similar to the previous finding, more than three-fourths (75.5%) of female participants who

wearing a headscarf reported significantly lower total 25(OH)D levels compared to 24.5% of female participants who do not wear a headscarf ($X^2 = 8.215$, $p = 0.016$).

In addition, Table 3 demonstrates that participants' vitamin D supplement intake was significantly associated with their total serum levels of 25(OH) ($X^2 = 7.642$, $p = 0.22$). Around three-fourths (71.5%) of participants who reported not taking vitamin D supplements reported significantly lower total 25(OH)D levels compared to 28.5% of participants who reported that they take vitamin D supplements. Furthermore, participants' working environment had a significant association with their total 25(OH) D levels. The majority (80.6%) of participants whose work environment is indoors reported significantly lower total 25(OH)D levels compared to 19.4% of participants whose work environment is outdoors ($X^2 = 6.238$, $p = 0.44$).

Discussion

The present study detected high prevalence of vitamin D deficiency among Kuwaiti adults, which confirms the findings of studies conducted in Arabian Gulf countries, including the United Arab Emirates, Oman, Saudi Arabia, Qatar, and in Kuwait^{22–26}. The comparable high incidence of vitamin D deficiency in the Gulf region populations may be due to weather conditions. These countries share a similar geographical setting and extreme hot and humid weather most of the year, which prohibits people's outdoor activities and daily lifestyle practices, which encourages them to spend their leisure time doing indoor activities more at indoor shopping malls, cafés, indoor gyms, or house-to-house visiting. Given the extremely hot temperature, people living in the Gulf region tend to restrict their outdoor activities to evenings after sunset which contributes to fewer hours of direct exposure to optimal sunshine necessary for endogenous synthesis of D3 (cholecalciferol) production and results in vitamin D deficiency^{27,28}.

Furthermore, the current study reveals that the prevalence of vitamin D deficiency was higher among Kuwaitis (both men and women) who wear conservative clothing and among women who cover their hair, which confirms the findings^{29–31} who conducted their research among Arabian Gulf women. The association between vitamin D deficiency and clothing style can be attributed to the fact that Gulf region populations share similar religious and cultural practices and conservative clothing styles. As part of their traditional and religious customs, most women begin to wear traditional conservative clothing at a young age that covers their whole body and hair, and most of the men wear long garments at a young age, covering their whole body and a headscarf to cover their head. The traditional clothing obscures direct sunlight from reaching individuals' skin, which affects the synthesis of vitamin D in the body.

The findings of the current study confirm findings, all of which show a significant association between individuals' socioeconomic status, excessive calorie intake, and

TABLE 3.
PARTICIPANTS' VITAMIN D RISK FACTORS AND 25(OH)D LEVELS

	25 OHD level				X ²	Sig
	Deficient (<50 nmol/L) N (%)	Insufficient (50–75 nmol/L) N (%)	Sufficient (>75–125/L) N (%)	Total N (%)		
Smoking habits						
Non smoker	130 (65.3)	27 (77.1)	23 (92.0)	180 (69.5)	10.296	0.036
Previous smoker	18 (9.0)	4 (11.4)	1 (4.0)	23 (8.9)		
Smoking	51 (25.6)	4 (11.4)	1 (4.0)	56 (21.6)		
Prevalence of eating fast food						
Non	37 (18.0)	11 (30.6)	8 (30.8)	56 (20.9)	12.682	0.048
Once a week	53 (25.7)	14 (38.9)	9 (34.6)	76 (28.4)		
2–6 times weakly	99 (48.1)	10 (27.8)	9 (34.6)	118 (44.0)		
Everyday	17 (8.3)	1 (2.8)	0 (0.0)	18 (6.7)		
Do your clothes covers your whole body?						
Yes	136 (65.7)	24 (66.7)	23 (88.5)	183 (68.0)	5.538	0.053
No	71 (34.3)	12 (33.3)	3 (11.5)	86 (32.0)		
Do you wear a head scarf (female)						
Yes	83 (75.5)	21 (91.3)	19 (100.4)	123 (80.9)	8.215	0.016
No	27 (24.5)	2 (8.7)	0 (0.0))	29 (19.1)		
Taking vitamin D supplement						
Yes	59 (28.5)	18 (50.0)	11 (42.3)	88 (32.7)	7.642	0.022
No	148 (71.5)	18 (50.0)	15 (57.7)	181 (67.3)		
Is your working environment outdoor?						
Yes	36 (19.4)	11 (31.4)	1 (4.5)	48 (19.8)	6.238	0.044
No	150 (80.6)	24 (68.6)	21 (95.5)	195 (80.2)		

high incidence of vitamin D deficiency^{32–34}. These findings can be due to the wide distribution of fast food restaurants in Kuwait with affordable prices and easy access, along with adoption of sedentary lifestyle practices among most Kuwaitis and lack of governmental food fortification policies for dairy products with vitamin D. This study also found that only one-third of the participants with lower serum 25(OH)D concentrations reported taking vitamin D supplements, confirming findings among Kuwaiti adults¹⁹. This finding necessitates more nutritional programs that teach the public about the food sources of vitamin D, and alternatively, about vitamin D supplements.

Unlike to the following research^{2,35–36} who reported high incidence of vitamin D deficiency among women, the current study found a high prevalence of vitamin D deficiency among Kuwaiti men, with significantly lower total 25(OH)D levels than Kuwaiti women. Our findings confirm findings among Luxembourg participants³⁷. This might be attributed to the differences in lifestyle behaviors between men and women such as smoking habit as a vitamin D risk factor that is culturally prohibited to Kuwaiti women and only permitted for men, as well as differences in physical activity, working environments, dietary habits, and exposure to the sun among men and

women. Contrary to our expectation, the current study did not detect a significant association between exposure to the sun and lower 25(OH)D levels which confirms the study findings¹⁶. This may be due to the fact that most of the activities that the participants are engaged in are indoors, along with their working environments that are also indoors, which minimizes the duration of exposure to sunshine during optimal times of receiving sun rays for vitamin D synthesis.

Conclusion

In conclusion, vitamin D deficiency is a major public health issue that affects individuals across all life stages, and from different nationalities. The present study demonstrates that vitamin D deficiency is common among Kuwaiti adults identifies many sociocultural risk factors. Continued effort to increase knowledge and awareness of vitamin D deficiency risk factors and health outcomes is needed through intensive educational programs. Campaigns about the main sources of vitamin D should be held by professional experts in medical and nutritional fields for the community. It is necessary to increase the population's knowledge on consuming fortified products and supplements and encourage them to pursue outdoor activities during their leisure time.

Limitations and Strengths

The present study is the first in Kuwait that highlights an association between vitamin D status and risk factors

of vitamin D deficiency from the medical anthropological perspective. It provides reliable information on serum 25(OH)D concentrations, duration and times of exposure to the sun, working environments, and clothing styles. However, since we have used a cross-sectional study design, our method did not allow for the long-term impact of other factors that could be potential confounders to vitamin D concentrations such as skin color, genetic factors, and dietary intake containing vitamin D-fortified foods. Therefore, longitudinal medical anthropological studies are needed to assess other risk factors that affect the synthesis of 25(OH)D concentrations, such as exposure to the sun, duration of exposure, time of day when an individual had exposure to the sun, amount of unprotected skin exposed to the sun, dietary habits, and barriers of outdoor activities. The following recommendations could be helpful to lessen the incidence of vitamin D deficiency among Kuwaitis population such as: (1) The Ministry of Health is recommended to link the immunization schedule of the children with the assessment of vitamin D 25(OH)D blood test levels and link the health screening for job recruitment among the elderly with the assessment of vitamin D 25(OH)D blood test levels, (2) Substitution of the fast food restaurants by healthy restaurants who provide organic food that includes in their menu a variety of soy products, seafood, protein foods, and seeds, (3) Governmental financial support for the local agricultural markets and encouraged them to produce organic food with vitamin D fortified products, and (4) Ministry of Health should adopted public health campaigns that advertise the importance of consuming fortified food specially dairy products with vitamin D among Kuwait's community.

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PREVALENCIJA I ČIMBENICI RIZIKA NEDOSTATKA VITAMINA D U UZORKU STANOVNIŠTVA IZ KUVAJTA: SOCIOKULTURNA STUDIJA

SAŽETAK

Unatoč velikoj količini sunčeve svjetlosti koju stanovnici Kuvajta primaju tijekom cijele godine u toj zemlji je prisutna velika prevalencija nedostatka vitamina D. Ova deskriptivna studija usmjerena je na utvrđivanje prevalencije nedostatka vitamina D i povezanih sociokulturnih svakodnevnih rizika u ponašanju 276 odraslih stanovnika Kuvajta na temelju njihovih razina 25-hidroksivitamina D 25(OH)D u krvnom serumu. Ispitanici su odabrani ne-slučajnim uzorkovanjem. Istraživanje poprečnog je provedeno pomoću strukturiranog upitnika. Prosječna razina vitamina D 25(OH)D u ispitanika bila je 23,44 nmol/L (SD = 10,89). Socijalni demografski čimbenici značajno povezani s nedostatkom vitamina D bili su: spol ($P < 0.05$), dob, mjesečni prihod, bračni status i zanimanje ($P < 0.01$). Čimbenici rizika svakodnevnog životnog stila i ponašanja sudionika koji su bili značajno povezani s nedostatkom vitamina D odnosili su se na radnu okolinu, naviku pušenja, brzu hranu, uzimanje prehrambenih dodataka ($P < 0.05$) i stil odijevanja ($P < 0.001$). Ova studija pokazuje da je nedostatak vitamina D uobičajen među odraslim stanovnicima Kuvajta i povezan s brojnim sociokulturnim rizičnim faktorima. Stoga su potrebni stalni napori za povećanjem znanja i svijesti o faktorima rizika nedostatka vitamina D i njegovim učincima na zdravlje.

English Language Learning Demotivation of University Students

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ABSTRACT

Theories on second language acquisition (SLA) differently estimate the importance of individual factors in language acquisition. Within those factors, the importance of motivation as well as demotivation is essential. Motivation is extremely important during demanding learning process. The first objective of this study is focused on determining the correlations between factors of demotivation (DM) during learning English as a foreign language (LEFL) with factors of motivation (M), as well as with chosen relevant variables. The second objective is focused on determining the differences between factors of demotivation during LEFL in relation to gender, year of study and programme of study. The study was conducted among students enrolled in 3-year undergraduate study programmes at the University of Dubrovnik. The Questionnaire for Measuring Type and Intensity of Motivation of Croatian Students for Learning English as a Foreign Language was applied as a research instrument. The results indicate the absence of statistically significant differences in DM factors, according to gender, programme and year of study. However, a statistically significant, moderately high and negative association of pragmatic-communicative and affective factors of motivation with demotivation has been established, while the other correlations between DM and M are very low. On the other hand, a statistically significant, negative and low correlation with DM factors has been found for the final grade students only. The findings suggest the need for further research based on a context-specific approach to understanding demotivation in LEFL.

Key words: demotivation, language learning, English, students

Introduction

The rapid changes in the contemporary way of life, induced by the globalisation processes, technological advances and mobility of people and assets brought attention to the importance of communication among people from all over the world. Attention has been drawn to learning foreign languages. The process of second language acquisition has proven to be complex and varied thus resulting in a plethora of theories, all of them aiming to better understand the language learning process. The success in SLA varies from individual to individual and it depends on many factors. Within numerous factors that have an impact on SLA, the importance of motivation as well as demotivation is essential. Recent theories on SLA focus on the importance of individual factors. Gardner¹ distinguishes the cognitive and affective characteristics of individual factors. He puts motivation, along with attitudes and anxiety, among the affective factors. According to Mihaljević Djigunović² it is commonly accepted nowadays that during the foreign language learning process both cognitive and affective learner qualities are activated.

Motivation in second language acquisition

Nevertheless, motivation is considered to be one of the most complex and most important factors which directly influence the language learning process and the learning results^{3,4}. Many researchers have attempted to come up with a comprehensive and single definition of motivation, but so far without success. In 2006, Gardner⁵ defined motivation as a general characteristic of the individual that applies to any opportunity to learn the language. His socio-educational model conceptualises motivation as the sum of the effort, desire to learn and positive attitudes⁵. More recently, Dörnyei³ defines motivation as the initial stimulus for learning language and the driving force to sustain the long and arduous learning process. He constructed the second language (L2) motivational self-system which comprises the ideal L2 self, ought-to L2 self and L2 learning experience³.

Demotivation in second language acquisition

On the other hand, demotivation is a relatively new and still under-researched area in the field of SLA. While mo-

tivation might be considered to have a positive influence on learning language, demotivation has the negative effect on success in SLA. It is important to look into demotivation as perhaps one of the main causes for the language learning failure. According to Oxford⁶, demotivation is a process that can be best understood by asking students to recall their learning experiences over a period of time. Dörnyei³, however, defines demotivation as concerning specific external forces that reduce or diminish the motivational basis of a behavioural intention or an on-going action. In other words, demotivation could be regarded as the negative counterpart of motivation³.

The previous research on demotivation focused mainly on identifying the demotivating factors. Gorham and Christophel conducted two separate studies on the students' demotivation for learning^{7,8}. One study implemented qualitative methodology, while the other was a quantitative study. Nevertheless, both studies provided rather consistent results. Namely, the participants mainly attributed demotivation to their teacher (two-thirds of demotivators were attributed to the teacher)^{7,8}. Thus, the teacher was the main demotivating factor and described as boring, unorganised and unprepared, but also unapproachable, biased and in some cases even insulting. Other factors included the teaching material, grading and the dislike for the language^{7,8}. Their general finding was that negative teacher behaviour was central to students' demotivation^{7,8}. Oxford attempted to identify demotivating factors by analysing information from written essays collected from 250 students over a period of five years⁶. The research findings identified four demotivators, out of which three were related to the teacher (personal relationship with the students, attitude towards the course or the material, style conflicts between teachers and students) and the fourth demotivator was identified as the nature of the classroom activities (irrelevance, overload and repetitiveness).⁶ Dörnyei in 2001 conducted a study focusing specifically on demotivated students, who were identified as demotivated according to their teachers and peers⁹. He conducted one-to-one interviews with the students asking them about their bad learning experiences. Nine main demotivating factors were identified⁹. The teacher was the most frequent source of demotivation in 40% of the cases. Other demotivating factors involved reduced self-confidence, inadequate school facilities, negative attitude towards L2, compulsory nature of study, interference with another foreign language (FL), negative attitude towards L2 community, attitudes of group members and course book⁹. Williams, Burden and Lanvers¹⁰ carried out a study to determine whether there was any relationship among students' demotivation with respect to gender, age and the language. The results of their study indicated that male students were more demotivated than their female peers, and that there was a negative relationship between age and level of motivation¹⁰. Muhonen¹¹ conducted a research on 91 students who were learning LEFL as a compulsory subject in Finland and were asked to do a writing task. This study identified 5 demotivating factors. The teacher was the primary source of demotivation (58.6%), specifically his/her teaching methods, lack of competence and

personality¹¹. Other factors were the learning material, learner characteristics, school environment and learner's attitude towards English language. Regarding gender, only slight differences were found, male students were just slightly more inclined to attribute demotivation to the teacher than the female ones¹¹. Trang and Baldauf¹² researched demotivation on a sample of 100 students learning English as a compulsory subject in Vietnam. The students were asked to recall their English learning experiences and to write an essay. Their findings revealed three main categories of demotives: context demotives, structure/format demotives and teacher behaviours¹². Consistent with the results of previous research on demotivation the study revealed that the largest source of demotives was related to teachers, in particular the teaching methods. The students were also demotivated due to poor pre-knowledge of English.

Croatian research on motivation and demotivation in second language acquisition

In Croatia, Mihaljević Djigunović^{2,13} carried out an extensive study involving three different sets of participants to construct an instrument for researching motivation for LEFL in Croatia. To determine the motivational types in the Croatian context, after a thorough research, an instrument for measuring the type and intensity of motivation was construed^{2,13}. These findings indicated three motivational types for learning English in Croatia: pragmatic-communicative motivation, affective motivation and integrative motivation, and two demotivators: the teaching setting demotivator and the learning difficulties demotivator. The teaching setting demotivator involved the teacher, teaching materials and teaching methodology, while the learning difficulties involved lack of basic pre-knowledge, seeing English as too complicated, etc¹³.

The scope of this study is focused primarily on the factors of demotivation (DM) during learning English as a foreign language (LEFL), among students enrolled in 3-year undergraduate study programmes at two different departments at the University of Dubrovnik.

Having taken into consideration the importance of demotivation in second language acquisition, the first set of objectives of this study are focused on determining the differences between factors of demotivation during LEFL in relation to gender, year of study and programme of study. The second objective is focused on determining the correlations between factors of demotivation (DM) during learning English as a foreign language with factors of motivation (M), as well as with chosen relevant variables.

Our initial hypothesis is that no differences in demotivation between students enrolled in two different study programmes (Maritime Department and Mass Communications Department) and between students from different years of study are to be expected, having in mind the similar socio-educational context. Finally, no differences in demotivation for learning EFL with regard to gender can be expected. Male students are predominantly enrolled in the Maritime Department, while the Mass Communications Department has significantly larger number of fe-

male students. In the maritime profession English is the standard communication language, while in the mass communications the importance of foreign languages, especially English as lingua franca is undeniable. On the other hand, we supposed that some specific factors were responsible for motivation and some other for demotivation. Therefore, we expected that demotivation factors should be positively intercorrelated and motivation factors should be positively intercorrelated, but without positive cross-correlations between motivation and demotivation factors.

Method

Participants

The entire purposeful sample included 172 participants, within which two sub-samples were included, comprising all the students enrolled in 3-year undergraduate study programmes at two University of Dubrovnik Departments: Maritime Department and Mass Communications Department. The first sub-sample ($N_1=107$) involved the students from the Maritime Department: 55 first year students, 27 second year students and 25 third year students, with median age 22. The students from the Maritime Department were mainly male students ($N=101$), with only 6 female students, which was to be expected having in mind the professional orientation traditionally preferred by men. The second sub-sample consisted of the students from the Mass Communications Department ($N_2=65$): 24 first year students, 23 second year students and 18 third year students. The median age of these students was 23. Most of the students were female ($N=45$), with 20 male students.

Instrument

The research instrument consisted of two parts. In the first part demographic data was collected: age, gender, the programme of study and the year of study. The second part was the Questionnaire for Measuring Type and Intensity of Motivation of Croatian Students for Learning English as a Foreign Language (TIMLEFL, by Mihaljevic Djigunovic²) used to measure three types of student motivation and two types of student demotivation for learning English as a foreign language. TIMLEFL is a self-evaluation instrument containing 38 statements. The students were to specify their agreement with each of the statement on a five-point Likert scale ranging from strongly disagree (1) to strongly agree (5). The instrument showed an acceptable reliability type internal consistency. The results

revealed that all original dimensions of the instrument showed satisfactory reliability, with Cronbach's alpha coefficients as follows: pragmatic-communicative motivation (0.84), affective motivation (0.73), integral (0.65) motivation, teaching setting demotivator (0.50) and learning difficulties demotivator (0.76). Particularly low, but still acceptable, was the reliability revealed for teaching setting as the demotivator.

Data collection and statistical analyses

The research was carried out at the University of Dubrovnik during regular English classes at the beginning of 2013 (students from both departments were tested within the same week). The participants were informed about the general purpose of the research without specifying the aim of the study to avoid bias. They were asked for sincere and accurate replies and all participants were granted anonymity. Relevant permissions from the Heads of Departments were timely obtained.

Statistical analysis was performed using the statistical package *SPSS 20.0*. To determine the differences in demotivation factors according to gender and programme of study, Mann Whitney U test was used, while Kruskal Wallis test was used to determine the differences according to year of study. Pearson correlation coefficients were used in the analysis of correlations between two types of demotivation and three types of motivation. Cronbach's alpha coefficients were used as the measure of reliability in certain subscales of motivation and demotivation for SLA. Paired samples t-test was used to test overall differences between two types of demotivation.

Results

TABLE 1
DESCRIPTIVE STATISTICS FOR DEMOTIVATION FACTORS FOR LEARNING ENGLISH AS A FOREIGN LANGUAGE

Demotivators	Minimum	Maximum	Mean	Std. Dev.
Teaching setting	1.22	4.33	2.46	0.53
Learning difficulties	1.00	4.00	1.94	0.75

The testing of differences of descriptive indicators by paired samples t-test (Table 1), clearly indicated that the teaching setting was a stronger source of demotivation than the learning difficulties ($t=10.27$; $p<0.01$).

TABLE 2
GENDER DIFFERENCES IN DEMOTIVATION FACTORS FOR LEARNING ENGLISH AS A FOREIGN LANGUAGE

Type of demotivation	Mann-Whitney U test (p)	M Male (120)	s Male (120)	M Female (52)	s Female (52)
Teaching setting	0.27	2.48	0.50	2.41	0.59
Learning difficulties	0.16	1.99	0.77	1.82	0.72

Legend: M= Mean; s= Standard Deviation

TABLE 3
DIFFERENCES IN DEMOTIVATION FACTORS FOR LEARNING ENGLISH AS A FOREIGN LANGUAGE IN RELATION TO THE PROGRAMME OF STUDY

Type of demotivation	Mann-Whitney U test (p)	M Maritime (107)	s Maritime (107)	M Mass Communications (65)	s Mass Communications (65)
Teaching setting	0.27	2.47	0.47	2.44	0.62
Learning difficulties	0.23	1.98	0.73	1.88	0.80

Legend: M= Mean; σ = Standard Deviation

TABLE 4
DIFFERENCES IN DEMOTIVATION FACTORS FOR LEARNING ENGLISH AS A FOREIGN LANGUAGE IN RELATION TO THE YEAR OF STUDY

Type of demotivation	Kruskal-Wallis test (p)	M 1 st year (79)	s 1 st year (79)	M 2 nd year (50)	s 2 nd year (50)	M 3 rd year (43)	s 3 rd year (43)
Teaching setting	0.34	2.39	0.49	2.50	0.50	2.54	0.63
Learning difficulties	0.65	1.90	0.75	2.02	0.79	1.92	0.72

Legend: M= Mean; σ = Standard Deviation

TABLE 5
CORRELATION BETWEEN DEMOTIVATION AND MOTIVATION FACTORS FOR LEARNING ENGLISH AS A FOREIGN LANGUAGE

Variables of motivation and demotivation	Pragmatic-communicative motivation	Affective motivation	Integrative motivation	Teaching setting	Learning difficulties
Pragmatic-communicative motivation	1.00	0.72**	0.34**	-0.18*	-0.53**
Affective motivation		1.00	0.44**	-0.04	-0.42**
Integrative motivation			1.00	0.07	-0.01
Teaching setting				1.00	0.50**
Learning difficulties					1.00

Legend: **correlation significant at $p < 0.01$; *correlation significant at $p < 0.05$; Shaded – correlations of demotivation factors

Gender differences for two types of student demotivation for LEFL were analysed. As shown in Table 2 there are no statistically significant gender differences in the types of student demotivation for LEFL.

The insight in Table 3 indicates there are no statistically significant differences in demotivation factors for learning English as a foreign language in relation to the programme of study.

Table 4 shows that there are no statistically significant differences in demotivation factors for learning English as a foreign language, in relation to the students' year of study.

The results of the correlation analysis, as shown in Table 5, clearly indicate the positive inter-correlation of demotivation factors and also positive inter-correlations of motivation factors. On the other hand, learning difficulties are moderately negatively correlated with two motivation factors, pragmatic-communicative motivation and affective motivation. There is a negative correlation between pragmatic-communicative motivation and teaching setting.

Discussion and Conclusions

The important role of individual factors in the language learning process is highly recognised nowadays. Demotivation is still an under-researched area and it is of utmost importance to carry out research of this phenomenon to fully understand the complex process of SLA. This research, while being merely a small contribution to the field, has confirmed the importance of researching demotivation for learning English as a foreign language in the Croatian socio-educational context. The hypothesis that some specific factors are responsible for motivation and some others for demotivation is strongly confirmed.

This research has shown that demotivation for learning English largely depends on the teacher, teaching materials and teaching methodology. The influence of the learning difficulties, such as lack of basic pre-knowledge and perception of EL as too difficult is not to be neglected as well. Our main finding is that different factors influence motivation for LEFL and demotivation. However, gender, the programme of study and the year of study

proved to be of no significance with respect to the level of demotivation.

The absence of gender differences is the finding which is consistent with some other studies on demotivation¹¹. The absence of differences in demotivation for SLA by the type of the programme of the study confirms our initial hypothesis that students from the Mass Communications Department can in general have the same level of demotivation for learning EFL as the students from the Maritime Department. The reasons may lie in the nature of the both studies. English in the maritime world is official language of the trade and may be viewed by majority of the students as instrumental, a tool for obtaining a job. This explanation could be also applied to the students from the Mass Communications Department as they will rely on their language skills in their future professional life within the European Union or worldwide. Finally, there were no statistically significant differences in demotivation factors for learning English as a foreign language in relation to the year of study. This fact confirms our initial hypothesis that the year of study is a relatively short time for any significant differences to be shown, about the level of demotivation for learning English as a foreign language. However, statistically significant correlations between demotivation and two types of motivation (pragmatic-communicative and affective) have been established. In our previous study¹⁴, female students demonstrated higher levels of motivation for learning English than male students. Moreover, regarding the motivational types and intensity, the programme of study played an important role as well, in the direction that students from the Mass Communication Department were significantly more motivated to learn English as FL, than students from the Maritime Department¹⁴. However, the year of study has

proven to be nonsignificant, with respect to the level of motivation¹⁴ or demotivation.

As discussed in our previous study¹⁴, the motivation for learning English largely depends on the students' awareness of the importance of English as the international language, as well as their pragmatic goals (employment, socialisation and integration into the global community). Hence, social factors at a more general level can affect motivation, such as the social dynamics or power relationship between the languages¹⁵. These factors could have similarly strong influence even on the factors of demotivation for learning FL.

The main benefit of this study is a fact that it is one of rare studies on demotivation for SFL learning in Croatia, especially in certain departments of the University of Dubrovnik. The shortcoming is purposeful and not so large sample of participants, with limited number of socio-demographic variables about the participants which are included in the poll. Having in mind that various learner attributes⁴ display a considerable amount of contextual and temporal variation (variation from time to time and from situation to situation), the authors believe the results from this research would best be compared and utilised in further research of demotivation in the Croatian classroom context, focusing on and studying other programmes of study at the University of Dubrovnik and other Croatian universities. Future studies should draw up on larger and more representative samples of student population to share more light on the student demotivation in learning EFL, with better balanced gender representation. Moreover, the teachers should be involved in the research, enabling thus the determination of best measures for reducing demotivation and increasing motivation of our students.

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DEMOTIVACIJA ZA UČENJE ENGLESKOG JEZIKA KOD SVEUČILIŠNIH STUDENATA

SAŽETAK

Teorije o ovladavanju inim jezikom (SLA) različito ocjenjuju značaj individualnih faktora u procesu usvajanja jezika. Motivacija, kao i demotivacija, jedan je od bitnih faktora. Neupitna je važnost uloge motivacije u dugotrajnome i zahtjevnome procesu učenja jezika. Glavni cilj ovoga rada jest utvrditi postoje li povezanosti između faktora demotivacije (DM) za učenje engleskoga kao stranoga jezika s faktorima motivacije (M), kao i s određenim relevantnim varijablama. Drugi cilj je utvrditi postojanje razlika između faktora demotivacije u odnosu na spol, godinu studija i smjer studija. U tu svrhu, istraživanje je provedeno među studentima trogodišnjih preddiplomskih studija na dva sveučilišna odjela: Pomorskom odjelu i Odjelu za komunikologiju. Podatci su prikupljeni pomoću Upitnika za mjerenje tipa i intenziteta motivacije hrvatskih učenika za učenje engleskoga kao stranog jezika. Rezultati pokazuju da nema statistički značajnih razlika u faktorima demotivacije u odnosu na spol, smjer i godinu studija. Međutim, utvrđena je statistički značajna, umjereno visoka i negativna povezanost uporabno-komunikacijske i afektivne motivacije s demotivacijom, dok su ostale korelacije između demotivacije i motivacije vrlo niske. Osim toga, statistički značajna negativna i niska korelacija faktora demotivacije utvrđena je samo za treću godinu studija. Rezultati ovoga istraživanja ukazuju na potrebu daljnjih istraživanja u različitim obrazovnim kontekstima kako bi se stekle spoznaje o demotivaciji za učenje engleskoga kao stranoga jezika.

Plural Pasts and Monolithic Present: Youth and Multicultural Heritage in Croatia

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ABSTRACT

The present paper seeks to explore the awareness and knowledge about past cultural diversity of young people in four Croatian cities (Pula, Rijeka, Zadar and Zagreb) with rich multicultural historical legacies. It is based on the results of a survey obtained from 631 high-school students in these cities. The survey focused on students' awareness and knowledge of plural cultural heritage in their respective cities and their ideas about what should be valued and how. It also included students' attitudes and beliefs about current diversity issues and intercultural values, which enabled the researchers to explore the association between their knowledge of heritage and their intercultural attitudes in more detail. The results obtained indicate that both the degree of awareness of past diversity and acceptance or rejection of intercultural values varied by specific spatial, historic and current socio-demographic contexts of the cities. While the overall findings suggest a comparatively strong association between heritage and intercultural values, some of them indicate that appreciation of past cultural diversity may also co-exist with current anti-multicultural attitudes. This has implications for education policy and strategies in facing the challenges of a decline in socio-political support for intercultural values in both Croatia and a wider European context.

Key words: *cultural heritage, multiculturalism, attitudes, youth, anthropology*

Introduction

In recent years, most European societies, and particularly the post-socialist countries of Europe, have witnessed a marked expansion of heritage as a field of discourse and practice. This concern for heritage preservation seems to be directly linked to globalisation processes and the perceived losses of traditional culture and lifeways, prompting societies to define themselves increasingly by their relationship to the past. At the same time, cultural pluralism, mutual recognition and tolerance are seen as important European values by which we should evaluate not only our present but also our past. While Europeanization processes have renewed scholarly interest in intercultural encounters and multilingualism and there is an increasing recognition that such current practices are not unprecedented, the official discourses and approaches are far away from accepting such processes as a historically-rooted phenomenon and part of common cultural heritage.

While there is a multiplicity of meanings and interpretations of heritage, most scholars agree that it denotes everything handed down to us from the past and infused

with present purposes.^{1,2} Critical heritage studies regard heritage as a social construction and a result of the process managed by the authorized heritage discourse that legitimizes certain experiences and identities. Although the value of cultural heritage is often seen as universal and eternal, Smith³ points to a very specific origin of such authorized heritage discourse, which goes back to the 19th century Western European upper-middle class ideology, and is thus »as much a discourse of nationalism and patriotism as it is of certain class experiences and social and aesthetic value«. Brett maintains that, heritage is a »contemporary form of popular history which cannot but be involved in pertinent questions about the nation, the state, the region, identity and culture«. ⁴According to Smith,³ the official selection of heritage is based on the authorized heritage discourse which views heritage as tangible, aesthetically pleasing material objects, sites, places or landscapes that are immutable, and which are interpreted within the canon of national myths and aimed at building the national identity. The authoritative power of this discourse emanates from relevant national institutions and international organizations, such as UNESCO. UNESCO

suggests that ‘Heritage is our legacy from the past, what we live with today, and what we pass on to future generations’,⁵ and it distinguishes between three types of heritage: cultural, divided into tangible and intangible, and natural heritage. The Convention for the Safeguarding of the Intangible Cultural Heritage defines ‘intangible cultural heritage’ as:

The practices, representations, expressions, knowledge, skills – as well as the instruments, objects, artefacts and cultural spaces associated therewith – that communities, groups and, in some cases, individuals, recognise as part of their cultural heritage. This intangible cultural heritage, transmitted from generation to generation, is constantly recreated by communities and groups in response to their environments, their interaction with nature and their history, and provides them with a sense of identity and continuity, thus promoting respect for cultural diversity and human creativity.⁶

As it can be seen from the above quote, intangible heritage is perceived as incorporating a wide range of non-material aspects, not only in the form of knowledge and practices but also as values, norms and belief systems. Accordingly some scholars even argue that all heritage is intangible as material or tangible heritage is not inherently valuable, but derives value and meaning from the present day cultural processes and activities that are undertaken around it.³ According to Graham et al.⁷ the term »heritage« has been recently broadened to such an extent that it includes almost any sort of inter-generational exchange or relationship, welcome or not, between societies as well as individuals.

The focus of heritage studies has recently shifted from the role heritage has in contemporary life on the actual processes that transform things, places, acts and experiences into heritage, especially having in mind that these processes can be »read« as a narrative of identity, politics and power.⁸ The rationale for taking such an approach lies in the belief that the selection, reproduction and consumption of heritage representations is never a fully transparent process. As a result of ideologically motivated processes of selection, heritage is partial and sometimes not true collection of the past chosen to be represented,⁸ but often one affected by nationalism, cultural elitism and social exclusion. Heritage is thus viewed as a process of meaning-making or »a mode of cultural production in the present that has a recourse to the past«.⁹ It is a set of practices involved in the construction and regulation of values, a discourse about negotiation, about using the past, and collective and individual memories, to negotiate new ways of being and to perform identities.³ People engage with heritage through »a process of communication and an act of making meaning in and for the present«.³ In the process of engagement, next generations may either appropriate certain set of past resources or contest their validity and even reject them.¹⁰ Both education of young generations and heritage practice need to »recognize and critically deal with issues of dissonance and the use of memory in the formation of heritage and identity«.³ The main mission of education is to preserve the past so that the young have

the cultural and intellectual resources they need to deal with the challenges of the future. Clearly, the process of meaning making resulting in either appropriation or contestation of past resources presupposes a body of knowledge, skills, and modes of expression which constitute the heritage of the cultivated classes.¹¹ For Bourdieu heritage is reproduced through education where under the assumption of common values between pupil and teacher the system acts to conceal its real function, namely, that of confirming and consequently legitimizing the right of the heirs to the cultural inheritance, and only when the heritage has taken over the inheritor can the inheritor take over the heritage.^{11,12} Education and knowledge are thus the preconditions for the appropriation of heritage by the inheritors in next generations that would enable them to draw on the insights from centuries of human experience in their engagement with both the present and the future.

Most European nations in response to the diversity caused by immigration strive to foster a strong national identity and the common core of the nation. In new nation-states, such as Croatia and other post-socialist countries, heritage is often perceived as a resource for promoting national sovereignty, and fostering the feelings of unified identity, belonging and continuity in both official discourses and school textbooks.¹ As argued by Penrose, such discourses are exclusionary as within newly empowered nation-states those »individuals and places which do not fit into the newly recognized nation’s self-construction will continue to be marginalized« or pressured »to conform to the definition«.¹³ In spite of declared values of inclusive democracy and multicultural education, by impressing upon children the values of the nation-state, schools play a critical role in its perpetuation by teaching a dominant historical narrative that celebrates the nation and contains dissonant aspects of the nation’s history that could undermine civic loyalty. During the past two decades, multicultural education has emerged as a vehicle for developing competencies to deal efficiently with such cultural processes and to raise multicultural awareness. This awareness might be defined as a set of beliefs and explanations that recognizes and values the importance of ethnic and cultural diversity in shaping lifestyles, social experiences, personal identities, and educational opportunities of individuals, groups, and nations.¹⁴ The formation of such beliefs depends on knowledge, that is never neutral but is influenced by human interests, and reflects the power and social relationships within society.

Urban multicultural heritage of Austria-Hungary

In view of the claim that the study of the past should inform our understanding of the present and expectations in the future, history education needs to focus on advancing students’ ability to construct meaningful and coherent narratives that have practical use for them, opening access to competing ideological and political ideas that offer different perspectives on the future.^{15,16} The restored memories of the multicultural past have the potential to

become such a resource for the negotiation and transformation of present values and identities and better orientation in time.¹⁷

The coexistence of regional, national and supranational allegiances in multi-ethnic and multilingual Austria-Hungary provides a proper context for the study of intercultural practices within the areas of Southeast Europe. The Empire was clearly a contact zone of migrants and travellers, where people drew on the practices of their various places of origin to organize social relations, and where contests over their multiple languages and cultural logics took place, along with intercultural dialogue. During the time of the Dual Monarchy, some towns in the region experienced vibrant urbanization, industrialization and modernization that facilitated mobility and immigration of military, civil servants, merchants, craftsmen, labourers and other professions from diverse parts of the Empire while better access to schooling and working possibilities attracted the inhabitants from the nearby villages. Thus, the town of Pula became Austria's main naval base and a major shipbuilding centre, Rijeka became a major commercial port of the Hungarian part of the Empire and developed several important industries. Zadar was the administrative capital of the province of Dalmatia, while Zagreb was the capital of Croatia and Slavonia. In addition to this, there were certain differences between the cities in terms of their political configuration: Zagreb as capital of Croatia and Slavonia continued to exercise autonomous powers although within the Kingdom of Hungary, Rijeka was governed directly from Budapest, while Pula and Zadar were part of the Austrian kingdom. From a demographic perspective, they all followed a relatively similar trajectory during the course of the 19th century and the beginning of the next century registering significant rates of population increase which was largely due to an extensive immigration.

At the beginning of the 20th century, all four cities transformed from small provincial towns into major urban centres characterized by a high degree of cultural, ethnic and linguistic diversity. This mixture of people was involved in transnational practices framed by variable political and discursive structures of power relations and a triple Italian, German and Hungarian hegemony. Nevertheless, cross-boundary interdiscursivity between national or non-national discourses was possible not only through migration, but also through the common use of multilingualism as a major form of communication. Croatian-Italian bilingualism was practiced in all three port cities, and the use of German in Zagreb was widespread. While today this legacy is visible in buildings or some monuments and coffee houses which remind us of the era of Austro-Hungary, its reproduction and appropriation is not straightforward. Due to wars and military conflicts and multiple regime changes, the ethnic composition of the cities and whole regions changed fundamentally. The cultural diversity of the cities either considerably diminished or vanished while generational memory has begun to fade into history making the representation of lost diversity more difficult.

The aim of this paper is to evaluate the attitudes of young people living in the four cities toward cultural diversity and cultural heritage with a focus on the legacy of Austria-Hungary. While analysing these various cities, we addressed the same set of questions considering that public appreciation of cultural heritage is closely tied to the value of heritage: What counts as 'heritage' for the young people? What is the role of heritage in terms of identity and cultural diversity? How is Austro-Hungarian heritage perceived and/or appropriated? Are there any indications that young people are able to overcome national narratives in favour of more cosmopolitan attitudes toward heritage and in their current life?

Methods

With the research questions in mind a quantitative survey was designed with a range of questions focusing on heritage and multiculturalism. The survey included one section of items to measure general knowledge and attitudes toward cultural heritage. It was aimed at students' perceived meaning of cultural heritage and its value for cultural identity and cultural diversity, learning history and tourism. A separate section of items measured these variables with respect to the specific case of the Austro-Hungarian heritage. The third sets of items measured students' general attitudes toward cultural diversity and intercultural practices.

The survey was administered to a sample of high-school students in their schools in four cities: Pula, Rijeka, Zadar and Zagreb. The sample included 631 students aged between 16–18 years (mean age 17.5). It is fairly well distributed across towns under study, with highest number of participants in Zagreb (36.8%) and the lowest in Rijeka (16.7%). Among students 51% were girls and 41% boys (Table 1). Most of the students were born in the town where they now attend school (87%), 3.5% of them were born in a wider town region, 5.2% were born in other places of Croatia, while 4.3% were born in other countries, mostly those that once made part of Yugoslavia. The highest number of students born in the town wider region was in Zadar, the highest number born in other parts of Croatia was in Pula and Zagreb, while the highest number of those born abroad was found in Rijeka (Table 2).

TABLE 1
DISTRIBUTION OF SAMPLE BY TOWN AND SEX

Town	Sex		
	Males	Females	Total
Pula	70	93	163
Rijeka	43	63	106
Zadar	48	82	130
Zagreb	98	134	232
Total	259	372	631

TABLE 2
DISTRIBUTION OF SAMPLE BY PLACE OF BIRTH

Town	Place of birth				Total
	Town county	Wider region	Croatia	Abroad	
	%				
Pula	89.4	0.6	6.8	3.1	100
Rijeka	82.1	2.8	4.7	10.3	100
Zadar	83.0	7.6	3.1	5.3	100
Zagreb	89.2	3.4	5.6	1.7	100
Total	87.0	3.5	5.2	4.3	100

TABLE 3
DISTRIBUTION OF SAMPLE BY SELF-DECLARED NATIONAL BELONGING

Town	National belonging			Total
	One nationality	Mixed	Undeclared	
	%			
Pula	64.42	28.22	7.36	100
Rijeka	66.98	24.53	8.49	100
Zadar	88.55	6.87	4.58	100
Zagreb	88.41	3.86	7.73	100
Total	78.67	14.22	7.11	100

One of the questions asked students about their national belonging. It offered students several possible answers: to declare their national belonging to one nationality (Croatian or otherwise), to express their belonging to two or more nationalities or to decline to declare their belonging to any nationality. The distribution of the sample by national belonging is given in Table 3. Overall, most of the total number of students declared that they belong to one nationality (mostly Croatian) (78.67%). A considerable number of students belonged to two or more nationalities (14.22%), while 7.11% declined to declare their nationality. As it can be seen from the table, there is an unequal distribution of different declarations of belonging across towns under study: the highest percentages of students affiliated with only one nationality were found in Zadar (88.55%) and Zagreb (88.41%), of those with mixed belonging in Pula and Rijeka, 28.22% and 24.23% respectively, and of those with undeclared nationality in Rijeka (8.49%). Such a distribution corresponds well to findings on national belonging in general population of the examined towns which indicate higher number of one-national Croatian belongings in Zadar and Zagreb (94.15% and 93.14%, respectively) and lower in Pula and Rijeka (70.14% and 82.52%, respectively).¹⁸

The survey responses were entered into a study database and analyzed through the use of SPSS (Statistical

Package for Social Sciences). Descriptive analyses were conducted for the overall sample. Frequencies and percentages were calculated for categorical variables, whereas means, standard deviations (SD), minimums, and maximums were calculated for Likert scale items and statistical analyses. Statistical significance testing was used throughout data to compare various sections of the sample (e.g. comparing different towns, males and females, by place of birth and by national belonging).

Results

Perceptions of what represents heritage

To gain a better understanding of the various perceptions the students have when it comes to heritage, respondents were firstly asked in an open question of the survey how they would define heritage. A majority of students define cultural heritage as something that we inherited from previous generations, using often the word tradition to explain it. Next, they were asked to identify features from an extensive list of both tangible and intangible types of heritage (including the built environment, folklore, languages, the cultural/lifestyle features) which according to them represent heritage. Figure 1 addresses this by identifying the features which are most commonly seen to be heritage by the students. It can be seen that most of the students chose monumental buildings and sites, excluding those that might be less appealing, like old industrial objects (factories, railway structures or bridges) or even cemeteries. On the other hand, the forms of intangible heritage chosen by the majority of students refer to traditional feasts and performances, songs and dances, and, interestingly, languages and dialects. Much lower percentage of students included lifestyle as a form of heritage, while only 6.6% of the students marked all types of heritage offered on the list.

For the majority of the students (74.41%), these ideas of a shared heritage are clearly linked to the cultural identity of their towns and contribute to their »place distinctiveness« or what makes the place identifiable and different. It may be said that specific types of heritage,

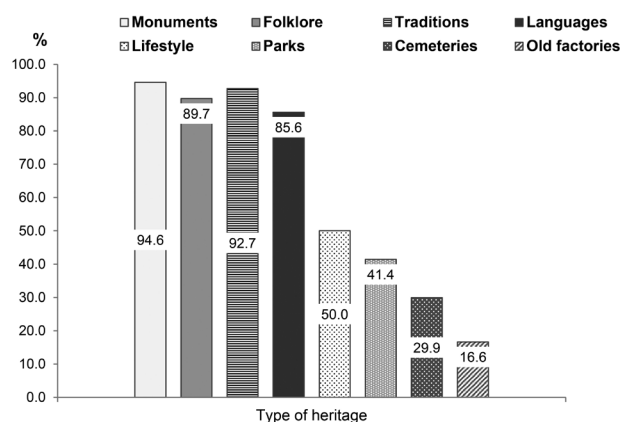


Fig. 1. Elements perceived as heritage by students.

Cultural heritage strengthens cultural identity

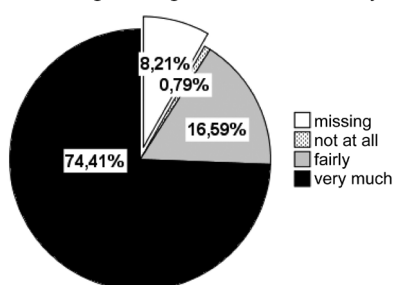


Fig. 2. Perceived importance of cultural heritage for the maintenance of cultural identity.

Cultural heritage contributes to cultural diversity

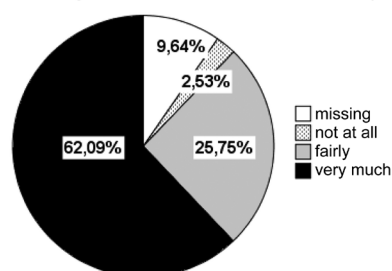


Fig. 3. Perceived contribution of cultural heritage to cultural diversity.

particularly in terms of more iconic landmarks, serve as shared points of reference and, are invested with symbolic power and shared meaning. This finding suggests that students give considerable importance to heritage and place as a means of anchoring and expressing their identities (Figure 2).

A somewhat lower number of students (62%) perceive heritage as contributing to cultural diversity, understood in terms of religion, ethnicity, gender, age, etc. This finding indicates a tendency to view heritage as a rather homogenous, unchangeable set of ideas, less affected by past migration processes (Figure 3).

To develop an understanding of the types of heritage by historical periods that the students consider important for the identity and cultural diversity of their towns, respondents were asked their views about each specific historical period. The results obtained are statistically significant by towns ($p < 0.1$) and they indicate that Austro-Hungarian period received high values in Zagreb, Rijeka and Pula as compared to Zadar, where students consider that heritages from Antiquity and Middle Ages are the most important markers of their town's identity. Interestingly, the students in Pula also regard heritage from Antiquity as most important, while Yugoslav socialist period turns out to be second by importance for cultural identity of Rijeka (Figure 4). Similar, statistically significant differences ($p < 0.1$) were obtained with respect to the students' perceived degree of contribution to current cultural diversity of their towns by historical periods. Again, the students in Pula and Rijeka consider the Austro-Hungarian period as the

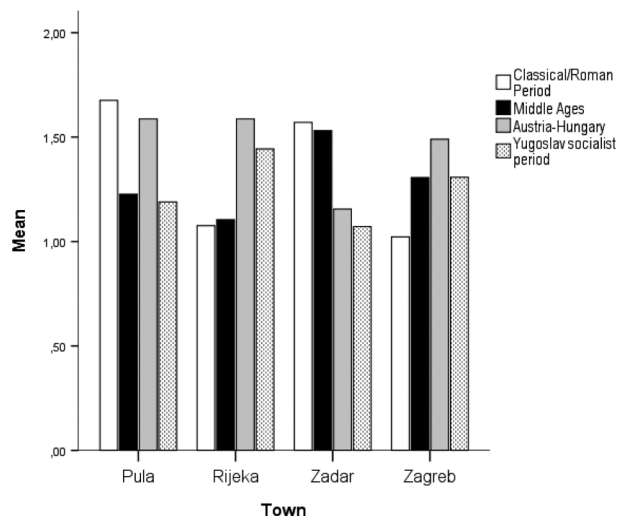


Fig. 4. Students'perceived importance of historical periods for current cultural identity of their town.

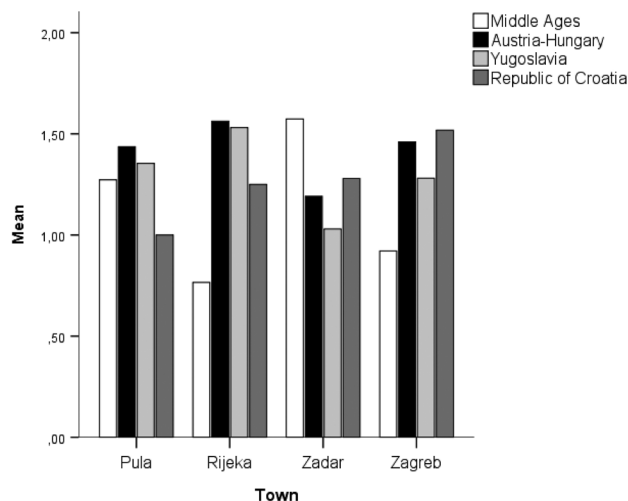


Fig. 5. Students'perceived degree of contribution to current cultural diversity of their towns by historical periods.

most important in terms of cultural diversity; those in Zadar regard the contribution of Antiquity as the most important while the students in Zagreb feel that the most recent period of the Republic of Croatia has brought most of cultural diversity to their town (Figure 5).

Attitudes toward Austro-Hungarian heritage

The next step in the analysis was directed toward the students' views of the multicultural legacy of the Austro-Hungarian Empire in terms of both cultural diversity and its symbolic value for the cultural identity of their towns. Table 4 shows the proportion of the students who think that Austro-Hungarian heritage is important for their respective towns. The findings reveal that only 46% of the students consider this part of the history as relevant con-

TABLE 4
PERCEIVED IMPORTANCE OF AUSTRO-HUNGARIAN
HERITAGE

Town	Do you think Austro-Hungarian heritage is important for your town?		
	No	Yes	Missing
	%		
Pula	9.8	67.5	22.7
Rijeka	18.9	53.8	27.3
Zadar	30.5	20.6	48.9
Zagreb	19.7	42.1	38.2
Total	19.3	46.1	34.6

tribution to their town's cultural diversity and part of its identity. While 19.3% of the students do not perceive at all the Austro-Hungarian legacy as part of the heritage, even 34.6% did not write anything to this question which indicates that they do not know whether this heritage is important or not for their towns (Table 4).

It was also imperative to determine how the students themselves self-evaluate their knowledge on and familiarity with Austria-Hungary. Figure 6 shows the proportion of the students who feel they have a good level of knowledge about this heritage. As it can be seen, only 9.4% of the students feel they have a good understanding of what this heritage means. A high 66.3% of the students admit that their knowledge about the Austro-Hungarian period is poor, while about 23% of the students are not sure about that. This level of understanding is similar across the sample, with no significant differences across towns under study. With that in mind, perceived understanding is notably higher amongst those from Pula and Rijeka, suggesting that this type of heritage is potentially more salient for these communities.

When we questioned their interest in Austria-Hungary, as many as 43% of the students claim that they are not at all interested in learning about this period as compared to 23% of those who are interested in this part of history, while even 33% of the students could not decide about that. This clearly indicates that both formal and informal education have not been efficient in transmitting the knowledge of this relevant part of multicultural heritage to the students (Figure 7). The distribution of findings related to the lack of knowledge about and interest in this part of heritage again showed that it is less pronounced in the towns of Pula and Rijeka, indicating that some more efficient forms of multicultural education are at work in these towns in terms of raising awareness among students about past multicultural experiences.

The analysis of attitudes toward specific positive (multilingualism, ethnic diversity, tolerance) and negative features (economic poverty, problematic ethnic relations, the prison of nations) of the Austro-Hungarian heritage performed by towns under study again showed variable distribution of findings. For the analysis the responses were

My knowledge about Austria-Hungary is poor

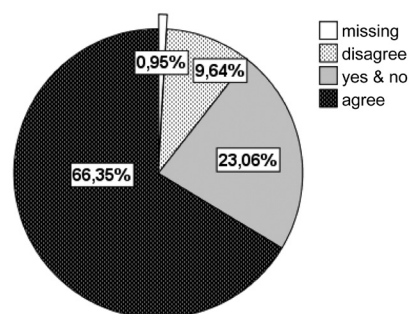


Fig. 6. Students' reported lack of knowledge about Austro-Hungarian heritage.

I am interested in Austria-Hungary

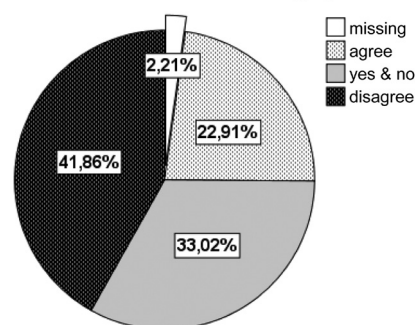


Fig. 7. Students' reported degree of interest in Austro-Hungarian heritage.

aggregated into two composite variables denoting positive (P2) and negative attitudes (N2) toward Austria-Hungary, and their mean values are shown in Figure 8. It can be seen from the figure that the overall negative attitudes are somewhat higher than the overall positive attitudes toward Austria-Hungary. However, although the overall findings indicate that the students do not think that it would be better if the Habsburg monarchy did not dissolve, as expected, more positive views of that part of history can be seen for the towns of Pula and Rijeka, as compared to Zadar and Zagreb (Figure 8). The findings are statistically significant by town and sex, with somewhat less pronounced negative attitudes of female students. As indicated by previous data shown in Table 4 and Figures 6 and 7, these findings are partly due to the lack of knowledge about this part of history.

Attitudes toward cultural diversity and multiculturalism

The analysis of students' general attitudes toward diversity shows that overall, a majority of students have a more positive than negative view of various forms of diversity, but there are hints of a concerned minority that is supportive of diversity with some important reservations. For the analysis the responses were aggregated into two composite variables denoting positive (P1) and negative

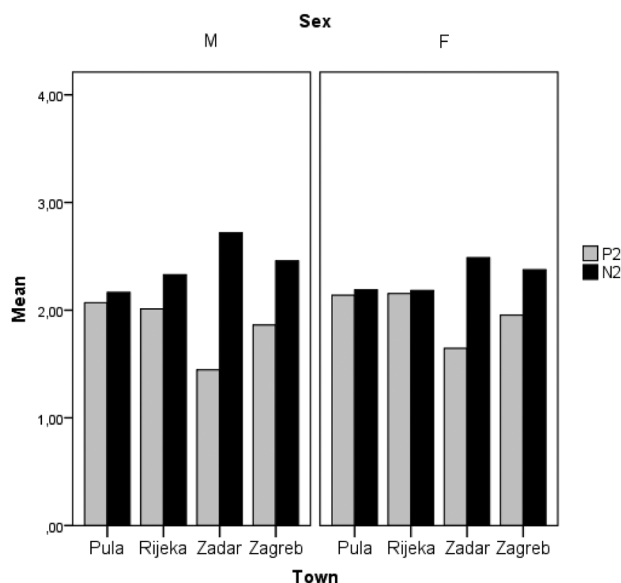


Fig. 8. Students' positive (P2) and negative (N2) attitudes towards Austro-Hungarian heritage by town and sex.

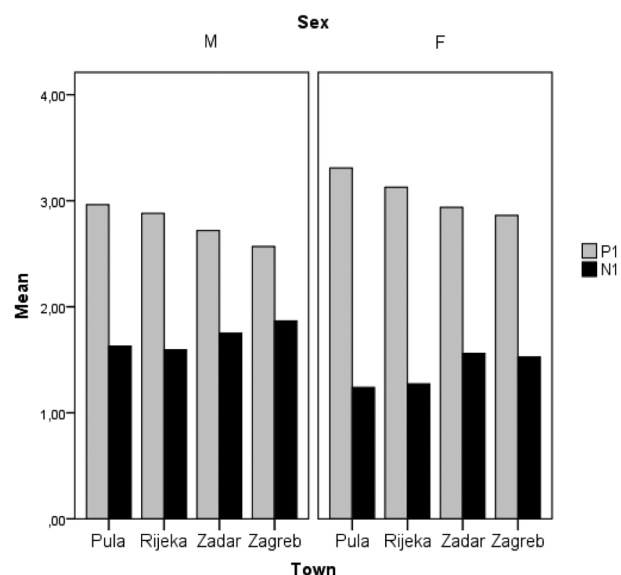


Fig. 9. Students' positive (P1) and negative (N1) attitudes towards cultural diversity by town and sex.

attitudes (N1), and their mean values are shown in Figure 9. A more detail examination of the students' positive attitudes toward cultural diversity reveals that overall most of the students are generally interested in meeting other cultures (89%) and would agree that ethnicity or nationality are less important in defining persons than their personal qualities (92%). However, a lower number of students are happy to see other ethnicities living in their town (60%). The lowest number of students think that the presence of different ethnicities enrich their town and country (35%), while in terms of intercultural contact students are less inclined to make friends with people belong-

ing to other ethnicities (48%). Statistically significant differences between examined towns reveal that a more substantial appreciation of cultural diversity and positive attitude toward intercultural contact are shown by youth in the towns of Pula and Rijeka in comparison to those from Zadar and Zagreb (Figure 9).

These findings are confirmed also by the analysis of negative attitudes toward cultural diversity which shows a similar overall distribution across towns. Although the overall values, as shown previously, are lower than those for positive attitudes, still more than 32% of students state that they are more comfortable with people of their own nationality and culture, while over 22% of the students think that immigrants of other ethnicities endanger their culture and their lifestyle. Again, the distribution varies across towns revealing that students from Pula and Rijeka are less inclined toward such attitudes than those from Zagreb and Zadar. Across these various questions, support for diversity is consistently higher and negative values lower among female students, although this tends to be a question of degree rather than of a completely different viewpoint (Figure 9).

Further analyses of students' attitudes towards cultural diversity indicated statistically significant differences by place of birth and by national belonging ($p < 0.1$). The support for diversity is significantly higher for the students born in other countries and those that immigrated to the towns under study from other parts of Croatia (Figure 10). As to the findings by national belonging, significantly higher appreciation of diversity is linked to students with mixed national belonging and those that decline to declare their nationality, while more negative attitudes toward diversity are found among students belonging to only one nationality (Figure 11).

To examine possible associations between the appreciation of the Austro-Hungarian heritage and general stu-

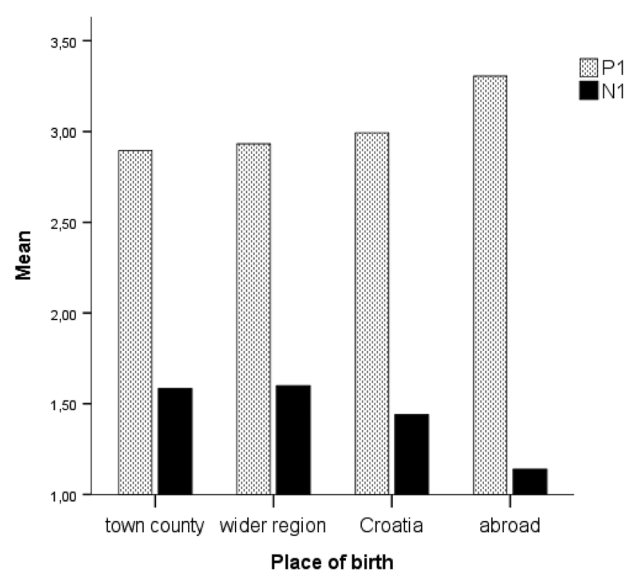


Fig. 10. Distribution of students' positive (P1) and negative (N1) attitudes towards cultural diversity by place of birth.

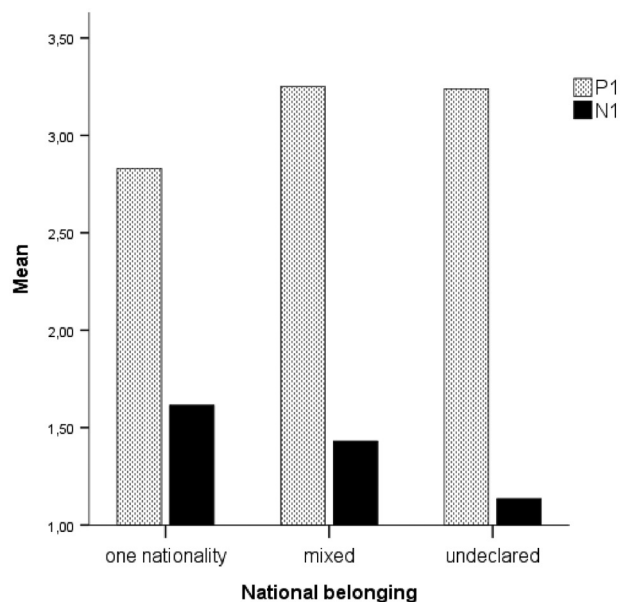


Fig. 11. Distribution of students' positive (P1) and negative (N1) attitudes towards cultural diversity by national belonging.

dents' attitudes toward cultural diversity as well as other variables the nonparametric Spearman correlation analysis was performed. As shown in Table 5, highly significant correlations were found between town, sex, place of birth and national belonging and attitudes toward cultural diversity. Importantly, a highly significant positive correlation was also found between positive attitudes toward Austro-Hungarian multicultural heritage and positive attitudes toward cultural diversity, while negative attitudes toward Austro-Hungarian heritage and lack of interest in this type of heritage were significantly and negatively associated with positive attitudes toward cul-

tural diversity. These findings were further confirmed by the analysis of mean values of positive and negative attitudes towards Austro-Hungarian heritage and towards cultural diversity across towns under study. Table 6 shows statistically significant differences between all towns for both types of attitudes. In terms of the appreciation of Austro-Hungarian attitudes significant differences were found between all towns except for Pula and Rijeka. As to attitudes toward cultural diversity there are significant differences between Pula and both Zadar and Zagreb, and between Rijeka and Zagreb. The findings of correlation analysis and of mean values across towns both indicate a strong association between the knowledge and appreciation of Austro-Hungarian heritage and general students attitudes toward cultural diversity and multiculturalism.

Discussion and conclusions

The analyses performed show that the students' definition of cultural heritage is related to selective processes at both general typological level and at the level of the origin of the cultural heritage, which is more linked to identification processes. The ideas of a shared heritage mostly focused on the tangible forms of historic built environment, and aesthetically pleasing material objects as the most visible sign of a link with the past. These findings fit what Smith³ calls the authorized heritage discourse referring to »a dominant Western discourse about heritage that works to naturalize a range of assumptions about the nature and meaning of heritage«, i.e. defines what heritage is, and what it is not. It is a »discourse which is concerned with the negotiation and regulation of social meanings and practices associated with the creation and recreation of 'identity'«, which is selective and exclusionary in nature. It is focused mainly on tangible heritage, which it defines as national treasure that people use to articulate common identity, especially national.³

TABLE 5
NONPARAMETRIC CORRELATION COEFFICIENTS FOR ATTITUDES TOWARDS CULTURAL DIVERSITY

Variables	Positive attitudes towards cultural diversity		Negative attitudes towards cultural diversity	
	SPEARMAN'S CORRELATION COEFFICIENTSa			
	Correlation coefficient	Sig. (2-tailed)	Correlation coefficient	Sig. (2-tailed)
Town	-.278**	.000	.159**	.000
Sex	.213**	.000	-.190**	.000
Place of birth	.105**	.008	-.075	.061
Nationality	.293**	.000	-.160	.000
Positive attitudes towards AH multicultural heritage	.218**	.000	-.117**	.003
Negative attitudes towards AH multicultural heritage	-.138**	.001	.066	.096
Lack of knowledge about AH	-.035	.375	-.005	.894
Lack of interest in AH	-.159**	.000	.066	.102

**Correlation is significant at the 0.01 level.

^a N= 611

TABLE 6
MULTIPLE MEAN COMPARISONS BETWEEN TOWNS (BONFERRONI TEST)

Dependent variable		Positive attitudes towards diversity (P1)			Negative attitudes towards diversity (N1)			Positive attitudes towards AH heritage (P2)			Negative attitudes towards AH heritage (N1)		
(I) Town	(J) Town	Mean Difference (I-J)	Std. Error	Sig.	Mean Difference (I-J)	Std. Error	Sig.	Mean Difference (I-J)	Std. Error	Sig.	Mean Difference (I-J)	Std. Error	Sig.
Pula	Rijeka	.13244	.07098	.375	.00348	.09265	1.000	.01195	.06980	1.000	-.06226	.06452	1.000
	Zadar	.31686*	.06657	.000	-.23854*	.08690	.037	.54723*	.06547	.000	-.40114*	.06051	.000
	Zagreb	.42286*	.05800	.000	-.26655*	.07572	.003	.18679*	.05705	.007	-.22760*	.05273	.000
Rijeka	Pula	-.13244	.07098	.375	-.00348	.09265	1.000	-.01195	.06980	1.000	.06226	.06452	1.000
	Zadar	.18442	.07421	.079	-.24202	.09687	.076	.53528*	.07298	.000	-.33888*	.06746	.000
	Zagreb	.29042*	.06663	.000	-.27003*	.08698	.012	.17485*	.06553	.047	-.16534*	.06058	.039
Zadar	Pula	-.31686*	.06657	.000	.23854*	.08690	.037	-.54723*	.06547	.000	.40114*	.06051	.000
	Rijeka	-.18442	.07421	.079	.24202	.09687	.076	-.53528*	.07298	.000	.33888*	.06746	.000
	Zagreb	.10600	.06191	.524	-.02801	.08082	1.000	-.36043*	.06089	.000	.17354*	.05628	.013
Zagreb	Pula	-.42286*	.05800	.000	.26655*	.07572	.003	-.18679*	.05705	.007	.22760*	.05273	.000
	Rijeka	-.29042*	.06663	.000	.27003*	.08698	.012	-.17485*	.06553	.047	.16534*	.06058	.039
	Zadar	-.10600	.06191	.524	.02801	.08082	1.000	.36043*	.06089	.000	-.17354*	.05628	.013

*. The mean difference is significant at the 0.01 level; 95% Confidence Interval

As regards the students' views on the links between cultural heritage, diversity and identity, the findings indicate a high degree of general appreciation of cultural diversity and of heritage as a marker of cultural identity. However, at the same time they also indicate ambivalent attitudes toward intercultural practices and some dimensions of diversity in terms of presence of other ethnicities. The specific findings on the attitudes toward Austro-Hungary as an important multicultural resource from the past contributing in a desirable way to the cultural diversity of the towns in question and their identities reveal clearly ambivalent attitudes about its symbolic value. This can be partly explained by a rather low level of awareness and knowledge about this period of history and multicultural legacy of Austria-Hungary.

The obtained differences between the towns indicate that recent dynamic immigration history and a long-term presence of other ethnicities contributes to positive appreciation of diversity and intercultural communication. The cities differ in history, size and geographic location, in the scale of demographic changes, and in the extent of the material destruction they suffered in the twentieth century. The findings might also be explained by the specific historical contexts of Pula and Rijeka and political configuration. Pula, as part of Istria, is characterized by the long-term co-existence with the Italian minority, supported also by the current official policy of bilingualism in Istria. Also, both Pula and Rijeka experienced considerable immigration from other parts of ex-Yugoslavia after the Second World War, as well as during the homeland war in the 90s, when they were less exposed to direct involvement in the war than Zagreb and particularly Zadar.

The results also point to possible influences on youth attitudes as a consequence of the effects of national politics on one hand, and globalization and Europeanization processes on the other, on local politics of memory within the cities in question. Memories of multicultural past in cities like Pula and Rijeka are considered as commodities for sale in tourism, and often as tools used by the local political elites to display that they were complying with the values promoted by the EU, so that it is not possible to measure to what extent differences in positive attitudes toward diversity represent pure performance and conformity and how much they reflect the real internalisation of these values.¹⁷

The lack of knowledge of potential past multicultural resources shown by the students has clear implications for multicultural education. As noted by some scholars, transformative academic education requires active inquiry to discover and include knowledge and perspectives that have previously been ignored or suppressed.¹⁹ This is the only way to help students with historical knowledge construction and the development of an inclusive concept of heritage which would acquaint young people with the legacy of the past as a resource to deal with current and future challenges.

Acknowledgement

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PLURALNA PROŠLOST I MONOLITNA SADAŠNJOST: MLADI I MULTIKULTURNA BAŠTINA U HRVATSKOJ

SAŽETAK

Rad istražuje stupanj informiranosti i znanja mladih o kulturnoj raznolikosti i interkulturalnim procesima u prošlosti u četiri hrvatska grada (Puli, Rijeci, Zadru i Zagrebu) sa bogatim multikulturalnim nasljedom. Temelji se na rezultatima upitnika 631 učenika i učenica srednjih škola u navedenim gradovima. Upitnik je bio usmjeren na informiranost i znanje učenika o pluralnom kulturnom nasljedu njihovih gradova i njihove stavove prema tom nasljedu. Također je uključivao i pitanja o stavovima učenika prema interkulturalnim vrijednostima i suvremenoj kulturnoj raznolikosti kako bi se istražila potencijalna povezanost između znanja o multikulturalnoj baštini i interkulturalnih vrijednosti. Dobiveni rezultati pokazuju statistički značajne razlike među ispitivanim gradovima u znanju o multikulturalnoj baštini i prihvaćanju ili odbacivanju interkulturalnih vrijednosti koje su povezane sa specifičnim prostornim, povijesnim i suvremenim socio-demografskim okolnostima pojedinih gradova. Uz utvrđenu općenito značajnu povezanost između znanja o baštini i interkulturalnih vrijednosti, neki rezultati pokazuju da poznavanje kulturne raznolikosti u prošlosti može supostojati s negativnim stavovima o multikulturalnosti. Ovi pokazatelji važni su za politiku obrazovanja i razvoj strategija u suočavanju sa sve izraženijim opadanjem socio-političke podrške multikulturalnim i interkulturalnim vrijednostima, kako u Hrvatskoj tako i u širem europskom kontekstu.

Unexpected Systemic Amyloidosis in Patient With Restrictive Cardiomyopathy

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ABSTRACT

Systemic amyloidosis is a rare disease and it is caused by deposition of insoluble abnormal fibrillary proteins known as amyloid in extracellular space. Protein deposits are components of immunoglobulins produced by plasma cells and B-lymphocytes in response to antigenic stimulation. Three different forms of amyloidosis can be distinguished, the primary (AL), secondary (AA) and family form. Localized amyloidosis occurs in the pancreas in type 2 diabetic patients and in patients on chronic hemodialysis. The clinical presentation in amyloidosis depends on the affected organs. Symptoms are non-specific and included fatigue, weight loss, nephrotic syndrome, restrictive cardiomyopathy with thickening of the interventricular septum and ventricular wall, peripheral neuropathy, hepatomegaly, macroglossia, purpura and bleeding diathesis. In these article we presented the unexpected systemic amyloidosis in a patient with restrictive cardiomyopathy and without malignant and hematological diseases who suffered from dyspnea, weakness, fatigue loss of appetite and cough. Diagnosis of systemic amyloidosis was made upon histological samples after autopsy.

Key words: amyloid, systemic amyloidosis, gastrointestinal tract, cardiomyopathy, kidney failure

Introduction

Systemic amyloidosis is a rare disease and it is caused by deposition of insoluble abnormal fibrillary proteins known as amyloid in extracellular space¹. Three different forms of amyloidosis can be distinguished, the primary (AL), secondary (AA) and family form. Localized amyloidosis occurs in the pancreas in type 2 diabetic patients and in patients on chronic hemodialysis^{1–4}.

This is a disease of older adults and the median age at diagnosis is about 60 years.

The clinical presentation in amyloidosis depends on the affected organs and the symptoms are usually nonspecific.

Case presentation

A 64 years old female presented to the University Hospital with two month history of progressive dyspnea, weakness, fatigue loss of appetite and cough. The patient had a past medical history of heart failure with pericardial and pleural effusion two months prior to presentation. The patient developed weakness, progressive dyspnea and a cough. The cardiological outpatient diagnosis and treatment indicated a progressive heart failure. MSCT and MR showed ascites and calcified lymph nodes in abdomen.

Myelogram was normal. Complete gastroenterology exam showed no signs of malignancy. Clinical condition of the patient was bad and despite all measures, parenteral diet and supporting therapy patient died of cardiac failure and malnutrition.

Autopsy showed ascites, pleural and pericardial effusion, pulmonary edema, dilated and increased heart, pancreas with fibrosis and calcification of the surrounding fatty tissue. Abdominal lymph nodes were enlarged and calcified. The small and large intestine were edematous. Histological examination showed amyloid deposits and thickening of the wall of the blood vessels in the myocard, thyroid gland, liver (Figure 1, 2 and 3), kidneys and lungs. Amyloid deposits were found in the wall of the submucosal blood vessels in the stomach, small and large intestine (Figure 4, 5 and 6). The pancreas was partially preserved, partially necrotic. The surrounding adipose tissue was necrotic with calcification and infiltrated by lymphocytes

Discussion

Amyloidosis is rare disease with incidence of approximately 6–10 case per milion persons a year. It is characterized by the extracellular deposition of abnormal fibrillar protein, which disrupts tissue structure and function.

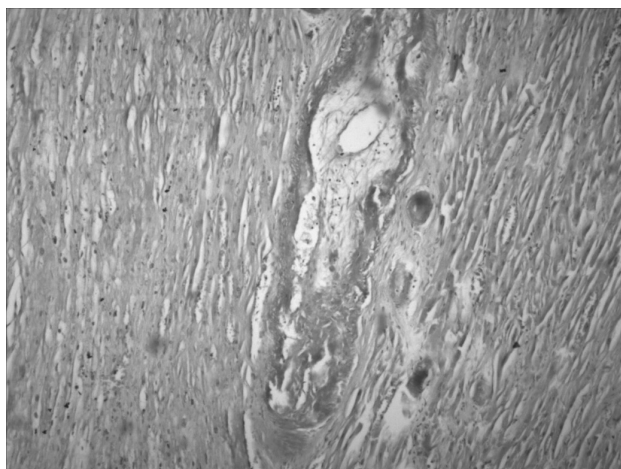


Fig. 1. Deposit of the amorphous eosinophilic material in wall of the heart blood vessels. HE 40x.

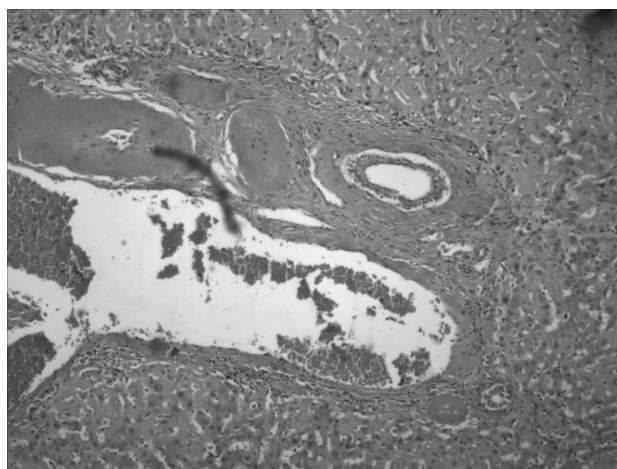


Fig. 3. Deposit of the amorphous eosinophilic material in wall of the liver blood vessels. HE 40x.

There are six types of amyloidosis: primary, secondary, hemodialysis-related, hereditary, senile and localized¹.

The most commonly affected organs are the heart and kidneys while the GI tract is rarely affected, but this can occur in primary and secondary form of the amyloidosis. The amyloid deposits in the GI tract are associated with the diarrhea, anorexia, weight loss, nausea and vomiting, dyspepsia, hemorrhage, steatorrhea or constipation^{5,6}.

Amyloid deposits in the stomach wall occur in less than 10% of patients. Endoscopic examination may show thickened gastric folds, gastric ulcers, hematomas, granular appearing mucosa and plaque-like lesion. Histological examination reveals amyloid deposition on the muscularis mucosa, submucosal vessels and muscularis propria in almost all areas of the gastric wall²⁻⁴.

Amyloid deposition in the GI tract is most prominent in the small intestine. Amyloid deposition is seen in the mucosa, the submucosal connective tissue, the muscularis

mucosa, and muscularis propria, within nerves and mostly within blood vessel walls. With increased deposition, a variety of symptoms, such as bleeding, motility disorders, and malabsorption, may occur and deposition within blood vessel walls may lead to bowel ischemia, mucosal atrophy and ulceration, and eventually perforation²⁻⁶.

Amyloidosis of the large intestine must be differentiated from inflammatory bowel disease, ischemic colitis, malignant diseases and collagenopathies. The descending and rectosigmoid colon are mostly involved. Endoscopic examination may show polypoid lesions, ulceration and petechial lesions. The symptoms include motility disorders or pseudoobstruction, acute obstruction, rectal bleeding and colonic dilatation^{2,7}.

In autopsy series, 56–95% of patients with amyloidosis had liver involvement. Clinical manifestations of hepatic amyloidosis are usually mild and included hepatomegaly with elevated alkaline phosphatase level, ascites which is

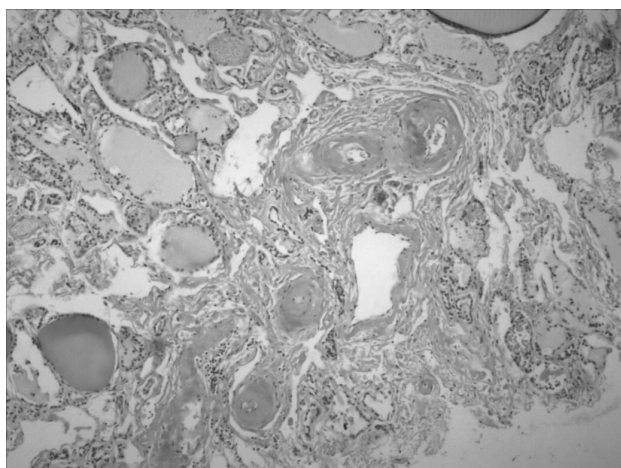


Fig. 2. Deposit of the amorphous eosinophilic material in wall of the thyroid gland blood vessels. HE 40x.

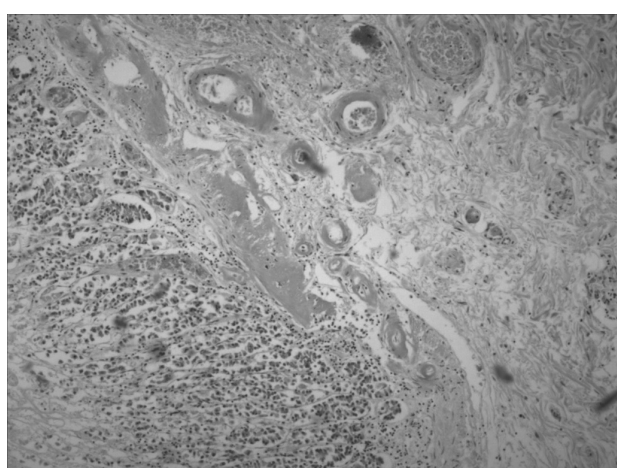


Fig. 4. Deposit of the amorphous eosinophilic material in wall of the stomach submucosal blood vessels. HE 40x.

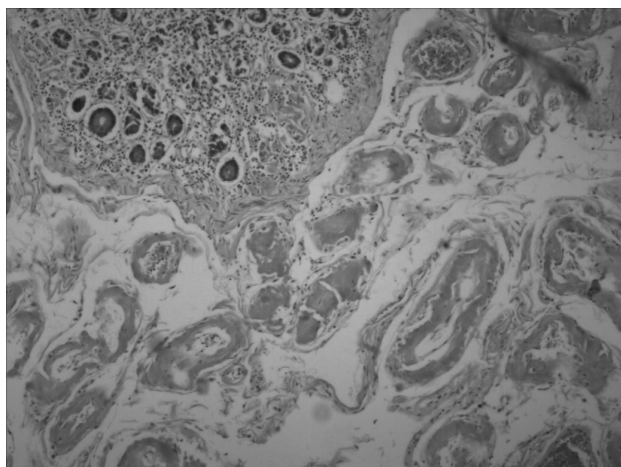


Fig. 5. Deposit of the amorphous eosinophilic material in wall of the small intestine submucosal blood vessels. HE 40x.

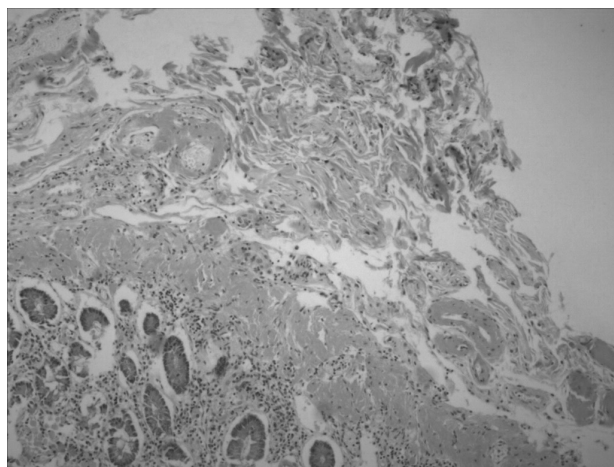


Fig. 6. Deposit of the amorphous eosinophilic material in wall of the large intestine submucosal blood vessels. HE 40x.

often due to cardiac failure and hypoalbuminemia and hyperbilirubinemia. Disposal of amyloid is periportal, leads to atrophy of hepatocytes, and when blocking sinusoids, portal hypertension occurs. The safety of liver biopsies is controversial and bleeding is the most common complication.⁸

Cardiac amyloidosis is characterized by amyloid deposits in the ventricles and atria, perivascularly, as well as in the conductive system. The heart amyloidosis leads to infiltrative / restrictive cardiomyopathy. Amyloidosis of the heart often coexists with significant dysfunction of other major organs and the presence of cardiac amyloidosis is often the worst prognostic factor^{9,10}.

Conclusion

In this article we presented the case of a patient who has been treated for a number of months by progressive heart failure and with non-specific symptoms by the GI tract. Clinical examination did not detect the cause of heart failure and progressive loss of weight and malnutrition. The patient died because of the multi-organ failure. The diagnosis of systemic amyloidosis was performed by examining samples obtained during autopsy. Systemic amyloidosis should be considered in patients with cardiomyopathy, proteinuria, hepatomegaly, and different and non-specific symptoms of GI tracts without specific endoscopic findings.

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NEOČEKIVANA SISTEMSKA AMILOIDOZA KOD BOLESNICE S RESTRIKTIVNOM KARDIOMIOPATIJOM:

SAŽETAK

Sistemska amiloidoza je rijetka bolest koju uzrokuje taloženje netopivih abnormalnih fibrilarnih proteina poznatih kao amiloid u izvanstaničnom prostoru. Proteinske naslage su komponente imunoglobulina koje proizvode plazmastanice i B-limfociti kao odgovor na antigensku stimulaciju. Razlikujemo tri osnovna oblika amiloidoze, primarni (AL), sekundarni (AA) i obiteljski oblik. Lokalizirani oblik amiloidoze javlja se u gušterači kod bolesnika s dijabetesom tipa 2 i kod pacijenata s kroničnom hemodijalizom. Klinička slika amiloidoze ovisi o zahvaćenim organima. Simptomi su nespecifični i uključuju umor, gubitak težine, nefrotski sindrom, restriktivnu kardiomiopatiju sa zadebljanjem interventrikularnog septuma i stjenke lijeve klijetke, periferne neuropatije, hepatomegaliju, makroglosiju, purpuru i dijatezu. U ovom radu prikazali smo neočekivani nalaz sistemske amiloidoze kod pacijentice s restriktivnom kardiomiopatijom i dispnejom, gubitkom apetita, kašljem i izraženom slabošću ali bez znakova malignih i hematoloških bolesti. Dijagnoza sistemske amiloidoze postavljena je na histološkim uzorcima nakon obdukcije.

From Bacteriophage to Antibiotics and Back

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ABSTRACT

Life is a phenomenon, and evolution has given it countless forms and possibilities of survival and formation. Today, almost all relationship mechanisms between humans who are at the top of the evolutionary ladder, and microorganisms which are at its bottom are known. Preserving health or life is not just an instinctive response to threat anymore; rather it is a deliberate action and use of knowledge. During major epidemics and wars which create great suffering, experiences of the man-disease (cause) relationships were examined, so we can note the use of bacteriophages in Poland and Russia before and during the Second World War, while almost at the same time antibiotic therapy was introduced. Since bacteriophages »tracked« the evolution of bacteria, the mechanism of their action lies in the prokaryotic cell, and it is not dangerous for the eukaryotic cell of human parenchyma. It is, therefore, necessary only to reuse these experiences nowadays when we are convinced that bacteria have an inexhaustible genetic and phenotypic resistance mechanism of their own. Antibiotics continue to represent the foundation of health preservation, but now they work together with specific viruses – bacteriophages that we can produce and apply in the context of multi-resistance, as well as for the preparation of new pharmacological preparations. This new approach promises knowledge and possibilities for a new antibiotic-bacteriophage model.

Key words: antibiotics, antibiotic resistance, bacteriophage, historical overview

History of Phage Therapy

During the cholera epidemics in 19th century India, around 20 million people lost their lives. Those who drank water or swam in the Ganges rarely contracted cholera¹. Hindu ritual baths take place in the Ganges River which, due to the monsoons, contains large quantities of phages from the ground. Although in the Hindu world the purpose of these baths is a physical and spiritual purification, their primary effect is therapeutic. In 1896, British bacteriologist Ernest Hanbury Hankin showed that water from the Ganges and Yamuna rivers contain some biological substance which destroys cholera cultures. It could pass through the Millipore filters which retained larger microorganisms such as bacteria². Two years later, Russian microbiologist Gamaley noticed a similar phenomenon³. However, it was not until 20 years later that Frederick Twort confirmed that the substance which passes through the filter can completely break bacteria, but cannot grow in their absence⁴. Twort described it as a substance secreted by microorganisms for a purpose unknown at the time^{5,6}. Twort's results were published in 1915 in The Lancet journal which is why the discovery of bacteriophage is

connected with his name⁷. Twort's work in The Lancet reminded Felix d'Herelle of his previous research conducted in Mexico and Tunis on treating the plague-infected locusts⁸. D'Herelle conducted the first application of bacteriophage as medication on soldiers who suffered from bacillary dysentery^{6,9,10}. Even the name 'bacteriophage' was suggested by d'Herelle, a combination of the word 'bacteria' and the Greek 'phagein' meaning to eat. D'Herelle believed that bacteriophages are viruses which destroy bacteria. His contribution is also evident due to the foundation of several centers for treatment via bacteriophage¹¹. For his idea of treating humans and animals against various bacterial diseases with bacteriophages, he was nominated for a Nobel Prize eight times but was sadly never awarded⁸.

Although d'Herelle's treatments of patients with bacteriophages conducted in a hospital in Paris were very successful, they were not immediately published¹². The first published study of bacteriophage treatment dates from 1921 when Richard Bruynoghe and Joseph Maisin announced that they used bacteriophage in the treatment of staphylococcal skin infection¹³. The treatment's positive results provided an incentive for additional research. All

of this also prompted a few companies to start producing phages commercially and to continue to use them to treat various bacterial infections. D'Herelle's laboratory situated in Paris produced five different phage preparations whose purpose was to treat bacterial infections⁶. In Poland, which also leads the way in bacteriophage research and application to treat humans today, the firsts cases of phage therapy were recorded in 1926/1927 at the Jagiellonian University Medical College Clinic of Surgery in Krakow^{10,14}. Despite criticism, research was continued at an institute in Wrocław, today known as The Institute of Immunology and Experimental Therapy PAS in Wrocław¹⁵. The Soviet Union used phage therapy during the Second World War when it was effectively used to treat soldiers^{8,16}. Since then in Georgia, a former Soviet Union Republic, phages have been used intensively and with some successful results at the George Eliava Institute in Tbilisi, Georgia. Currently, the Institute has accomplished impressive results in the treatment of wounds and bedsores, ulcers, burns, and purulent osteomyelitis. Phage preparations can also be bought in their pharmacy and used to treat infections caused by *Streptococcus*, *Escherichia coli*, and *Pseudomonas*^{17–19}. Phage intended for therapeutic treatments were also produced in the United States of America. In 1940, the Eli Lilly and Co. company produced seven phage preparations intended for human use^{6,20}.

The majority of early bacteriophage research was based on preventing bacterial infections¹⁰ and were conducted in the former Soviet Union or Poland. It was not until the end of the 1980s that bacteriophages were discovered 'again' and research on animals started^{21,22}. Research on humans began in the 2000s. Results of the first phase of randomized clinical research carried out with bacteriophage in the USA were published in 2009²³.

Emergence of Antibiotics

By imitating the relations in microbiocenosis, complex pharmaceutical products were created – drugs that in very small doses and concentrations suppress or destroy pathogens in humans and animals. They are called antibiotics, and their mechanisms of action includes: damaging the cell wall, disabling production of essential bacterial protein, blocking transporting cell wall's mechanism, blocking cell reproduction, blocking bacterial enzymes, etc.^{24,25}. Antibiotics are drugs that are selectively toxic to bacteria, and nontoxic or acceptable toxic for the host's organism.

The founder of modern antimicrobial therapy is Alexander Fleming who in 1928 published his research on growth inhibition of *Staphylococcus aureus* on a feeder contaminated with molds *Penicillium notatum*. The discovery of penicillin was followed by the discovery of sulphonamide in 1932, streptomycin in 1943, chloramphenicol in 1946, erythromycin in 1948, vancomycin in 1953, rifampin in 1957, ciprofloxacin in 1961, daptomycin in 1986, bedaquiline in 1997²⁴.

More than 4000 antibiotics were isolated from microbial sources, and more than 30000 synthetic and semi-synthetic ones were produced by natural origins (of same

or similar compositions and operating principles). In clinical practice only about 100 various antibiotics are used because of the harmful consequences they provoke in the patients or because their wider usage would be too expensive^{26,27}. Therefore, the theoretical value of antibiotics depends only upon their activity, i.e., their efficiency, speed, simplicity, availability, and safety which can fall within these characteristics: selective toxicity (bacterial cell versus host cell), therapeutic ration, tolerance, pharmacokinetics, and pharmacodynamics²⁸. Antibiotics are divided according to their mode of operation, effect, mechanism of action, and chemical composition.

Antibiotics had soon become an irreplaceable cure for an increasingly large number of bacterial diseases while research and creation of new antibiotics promised a care-free future²⁴. However, already after twenty years of the systematic antibiotic application, it was noted that infections with some agents responsible for diseases (gram positive and gram negative bacteria) could not be overcome with existing antibiotics anymore, and synthesis of new, mostly selective antibiotics ensued²⁹. Combinations or alternative antibiotics were used, which led to panic at the end of the last century. In fact, some types of bacteria, using their genome which was upgraded during billions of years, create resistance against the most commonly used antibiotics, and soon afterward against all available ones²⁶.

Countless attacks, both biological and environmental, 'teach' the bacteria which then create a resistance mechanism against most antibiotics by producing new proteins which block the antibiotics or repair damaged systems in the cytoplasm, and can also change cell wall structure as the first line of defense (the most effective barrier). Therefore, bacterial resistance and understanding its mechanisms becomes the subject of study while new antibiotics are synthesized simultaneously^{30,31}.

Resistance may be natural, i.e., permanently displayed through the 'wild' phenotype, or acquired, displayed through mutations or new gene acquisition²⁴. The inherited – natural resistance is exhibited by bacteria by making it more difficult for antibiotics to enter the gram-negative cell of a bacteria. In that way, lipopolysaccharide membranes stop the entry of penicillin G, macrolides, and vancomycin while some inactivate antibiotics via bacterial enzymes (e.g., *Klebsiella* which has beta-lactamase). An example of natural resistance is the absence of affinity between antibiotics and the target location or the absence of the target location. Therefore mycoplasmas, which do not have a cell wall, are resistant to beta-lactamase, antibiotics which influence cell wall synthesis³².

Acquired resistance is far more dangerous than the natural since it exhibits little known mechanisms which bacteria can develop, including gene modification. Cross-resistance is the simultaneous resistance to all antibiotics in the same group (e.g., all beta-lactamase antibiotics)³³. Multiple resistance is a resistance to various groups of antibiotic combinations of several different and independent resistance mechanisms^{25,34}.

Bacteria develop acquired resistance through horizontal and vertical gene transfer, and recombination mechanisms. Horizontal gene transfer is a direct transfer of ge-

netic material between biologically unrelated species, while in the vertical gene transfer genetic material passes from the parents to their daughter's cells. Gene recombination mechanism, when bacteria exchange genes via third parties, can occur between two bacteria by the process of transformation, conjugation, and transduction. By recombination, bacteria acquire new genes and therefore become resistant to antibiotics they have never been treated with. Especially 'dangerous' way of gene transfer is conjugation, i.e., gene transfer during sexual reproduction (copulation) between different species of bacteria (parts of plasmids being transferred)^{25,35}. Therefore, bacteria acquire new genes which can produce or enhance resistance to antibiotics they never had direct contact with.

Because of all this, in the 21st century antibiotics can be as efficient with treating and stopping the spread of contagious bacterial diseases as they were two centuries ago. More and more multiresistant species appear making likely that even the most simple operations may become fatal because of postoperative infection with the aforementioned multiresistant bacteria³⁴. The most dangerous types may be both gram-positive and gram-negative bacteria: *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, Penicillin resistant, *Streptococcus spp* (PRSP), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant, *Enterococcus* (VRE), extended-spectrum beta-lactamases bacteria (ESBLs). However, the biggest danger lies in the resistance of gram-negative (intestinal bacteria) against carbapenems: imipenem, meropenem, ertapenem³⁶.

Apart from the aforementioned large problems regarding antibiotics (except increasingly larger bacteria resistance), the frequent implementation of inadequately researched or tested antibiotics brings about new dangers. This is why FDA (Federal Agency of the United States) notes that there is a real danger of antibiotics (ciprofloxacin, ofloxacin, norfloxacin, gemifloxacin, moxifloxacin and others) with regard to the permanent nerve, kidney, and eye damage; while fluoroquinolones present a risk for children, senior citizens, and pregnant women³⁷. It is therefore obvious that bacteria have a genetic potential and resistance mechanisms against antibiotics because they have encountered similar attacks during their evolution, which resulted in the development of new defense mechanisms²⁵. When the questionable and almost inefficient use of antibiotics against bacterial infections from biofilm is added, it becomes evident that a return to the drawing board is needed and the relations in microbiocenosis, in which bacteria have a powerful and well-known enemy – the bacteriophage, need closer attention. Let us then look at how bacteriophage may aid science and medicine to regain balance and to provide the human race with a carefree life and progress.

A Natural Antibiotic – The Return of the Bacteriophage

As always, when we cannot solve existing problems in the usual way, we look for a solution by going back to the drawing board. Luckily, we do not have to go back to the

very beginning because there are enough knowledge, experience, and results which claim that bacteriophages can be considered in the change of treating bacterial and viral diseases^{38,39}. If we remember the limitations and obstacles to treating diseases with antibiotics, it will become evident that these problems occur because bacteria acquire new attributes. Via transformation, transduction, and conjugation – the mechanisms of gene recombination, bacteria acquire the ability to respond to antibiotics since they are not as adjusted and reshaped as bacteria, i.e., antibiotics are outdated answers.

Bacteriophages are similar to viruses; they can modify their genome twice as fast as prokaryote cells. Treating bacterial diseases on the principle of parasitic antibiosis with the aid of phage preceded the use of antibiotics. Therefore, only the principle of mimicking natural balance can offer incessant search for new right answers. Bacteriophages decipher bacteria molecule's signals in biofilm, hydrolyze the intercellular barrier by disintegrating the extracellular polymeric substances (EPS), adhere to bacterial receptors and »infiltrate« their genes into bacterial protoplasm, and may even disintegrate the protein-protective mechanism in the bacteria itself⁴⁰.

In vitro studies of phage relationship on plankton and biofilm cells confirm that the selected course is correct, and the use of bacterial phage in the prevention of biofilm formation on endoprosthesis and catheters suggests a possible effective use of phage *in vivo*. Ethical committees allow the application of bacteriophage in the treatment of animals and humans in the terminal phases of infections with multi-resistant types of bacteria. Due to a series of ethical and moral doubts, such application is termed a test, not a treatment for prudential reasons.

By declaring that bacteriophage preparations are a drug, their application is additionally prevented without clinical trials which are too short and too expensive in the search for radical change in the accustomed way of treatment of some bacterial diseases^{41,42}. It is to be believed that despite some justified, but also some unjustified limitations, the most optimal solutions will be found because everything else would lead to an increasingly likely defeat against more and more serious forms of a significant portion of bacterial diseases and infections.

Phage is very effective in treating animals, especially fish and poultry, even for industrial purposes, for plant pathogens growth control (bio-controllers), and in food protection before conservation^{38,43–46} (Table 1). Experience outside of human application has significantly contributed to the so-called Declaration of Helsinki in 2005, under which bacteriophage therapy is permitted on humans and animals for experimental treatment⁴⁷. Phage therapy allows the treatment of many bacterial infections that have not yet been overcome by the use of traditional pharmacologic agents^{14,48}. Some examples of phage application for human and animal treatment or use in preventive protection of endoprosthesis, catheter, and other areas that can be contaminated by biofilms illustrate current experience and knowledge of real possibilities and directions in the treat-

TABLE 1
LIST OF COMPANIES THAT PUBLISH THE USE OF BACTERIOPHAGE AS BASIC TECHNOLOGY⁶⁹

	Company name	Country	Website
Preclinical phage therapy research and development			
1.	AmpliPhi Biosciences	SAD	www.ampliphibio.com
2.	Enbiotix	SAD	www.enbiotix.com
3.	Fixed Phage	UK	www.fixed-phage.com
4.	InnoPhage	Portugal	www.innophage.com
5.	Intralytix	SAD	www.intralytix.com
6.	Pherecydes Pharma	France	www.pherecydes-pharma.com
7.	Technophage	Portugal	www.technophage.pt
The development of products competent phages			
8.	AvidBiotics	SAD	www.avidbiotics.com
9.	Eligo Bioscience	France	http://eligo.bio/
10.	Enbiotix	SAD	www.enbiotix.com
11.	Phico	UK	www.phicotx.co.uk
Phage product distribution			
12.	Biochimpharm	Georgia	http://biochimpharm.ge/
13.	Imbio	Russia	http://home.mts-nn.ru/~imbio/
Patient phage therapy			
14.	Center for Phage Therapy	Poland	www.iitd.pan.wroc.pl/en
15.	Eliava Phage Therapy Center	Georgia	http://eliavaphagetherapy.com/
16.	Phage Therapy Center	Georgia	www.phagetherapycenter.com
17.	Phage International	SAD	www.phageinternational.com
Phage-mediated biocontrol			
18.	APS Biocontrol	UK	http://apsbiocontrol.com/
19.	Epibiome	SAD	www.epibiome.com
20.	Intralytix	SAD	www.intralytix.com
21.	Omnilytics	SAD	www.omnilytics.com
22.	Phage Biotech	Israel	www.phage-biotech.com
23.	Phagelux	China	www.phagelux.com
24.	Technophage	Portugal	www.technophage.pt
Phage lysate products			
25.	Delmont	SAD	https://delmontlabs.com/
Development of enzybiotics			
26.	GangaGen	SAD/India	www.gangagen.com
27.	Lysando GmbH	Germany	www.lysando.com
28.	New Horizons Diagnostics	SAD	www.nhdiag.com
Phage-based bacterial detection technologies			
29.	Sample6	SAD	www.sample6.com
Phage-associated industrial contamination			
30.	Phage Consultants	Poland	www.phageconsultants.com

ment and prevention of bacterial and viral diseases^{7,49}. (Table 1)

The problems of phage treatment are that they are recognized by the immune system as foreign matter, and are

not able to reproduce with ease. Thus, phage does not succeed in multiplying by a large enough number to infect a sufficient number of bacteria. On the other hand, phage is not used as often largely due to the pharmaceutical indus-

try where antibiotic production is increasing even at the expense of large investments in research and synthesis of new types⁵⁰.

Phagotherapy is a term that will be used in further consideration as it implies a variety of procedures that are used in chemotherapy, radiotherapy, which are usually used in treatment⁵¹. The name of the drug is phage, and the bacteriophage, in the strict sense, will be referred to as therapeutic phage (lithic phage).

Clinical Application of Bacteriophage

Resistance of some gram-negative bacteria (*P. aeruginosa*, *E. coli*, *Klebsiella*) and some gram-positive ones (*S. aureus*, *Streptococcus sp.*, *Enterococcus*), as a cause of severe diseases and septic states, was provoked, among other things, by inadequate and unsuitable therapeutic doses, mutation ability, or as a result of the difficult circumstances of treatment of immunocompromised patients⁵². These are causal reactions which significantly reduce the overall resistance of infected patients and increase their likelihood of secondary infections with the same resistant types. The application of phagotherapy is already apparent today in people with diabetes infected with MRSA, those infected with *Burkholderia cepacia* (BCC), and those suffering from cystic fibrosis (CF)^{53–56}.

Cystic fibrosis is a disease of the lower respiratory system with chronic duration, and it is genetically conditioned⁵⁷. CF is a result of the mutation of the CFTR (cystic fibrosis transmembrane conductance regulator) gene with more than 1,500 known mutations. The most common result is the loss of amino acid phenylalanine in the protein regulating the transport of chlorine through the cell membrane⁵⁸. Due to this process, a very viscous and sticky secretion occurs in the lower airways and prevents normal coughing and removal of bacteria. In such patients, therefore, infections with gram-negative bacteria are very common, predominantly with *Pseudomonas aeruginosa*⁵⁹. It has been shown that the cause of these infections is *Burkholderia cepacia*, also a gram-negative bacteria, which is also resistant to antibiotics⁶⁰. BCC infections are the second leading cause of death among hospital patients (the mortality rate is around 20% in Canada)⁶¹. It spreads rapidly among lying patients, especially patients suffering from CF, and in the acute phase it results in septicemia, heart failure, and significantly reduces life expectancy. Phagotherapy was performed on mice infected with isolated strains of BCC from immunocompromised patients with cystic fibrosis. Isolated strains of BCC are highly resistant to antibiotics, and completely to rifampicin, ampicillin, cefepime, and others⁶².

Inflammation of *B. cepacia* and phagotherapy using the aerosol in the lung was first performed with several different phages⁵⁴. Aerosol *B. cepacia* was applied 24 hours before the aerosol phage, and two days after treatment, significant and dramatic reduction in the number of bacteria (logarithmic reduction rate) was observed. Before infection, the mice were immunocompromised with cyclophosphamide (CPA) which causes leukopenia, and

the significant reduction of *B. cepacia* could not be attributed to the immune system of the host⁶³. Phage therapy in mice infected with BCCs strains from patients with CF is very effective and could be applied to humans.

In mice, co-therapy of lithic bacteriophage and linezolid were also implemented in the treatment of diabetic foot infection caused by MRSA⁵⁶. In annual diabetic patients, there is a chance that the »diabetic foot« disease will occur in 1%, and the pathogenesis mechanism includes neuropathies, infections, and ischemia. The person with diabetes does not feel pain in the area of the lesion, and the anti-inflammatory response of the organism is suppressed, making patients aware of infection only at the wound drainage stage⁶⁴. Exceedingly wide-spread mixed type infections are a major problem because the cause of the infection is very often resistant (multi-resistant). One of the most common causes is MRSA.

A very effective antibiotic – linezolid (oxazolidinone)⁶⁴ can be used for the treatment of MRSA purulent infections in the heel for diabetics, and the experiment was intended to confirm the existence of a similar agent – the therapeutic phage, which is best suited for *in vivo* administration⁵⁶. This research was also performed on mice. The rear heels of diabetic mice were infected with MRSA clinical isolates, identified by gram response and biochemical probes. Therapeutic phage MR-10 was injected into the rear foot of diabetic mice. Chhibber et al., through this study demonstrated the efficacy of phage and antibiotic linezolid therapy⁵⁶. Pharyngeal and linezolid therapy gives the best results, and it is possible to conclude that combined therapy reduces the occurrence of resistance, simplifies and reduces the application. Also, it has been shown that phages allow antibiotics to be more effective, i.e., they accelerate their entry into the bacteria and thus become an effective alternative to the treatment of MRSA infections in people with diabetes.

This experience of *in vivo* co-therapy is consistent with *in vitro* experiments on the combined effect of bacteriophage and antibiotics on resistant strains of *E. coli* and *P. aeruginosa* in particularly hard conditions - in biofilm⁶⁵. Biofilm is a community of prokaryotic cells coated with the extracellular polymer matrix. It is protected from antimicrobial drugs and acts as a matrix for the establishment and development of slow-growing subpopulations of resistant bacteria and specific gene expression⁶⁶. In order to reduce the risk of developing resistant cells, combined therapy is often used. This primarily refers to the combination of multiple antibiotics (tobramycin + clarithromycin, daptomycin + clarithromycin). Likewise, phage cocktail used as pre-treatment can significantly reduce the development of biofilm⁶⁶. Combinations of antibiotics and phage in therapy more effectively prevent the development of biofilms or reduce already formed biofilm matrix for one to two logarithms⁶⁶. There are no reports of resistance in biofilm treated with phage and antibiotic combination.

Study of co-therapy of bacteriophage and the antibiotic tobramycin on the biofilm of mixed culture of *E. coli* and *P. aeruginosa* has shown that such combined treatment reduces the number of resistant bacteria⁶⁵. *E. coli* and *P.*

aeruginosa cultures were used, T4 bacteriophage was used to infect *E. coli*, and bacteriophage PB-1 was used to infect *P. aeruginosa*. Tobramycin was used as the antibiotic. Tobramycin is an aminoglycoside bactericide derived from *Streptomyces tenebrarius*, (tetrasaccharide structures, streptamine structures). It is an inhibitor of bacterial protein synthesis and an inactivator of microbial enzymes. It is more effective against gram-negative bacteria, but in a broader spectrum also against some gram-positive bacteria⁶⁶. Coulter et al. have shown that phage and antibiotics act to reduce resistant bacteria. In monoculture of *E. coli* treated with tobramycin or T4 phage, the reduction is about 39%, and their combination reduces the number of resistant bacteria by 99%⁶⁵. In monoculture of *P. aeruginosa*, the individual reductions for both T4 phage and tobramycin are about 60%, while their combination reduces the number of resistant bacteria by about 99%⁶⁵. Since the study was conducted in pure bacterial cultures, the conclusion should also include the results of the combined effect of tobramycin and the associated specific therapeutic phage for treatment in polymicrobial biofilm infection.

In some studies, it has been observed that in biofilms with mixed cultures, specific phages are not fully effective, probably due to the different types of EPS produced by those types, and the specific phage does not have an effective response to that nonhomogeneous matrix. This is why the research done in the Qvin Astrid military hospital in Belgium on patients with colonized burns is very insightful⁶⁷. Infected wounds were treated with phage cocktail (phage active against *P. aeruginosa* and *S. aureus*). In controlled conditions, the examination was conducted on 9 persons and 10 burns. Selected phage cocktail BFC-1 intended for the treatment of *P. aeruginosa* and *S. aureus* (phage from the families *Myoviridae*, *Podoviridae*, and *ISP Myoviridae*) was at a concentration of 109 units/ml purified from endotoxin. Previously classical bacteriological identification had established that the wounds were infected with *P. aeruginosa* and *S. aureus*. Directly before

applying BFC-1 phage to the colonized burns, the wounds were divided into two equal parts, one half being treated with standard therapy. Thus, patients with suspected MDR strains of *P. aeruginosa* received amikacin in combination with ceftazidime and meropenem, and patients with suspected MDR strains of *S. aureus* were treated with vancomycin and linezolid. The other half of the same patients' wounds were treated with BFC-1 phage cocktail at 1 ml sterile and endotoxin purified by spraying system and on average 0.03ml per square centimeter of the wound (or about 10⁷ phages). The surface of the wounds was approximately 95 cm² on average, and after 2 to 3 hours the biopsy was performed on each. After the initial colonization of the wounds from the homogenized sample after the final biopsy, taken after the BFC-1 phage treatment, only a small bacterial load was observed while unchanged bacterial loads were observed in wounds treated with antibiotics. Rose et al. undoubtedly confirmed the effectiveness of phagotherapy on burns⁶⁸.

Conclusion

The question is whether phage can be the second line of defense, the line that all the more powerful pathogens cannot overcome? Of course they can, because their desirable and positive effect is incomparably greater than the possible unwanted consequences. Science is constantly discovering new forms and preparations of phagotherapy, gene therapy, antibiotic co-therapy, diagnostics, disinfection, decontamination, and neutralization of biological toxins. The ability to self-duplicate, the specificity of action, the harmlessness, the high degree of desirable mutation, and availability obligate scientists to carefully study the relationship between humans, diseases, and treatment (phage), as well as insufficiently clear *in vivo* dynamics of phage, immune response, and activation of defense mechanisms. It is obvious that the main principle of nature is to find answers in the same place where questions arise.

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OD BAKTERIOFAGA DO ANTIBIOTIKA I NAZAD

SAŽETAK

Evolucija je životu kao fenomenu, dodijelila bezbrojne oblike i mogućnosti opstanka i nastajanja. Danas su poznati gotovo svi mehanizmi odnosa čovjeka na vrhu evolucijske ljestvice s mikroorganizmima koji su na njezinom začetku. Očuvati zdravlje, odnosno život, nije više samo instinktivni odgovor na ugrozu, nego smišljeno stvaranje i korištenje znanja. U okolnostima velikih epidemija i ratova koji stvaraju veliku patnju, primijenjena su iskustva tih odnosa čovjek-bolest (uzročnik), pa tako bilježimo uporabu bakteriofaga u Poljskoj i Rusiji prije i za vrijeme Drugog svjetskog rata, a gotovo istovremeno uvedena je i antibiotska terapija. Budući da su bakteriofagi – »pratili« evoluciju bakterija, mehanizam njihova djelovanja počiva na prokariotskoj stanici, što ih čini neopasnim za eukariotsku stanicu ljudskog parenhima. Potrebno je dakle samo vratiti ta iskustva u današnje vrijeme kada smo se uvjerali da bakterije imaju neiscrpan vlastiti genski i fenotipski rezistencijski mehanizam. Antibiotici i dalje predstavljaju temelj očuvanja zdravlja, ali sada u kombiniranom djelovanju sa specifičnim virusima – bakteriofagima koje možemo proizvesti i primijeniti u okolnostima multirezistencije, ali i za pripremu novih farmakoloških pripravaka. Dakle, treba se usmjeriti na ta nova znanja i mogućnosti »sve do novog modela antibiotik-bakteriofag«.

Physiological Basis of Human Longevity: the Concept of a Cascade of Human Aging Mechanism

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ABSTRACT

The research is devoted to the fundamental issue of medicine and biology – the study of factors limiting the life span of a person. As a model, the system of adaptation of the human body to the forces of natural gravity is chosen, the disadaptation to which manifests itself in falls and everyday injuries. The object of the study was the selection of severe fractures of bone tissue due to fall, taken in the age aspect. Statistical and meta-analytical research methods were used. It is shown that the age-related increase in mortality due to household falls, coming to severe bone fractures, is non-linear and increases in geometric progression. As a result of the coincidence of the age characteristics of bone fragility and age-related kidney function, an assumption is made about the role of involution of the renal tissue in the development of osteoporosis in the elderly and the need for a new approach to the prevention of osteoporosis and domestic injuries.

Key words: gravity, vestibular function, fall, osteoporosis, hip fracture, aging, cascade mechanism.

Introduction

This work continues the study of patterns forming a space-time continuum based on human health reliability theory postulates complex systems^{1,2}. In this study, the most common system of aging patterns were recorded, accompanying the process with different kinetics:

- linear nature of the accompanying morphological changes
- non-linear nature characterizing fading functionality of organs and systems with age¹. A space-time continuum in the present context is represented as biosocial continuous chain of events accompanying and forming individual structure and the speed of the life cycle in time and space according to various scenarios.

The analysis of numerous data on the age characteristics of the human body^{3–7} reflects a variety of approaches to the study of the causes and patterns of age-related processes. Comparative approach, based on the analysis of quantitative data on the state of organs and systems of the human body in different periods of his life, revealed a number of interesting facts. The abundance of factual material collected by the researchers create self-age process

management capabilities. This, apparently, explains the explosive interest of people to the »anti-aging« problem. Very popular in the world have a different formula to determine the »passport« and »biological« age, which caused some researchers ironic attitude to the »rejuvenating websites«⁸. The main emphasis in the study of 'biological' age of the author's case in the comparison of the data of numerous physiological and biochemical research laboratory customers were to become in healthy people. This so-called age norms of health indicators. According to most of them international consensus has been reached. Similar studies are »cross« type of statistical research. How to react to a particular body for future trials of life, such studies can not answer to give. To do this, you need to deploy on time, »longitudinal studies« research. According to most of them international consensus has been reached. Similar studies are »cross« type of statistical research. How to react to a particular body for future trials of life, such studies can not answer to give. To do this, you need to deploy on time, »longitudinal studies« research. According to most of them international consensus has been reached. Similar studies are »cross« type of statistical research. How to react to a particular body for future trials of life, such studies can

not answer to give. To do this, you need to deploy on time, »longitudinal studies« research.

Thus, evaluation of the age of the kinetics of the changes in health becomes an important theoretical and practical significance, which is responsible for the relevance of the chosen direction in this paper.

The Aim of the present study was to clarify the STC health laws based on the kinetic approach to the evaluation of certain key clinical and metabolic processes in the space-time continuum system, ensuring human activity throughout life and dwindling when aging.

The object of the study is the relationship of metabolic processes and clinical outcomes maladjustment to the forces of gravity in the STC system, as well as their analysis in terms of the kinetic features. We used meta-analytic, deductive and conceptual methods.

Methods

Under the kinetics of a process implies the character of changes of elements in any system. Formal kinetics, particularly considering methods for finding the dependence of the chemical reaction rate on the concentration of the reactants⁹. To distinguish linear and linear processes described respective laws¹⁰. The importance of understanding the differences line by nonlinear laws is that reflecting the processes taking place in a particular system, they also reflect the specifics of the regulatory mechanisms inherent in this system. Thus, with the kinetics described by a linear law, the incoming external disturbing signal is generated proportional response. When the kinetics, reflecting the non-linear law, the answer may not reflect the scale of the input signal.

An important distinction of these processes is their degree of control on the part of the system. For a system with a linear process of changing the size of deflection – it merely changes the scope, but not the means of action. At occurrence of nonlinear processes»in the system may vary considerably and will form action until fundamental processes picture quality changes«¹⁰.

One of the forms of the nonlinear nature of the process is a cascade. Cascade mechanism is characterized by a sequence of well-determined events where the primary event causes the next. This subsequent event, in turn, is the cause of a new event, considered as a consequence of the subsequent. Cascading mechanisms are well represented in biology¹¹, at the level of the metabolism of living organisms. In these cases, a protein at a specific stage of existing as a substrate under the influence of impact (enzymatic, electrical, biochemical) becomes an active enzyme which acts on another protein as a substrate, turning it in an active enzyme. Such a sequence may involve several steps or stages.

A striking example of the cascade in biology is a cascade of enzymatic reactions that accompany the process of blood coagulation, activation of immune responses in allergy, explosive nature of lipid peroxidation upon depletion of antioxidant defense and other processes.

Thus, along with the evolution Process specific for homeostatic functions well traceable throughout the human spatio-temporal continuum, there are risks out of control of a number of reactions followed an exponential character homeostasis disorders. Lack of knowledge about these risks creates uncertainty – »a state of complete or partial lack of information necessary for an understanding of the events, their consequences and their probabilities«^{12,13}.

All the above features of the course of metabolic processes in biological systems can be illustrated by the example of age-related disorders of the human body is constantly adapting to a force acting in the world as gravity.

As part of classical mechanics describes the gravitational attraction the law of universal gravitation Newton, which states that the force of gravitational attraction F between the two material points weight $\{m_1\}$ and m_2 , separated by distance r is proportional to both masses and inversely proportional to the square of the distance – that is:

$$F = G (m_1 * m_2) / r^2,$$

where G – gravitational constant, Equal to approximately $6.67 \times 10^{-11} \text{ m}^3 / (\text{kg s}^2)$ ¹⁴.

The gravitational effect on the level of organs and tissue does not stop throughout the existence of the body. Arterial and venous circulation, movement of food through the digestive tract, urinary excretion, the formation of edema, stagnation in the lungs, the work of the vestibular apparatus, the formation of blood clots in the vessels of the lower limbs, blood rheology and erythrocyte sedimentation rate – this is not a complete list of the participation of gravitational forces in biological and pathological processes.

The influence of gravitational waves and propagates in a microcosm. At this level, because of the principle of equivalence of mass and energy, the atomic structure of matter acquire their specific gravitational charge. Under certain environmental conditions the gravitational interaction of microparticles becomes electromagnetic force reaching gigantic values with equal gravitational electric charge¹⁵.

Disadaptation of the human body to the gravitational forces of the Earth appears in the body balance disorders and falls. There is a wide class of disorders of vestibular function in humans (N81), including at ICD-X following disorders: N81.0 (Meniere's disease). R81.1. (Benign paroxysmal vertigo), N81.2 (Vestibular neuronitis), N81.3 (Other peripheral vertigo: Lermuaye syndrome, otic, otogenic, peripheral vertigo) N81.4 (Central origin vertigo), and others.

And if functional disorders of the vestibular function of the brain, such as dizziness, is quite difficult to quantify due to lack of medical facilities in the mandatory statistics such states, the age estimates of the number of falls involving injuries, can be carried on the balance sheet statistics trauma centers and services.

Ranked Among the different types of bone fractures associated with age-related changes in bone metabolism, hip fractures is most dangerous.

The analysis of numerous research data shows wide geographical variation frequency hip fractures worldwide. The higher frequency hip fractures submitted in industrialized countries than in developing countries. The highest rates of disease are seen in Northern Europe and the United States and the lowest in Latin America and Africa. Asian countries such as Kuwait, Iran, China and Hong Kong, show the average hip fracture. There is also a gradient from north to south, observed in the European studies, and confirmed by the United States¹⁶.

Asian auditor's report, published in Beijing in 2009¹⁷. It reflected the growing nature of the problems associated with the proliferation hip fractures and Asia.

Swedish scientists estimated the total number of hip fractures in men and women (Code ICD-X S72.0) in 1990 worldwide was 338 000 and 917 000 people respectively, a total of 1.26 million. They suggested that in case of preservation of the age and sex incidence of the number of hip fractures will increase approximately double to 2.6 million by 2025 and four – to 4.5 million by 2050¹⁸. According to the amendment to increase the average length of people in the future¹⁹ to increase the frequency hip fractures up to 6.26 million. 2050.

In the US, more than 1.6 million people annually admitted to the hospital due to severe bone fractures. Including: 700 000 vertebral fractures; up to 400,000 hip fractures; 250,000 wrist fractures, and more than 300 000 different combination of fractures. health care costs for treatment of fractures up to 38 million dollars a day (according to the hospitals and nursing homes). 80% fractures reported in women (ratio of male: female = 1: 4)²⁰.

Hip fracture risk in women is equal to its combined risk of developing breast cancer, uterus and ovaries. 17% of men who have reached 90 years, suffering a fracture of the proximal femur. Approximately 25% of the injuries are fatal within a year after a hip fracture. Almost 70% of those experiencing acute postoperative stage are forwarded to a nursing home, as in need of constant care, and only 25% of victims of hip fractures restore the previous level of independence. The rest of the survivors are in different classes of disability requiring supportive care at home or in nursing homes.

According to russian authors^{21–23} in the country every 5 minutes there is a hip fracture caused by osteoporosis. In this case, up to 90% of hip fractures occur in those over 65 years. In some regions of the Russian Federation, the mortality within 6 months after hip fracture reaches 55%.

WHO forecasts are disappointing: in 2050 it is expected that the number of victims of a hip fracture women will increase by 240%, men – 310%. Only 10% of patients after a hip fracture return to previous activity²³.

In the above Louise Margolis statistical data from the US²⁰ draws attention to the remark about the exponential growth of hip fracture risk with age. A number of authors noted a linear decrease in calcium content and bone density with age.

So after the onset of menopause (pre-, peri - and post-menopausal women), aged 50–51 years, women lose up to 2–3% of their bone mass in the first 5 years of endocrine adjustment. Then calcium loss rate is reduced, so that the bone density in women is reduced by about 30% between the ages of 50 to 80 years, during which time they lose up to 20% of their bone mass after menopause^{20,24,25}.

The term »exponential growth« entered our lexicon to refer to fast and, as a rule, unrestrained increase. It stands for »growing rate of increase values of a measur-

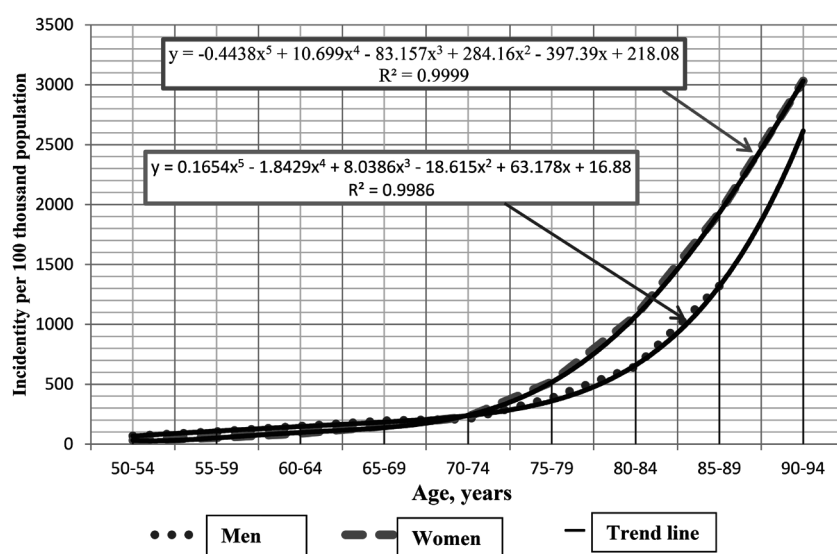


Fig. 1. The exponential increase in injuries associated with hip fracture in men under 89 years and women up to 94 years in Russia. After this age marked decrease in the occurrence of fractures, due to the sharp restriction of physical activity at this age.

able phenomenon²⁶. For any exponentially growing events assertion – »the more important quantity takes, the faster it grows²⁷.

A similar pattern was found in a large study on the prevalence hip fractures and held in the Russian Federation in 1992–1997. Study of 16 russian cities were involved²⁸. Under observation were 1,749,274 people over the age of 50 years. Reconstruction of the table data of this work, graphic material shows pronounced exponential nature of the growth of age disadaptation man to the forces of gravity (Fig. 1).

This statistical study reflects a phenomenon which should not be linear, but exponential. The most distinct Similar processes are described mathematical approximation related to the polynomial approximation²⁹. Thus, the trends of graphic images in Fig. 1 more fully described / approximated by the following mathematical expressions calculated Microsoft Excel 2007 program:

$$y_{\text{wom}} = -0,4438x^5 + 10,699x^4 - 83,157x^3 + 284,16x^2 - 397,39x + 218,08$$

$$y_{\text{men}} = 0,1654x^5 - 1,8429x^4 + 8,0386x^3 - 18,615x^2 + 63,178x + 16,88$$

The obtained mathematical expressions passport age of the person (x) Serves as an argument to form a polynomial power function (y) – annual amount of fresh Hip fracture cases per 100 thousand population.

The exponential growth of accidents with Hip fracture accompanied by a similar increase in mortality of people from this injury. Fig. 2 shows a graphic reconstruction of tabular data research³⁰ on the level of mortality of people of different ages from Hip fracture. According to the authors of this work, the mortality rate in the total group of men and women during the first year after a hip fracture in men amounted to – 26.8% in women – 19.7%. The overall mortality rate for persons over 65 years was 21.2%. Calculations show that the mortality rate value increases in a quadratic and cubic extent depending on the chronological age of women (see Fig. 2).

Thus, for example, women's health, it can be noted that the loss of 20% of calcium by bone tissue in post-meno-

pausal period²⁰ accompanied by a 20-fold increase in mortality in the first year after hip fracture³⁰. Such exponential growth in the United States from Hip fracture age mortality observed in the study³¹: mortality from hip fractures in men and women aged 65–74 years less than 75–84 years 1.5 times and less than 2.5 times, than in people over 85 years.

Discussion of the Results

The above facts do not explain the exponential growth Hip fracture and mortality from it only one mechanism of osteoporosis, develop gradually, with the linear nature of the composition and changes in bone structure. This contradiction can be explained by taking into account the impact of comorbid diseases, existing and emerging, regardless of the underlying disease³², and the accompanying process of osteoporosis in men and women. In this case, according to the principles of the theory of stability of complex systems, all accompanying diseases as indicators of failure or disorders accompanying processes are mutually potentiate each other, sharply or exponentially increasing the risk of occurrence Hip fracture and poor outcome.

The guide Mayo Clinic (USA) on risk factors in Hip fracture³³. The following presents Hip fracture comorbid diseases:

- Acute myocardial infarction or of myocardial history;
- Cancer or metastatic carcinoma;
- Cerebrovascular disorders;
- Chronic lung disease;
- Chronic renal insufficiency;
- Congestive heart failure;
- Dementia;
- Diabetes with or without complications;
- Liver moderate or severe;
- Paralysis;
- Peripheral vascular disease;
- Rheumatological disease;
- Peptic ulcer disease;
- Cirrhosis of the liver.

In the OM Lesniak (2017) proposed a classification related osteoporosis factors³⁴. The author highlights:

- modifiable factors: alcohol abuse, smoking, excessive physical exercise;
- malnutrition factors: malnutrition and low body mass index (BMI), malabsorption, vitamin D.
- diseases and their treatment: chronic kidney disease, chronic obstructive pulmonary disease, late onset of puberty, an excess of glucocorticoids (endogenous or exogenous), HIV infection and treatment with protease inhibitors, hypercalciuria, hypogonadism (including androgen-deprivation therapy), inflammatory joint diseases, mastocytosis, multiple myeloma, osteogenesis imperfecta, primary hyperparathyroidism, hyperthyroidism.

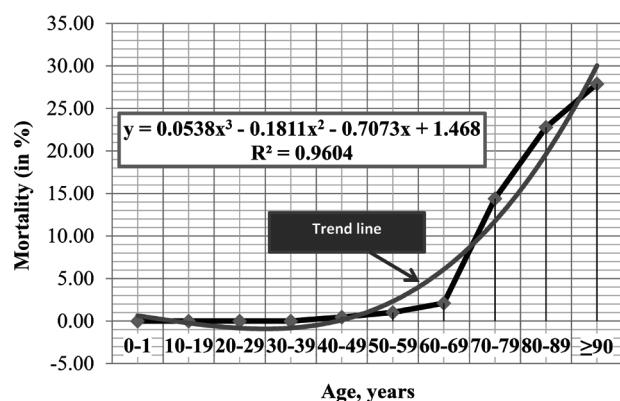


Fig. 2. Age dynamics of mortality Hip fracture.

It becomes obvious that the age-violation of human adaptation to the forces of gravity ends with the inevitable fall, followed by an adverse outcome when damaged bones of the skull, spine or hip. By itself, the specific cause of the fall may be random, but the drop is not very random. It is prepared by numerous elements clinical disorders in human osteoporotic patient: chronic kidney disease deficiency of vitamin D, alcohol abuse and multiple accompanying chronic conditions («Comorbidity cascade»).

Surprisingly, – informs «Live Science» site^{35,36}: – but according to the American Association of Neurological Surgeons (the AANS) is the most common way to die after 65 years – the fall (emphasis added). For example, according to the US Commission on the Consumer Product Safety (CPSC), in 2006 more than one million people a year applied to the emergency room in US hospitals about head injuries related to accidents that have occurred in the conditions of life. Among the most common indirect causes of elderly falls called: floors or floor coverings (260,983 cases); ladders or steps (114,752); beds (101,548); tables (70,452); chairs (50 482); baths or showers (34.446); a door (32 009); carpets (14 867); shelves, balconies, open floor (13, 994); toilets (11,246); Bunk bed (10 778); countertop (10 683); door thresholds or CT box (10 014) and other elements of premises³⁵. The AANS has an extensive list of tips to prevent head injuries³⁶.

As can be seen from the list, sooner or later, the elderly comes psychophysical disadaptation to the outside world, including family life. And if a child, imperfect vestibular system leads to frequent falls baby makes the first steps, and each episode of the fall served as adjusted subsequent movements in old age any fall, jump, slipping often lead to dramatic consequences caused disadaptation to gravity – fall and increased fragility of osteoporotic bone with subsequent life-threatening numerous clinical complications (based on the cascade).

Comorbid diseases of aging (sclerotic lesions of cerebral blood vessels, endocrine disorders, cardiovascular diseases, etc.) violate the central nervous system and mental

activity: reduced cognitive human performance falls vision, lost his care and caution, slows the rate of reaction to sudden event. Comorbid disease form comorbid cascade that promotes disruption of function and subsequent vestibular fact – fall elderly person. But the real cause of domestic bone fractures is osteoporosis.

In turn, extensive comorbid cascade of clinical manifestations preceded by a cascade of metabolic disorders, often goes unnoticed, «linear» and not cause concern among healthcare professionals.

Projection of these faults on PVC allows conceptually determine the kinetics of age following adaptive violations against the gravitational force (Fig. 3).

Thus, bone fractures have occurred through no fault of external causes, due to the internal metabolic processes that destroy, after a person's age, skeletal resistance to gravity loads. In this case, hidden from prying eyes, causing no complaints change dramatically over a few years, significantly change the quality of life and increases their risk of adverse outcomes in violation of the functions of the vestibular apparatus.

Offered in Fig. 3 cascade model naturally raises the question of timely preventive measures against osteoporosis. Of all the «bouquet» comorbid diseases contact cascade reasonably allocated destruction of kidney tissue, the most important with regard to the regulation of bone metabolism, as in the previous work¹, we have shown the difference between the morphologic and functional speed of aging. In this case, impairment of renal function remarkably accurately resemble an exponential increase in injuries associated with hip fracture (Fig. 1).

It is known that the kidney regulates a number of important processes of vital bone: calcium homeostasis, and phosphorus metabolism of vitamin D. When they tackle formed secondary osteoporosis clinic³⁷. The kidneys are the target organ and base degradation of parathyroid hormone (PTH) is synthesized alpha hydroxylase, which is formed with a biologically active form of vitamin D – 1,25 (OH) 2D3. It is shown that already at an early stage glomerulo-

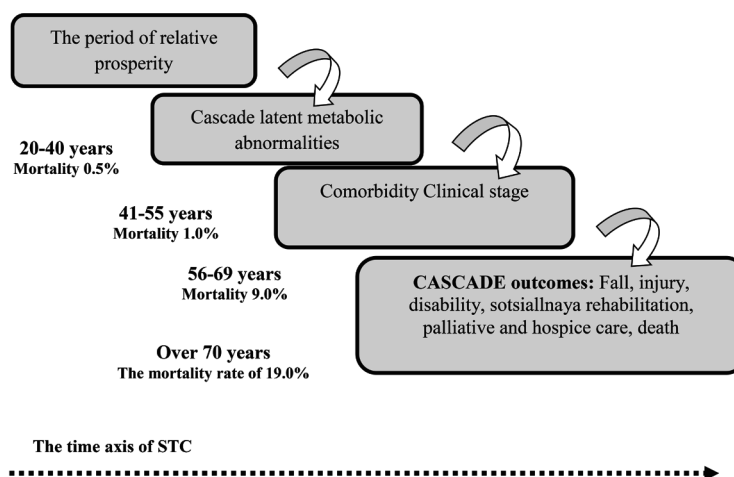


Fig. 3. Cascade mechanism of maladjustment to the effects of gravity.

nephritis, conditions are created for disorders of calcium metabolism, exacerbated in chronic renal failure. Reduced ionized calcium is proportional reduction of glomerular filtration. The negative effect on calcium homeostasis provides and hyperphosphatemia. In chronic renal failure (CRF) in the occurrence of hypocalcaemia participates calcium and protein-bound fraction. In the pathogenesis of reducing total and ionized calcium in renal disease play an important role not only disruption of synthesis 1,25(OH)₂D₃ but deficiency 25(OH)D₃ due proteinuria³⁷.

Kidney transplantation also revealed no significant advantages in respect of bone metabolism, since the use of immunosuppressive drugs gives rise to a secondary cortico-induced osteoporosis³⁷.

Conclusion

Human aging process is accompanied by a progressive decrease of adaptation to external influences, in particular to the forces of gravity.

Process disadaptation to gravity is shown by both the nervous and mental activity (disorders of the vestibular

apparatus and the fall), and on the part of the skeletal system (increased bone fragility due to osteoporosis).

Disadaptation process is exponential.

This nature of age-related changes of human kidney suggests the existence of close functional link between adequate working kidney nephrons and bone metabolism.

Changes disadaptation to gravitational effects are cascading nucleating in a period of relative prosperity through latent metabolic abnormalities that give rise to a new stage of co-morbid diseases, which in turn give rise to a cascade of clinical outcome – many complications after bone fractures resulting from falls.

Focused monitoring of renal function is able to provide substantial assistance in the organization of preventive measures against latently emerging osteoporosis.

Threaded type of change in the space-time continuum of health logically assumes a non-linear nature of the corrective action to maintain homeostasis. A particular case of such a »cascade therapy« may be used in the practice of several chronic diseases, pulse therapy, »involves the introduction of ultra-high doses of drugs in a very short time in lesions of connective tissue« and other diseases^{38,39}.

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FIZIOLOŠKA OSNOVA LJUDSKE DUGOVJEČNOSTI: KASKADNI KONCEPT LJUDSKOG STARENJA

SAŽETAK

Istraživanje je posvećeno temeljnom pitanju medicine i biologije - proučavanju čimbenika koji ograničavaju ljudski životni vijek. Kao model, odabran je sustav prilagodbe ljudskog tijela silama prirodne gravitacije, čije pomankanje se manifestira u padovima i svakodnevnim ozljedama. Cilj studije bio je odabir teških lomova koštanog tkiva uslijed pada, s obzirom na dob. Upotrijebljene su statističke i meta-analiitičke metode istraživanja. Rezultati pokazuju da je porast mortaliteta zbog pada u kući, koji uzrokuje teške frakture kostiju, nelinearan i da se povećava geometrijski. S obzirom na podudaranje u povezanosti dobi i fragilnosti kostiju i povezanosti dobi s funkcijom bubrega, može se pretpostaviti da involucija bubrežnog tkiva ima određenu ulogu u razvoju osteoporoze u starijih osoba te da je potreban novi pristup prevenciji osteoporoze i ozljeda u kući.

A Decade of the Common *FTO* rs9939609 Polymorphism: A Systematic Review

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ABSTRACT

Several association studies focusing on *FTO* gene polymorphisms have been published in the past years; however, the association between *FTO*-related conditions and *FTO* gene variants remains unexplained. Population genetics and association studies of different populations provide a valuable tool for further research. Thus, the aim of this systematic review is to summarize current knowledge on the *FTO* SNP rs9939609, and its association with presumably related conditions. The study included original research articles collected from PubMed and ResearchGate databases that were published in the period between 2007 and November 2017, and that provide information on rs9939609 mutant allele frequency and its probable association with any condition suspected of being related to the mutant allele. Genotype data was extracted and analyzed, and missing data was obtained from secondary sources. Short summaries of relevant studies from primary sources are organized in an overview table. The results of the systematic review suggest that mutant allele A is the most prevalent in European populations and least frequent on the Far East. In addition, it has been concluded that allele A is a good tool for the prediction of an increased risk of higher-than-normal BMI in a person carrying it, as well as that allele A should be further analyzed as a possible risk marker for type 2 diabetes mellitus and polycystic ovary syndrome development.

Keywords: *FTO*, rs9939609, body mass index (BMI), type 2 diabetes mellitus (T2DM), polycystic ovary syndrome (PCOS), population genetics

Introduction

Since obesity and many other weight-related disorders have reached epidemic levels, there is a large interest in the causes of excess body weight as well as various disorders that share this specific symptom. The *FTO* (Fat mass and obesity-associated protein) gene, which was identified as one of the possible genetic triggers, is a large gene containing nine exons that span over 400 kb on the long arm of chromosome 16 at position 12.2, and encodes for 2-oxoglutarate-dependent dioxygenase which functions in the repair of alkylated DNA and RNA by oxidative demethylation. It was first identified in the mouse genome on chromosome 8, and is strongly conserved across various vertebrate species¹⁻⁵. *FTO* was shown to act as a demethylase with a strong preference for 3meU (methyluracil) and 3meT (mononucleotide 3-methylthymidine) in single-

stranded RNA and DNA, respectively⁶. In addition, the gene plays an important role in global metabolism and maintaining homeostasis, and is known to be unequivocally associated with adiposity and body fat accumulation control, as well as the control of adipocyte differentiation into white or brown fat cells⁷. *FTO* variants can interact with *IRX3* (Iroquois homeobox 3) and even cause an increase in *IRX3* and *IRX5* (Iroquois homeobox 5) expression during adipocyte differentiation⁸.

The *FTO* gene has 68 identified single nucleotide polymorphisms (SNPs)⁹. The *FTO* rs9939609 SNP is one of the ten SNPs in the first intron of the *FTO* gene, which was found to be associated with increased weight, raised body mass index (BMI) and related traits⁷. The *FTO* rs9939609 SNP has two alleles, T (wild-type) and A (mutant), which were shown to be associated with the development of obesity¹⁰.

The link between *FTO* and eating disorders and type 2 diabetes mellitus

Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) susceptibility genes and multiple SNPs located in the first intron of *FTO* were identified to be associated with T2DM in 2007⁷, which led to a number of studies examining the effect of *FTO* SNPs on this and related conditions.

The rs9939609 SNP was found to be connected to an increased risk of T2DM, although this influence could also be explained by its effect on BMI in affected and unaffected individuals¹¹, since *FTO* was also found to influence appetite and food intake¹². Furthermore, Gerken et al. suggested that, since present particularly in hypothalamic nuclei governing energy balance, *FTO* mRNA levels in the arcuate nucleus might be regulated by feeding and fasting⁶. It has also been reported that *FTO* SNPs are associated with insulin resistance and that association can be observed even after the adjustment of BMI¹³.

The function of the *FTO* protein product has not been completely understood yet. Most studies are association studies, and mechanisms of relations between gene variants and traits remain obscure¹⁴. Numerous studies confirmed that the *FTO* genotype has an impact on BMI, which in turn has an effect on metabolic traits. However, there is still a lack of linkage between the genotype and the resulting metabolic trait.

A recent study depicted that the *FTO* gene has a rapid turnover in the pancreatic β cells involved in the regulation of insulin secretion under glucose stimulation¹⁵. Additionally, according to the same study, *FTO* plays an important role in the function regulation of pancreatic β cells. However, the functional role of *FTO* in pancreatic β cells and related molecular mechanism are still unclear.

As the strongest common genetic determinants of adiposity, *FTO* variants partially act by influencing dopaminergic signaling in the brain and promote increased food intake¹⁶. The *FTO* A allele is linked to central insulin resistance, and peripheral insulin sensitivity regulation is controlled by central insulin¹⁷. Dopamine neurons also express *FTO* proteins and inactivation of the *FTO* gene impairs dopamine receptor type 2 (*D2R*) and type 3 (*D3R*; collectively, 'D2-like receptor')-dependent control of neuronal activity and behavioral responses¹⁸.

There appears to be an interaction between *FTO* and *ANKK1* polymorphisms that are responsible for midbrain activity during reward learning as both *FTO* and *ANKK1* confer risk for obesity by their influence on D2 receptors¹⁹. *ANKK1* indicates reduced D2 receptor availability, which was shown to lead to an *FTO* variation-related increase in body fat and waist circumference, as well as reduced peripheral insulin sensitivity¹⁶. This event confers an increase in the risk of obesity in the presence of any of the two risk alleles.

Eating disorders

FTO was recently identified as a potential candidate for the molecular association with eating disorders (ED).

A study published in 2017 suggests that individuals with the A allele are more likely to develop ED or to express emotional eating^{8,20}. The two *FTO* SNPs assumed to have the largest effect on BMI variations between individuals are rs9939609⁷ and rs9930506²¹. The recent genome-wide association study (GWAS) for BMI, which included data from up to 247,796 European individuals, found rs1558902 to be the most significantly associated SNP ($PGWAS < 10^{-60}$)²².

FTO and polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is frequently accompanied by insulin resistance and glucose intolerance²³. In addition, 38% to 88% of women with PCOS are overweight or obese²⁴. Consequently, T2DM and PCOS share common symptoms, which led to the investigation of the connection between *FTO* gene variants and individuals' risks of PCOS. Recently published studies have shown that the *FTO* gene variants might have large effects on obesity, insulin resistance and glucose intolerance among patients with PCOS phenotypes^{25,26}. The association between *FTO* and PCOS, however, still remains unclear and decrease in severity of phenotypic manifestations of PCOS is often linked to BMI improvement with weight loss²⁷. In a study completed in 2014, Louwers et al. demonstrated a lack of connection between 12 BMI risk alleles and PCOS²⁸.

The effect of *FTO* rs9939609 combined with *MC4R* rs17782313 has been discussed in relation to PCOS after studies on their effect on obesity²⁹. A ten-kg weight difference was reported between AA and TT homozygotic PCOS carriers³⁰. In addition, the *FTO* rs9939609 polymorphism

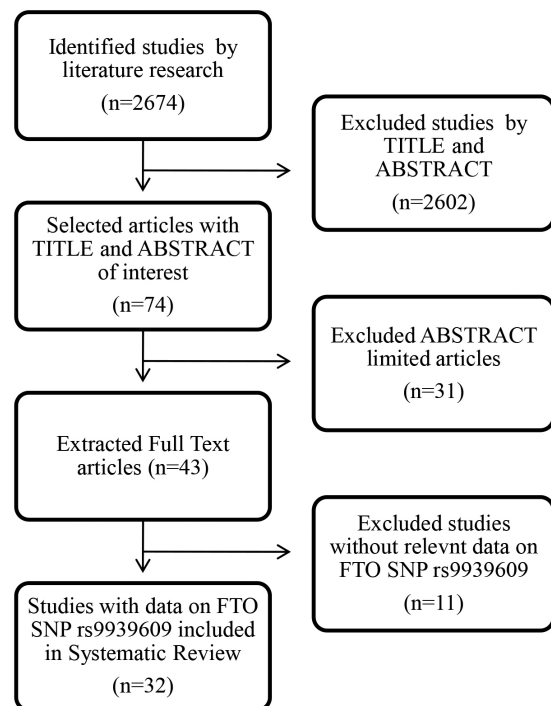


Figure 1. A flowchart for systematic review

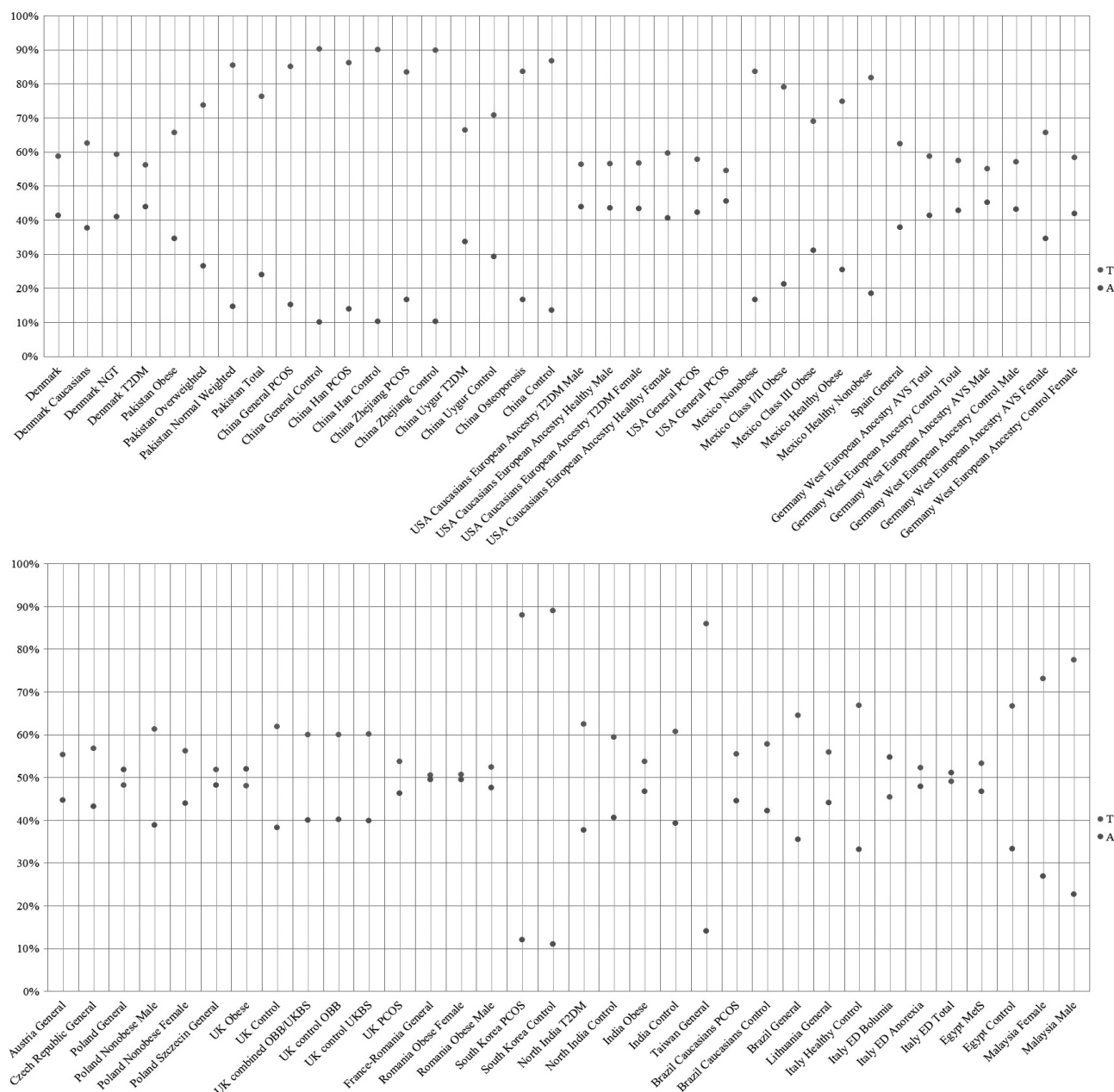


Figure 2. A graph depicting the frequencies of T and A alleles of *FTO* SNP rs9939609 in worldwide populations. Results were obtained from original research articles. Allele frequencies were found to be similarly abundant in Caucasian populations

was found to be significantly associated with the risk of PCOS in Asians and Caucasians³¹. Li et al. (2013) also found that the mutant allele A of rs9939609 leads to a higher risk of PCOS ($P_{\text{combined}} = 1.26 \times 10^{-11}$, ORs = 1.44; $P_{\text{meta}} = 6.89 \times 10^{-12}$), even after the adjustment for differences in BMI ($P = 1.82 \times 10^{-6}$)³².

We have therefore aimed to collect previously published data regarding the mutant allele A in different regions around the world and to summarize them in the form of a systematic review in order to investigate whether T2DM and PCOS onset and increase in BMI might be related to rs9939609 genotype.

Materials and Methods

This review is based on research papers collected from relevant databases. Terms »FTO«, »SNP«, »diabetes«, »in-sulin«, »resistance«, »PCOS«, »gene«, »variant«, »BMI«, and »hypothalamus«, were searched in PubMed and ResearchGate with a focus on papers published from the period of 2007 up to the end of November 2017.

In order to analyze the abundance of the mutant *FTO* allele, the authors searched for genotyping data of particular populations in the articles. From 46 papers, 14 studies concentrated on non-target SNPs or contained

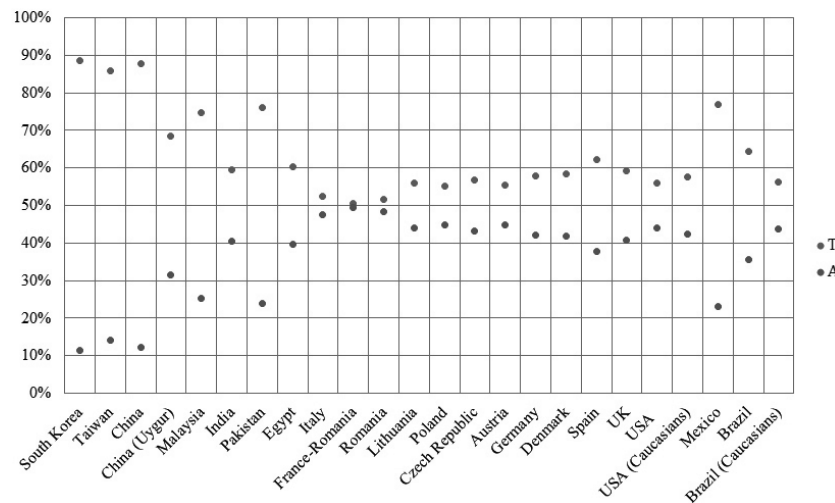


Figure 3. All *FTO* allele frequencies were plotted together in the following graph, and present over 24 worldwide populations

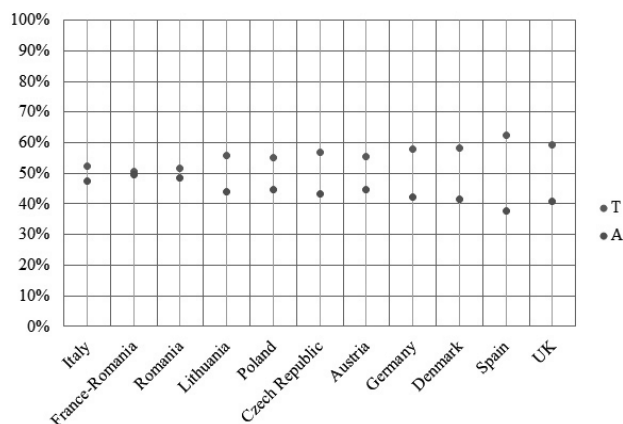


Figure 4. Higher-than-average distribution of the mutant allele *A* throughout Europe. More specifically, Romania and Italy reveal a statistically significant association between investigated *FTO* related disorders and mutant allele *A* as the groups in these populations are rich in that allele.

data which was not represented adequately for further analysis, and were eliminated as non-eligible studies. Therefore, a total of 32 studies were accepted for this review. Data extraction was performed in order to investigate the frequency of mutant alleles in specific regions. All selected articles provided data on the distribution of *FTO* alleles in the studied population. Exclusion and inclusion criteria are briefly explained in the Figure 1.

In order to investigate the possibility of *FTO* rs9939609 being an indicator for mutant allele-related disorders, *A* allele distribution was compared among populations in the world and data was linked to associated disorders for particular populations. Participants in the included studies were patients with conditions compared to control healthy subjects, or overweight/obese individuals compared to underweight/normal weight individuals. Different types of

genotyping methods were used, such as Taqman allelic discrimination^{33,34}, restriction enzyme length polymorphism (RFLP)^{35,36} with restriction enzyme *ScaI*³⁷, TaqMan assays^{38–42}, Qiagen^{32,40,43,44}, tetra-primer amplification refractory mutation system polymerase chain reaction whole on peripheral blood samples (ARMS-PCR)⁴⁵ or agarose gel electrophoresis^{46,47}. For result generation, we used sample sizes, genotype frequencies, allele frequencies, region and year of study publication.

In the first data extraction, a total of 32 data sets were obtained. Reorganization of obtained data provided distinguished data sets according to investigated populations, regions, and mutant allele-associated disorders. Results are presented in graphical form.

Results and Discussion

Most association studies reporting the effects of *FTO* variants were concerned with the gene variant effects on obesity and PCOS. A summary of all performed studies is given in Table 1.

In order to investigate the possibility of *FTO* rs9939609 being an indicator of mutant allele-related disorders, *A* allele distribution was compared among worldwide populations, and data was associated to the disorders of interest in those same populations. The data extraction provided 32 cohorts with genotype and allele frequencies from a total of 24 different populations. The highest mutant allele *A* frequency (49.5%) was observed in a combined French and Romanian population study³⁰. The lowest *A* allele frequency was reported in South Korean populations⁴⁸ in a study conducted by Kim et al., followed by the Chinese population^{29,32,49,50} and the Taiwanese population⁴⁰.

In the investigation of allele frequencies among the study populations (Figure 2), it was observed that the highest frequency of *A* allele is present in European population^{8,13,30,33,41,44,45,47,51–54}, populations of European descent

TABLE 1
AN OVERVIEW OF THE STUDIES ON *FTO* SNP RS9939609

Study population	Scope	Findings of interest	Reference
UK Obese, UK Control	ED	UK children participated in research regarding food intake. They were genotyped for rs9939609 and their eating behavior was analyzed. There was no significant relation between <i>FTO</i> genotype and any physical activity characteristics. However, it was shown that A allele is likely affecting appetite.	[34]
Czech Republic General Population	PCOS	Authors proposed that the <i>FTO</i> gene is associated with higher OGTT-stimulated glycaemia and leptin levels besides obesity-related quantitative traits. In this study, samples of participants from the Czech Republic were included. The most present genotype in population was TA, and mutant allele frequency was 43.2%.	[58] Retrieved from [30]
Mexico Nonobese, Mexico Class I/II Obese, Mexico Class III Obese, Mexico Obese Healthy, Mexico Nonobese Healthy, Mexico T2DM, Mexico Healthy	BMI	Subcutaneous and visceral adipose tissue biopsies from class III obesity patients were investigated for <i>FTO</i> and <i>ABCA1</i> mRNA expression. This was the first report giving evidence of <i>FTO</i> and <i>ABCA1</i> variant interactions affecting BMI. <i>FTO</i> and <i>ABCA1</i> risk alleles revealed a lack of significant association with BMI or WC.	[38]
USA Caucasians European Ancestry T2DM Male, USA Caucasians European Ancestry Healthy Male, USA Caucasians European Ancestry T2DM Female, USA Caucasians European Ancestry Healthy Female	Obesity	Study subjects were genotyped for rs9939609 in a larger research cohort. Measurements also included plasma adiponectin and leptin for both diabetic men and women. The authors observed a decrease in the level of association between rs9939609 and BMI in individuals older than 65.	[36]
Denmark Treatment Naïve, Denmark Caucasians, Denmark NGT, Denmark T2DM	T2DM	Danish participants from five different study groups were genotyped for <i>FTO</i> rs9939609. It was confirmed that this variation is associated with T2DM even without BMI adjustment.	[33]
France and Romania General Population	PCOS	From a study completed by Wojciechowski (2012), combined genotypes of French and Romanian populations previously published in Attaoua (2008) were analyzed for a total of 189 genotypes. Attaoua (2008) reported very similar incidence in both wild and mutant alleles. The most present genotype was TA. Attaoua (2008) reported association between MetS and PCOS.	[59] Retrieved from [30]
China PCOS, China Control	PCOS	PCOS patients and healthy controls participated in this case-control association study of linkage between <i>FTO</i> rs9939609 and PCOS development. The difference in allele frequencies between the groups was statistically significant with a higher frequency of mutant allele in the PCOS group. The mutant allele was found to influence PCOS development by causing an increase in the carrier's BMI.	[50] Retrieved from [31]
Poland General Population	PCOS	PCOS patients were the subject of anthropometric measurement, euglycaemic–hyperinsulinaemic clamp, oral glucose tolerance tests and sex hormone assessments. The study group was also genotyped for the <i>FTO</i> rs9939609 polymorphism. The <i>FTO</i> gene risk variant seems to have more impact on obesity and related traits in patients with PCOS than in others.	[52]
Austria General Population	PCOS	The impact of <i>FTO</i> variants on metabolic and endocrine parameters in PCOS women were investigated in PCOS patients. Alongside genotyping, metabolic and hormonal measurements, oral glucose tolerance test, hirsutism score, and lipometry were performed. The A allele was associated with a significant increase in free testosterone, BMI, 2-hour glucose, 1-hour insulin, and AUCins, as well as body fat percentage. The authors demonstrated that rs9939609 influences hyperandrogenemia and anthropometric parameters in women with PCOS.	[54]
UK combined OBB/UKBS, UK Control OBB, UK control UKBS, UK PCOS	PCOS	This was a genetic association study of the <i>FTO</i> rs9939609 variant with case–control analyses conducted on UK PCOS patients. Authors provided the first report about predisposing variants to common obesity, which also may lead to PCOS, mediated through adiposity. Authors reported a significant association between <i>FTO</i> genotype and PCOS status.	[60]

USA General Population, USA General population	PCOS	Subjects from families having a history of PCOS had samples taken for genotyping of 15 gene polymorphisms. Transmission/disequilibrium test (TDT) was used to assess linkage between PCOS and gene variants. In the independent case-study, PCOS women and healthy women were also analyzed. SNPs in <i>FTO</i> seemed to be associated with BMI in patients with PCOS, but to the reproductive phenotypes of PCOS.	[39]
Malaysia Female, Malaysia Male	Obesity	The aim of this study was to analyze the association of rs9939609 and obesity among 9- to 12-year-old children in Malaysia. No association between <i>FTO</i> rs9939609 polymorphism and BMI categories ($p=0.9014$), WC ($p=0.6828$), body fat percentage ($p=0.9011$), or gender ($p=0.3345$) was confirmed.	[61]
China Han PCOS, China Han Control	PCOS	A two-staged study involving PCOS patients and controls from an earlier GWAS, and an independent group of PCOS patients and controls was investigated for the relation between PCOS development and <i>FTO</i> gene variants. The influence of the SNP study was confirmed in both obese and non-obese groups.	[32]
Spain General population	Metabolic disturbance	Patients subsequently treated with pegIFNa/RBV therapy participated in this cross-sectional study. The results imply that the subjects carrying the risky AT/AA genotype of rs9939609 had higher odds of metabolic disturbances and did not successfully respond to HCV therapy.	[53]
India Obese, India Control	T2DM	The individuals were a subject of investigation of <i>GSTM1</i> , <i>GSTT1</i> and <i>FTO</i> polymorphism association with T2DM. The authors suggested further research, as significant association between the condition and <i>FTO</i> variants was not found.	[35]
South Korea PCOS, South Korea Control	PCOS	The objective of this study was to investigate the association between <i>FTO</i> polymorphism and PCOS in non-obese women. It was concluded that this gene variant affects the development of PCOS by acting to increase BMI, but that rs9939609 is not the main determinant for developing PCOS throughout the lifetime.	[48]
Pakistan Obese, Pakistan Overweight, Pakistan Normal, Pakistan Total	BMI	To find if there is an association between <i>FTO</i> gene rs9939609 polymorphism and BMI status of Karachi adolescents, subjects were classified as either normal, overweight or obese. The results have shown that 59.3% tested individuals had genotype TT, 32.6% were heterozygous, and 7.3% had the AA genotype. The results confirmed an increased risk of obesity in adolescents with genotype AA.	[37]
China Zhejiang PCOS, China Zhejiang Control	PCOS	This study, enrolling PCOS patients and controls, were looking for a link between <i>FTO</i> rs9939609 and <i>MC4R</i> rs17782313 polymorphisms and PCOS and obesity-related traits. The effect of <i>FTO</i> rs9939609 variant on PCOS development was confirmed, although through major influence on BMI.	[29]
China Uyghur T2DM, China Uyghur Control	T2DM	T2DM patients and healthy controls participated in this study to examine the correlation between <i>FTO</i> , T2DM and BMI. The authors concluded that A allele of rs8050136 and rs9939609 (both from <i>FTO</i> gene) may be associated with the T2DM and obesity prevalence.	[46]
Brazil Caucasians PCOS, Brazil Caucasian Control	PCOS	The study included PCOS patients and women with regular ovulation cycles. The correlation of <i>FTO</i> SNPs rs9939609 and rs8050136 with PCOS incidence was not found, while the presence of the risk allele in both SNPs was associated with higher fasting glucose levels.	[55]
Germany West European Ancestry AVS Total, Germany West European Ancestry Control Total, Germany West European Ancestry AVS Male, Germany West European Ancestry Control Male, Germany West European Ancestry AVS Female, Germany West European Ancestry Control Female	AVS	Impact of the <i>FTO</i> genotype was investigated in German patients with AVS and controls. The authors reported that association of rs9939609 with AVS was independent of BMI and other variables, such as diabetes mellitus.	[45]
Brazil General Population	Obesity	In the cross-sectional study, samples from children between the ages of 7 and 17 were genotyped for the study <i>FTO</i> SNP, along with recording information on the family history of obesity. The results implied that children with AA genotype were overweight/obese in 57.4% of all cases. On the other hand, TA and TT had lower percentage of overweight/obese children (33.1% and 28.9%, respectively).	[43]

Lithuania General Population	Obesity	Lithuanian individuals between 25 and 64 years were genotyped for rs9939609 in order to investigate the association between genotypes and obesity, and metabolic syndromes. The study confirmed a relationship between AA gene variants and high BMI.	[41]
Taiwan General Population	Obesity	rs9939609 genotyping was done on Taiwanese adults to show that the TT genotype is the most abundant (73.3%), while AA was present in 1.5% study participants. Results prove that the <i>FTO</i> rs9939609 SNP may be linked with the risk of obesity.	[40]
China Osteoporosis, China Control	Osteoporosis	Two <i>FTO</i> SNPs, rs7206790 and rs9939609, were analyzed in the study of osteoporosis risk. The osteoporosis patients and healthy controls were genotyped. It was shown that rs9939609 SNP does not relate to the risk of osteoporosis development during the lifetime.	[49]
Poland Nonobese Male, Nonobese Female	BMI	<i>FTO</i> genotyping of non-obese Poles, 20 to 40 years of age, was performed to study four variants (rs9939609, rs9930506, rs1421085 and rs1121980). For rs9939609, both female and male Poles revealed the highest frequency of AT genotype, while AA genotype was the least abundant. No significant differences were found between fat distribution indicators related to the haplotypes of the <i>FTO</i> gene in individuals with normal BMI.	[44]
Romania Obese Female, Romania Obese Male	Obesity	In order to detect if there is any association between <i>FTO</i> variants and anthropometric and metabolic characteristics, Romanian children (obese and non-obese), were tested for allele variants. The results confirmed a significant association between obesity and rs9939609 genotype.	[51]
Poland Szczecin General	Metabolic disorders	The study involved Caucasian men aged 50–75 years. Higher concentrations of TCh and LDL were found in men with the <i>FTO</i> rs9939609 SNP, even though MetS, T2DM, HT, and obesity were not related to it.	[47]
North India T2DM, North India Control	Obesity	Obese and non-obese individuals were genotyped for rs9939609, along with measurement of fasting glucose and insulin levels, lipid profile, percentage body fat, fat mass and fat free mass. Significant association between <i>FTO</i> rs9939609 and obesity defined by BMI was reported.	[57]
Italy Healthy Control, Italy Eating Disorders Bolumia, Italy Eating Disorders Anorexia, Italy Eating Disorders Total	ED	The authors proposed a model of genetic variant interaction with eating disorders. The ED patients and healthy control subjects were genotyped for SNP rs9939609 and mutant allele A was confirmed to be associated with a binge eating behavior.	[8]
Egypt MetS, Egypt Control	MetS	This is the first study reporting rs9939609 as the genetic risk factor for metabolic syndrome in Egyptian populations. Individuals with risk genotype (AA) have significantly higher ALT levels, which remained significant after the correction for BMI and serum triacylglycerols. However, the result was not statistically significant following conservative Bonferroni correction.	[42]

T2DM=Type 2 diabetes mellitus, *FTO*=Fat mass and obesity-associated protein, PCOS=Polycystic ovary syndrome, AVS=Aortic valve stenosis, SNP=Single nucleotide polymorphism, BMI=Body mass index, WC=Wrist circumference, ALT=Alanine aminotransferase, HCV=Hepatitis C virus, *ABCA1*=ATP-binding cassette transporter, MetS=Metabolic syndrome, LDL=Low density lipoprotein, TCh=Total Cholesterol, HT= Hypertension, AUCins =Area under the curve insulin, TDT=Transmission/disequilibrium test, *NEGR1*=Neural growth regulator 1, *MC4R*=Melanocortin 4 receptor, ED=Eating disorders, *TCF7L2*=Transcription Factor 7 like 2, *INSIG2*=Insulin induced gene 2, IR=Insulin resistance

on other continents⁵⁵ or in populations that are likely to be of European descent⁴⁶.

In order to analyze A allele distribution in more depth, allele frequencies from different study cohorts were added together and joint results are presented in Figure 3. The allele distribution patterns imply that geographically close populations exhibit similar allele frequencies for rs9939609 SNP. Considering the fact that European populations are rich in *FTO* allele A, those populations were extracted from the dataset and plotted on a separate graph. In this way, it has been shown that alleles T and A have similar distribution throughout Europe (Figure 4).

South Korean, Taiwanese, and Chinese populations have similar patterns of A allele distribution. On the other hand, Uygur population, even though settled in China, shows a higher incidence of the mutant allele (Figure 5). According to earlier research on the Uygur population from China, this population truly differs from its neighboring populations, as confirmed in the present study (Figures 5 and 6a) using the *FTO* SNP rs9939609. Dogan et al. (2014) analyzed Uygurs in a study of 46 worldwide populations on the basis of autosomal short tandem repeats (STRs) to confirm its uniqueness in the region⁵⁶. On the other hand, it is interesting to note that Egyptian and

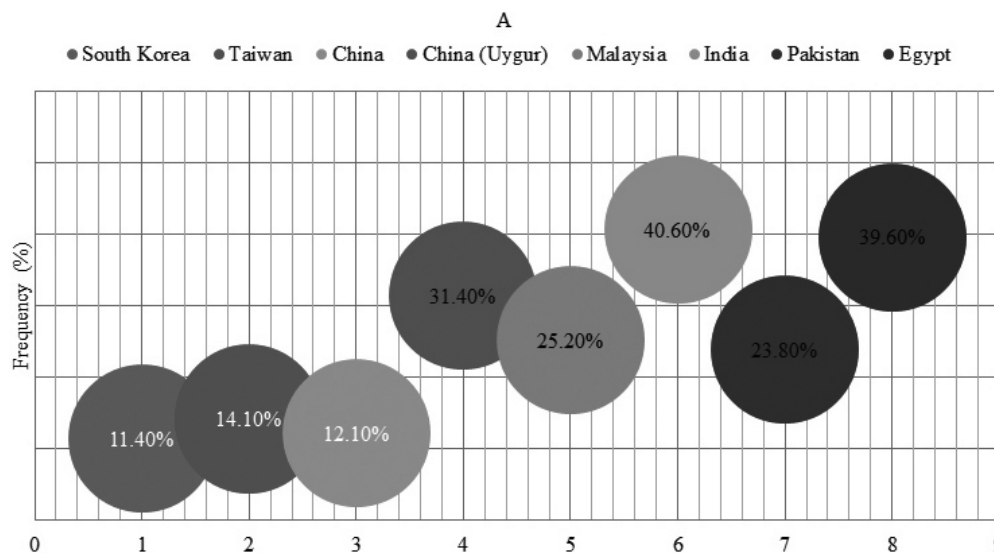


Figure 5. Uygur and Egyptian populations differ from their neighboring populations in terms of A allele frequency distribution.

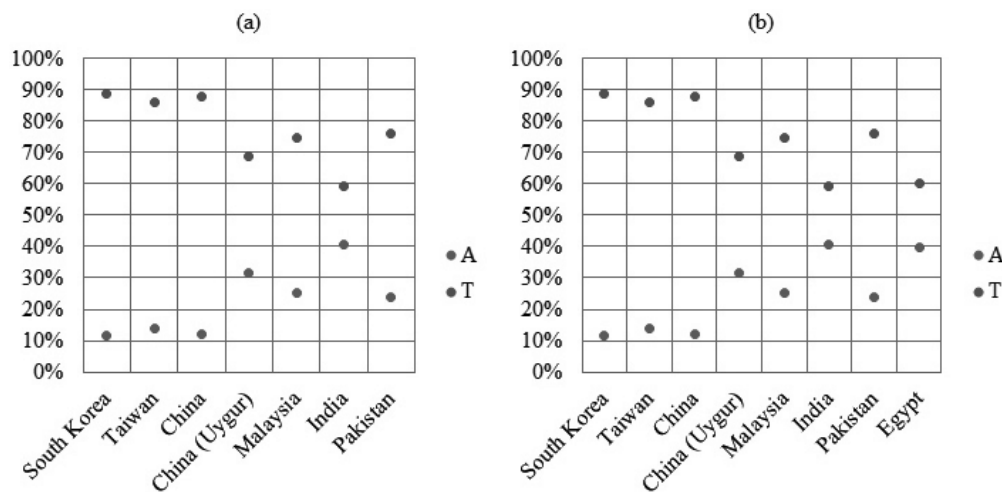


Figure 6. (a) The distribution of rs9939609 alleles in Asian populations with a significant increase of mutant allele A frequency in Indian and Uygur populations. (b) The comparison of rs9939609 allelic frequencies in Egypt and Asian countries.

Indian populations have similar frequencies of A and T alleles, while Pakistani population tends to differ from these two (Figure 6b). However, it was not feasible to assess the allele frequency ratios in Egypt and its closest neighbors, as Egypt is the only population of Arabs analyzed in this study (Figure 6b).

Brazilians of European origin exhibit results similar to those from European and US populations^{36,55}. In addition, it has been observed that Brazilian Caucasian subpopulation differs by 8.2% from other Brazilian populations in terms of mutant allele A frequency. The lowest incidence of *FTO* allele A in the American continent was observed in Mexico (Figure 7).

The focus of extensive research has been the relation between rs9939609 and T2DM^{7,33,46,57}. Numerous studies have been conducted on *FTO* resulting in confirmed effects

of gene variants on weight and BMI. A limitation of the present study is that data on *FTO* variants and their association with T2DM were obtained from only four populations, namely Denmark, US Caucasian, Uygur, and North Indian. However, it was observed that allele A frequencies were similar in the T2DM groups and in control groups in all populations (Figure 8). In Denmark, the T2DM group had a 6.3% higher frequency of allele A when compared to the control. The mutant allele was more prevalent than wild-type allele T in the other three populations with a frequency difference of 4.4%, 3% and 0.5% in Uygur, Indian and US Caucasian populations, respectively (Figure 8).

Despite the fact that the overall frequency of allele A is rather low in Chinese population, PCOS patients from the same country tend to have a significant increase in the frequency of this allele. On the other hand, Brazilian popu-

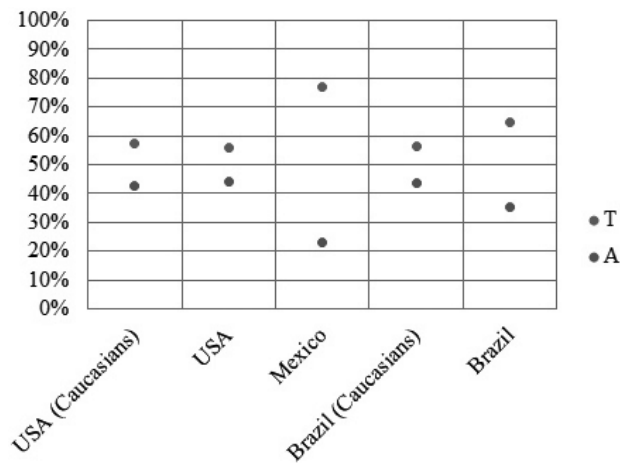


Figure 7. The Mexican population has the lowest incidence of mutant FTO allele A in the Americas. Also, a difference in allele frequency between general Brazilian populations and Caucasian Brazilians and Europeans settled within the US has been observed.

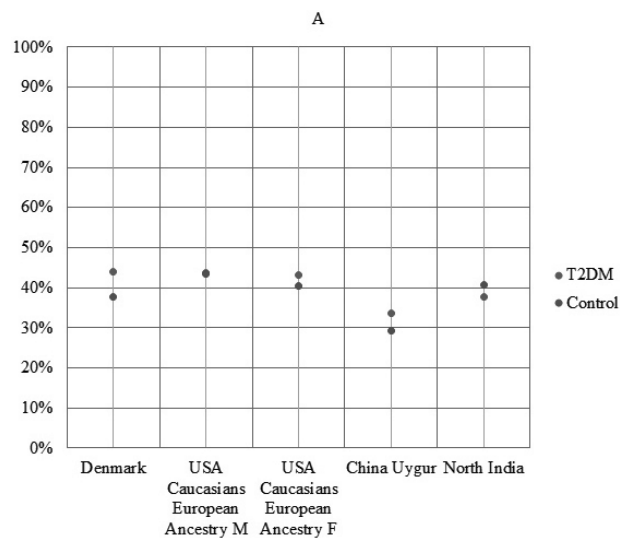


Figure 8. Only four studies investigating the association between FTO SNP and T2DM fulfilled the inclusion criteria for the present meta-analysis and were therefore included in the study. In all four populations, dramatic differences in allele frequencies between control samples and patients were not observed.

lations have a relatively high allele A frequency, however the difference between PCOS patients and controls is far less pronounced than in Chinese population (Figure 9).

Villalobos-Comparán et al. (2008) concluded that rs9939609 has been associated only with class III obesity and not with overall obesity or BMI in admixed Mexican adults³⁸. The data available for the present analysis allowed the comparison of obese individuals with controls in only three populations (UK, Mexican, and Indian)^{34,38,57}. On the basis of this limited dataset, it has been concluded

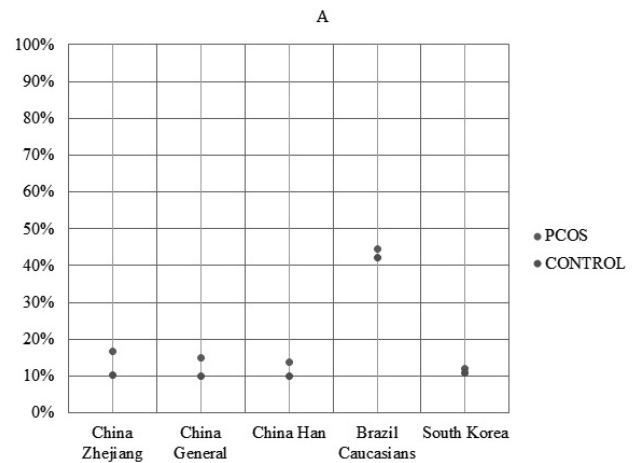


Figure 9. In terms of the relations between PCOS and allele A of the FTO polymorphism, China has the most pronounced differences between patients and controls. The remaining three populations do not reveal any significant differences.

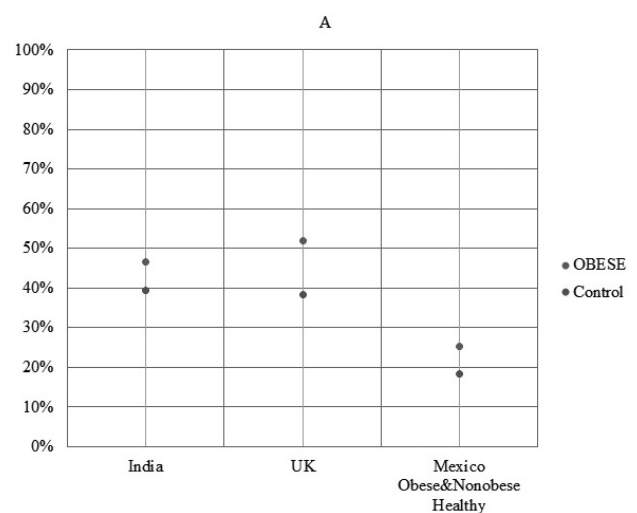


Figure 10. Although differences in allele A frequency has been noted between obese persons and controls in all three populations, it is important to note that T2DM frequency positively correlates with allele frequency as well, thus making it harder to generate final conclusions on FTO-obesity association.

that the obese and non-obese groups within the study populations positively correlate with A allele frequency in those populations. The most prominent difference in allele A frequency between obese and non-obese individuals was observed in the UK with allele A being 14.69% more prevalent in obesity group.

Conclusions and Future Perspectives

The main objective of the present study was to collect and discuss previously published data on FTO SNP

rs9939609, which has been associated with weight-related health conditions. The results of original studies show a higher incidence of mutant A allele in European populations, as well as in populations of European origin inhabiting other continents when compared to the other regions of the world. In addition, it has been found that the frequency of the mutant allele of this polymorphism positively correlates with increased BMI in study participants. Other conditions that were suspected of being associated with rs9939609 are PCOS and T2DM. However, as obe-

sity is usually one of the most prominent symptoms of both PCOS and T2DM, there is a necessity of normalizing patients' data according to BMI. In addition, there is a need for selecting patients and healthy controls with similar demographic and other parameters in larger study cohorts, which would enable researchers to follow the association between *FTO* polymorphism and a single condition. This is especially true for Arabic populations, as studies on these populations are scarce in current literature.

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DESETLJEĆE UČESTALOG FTO RS9939609 POLIMORFIZMA

SAŽETAK

Više studija povezanosti polimorfizama FTO gena su objavljene u proteklih godina; međutim, povezanost između raznih obilježja i i varijanata gena FTO-a ostaje neobjašnjena. Populacijska genetika i studije povezanosti u različitim populacijama pružaju vrijedne podatke za daljnja istraživanja. Stoga je cilj ovog sustavnog pregleda sažeti dosadašnje spoznaje o FTO SNP rs9939609. Rezultati sustavnog pregleda upućuju na to da je mutantni alel A najčešći u europskim populacijama i najrijeđi na Dalekom Istoku. Nadalje, zaključeno je da je alel A dobar indikator za predviđanje povećanog rizika od povišenog BMI-a kod osobe koja ga nosi, kao i da se alel A treba dalje analizirati kao mogući marker rizika za dijabetes melitus tip 2 te razvoj sindroma policističnih jajnika.

