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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

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GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией и Международной академией наук, образования, искусств и естествознания (IASEIA) США с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения.

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GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

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При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

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ავტორთა საყურადღებოდ!

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1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

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5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

Содержание:

SIRT1 CONTRIBUTES TO POLARIZATION OF PERIPHERAL BLOOD MONOCYTES BY INCREASING STAT6 EXPRESSION IN YOUNG PEOPLE WITH OVERWEIGHT AND LOW-RISK OBESITY

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Obesity contributes to the formation of low-intensity systemic inflammation with major participation of monocytes/macrophages [4,5]. The true heterogeneity and versatility of these cells is manifested by rapid phenotypic and functional switching in response to microenvironmental signals. There are three ways to promote polarization: epigenetic and cellular pathways that prolong or reduce the development and viability of macrophages, tissue microenvironment and external factors such as products of microorganisms and cytokines, released during inflammation [16].

Based on the phenotype and secreted cytokines, two main types of macrophages have been identified: classically activated M1 and alternatively activated M2. The M1 phenotype is stimulated with microbial products or proinflammatory cytokines – interferon-γ ligands (γIFN), tumor necrosis factor (TNF) or Toll-like receptor (TLR) – lipopolysaccharide (LPS), γIFN. M1 cells have a proinflammatory function mediated by the secretion of cytokines TNFα, IFN type I, interleukins (IL) 1β, 6, 12 and others, producing reactive oxygen species and nitrogen to ensure effective microbial destruction, expressing the surface markers MHC-I/II, CD80 and CD86 [8].

M2 is a "rest" phenotype that inhibits Th1/M1-induced inflammation, promotes tissue regeneration and is observed in cases of infection-free healing, and mediates Th2-related pathologies such as asthma and helminthiasis. The M2 fraction can be induced by canonical stimuli IL-4, IL-10, IL-13 and arginase-1 (Arg-1) and transforming growth factor β (TGFβ).

M2 macrophages are characterized by a high level of secretion of anti-inflammatory cytokines, such as IL-10 and a very low level of pro-inflammatory ones, such as IL-12 [18].

Under different pathophysiological conditions, the same signaling pathway may be involved in the polarization of M1 or M2 macrophages. Alternative macrophage activation regulates systemic inflammation and plays an important role in the development of metabolic disorders, though, the molecular mechanisms of regulation of macrophage phenotype switching have not been fully studied.

One of the essential ways to implement epigenetic mechanisms is posttranslational modification of histone and nonhistone proteins by their deacetylation using the sirtuin family SIRT1-7 [24]. SIRT1 is the nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase class III, which epigenetically reprograms inflammation by deacetylation of histones and transcription factors – nuclear factor κB (NF-κB) and activator protein 1 (AP-1), leading to transcriptional repression of inflammatory-related genes [26]. SIRT1 regulates polarization of macrophages by controlling the inhibition of the M1 subpopulation and stimulating the activation of M2 macrophages [27].

SIRT1 may be involved in alternative macrophage activation. It has been found that the expression of SIRT1 is higher in anti-inflammatory macrophages of the M2 phenotype, SIRT1 deficiency coordinates the stimulating conversion of M1 macrophages and inhibits alternative M2 activation [27].

Therefore, the aim of our study was to determine the regulatory role of SIRT1 in M1/M2 polarization of peripheral blood monocytes in young people with overweight and Class I obesity.

Material and methods. The study was conducted with the permission of the Commission on Bioethics of the Ukrainian Medical Stomatological Academy. Informed consent was signed by all subjects.

30 subjects of both gender, aged 18-25 years have been examined. Anthropometric studies with the calculation of body mass index (BMI) according to the formula: BMI =body weight (kg)/ height $(m)^2$ have been made. Groups were formed by the BMI: the subjects with normal body weight (n=10, BMI 18.50–24.99 kg/m^2), the subjects with overweight (n=10, BMI 25.00–29.99 kg/m^2), the subjects with Class I obesity (n=10, BMI 30.00–34.99 kg/m^2).

Peripheral blood mononuclear suspension was isolated from heparinized blood according to conventional technique by density gradient centrifugation of phycol-verografin (ρ=1.077 g/ml^3, Granum, Ukraine) followed by double washing in sterile 0.9% NaCl.

Monocytes were isolated by adhesion on the plastic plates in RPMI-1640 medium with L-glutamine and sodium bicarbonate (Sigma-Aldrich, USA), resuspended, using, upon counting completion, suspension with a concentration of not less than $3\text{-}5\times10^6$ cells/ml, which in a volume of 0.5 ml was transferred to the wells of the 24-well sterile plates.

E. coli lipopolysaccharide (LPS) (Sigma-Aldrich, USA) at a dose of 100 ng/mL and γIFN (Ingaron, Pharmaclone, Russia) at a dose of 100 ng/mL were used to induce polarization of macrophages by the M1 phenotype [11,14]. To induce polarization of macrophages by the M2 phenotype, recombinant human IL-4 (Sino Biological, Life Technolohies, USA) was added to the incubation medium at a dose of 20 ng/mL [14]. Unstimulated monocytes/macrophages were used as controls.

Cells and supernatant were selected for the study on day 3 and 7 of incubation at 37°C in an atmosphere with 5% CO_2. Cell suspensions were processed under sterile conditions.

The level of the *stat1*, *stat6* and *sirt1* gene expression was determined by the polymerase chain reaction (PCR) in real-time PCR. The total RNA was isolated from a biological sample using a set of reagents for isolation and purification of RNA with a magnetic sorbent (UkrGenTech, Ukraine).

Determination of *stat1*, *stat6* and *sirt1* gene expression was performed using the DT-light detection amplifier (DNA-Technology, Russia). The sequencing primers are shown in Table 1; the b-actin gene (*Actin beta*, *ACTB)* was used as a reference gene [20,21,17].

For data analysis, the relative Ct method was used, calculated by the formula $2^{-\Delta Ct}$.

Table 1. Primers for determining gene expression

Gene	Sequencing primers
stat1	F: 5' - CCAAAGGAAGCACCAGAGCC - 3' R: 5' - AGAGCCCACTATCCGAGACACC - 3'
stat6	F: 5′ - CTTTCCGGAGCCACTACAAG - 3′ R: 5′ - AGGAAGTGGTTGGTCCCTTT - 3′
sirt1	F: 5' - TCAGTGTCATGGTTCCTTTGC - 3' R: 5' - AATCTGCTCCTTTGCCACTCT - 3'
ACTB	F: 5' - GACAGGATGCAGAAGGAGATTACT - 3' R: 5' - TGATCCACATCTGCTGGAAGGT - 3'

The level of IL-6 and IL-10 cytokines in the cell supernatant was determined on day 7 of incubation; high-sensitivity C-reactive protein (hsCRP) (Vector-Best, Russia) and serum TGFβ1 (Affimetrix, eBioscience, Austria) was determined using the sets of reagents for solid-phase enzyme-linked immunoabsorbent assay in compliance with the manufacturer's instructions. The results were recorded using the LabLine-026 analyzer.

Statistical data processing was performed using the STATISTICA 10.0 (StatSoft Inc., USA) and GraphPad Prism 8.00 (GraphPad Software Inc., USA) software. Data are presented in the form of arithmetic mean (M) and its mean accuracy (m), the median (Me), upper and lower quartiles (Q1-Q3). The Shapiro-Wilk test was used to verify the normality of the data distribution. Statistical processing was performed using the nonparametric even Wilcoxon test and the odd Mann-Whitney test. Spearman's correlation analysis was used to determine the correlation between the rates. Differences at $p<0.05$ were considered statistically significant.

Results and discussion. During incubation for 3 days, a significant increase in the level of *stat1* gene expression was observed in cells, stimulated with M1 and M2 phenotype, in the subjects of all study groups (Table 2). The most significant increase was observed in LPS and γIFN-stimulated cells compared to the expression level in unstimulated cells. Upon stimulation of IL-4 monocytes, a significantly higher level of *stat1* expression was determined in cells of the subjects with normal weight and Class I obesity, compared to unstimulated cells.

The level of *stat6* gene expression was significantly higher in LPS and γIFN-stimulated cells in the subjects with normal weight and Class I obesity. A significant increase in expression level of IL-4-stimulated cells was found in the subjects with overweight and Class I obesity.

The *sirt1* gene expression was significantly increased in LPS and γIFN-stimulated and IL-4-stimulated macrophages in cells of the subjects of all study groups, compared to the corresponding rates of unstimulated cells.

Notably, the rates of *sirt1* expression in IL-4-stimulated cells were higher compared to LPS and γIFN-stimulated cells. In cells of the group with normal body weight, this difference was significant.

The maximum rate of the expression level was observed in the cells of the subjects with Class I obesity compared to unstimulated cells (1.084 (0.574 – 5.650)$^{-\Delta Ct}$ and 0.501 (0.203 – 0.877)$^{-\Delta Ct}$, p=0.005) (Table 2).

At the next stage, the level of *stat1, stat6* and *sirt1* gene expression in cells incubated for 7 days was determined (Table 2). In LPS and γIFN-stimulated cells, the level of *stat1* gene expression was significantly higher in cells of all groups compared to the expression level in the corresponding unstimulated cells.

The level of *stat1* gene expression in IL-4-stimulated cells of all groups is significantly higher compared to unstimulated cells. The highest level of expression was determined in cells of the group with Class I obesity compared to the rates in unstimulated cells (0.206 (0.036 – 0.466)$^{-\Delta Ct}$ and 0.109 (0.029 – 0.217)$^{-\Delta Ct}$, respectively, p=0.005).

The study of *stat6* gene expression in LPS and γIFN- stimulated macrophages has shown its significantly higher level in the cells of the subjects of all study groups compared to the corresponding rates of unstimulated cells.

The level of *stat6* gene expression in IL-4-stimulated macrophages was significantly higher in the cells of subjects with overweight and Class I obesity compared to unstimulated cells. The highest expression rate was observed in IL-4-stimulated macrophages in the obese subjects (0.017 (0.010 - 0.072)$^{-\Delta Ct}$ and 0.008 (0.006 - 0.041)$^{-\Delta Ct}$, respectively, p=0.005).

In cells of the subjects with normal body weight, the level of *stat6* gene expression in IL-4-stimulated cells was significantly lower than that compared to LPS- and γIFN-stimulated macrophages. The level of *sirt1* gene expression was significantly higher in LPS and γIFN-stimilated macrophages and IL-4-stimulated macrophages of cells of the subjects of all study groups compared to unstimulated cells.

Subsequently, we compared the levels of the *stat1, stat6,* and *sirt1* gene expression between the study groups (Table 3). No significant difference in the level of the *stat1* gene expression was found.

When incubated for 3 days, a significantly higher level of *stat6* gene expression was detected in IL-4-stimulated cells in subjects with overweight compared to the control group (0,008 (0,005 – 0,009)$^{-\Delta Ct}$ and 0,003 (0,002 – 0,005)$^{-\Delta Ct}$, respectively).

Significantly higher level of *stat6* gene expression was also found in unstimulated cells, LPS and γIFN- stimulated macrophages and IL-4- stimulated macrophages in obese subjects compared to the corresponding cells of the subjects with normal body weight.

Incubation for 7 days showed a significantly higher level of *stat6* gene expression in the IL-4-stimulated cells of the overweight subjects compared to the control group. In the subjects with Class I obesity, the *stat6* gene expression was significantly higher in the unstimulated cells and in IL-4-stimulated cells, compared to the control group.

The *sirt1* gene expression during incubation for 3 days in overweight subjects exceeded the data of the control group in the unstimulated cells, LPS and γIFN-stimulated cells and IL-4-stimulated cells. In cells of the subjects with Class I obesity the *sirt1* gene expression exceeded the data of the control group in unstimulated cells, LPS and γIFN-stimulated cells and IL-4-stimulated cells.

Table 2. The level of stat1, stat6, sirt1 gene expression in monocytes/macrophages of the study groups (Me (Q1-Q3))

Groups	***Stat1*, $2^{-\Delta Ct}$**			***Stat6*, $2^{-\Delta Ct}$**			***Sirt1*, $2^{-\Delta Ct}$**		
	Unstimulated cells	**LPS- and γIFN-stimulated cells**	**IL-4-stimulated cells**	**Unstimulated cells**	**LPS- and γIFN-stimulated cells**	**IL-4-stimulated cells**	**Unstimulated cells**	**LPS- and γIFN-stimulated cells**	**IL-4-stimulated cells**
	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10
on day 3 of incubation									
Subjects with normal body weight	0,098 (0,039 - 0,177)	0,199 (0,051 - 0,287) **p=0,005**	0,121 (0,041 - 0,267) **p=0,005** p1=0,406	0,002 (0,002 - 0,004)	0,005 (0,003 - 0,007) **p=0,005**	0,003 (0,002 - 0,005) p=0,241 p1=0,059	0,003 (0,002 - 0,005)	0,003 (0,002 - 0,005) **p=0,005**	0,006 (0,006 - 0,007) **p=0,008 p1=0,028**
Subjects with overweight	0,032 (0,022 - 0,102)	0,046 (0,034 - 0,250) **p=0,005**	0,035 (0,029 - 0,154) p=0,161 p1=0,290	0,004 (0,002 - 0,005)	0,005 (0,003 - 0,007) p=0,075	0,008 (0,005 - 0,009) **p=0,005** p1=0,096	0,024 (0,014 - 0,051)	0,028 (0,019 - 0,054) p=0,008	0,040 (0,024 - 0,083) **p=0,005** p1=0,308
Subjects with Class I obesity	0,071 (0,017 - 0,217)	0,131 (0,029 - 0,330) **p=0,005**	0,074 (0,027 - 0,267) **p=0,008** p1=0,496	0,095 (0,083 - 0,117)	0,159 (0,109 - 0,177) **p=0,005**	0,210 (0,154 - 0,354) **p=0,005** p1=0,064	0,501 (0,203 - 0,877)	0,559 (0,250 - 1,310) **p=0,005**	1,084 (0,574 - 5,650) **p=0,005** p1=0,212
on day 7 of incubation									
Subjects with normal body weight	0,092 (0,027 - 0,154)	0,118 (0,047 - 0,267) **p=0,005**	0,189 (0,047 - 0,330) **p=0,005** p1=0,406	0,003 (0,002 - 0,004)	0,006 (0,005 - 0,01) **p=0,005**	0,004 (0,003 - 0,006) p=0,059 **p1=0,049**	0,002 (0,001 - 0,003)	0,003 (0,002 - 0,004) **p=0,005**	0,004 (0,003 - 0,005) **p=0,005** p1=0,096
Subjects with overweight	0,032 (0,021 - 0,077)	0,056 (0,036 - 0,165) **p=0,008**	0,08 (0,047 - 0,217) **p=0,005** p1=0,273	0,005 (0,003 - 0,01)	0,007 (0,005 - 0,015) **p=0,008**	0,009 (0,008 - 0,027) **p=0,005** p1=0,112	0,019 (0,014 - 0,047)	0,035 (0,021 - 0,063) **p=0,005**	0,048 (0,025 - 0,095) **p=0,005** p1=0,364
Subjects with Class I obesity	0,109 (0,029 - 0,217)	0,128 (0,031 - 0,250) **p=0,008**	0,206 (0,036 - 0,466) **p=0,005** p1=0,273	0,008 (0,006 - 0,041)	0,011 (0,008 - 0,072) **p=0,008**	0,017 (0,010 - 0,072) **p=0,005** p1=0,241	0,483 (0,287 - 0,662)	0,685 (0,435 - 0,758) **p=0,005**	0,877 (0,819 - 1,650) **p=0,005** p1=0,089

notes: P – the significance of differences between the expression rates in LPS and γIFN-stimulated cells, IL-4-stimulated cells and unstimulated cells; p1 – the significance of differences between the expression rates in cells stimulated with LPS and γIFN and IL-4

Table 3. The level of the stat1, stat6, sirt1 gene expression in monocytes/macrophages of the study groups (Me (Q1-Q3))

Rates	Subjects with normal body weight n=10	Subjects with overweight n=10	Subjects with Class I obesity n=10
***Stat1*, $2^{-\Delta Ct}$**			
Unstimulated cells, 3 days of incubation	0,098 (0,039 - 0,177)	0,032 (0,022 - 0,102) p=0,450	0,071 (0,017 - 0,217) p=0,91 p1=0,821
LPS- and γIFN-stimulated cells, 3 days of incubation	0,199 (0,051 - 0,287)	0,046 (0,034 - 0,250) p=0,496	0,131 (0,029 - 0,330) p=0,821 p1=1,000
IL-4-stimulated cells, 3 days of incubation	0,121 (0,041 - 0,267)	0,035 (0,029 - 0,154) p=0,273	0,074 (0,027 - 0,267) p=0,623 p1=0,623
Unstimulated cells, 7 days of incubation	0,092 (0,027 - 0,154)	0,032 (0,021 - 0,077) p=0,385	0,109 (0,029 - 0,217) p=0,623 p1=0,521
LPS- and γIFN-stimulated cells, 7 days of incubation	0,118 (0,047 - 0,267)	0,056 (0,036 - 0,165) p=0,521	0,128 (0,031 - 0,250) p=0,97 p1=1,000
IL-4-stimulated cells, 7 days of incubation	0,189 (0,047 - 0,330)	0,08 (0,047 - 0,217) p=0,496	0,206 (0,036 - 0,466) p=0,678 p1=0,734
***Stat6*, $2^{-\Delta Ct}$**			
Unstimulated cells, 3 days of incubation	0,002 (0,002 - 0,004)	0,004 (0,002 - 0,005) p=0,227	0,095 (0,083 - 0,117) **p=0,0002** **p1=0,0002**
LPS- and γIFN-stimulated cells, 3 days of incubation	0,005 (0,003 - 0,007)	0,005 (0,003 - 0,007) p=1,000	0,159 (0,109 - 0,177) **p=0,0002** **p1=0,0002**
IL-4-stimulated cells, 3 days of incubation	0,003 (0,002 - 0,005)	0,008 (0,005 - 0,009) **p=0,005**	0,210 (0,154 - 0,354) **p=0,0002** **p1=0,0002**
Unstimulated cells, 7 days of incubation	0,003 (0,002 - 0,004)	0,005 (0,003 - 0,01) p=0,597	0,008 (0,006 - 0,041) **p=0,013** p1=0,112
LPS- and γIFN-stimulated cells, 7 days of incubation	0,006 (0,005 - 0,01)	0,007 (0,005 - 0,015) p=0,706	0,011 (0,008 - 0,072) p=0,054 p1=0,082
IL-4-stimulated cells, 7 days of incubation	0,004 (0,003 - 0,006)	0,009 (0,008 - 0,027) **p=0,005**	0,017 (0,010 - 0,072) **p=0,002** p1=0,054

Sirt1, $2^{-\Delta Ct}$			
Unstimulated cells, 3 days of incubation	0,003 (0,002 - 0,005)	0,024 (0,014 - 0,051) **p=0,0002**	0,501 (0,203 - 0,877) **p=0,0002** **p1=0,0003**
LPS- and γIFN-stimulated cells, 3 days of incubation	0,003 (0,002 - 0,005)	0,028 (0,019 - 0,054) **p=0,0002**	0,559 (0,250 - 1,310) **p=0,0002** **p1=0,0003**
IL-4-stimulated cells, 3 days of incubation	0,006 (0,006 - 0,007)	0,040 (0,024 - 0,083) **p=0,0003**	1,084 (0,574 - 5,650) **p=0,0003** **p1=0,0003**
Unstimulated cells, 7 days of incubation	0,002 (0,002 - 0,003)	0,019 (0,014 - 0,047) **p=0,0002**	0,483 (0,287 - 0,662) **p=0,0002** **p1=0,0002**
LPS- and γIFN-stimulated cells, 7 days of incubation	0,003 (0,002 - 0,004)	0,035 (0,021 - 0,063) **p=0,0002**	0,685 (0,435 - 0,758) **p=0,0002** **p1=0,0002**
IL-4-stimulated cells, 7 days of incubation	0,004 (0,003 - 0,005)	0,048 (0,025 - 0,095) **p=0,0002**	0,877 (0,819 - 1,650) **p=0,0002** **p1=0,0002**

notes: here and thereafter in Table 4: p – the significance of differences between the rates of groups with overweight, Class I obesity and subjects with normal body weight;
p1 – the significance of differences between the rates of groups with overweight and Class I obesity

Table 4. stat1/stat6 gene expression ratio (M±m)

Rates	Subjects with normal body weight n=10	Subjects with overweight n=10	Subjects with class I obesity n=10
3-day long incubation			
stat1/stat6 ratio, LPS- and γIFN-stimulated cells	45,83 ± 9,28	37,76 ± 13,72 p=0,45	1,07 ± 0,29 **p=0,002** **p1=0,0003**
stat1/stat6 ratio, IL-4-stimulated cells	52,67 ± 12,59	13,20 ± 3,58 **p=0,045**	0,51 ± 0,14 **p=0,0003** **p1=0,0003**
7-day long incubation			
stat1/stat6 ratio, LPS- and γIFN-stimulated cells	17,15 ± 5,40	18,94 ± 6,69 p=0,91	10,76 ± 3,81 p=0,308 p1=0,241
stat1/stat6 ratio, IL-4-stimulated cells	37,82 ± 8,87	12,12 ± 3,71 **p=0,026**	14,05 ± 5,66 **p=0,038** p1=0,791

7-day-long incubation of cells of the subjects with overweight was characterized by a significantly higher level of *sirt1* gene expression compared to the control group in unstimulated cells, LPS and γIFN-stimulated cells and IL-4-stimulated cells. In cells of the subjects with Class I obesity the *sirt1* gene expression was also significantly higher in unstimulated cells, LPS and γIFN-stimulated cells and IL-4-stimulated cells, compared to the control group.

To determine the direction of polarization of macrophages, the *stat1* to *stat6* gene expression ratio was calculated (Table 4).

It has been established that the *stat1/stat6* ratio in LPS and γIFN-stimulated cells, incubated for 3 days, was by 97.67% significantly lower in the subjects with Class I obesity compared to the subjects with normal body weight and by 97.17% lower compared to the subjects with overweight.

In the subjects with overweight, the *stat1/stat6* ratio in IL-4-stimulated cells was significantly lower compared to cells of the control group. The most significantly low *stat1/stat6* ratio was found in the IL-4-stimulated cells of the subjects with Class I obesity (0.51 ± 0.14 vs. 52.67 ± 12.59 in group with normal body weight and 13.20 ± 3.58 subjects with overweight, respectively).

In cells incubated for 7 days, the difference in the ratio was smaller (Table 4). In the subjects with overweight and obesity, the *stat1/stat6* in IL-4-stimulated cells was significantly lower by 67.95% and 65.50%, respectively, compared to the corresponding ratio in the cells of the subjects with normal body weight.

Subsequently, on day 7 of incubation, the level of cytokines in the cell supernatants was determined. In the subjects with Class I obesity, the level of IL-6 in supernatants of LPS and γIFN-stimulated cells was by 0.12% significantly higher compared to unstimulated cells.

The level of IL-6 in the supernatants of IL-4-stimulated cells of the subjects with normal body weight and overweight was significantly lower by 0.18% and 5.5%, respectively, compared to LPS and γIFN-stimulated cells.

The comparison between the study groups has shown that in the supernatants of LPS and γIFN-stimulated cells of the obese subjects, the level of IL-6 was by 3.34% significantly higher compared to the subjects with normal body weight (Fig. 1A).

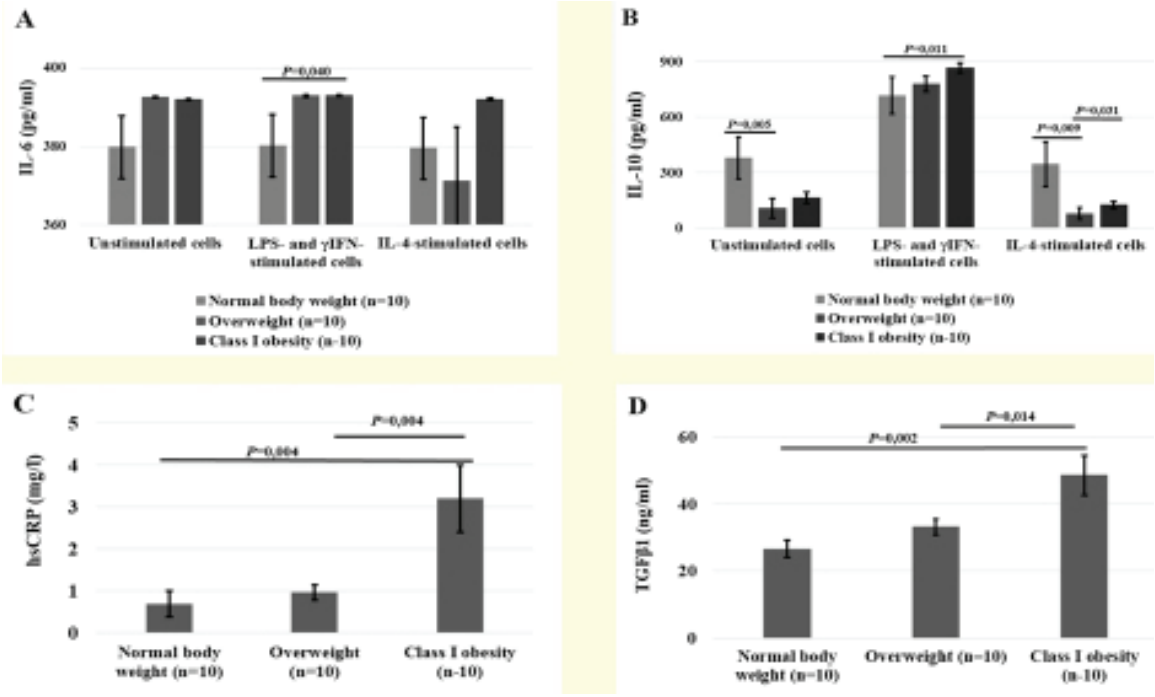

Fig. 1. The level of cytokines and biomarkers in the biological fluids of the subjects: (A) Il-6 in cell supernatants; (B) Il-10 in cell supernatants; (C) hsCRP in serum; TGFβ1 in serum

The study of IL-10 in the overweight subjects has shown that in the supernatants of unstimulated cells, the determined level of IL-10 was by 72.13% significantly lower compared to the subjects with normal body weight (Fig. 1B).

In supernatants of LPS- and γIFN-stimulated cells of the subjects with Class I obesity, the level of IL-10 was by 20.67% significantly higher compared to the corresponding rate of the group with normal body weight. The level of IL-10 in the supernatants of IL-4-stimulated cells in the overweight subjects was by 77.62% and 60,15% significantly lower compared to the subjects with normal body weight and the subjects with Class I obesity, respectively.

The level of hsCRP and TGFβ1 in the blood serum of the subjects was further determined. The level of hsCRP was significantly higher in the subjects with Class I obesity by 370.59% and 236.84% compared to the subjects with normal body weight and overweight, respectively (Fig. 1C). The concentration of TGFβ1 in the blood serum of the subjects with overweight and normal body weight did not differ significantly. In the obese subjects, the level of TGFβ1 was significantly higher by 82.19% and 46.36% compared to the subjects with normal body weight and overweight, respectively (Fig.1D).

Subsequently, correlation between the levels of *sirt1* gene expression in the dynamics of incubation in individuals with different body weight has been studied (Fig. 2).

Correlation analysis in the subjects with normal body weight revealed a positive strong correlation between *sirt1* gene expression in cells stimulated with IL-4 for 3 and 7 days (r=0.751, p=0.023) (Fig. 2A).

In the subjects with overweight, a positive strong correlation between *sirt1* expression in cells stimulated with LPS and γIFN for 3 and 7 days was determined (r=0.733, p=0.020) (Fig. 2, B); a moderate correlation between *sirt1* expression in unstimulated cells, 3 days of incubation and stimulated with IL-4 for 7 days (r=0.652, p=0.046) (Fig. 2C) and *sirt1* expression in cells stimulated with IL-4 for 3 and 7 days was determined (r=0.663, p=0.042) (Fig. 2D). The subjects with Class I obesity showed positive strong correlation between *sirt1* expression in LPS and γIFN-stimulated cells for 3 and 7 days (r=0.742, p=0.018) (Fig. 2E) and the moderate *sirt1* expression in IL-4 stimulated cells for 3 and 7 days (r=0.669, p=0.040) (Fig. 2F).

At the next stage the correlation between the rates of *sirt1* and *stat6* gene expression have been determined. In the subjects with normal body weight, a positive moderate correlation between *sirt1* gene expression in cells stimulated with IL-4 for 7 days and *stat6* in cells stimulated with IL-4 for 3 days was found (r=0.637, p=0.026) (Fig. 3A).

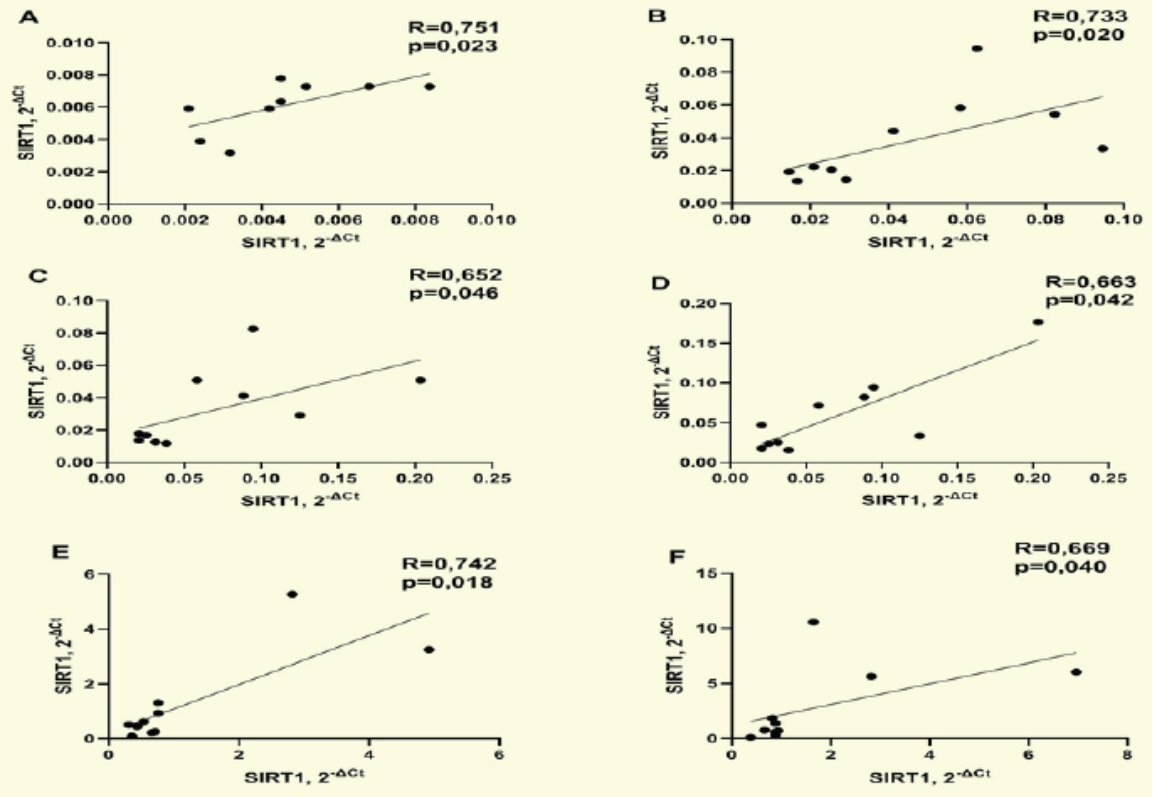

Fig. 2. Correlation analysis sirt1 expression during incubation under conditions:
(A) Il-4-stimulated for 3 and 7 days (normal body weight); (B) LPS- and γIFN-stimulated for 3 and 7 days (overweight);
(C) Il-4-stimulated for 7 days and unstimulated cells (overweight); (D) Il-4-stimulated for 3 and 7 days (overweight);
(E) LPS- and γIFN-stimulated for 3 and 7 days (class I obesity); (F) Il-4-stimulated for 3 and 7 days (class I obesity)

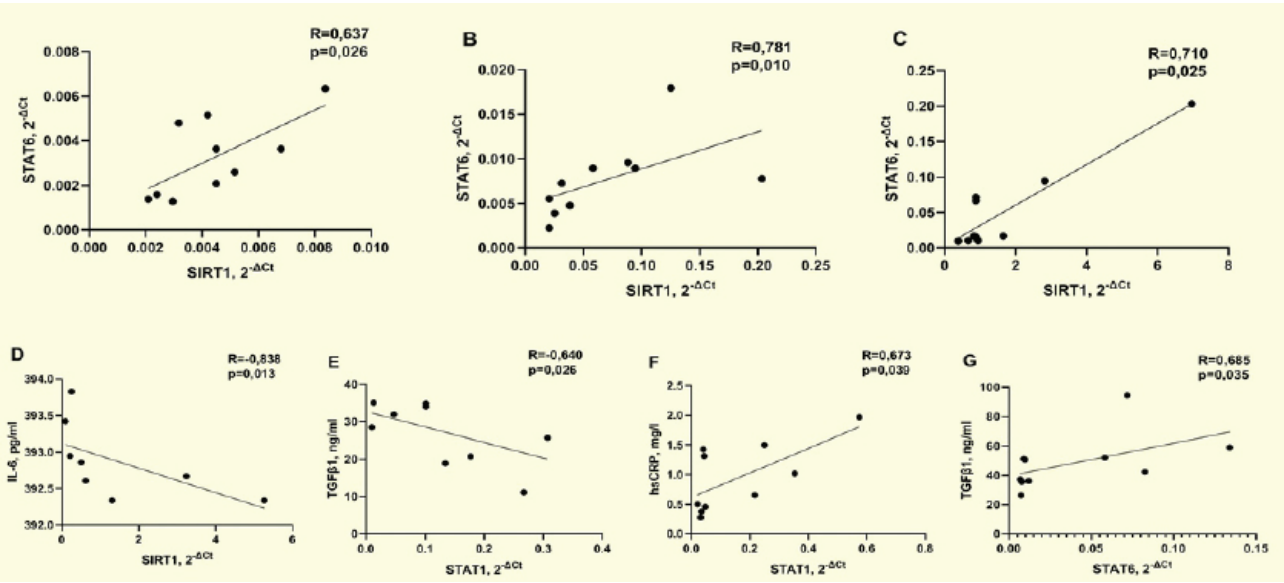

Fig. 3. I. Correlation analysis sirt1 with stat6 expression under conditions:
(A) Il-4-stimulated for 7 and 3 days (normal body weight);
(B) Il-4-stimulated for 7 and 3 days (overweight);
(C) Il-4-stimulated for 7 and 3 days (class I obesity).
II. Correlation analysis sirt1, stat1 and stat6 expression with cytokines and biomarkers under conditions:
(D) LPS- and γIFN-stimulated for 3 days (class I obesity);
(E) LPS- and γIFN-stimulated for 7 days (normal body weight);
(F) LPS- and γIFN-stimulated for 3 days (overweight);
(G) LPS- and γIFN-stimulated for 7 days (class I obesity)

In the subjects with overweight, positive strong correlations were formed between the *sirt1* expression in cells stimulated with IL-4 for 7 days and *stat6* in cells stimulated with IL-4 for 3 days (r=0.781, p=0.010) (Fig. 3B).

In the subjects with Class I obesity, positive correlations between the *sirt1* expression in cells stimulated with IL-4 cells for 7 days and *stat6* in cells stimulated with IL-4 for 7 days were found (r=0.710, p=0.025) (Fig. 3C).

Also, in obese subjects, a negative strong correlation between the *sirt1* expression in cells stimulated with LPS and γIFN for 3 days and IL-6 in the supernatant of cells stimulated with LPS and γIFN (r=-0.838, p=0.013) was observed (Fig. 3 D).

The studies of correlation between the level of expression and serum parameters in the subjects with normal body weight showed the presence of negative moderate correlation between *stat1* expression in cells stimulated with LPS and γIFN for 7 days and serum TGFβ1 (r=-0,640, p=0,026) (Fig. 3E).

In the group with overweight, a positive moderate correlation between *stat1* expression in cells stimulated with LPS and γIFN for 3 days and hsCRP was observed (r=0.673, p=0.039) (Fig. 3F). In obese subjects, a positive moderate correlation between the *stat6* expression in cells stimulated with LPS and γIFN for 7 days and serum TGFβ1 was observed (r=0.685, p=0.035) (Fig. 3G).

Obesity is currently recognized as one of the major health problems worldwide. Obesity leads to the development of chronic low-intensity systemic inflammation, the main factors of which are an increase in the number of proinflammatory subpopulations of macrophages in adipose tissue and deregulated production and functioning of adipose tissue hormones and cytokines.

Normally, in adipose tissue, macrophages are one of the main types of immune cells, most of them belong to the "alternatively activated" type 2 macrophages – M2, the M2:M1 ratio is constituted approximately 4:1 [25]. In obesity, the infiltration by monocytes, which differentiate into macrophages, increses. This leads to polarization with the formation of a pro-inflammatory phenotype, the development of adipose tissue inflammation and insulin resistance [5].

SIRT1, as a key regulator of metabolism, is responsible for a number of important processes – regulation of inflammation mediated by deacetylation of NF-κB, metabolism and stress through Forkhead Box Protein O (FOXO) and lipid metabolism mediated by Sterol Regulatory Element-Binding Protein (SREBP) [1,12]. SIRT1 regulates the activity of transcription factors that are key participants in the inflammatory processes. In particular, SIRT1 interacts directly with the RelA/p65 NF-κB subunit and deacetylates lysine 310, an important site for NF-κB activity [28].

Given the important role of SIRT1 in the regulation of inflammation, we investigated how SIRT1 affects the process of M1/M2 polarization of peripheral blood monocytes in young people depending on the body weight. We used a methodological approach to determine the regulatory role of SIRT1 in the formation of the polarization profile of peripheral blood monocytes mediated by the major transcription factors of the signaling cascade STAT1 and STAT6 in the subjects with overweight and Class I obesity. The level of expression of these factors under conditions of stimulation of polarization on the M1 or M2 profile, the production of cytokines directly by cells and their level in serum have been studied.

We obtained data indicating an increase in the level of the *sirt1* gene expression with weight gain. The *sirt1* expression was significantly higher in the cells of obese > overweight > healthy subjects. The highest rates of *sirt1* expression have been found in IL-4-stimulated cells of the subjects with Class I obesity.

An increase in the expression level is observed in the dynamics of cell incubation, which is confirmed by the formation of positive strong and moderate correlation between the expression level during the incubation for 3 and 7 days. Notably, it is observed in the subjects with normal body weight in cells stimulated with IL-4 for 3 and 7 days, and in the subjects with overweight and Class I obesity in LPS and γIFN-stimulated cells.

Changes in the level of *sirt1* expression were identified in some diseases, and in obesity and diabetes a decrease in the *sirt1* level was more common [3].

Given the dependence of SIRT1 activity on the nutrient status, it has been observed that weight loss in dietary and exercise restriction in overweight patients is accompanied by increased *sirt1* expression in peripheral blood mononuclear cells [7].

STAT6 is a key transcription factor for IL-4/IL-13 polarization by the M2 phenotype. According to Palma A. et al [19], in the presence of IL-4 there is a rapid expression of major regulators of M2a (STAT6, Peroxisome Proliferator-Activated Receptor γ (PPARγ) and Jumonji Jomain-Containing Protein D3 (JMJD3)), IL-10 production along with slow decline in IL- 12 production.

We have found that the level of *stat6* gene expression was maximally expressed in the subjects with normal body weight in stimulation with LPS and γIFN, and in the subjects with overweight and obesity in stimulation with IL-4. The comparison between the groups showed a significantly higher level of *stat6* expression in obese subjects in unstimulated cells and in macrophages stimulated by the M1 and M2 profile (3 days) and M2 (7 days). No significant differences in *stat1* expression between the groups with different body weight were detected.

Notably, the highest increase in both *stat6* and *sirt1* expression was observed in IL-4-stimulated macrophages. Positive strong and moderate correlations for IL-4-stimulated cells were identified between the *sirt1* and *stat6* expression, confirming the regulatory effect of SIRT1 and promoting polarization toward M2 phenotype formation by peripheral blood monocytes in the subjects with overweight and Class I obesity.

Antagonism has been established between STAT6 and STAT1, described for polarization of Th1 and Th2 cells by γIFN and IL-4, respectively [23]. Mutually antagonistic relationships link the transcription factors NF-κB and STAT6 and STAT1 and STAT6 in a way that the anti-inflammatory factor STAT6 helps to suppress the pro-inflammatory transcription factor STAT1 and NF-κB [2].

Calculation of the *stat1/stat6* expression ratio to assess the correlation between pro- and anti-inflammatory signaling pathways showed that even under conditions of non-critical weight gain, the ratio decreases, indicating redistribution of polarization towards the anti-inflammatory phenotype. The lowest rates of the *stat1/stat6* ratio were determined in the cells of the subjects with Class I obesity, stimulated with LPS and γIFN and IL-4 for 3 days. Also, the rate of *stat1/stat6* in the cells of the obese subjects, stimulated with IL-4 for 7 days, is significantly lower than in the subjects with normal body weight.

Levels of pro- and anti-inflammatory cytokines were determined to confirm the formation of the corresponding polarization profile. Weight gain is accompanied by the increase in the size and/or number of adipocytes, which is accompanied by the increase in production and the appearance of higher concentrations of cytokines IL-1β, IL-6, TNF-α [9].

We have found that the supernatants of LPS and γIFN-stimulated cells of the obese subjects had significantly higher level of IL-6 compared to the subjects with normal body weight. Correlation analysis showed a negative correlation between IL-6 secretion and SIRT1 expression in LPS- and γIFN-stimulated cells. Smith T.D. et al (2016) have reported that LPS and γIFN-stimulated macrophages showed the highest secretion of inflammatory cytokines, including IL-6, compared to levels in supernatants of unstimulated cells or cells exposed to IL-4 [22].

IL-10 is an important anti-inflammatory cytokine produced by T-cells and activated monocytes/macrophages. An increase in the level of IL-10 in cells stimulated with the M2 phenotype has been shown [22]. The findings show a significant increase in the level of IL-10 in the supernatants of LPS and γIFN-stimulated cells in the subjects with Class I obesity compared to the normal weight group and a decrease in the supernatants of IL-4- stimulated cells in the subjects with overweight compared to the subjects with normal body weight.

To identify signs of chronic systemic inflammation in the subjects, we determined the level of serum hsCRP and TGFβ1.

CRP is an acute phase protein that is involved in the immune response, its level increases with tissue damage, obesity, cardiovascular disease, stroke, infections and inflammation. Serum CRP levels are positively correlated with BMI [15]. Mahassni S.H., Bashanfar N.O. [13] report about changes in CRP and proinflammatory adipokines in young healthy individuals with overweight and obesity with minimal changes in the immune system and blood.

We have found that the level of hsCRP in the serum of the subjects with Class I obesity was significantly higher compared to the subjects with normal body weight and overweight, which are in concordance with literature data.

TGFβ1 is a polypeptide with potent immunosuppressive functions, a member of the cytokine family of transforming growth factor β. TGFβ1 elevation occurs due to infiltration and activation of macrophages in the adipose tissue. Chielle E.O. et al. [6] report that an increase in serum TGFβ1 is observed in the overweight subjects and especially in obese individuals regardless of the gender. TGFβ is positively correlated with body weight and BMI in obese women [10].

We obtained similar data, according to which the level of TGFβ1 in the serum of the subjects with Class I obesity was significantly higher compared to the subjects with normal body weight and overweight. Correlation analysis showed a positive correlation between TGFβ1 and *stat6* expression (p=0.035) in contrast to a negative correlation between *stat1* (p=0.026) in the subjects with normal body weight.

Thus, the findings show that in peripheral blood monocytes of the subjects with overweight and Class I obesity, SIRT1 implements a regulatory role mediated by the signaling cascade of the STAT6 transcription factor with the direction of polarization towards the anti-inflammatory phenotype. Significantly higher level of *sirt1* gene expression in unstimulated cells, in stimulation and its increase during the incubation period, indicate a possible preconditioning of peripheral blood monocytes, which counteracts the formation of the proinflammatory phenotype before recruiting monocytes into the adipose tissue. This effect occurs in people with overweight and low-risk obesity, without signs of metabolic pathology in the presence of minor significant changes in markers of systemic inflammation.

Conclusions. SIRT1 promotes M2 polarization of peripheral blood monocytes toward the anti-inflammatory phenotype in young individuals with overweight and Class I obesity mediated by increased expression of the *stat6* gene. The direction of polarization toward the anti-inflammatory phenotype is indicated by a decrease in the *stat1/stat6* ratio and the formation of correlations between *sirt1* and *stat6* expression for LPS and γIFN- stimulated cells and IL-4-stimulated cells.

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SUMMARY

SIRT1 CONTRIBUTES TO POLARIZATION OF PERIPHERAL BLOOD MONOCYTES BY INCREASING STAT6 EXPRESSION IN YOUNG PEOPLE WITH OVERWEIGHT AND LOW-RISK OBESITY

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Obesity contributes to the formation of low-intensity systemic inflammation with major participation of monocytes/macrophages. Nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase class III SIRT1 regulates the polarization of macrophages, controlling the inhibition of the M1 subpopulation and stimulating the activation of M2 macrophages.

The aim of our study was to determine the regulatory role of SIRT1 in M1/M2 polarization of peripheral blood monocytes in young people with overweight and Class I obesity.

30 subjects of both gender, aged 18-25 years have been examined. Groups were formed by the BMI: the subjects with normal body weight (n=10, BMI 18.50–24.99 kg/m^2), the subjects with overweight (n=10, BMI 25.00–29.99 kg/m^2), the subjects with Class I obesity (n=10, BMI 30.00–34.99 kg/m^2). Peripheral blood mononuclear suspension was isolated from venous blood. E. coli lipopolysaccharide (LPS) at a dose of 100 ηg/mL and γ-interferon (γIFN) at a dose of 100 ηg/mL were used to induce polarization of macrophages by the M1 phenotype. Unstimulated monocytes/macrophages were used as controls. The level of the *stat1*, *stat6* and *sirt1* gene expression was determined by Polymerase Chain Reaction Real-time PCR.

The findings showed an increase in the level of the *sirt1* gene expression with weight gain. The highest rates of *sirt1* expression were found in IL-4-stimulated cells of the subjects with Class I obesity. It has been concluded that SIRT1 promotes M2 polarization of peripheral blood monocytes toward the anti-inflammatory phenotype in young people with overweight and Class I obesity, mediated by increased *stat6* gene expression. The direction of polarization toward the anti-inflammatory phenotype is indicated by a decrease in the *stat/stat6* ratio and the formation of correlation between the *sirt1* and *stat6* expression in LPS and γIFN-stimulated cells and IL-4-stimulated cells.

Keywords: macrophages, macrophage polarization, SIRT1, STAT1, STAT6, overweight, Class I obesity.

РЕЗЮМЕ

SIRT 1 СПОСОБСТВУЕТ ПОЛЯРИЗАЦИИ МОНОЦИТОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ ПУТЕМ УСИЛЕНИЯ ЭКСПРЕССИИ STAT6 У МОЛОДЫХ ЛИЦ С ПОВЫШЕННОЙ МАССОЙ ТЕЛА И ЛЕГКИМ ОЖИРЕНИЕМ

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Ожирение способствует формированию системного воспаления низкой интенсивности, основными участниками

которого становятся моноциты/макрофаги. Никотинамидадениндинуклеотид (NAD^+)-зависимая деацетилаза III класса *sirt1* регулирует поляризацию макрофагов, осуществляя контроль за подавлением субпопуляции M1 и стимулируя активацию M2 макрофагов.

Целью исследования явилось определение регуляторной роли *sirt1* в M1/M2 поляризации моноцитов периферической крови у молодых лиц с повышенной массой тела и ожирением I степени.

Обследовано 30 человек обоего пола в возрасте 18-25 лет. По индексу массы тела (ИМТ) сформированы группы с нормальной массой (n=10, ИМТ 18,50-24,99 кг/м^2), с повышенной (n=10, ИМТ 25,00-29,99 кг/м^2), с ожирением I степени (n=10, ИМТ 30,00-34,99 кг/м^2). Суспензию мононуклеаров периферической крови выделяли из венозной крови. Для индукции поляризации по фенотипу M1 использовали липополисахарид (LPS) E. coli 100 нг/мл и γ-интерферон (γIFN) 100 нг/мл, по фенотипу M2 - IL-4 20 нг/мл. Контролем были нестимулированные моноциты/макрофаги. Уровень экспрессии генов *stat1*, *stat6* и *sirt1* определяли методом полимеразной цепной реакции в режиме реального времени.

Полученные данные свидетельствуют о росте уровня экспрессии гена *sirt1* в соответствии с повышением массы тела. Наибольшие значения экспрессии *sirt1* определены у лиц с ожирением I степени в клетках, стимулированных IL-4. Делается вывод, что *sirt1* способствует M2 поляризации моноцитов периферической крови в сторону противовоспалительного фенотипа у молодых лиц с повышенной массой тела и ожирением I степени опосредованно усилением экспрессии гена *stat6*. О направлении поляризации в сторону противовоспалительного фенотипа свидетельствует уменьшение величины соотношения *stat1/stat6* и формирование корреляционных связей между экспрессией *sirt1* и *stat6* для клеток, стимулированных LPS и γIFN и IL-4.

რეზიუმე

SIRT 1 ხელს უწყობს პერიფერიული სისხლის მონოციტების პოლარიზაციას STAT6 ექსპრესიის გაძლიერებით ახალგაზრდებში სხეულის მაღალი წონით და სიმსუქნით

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უკრაინის სამედიცინო სტომატოლოგიური აკადემია, პოლტავა, უკრაინა

სიმსუქნე ხელს უწყობს დაბალი ინტენსივობის სისტემური ანთების ჩამოყალიბებას, რომლის ძირითადი მონაწილეები არიან მონოციტები/მაკროფაგები. ნიკოტინამიდადენინდინუკლეოტიდ (NAD+)-დამოკიდებული III კლასის დეაცეტილაზა SIRT1 არეგულირებს მაკროფაგების პოლარიზაციას, აკონტროლებს M1 სუბპოპულაციის დათრგუნვას და ასტიმულირებს M2 მაკროფაგების აქტივაციას.

კვლევის მიზანს წარმოადგენდა SIRT1 რეგულატორული როლის განსაზღვრა პერიფერიულ სისხლის მონოციტების M1/M2 პოლარიზაციაში ახალგაზრდებში სხეულის მაღალი წონით და I ხარისხის სიმსუქნით.

გამოკვლეულია ორივე სქესის, 18-25 წლის 30 პაციენტი. სხეულის მასის ინდექსის (BMI) მიხედვით ჩამოყალიბდა შემდეგი ჯგუფები: ნორმალური წონით (n=10, BMI 18,50-24,99 კგ/მ^2), მომატებული წონით (n=10, BMI 25,00-29,99 კგ/მ^2) და I ხარისხის სიმსუქნით (n=10, BMI 30,00-34,99 კგ/მ^2). ვენური სისხლიდან გამოყოფდნენ პერიფერიული სისხლის მონონუკლეარების სუსპენზიას. M1 ფენოტიპის მიხედვით პოლარიზაციის გამოწვევისათვის იყენებდნენ E. coli ლიპოპოლისაქარიდის (LPS) 100 ნგ/მლ და γ-ინტერფერონს (γIFN) 100 ნგ/მლ, M2 ფენოტიპის მიხედვით - IL-4 20 ნგ/მლ. კონტროლს წარმოადგენდნენ არასტიმულირებული მონოციტები/მაკროფაგები. Stat1, stat6 და sirt1 გენების გამოხატვის დონე განისაზღვრა პოლიმერაზული ჯაჭვური რეაქციის მეთოდით რეალური დროის რეჟიმში.

მიღებული მონაცემები მოწმობს Sirt1 გენის ექსპრესიის დონის ზრდაზე შესაბამისად სხეულის მასის ზრდისა. Sirt1 ექსპრესიის მაჩვენებლების ყველაზე დიდი მნიშვნელობა განისაზღვრა პირებში I ხარისხის სიმსუქნით. გამოტანილია დასკვნა, რომ SIRT1 ხელს უწყობს პერიფერიული სისხლის მონოციტების M2 პოლარიზაციას ანთებისსაწინააღმდეგო ფენოტიპის მიმართულებით ახალგაზრდებში სხეულის მაღალი წონით და I ხარისხის სიმსუქნით, რაც გაშუალედებულია stat6 გენის ექსპრესიის გაძლიერებით. ანთებისსაწინააღმდეგო ფენოტიპის მიმართულებით პოლარიზაციაზე მოწმობს stat1/stat6 თანაფარდობის შემცირება და კორელაციური კავშირების ჩამოყალიბება sirt1 და stat6 ექსპრესიებს შორის უჯრედებში, რომლებიც სტიმულირებულია LPS, γIFN და IL-4.