

# Influence of Antirisk Factors of Cardiovascular Diseases on Intracellular Metabolism of Neutrophils in Men with Obesity

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## Abstract

**Background:** The factor promoting development of oxidative stress at obesity can be the neutrophils. **Objective:** To study the influence antirisk factors of cardiovascular diseases on intracellular metabolism of neutrophils in men with peripheral obesity. **Methods:** In 103 male patients aged 23 to 64 years without coronary heart disease, we studied the presence obesity, antirisk factors of cardiovascular diseases, and the metabolic activity of neutrophils. **Results:** It is identified that obesity in men associates with more rare use of crude vegetables or fruits every day, and also low physical activity. The daily uses of crude vegetables or fruits, and also the increase of physical activity in obese men promote increase intracellular activity of antioxidative protection at neutrophils. **Conclusion:** The antirisk factors of cardiovascular diseases increase activity antioxidative protection of neutrophils in men with peripheral obesity, reducing the probability of development of oxidative stress.

## Keywords

Obesity, Neutrophils, Reactive Forms of Oxygen, Antioxidance

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## 1. Introduction

The data are received that in the USA in 2013, 154.7 million adult people and 23.9 million children are having overweight or obesity [1]. It is believed that by 2015 year approximately 2.3 billion adult people will have overweight and more than 700 million will have obesity. It is shown that the risk of premature death in persons

with 40% of overweight is 2 times higher in comparison with the people, who have average body weight [2].

Today it is proved, that oxidative stress, caused by non-controlled production of free radicals, plays the important role in the development of atherosclerotic vascular lesions, which is the main cause of development of cardiovascular diseases [3]. The source of active forms of oxygen at obesity belonging to the basic modifiable risk factors of cardiovascular diseases can be the neutrophils. In patients with III degree of obesity, the increase of formation of oxygen radicals of neutrophils is accompanied by the decrease in activity intracellular antioxidative protection that can promote development of oxidative stress [4]. The INTERHEART case—control study, which had the benefit of a very large sample size, in addition to the risk factors of cardiovascular diseases also revealed antirisk factors—sufficient use of vegetables and fruits, regular reception of small doses of alcohol, and regular physical activity [5].

The objective was to study the influence of antirisk factors of cardiovascular diseases on intracellular metabolism of neutrophils in men with peripheral obesity without coronary heart disease.

## 2. Participants and Methods

### 2.1. Participants

103 male aged 23 to 64 years (mean  $\pm$  SD age:  $47.1 \pm 8.1$  years) which were hospitalized in hospital for professional survey have been examined. In order to exclude coronary heart disease in all patients, the electrocardiography was performed, along with bicycle ergometry, Holter electrocardiography monitoring, and echocardiography. For the diagnosis of peripheral obesity, body mass index was calculated. The consumption the crude vegetables or fruits every day, intake of alcohol, physical loadings of medium intensity at work, and employment by sport or presence active leisure in participants by means of questionnaire was studied.

### 2.2. Methods

The material for this study was peripheral blood analysis which was performed at admission of patients to the hospital with the consent of the Ethical Committee. Neutrophils were isolated from heparinised blood in Ficoll-Verographin double density gradient 1.077 and 1.092 g/cm<sup>3</sup>. The second interface cells contained 95% of neutrophils. The number of neutrophils in the cell suspension was counted in a cell with Goryaev vivo staining with methylene blue in 3% acetic acid (Turk dye) to determine viable cells. Viability of phagocytes, estimated by trypan test was more than 90%. In order to achieve a concentration of  $5 \times 10^6$  neutrophils in 1 ml of cell suspension was diluted with medium 199.

The test of spontaneous and stimulated nitro blue tetrazolium reduction (**NBT-test**) was performed by means of quantitative spectrophotometric method using 0.2% nitro blue tetrazolium in phosphate buffer, fixing neutrophils with methanol and dissolving in mixture of reduced diformazan potassium hydroxide and dimethylsulfoxide 3:5 volume mixture [6]. Suspension of phytohemagglutinin (*Phaseolus vulgaris*, “PanEco”, USA) was used as inducer of neutrophils redox metabolism.

Myeloperoxidase activity in neutrophils was studied using a 0.04% solution ortofenilendiamin in phosphate buffer pH 5.0, with the addition of 0.33% hydrogen peroxide solution at a ratio of 20:1 by volume. The reaction was stopped after 10 minutes of 10% sulphuric acid solution. Photometry was carried out at  $\lambda = 492$  nm [7].

The determination of glutathione reductase activity in neutrophils was evaluated by method on degree of oxidation of NADH. In the control and skilled tests containing 0.5 ml of suspension neutrophils 1.5 ml of distilled water, 0.1 ml NADH and 0.3 ml buffer pH 8 was added. The reaction started by addition 0.2 ml of 0.033 mM solution oxidized glutathione. The tests were left at temperature 37°C for 30 minutes. To stop the reaction the test was placed in refrigerator for 10 minutes at temperature 4°C. Photometry was carried out at  $\lambda = 340$  nm [8].

The determination of catalase activity in neutrophils was based on the ability of hydrogen peroxide to form salts with molybdenum stable colored complex. The reaction was run by adding 0.03% hydrogen peroxide solution and was stopped after 10 minutes, adding a 4% solution of ammonium molybdate. In control test instead of neutrophils distilled water was brought. The color intensity was measured at  $\lambda = 410$  nm against a control sample of distilled water [9].

### 2.3. Statistical Analyses

Statistical data processing was performed using the package Statistica 8.0 (StatSoft, Inc., USA). The data for categorical variables are expressed as absolute values and percents, and the data for continuous variables are ex-

pressed as the median with the 25% - 75% inter quartile range. Differences between groups were considered statistically significant at  $P < 0.05$ .

### 3. Results

#### 3.1. General Characteristics of the Study Participants

Obesity was encountered in 79 (76.7%) evaluable patients, of whom 49 (62%) revealed I degree of obesity (body mass index 30 - 34.9 kg/m<sup>2</sup>), 23 (29.1%)—II degree of obesity (body mass index 35 - 39.9 kg/m<sup>2</sup>), and 7 (8.9%) patients—III degree of obesity (body mass index  $\geq 40$  kg/m<sup>2</sup>). The 31 (30%) men were used the crude vegetables or fruits every day. The alcohol was taken 97 (94.2%) patients, from which 12 (12.4%) men some times a week and 32 (32.9%) men some times a month was intake. The 92 (89.3%) participant had physical loadings of medium intensity at work, and 32 (31%) men were engaged sport or had active leisure.

The comparative characteristics of the study participants are presented in **Table 1**. The alcohol some times a month by men with obesity used authentically more often ( $P < 0.05$ ). Obese men authentically less often had physical loadings of medium intensity at work ( $P < 0.05$ ), they were engaged sports or had active leisure, and also used the crude vegetables or fruits every day less often.

#### 3.2. Characteristics of the Obese Men Depending on the Use of Crude Vegetables or Fruits Every Day

The use of crude vegetables or fruits every day by men with obesity was accompanied authentically higher parameters of the spontaneous NBT-test ( $107.8 > 95.7$  mM NBT reduced;  $P = 0.04$ ) and catalase activity ( $344.3 > 333.7$  mCat/l;  $P = 0.03$ ) at neutrophils (**Table 2**). In men using crude vegetables or fruits every day also was observed authentically lower levels of glucose ( $4.8 < 5.2$  mM/l;  $P = 0.02$ ) and triglycerides ( $2 < 2.7$  mM/l;  $P = 0.01$ ) in blood.

#### 3.3. Characteristics of the Obese Men Depending on the Employment by Sports or Presence Active Leisure

In obese men which were engaged sports or had active leisure has been revealed authentically higher glutathione reductase activity ( $314.9 > 298.8$  nM·l<sup>-1</sup>·sec<sup>-1</sup>;  $P = 0.01$ ) at neutrophils (**Table 3**), and also lower level of uric acid in blood ( $6.5 < 7$  mg/dl;  $P = 0.02$ ).

Presence of physical loadings of medium intensity at work and intake of alcohol did not cause authentically changes of intracellular metabolism of neutrophils.

**Table 1.** Comparative characteristics of the study participants.

Parameter	No Obesity (n = 24)	Obesity (n = 79)	P
The use of crude vegetables or fruits every day	8 (33.3)	23 (29.1)	>0.05
The intake of alcohol:	22 (91.6)	75 (94.9)	>0.05
- Some times a week	3 (13.7)	9 (12)	>0.05
- Some times a month	3 (13.7)	29 (38.7)	0.02
Physical loadings of medium intensity at work	24 (100)	68 (86)	0.02
Employment by sports or presence active leisure	9 (37.5)	23 (29.1)	>0.05

**Table 2.** Comparative characteristics of the obese men depending on the use of crude vegetables or fruits every day.

Parameter	The use of crude vegetables or fruits every day		P
	No (n = 49)	Yes (n = 20)	
Spontaneous NBT-test, mM	95.7 (77.8; 107.8)	107.8 (94.5; 112.8)	0.04
Stimulated NBT-test, mM	107.1 (88.2; 112.4)	108.5 (107.1; 113.5)	>0.05
Myeloperoxidase, SED	5.4 (4; 7.4)	5.5 (4.5; 7.7)	>0.05
Glutathione reductase, nM·l <sup>-1</sup> ·sec <sup>-1</sup>	302.8 (281.4; 365.8)	304.4 (284.8; 366.5)	>0.05
Catalase, mCat/l	333.7 (241.7; 392.9)	344.3 (285.8; 400.9)	0.03

**Table 3.** Comparative characteristics of the obese men depending on the employment by sports or presence active leisure.

Parameter	Employment by sports or presence active leisure		P
	No (n = 46)	Yes (n = 23)	
Spontaneous NBT-test, mM	95.7 (78.8; 108.5)	106.7 (88.9; 109.2)	>0.05
Stimulated NBT-test, mM	107.8 (94.2; 112.4)	108.7 (93.9; 112.9)	>0.05
Myeloperoxidase, SED	5.4 (4; 7.2)	5.8 (4.3; 7.5)	>0.05
Glutathione reductase, nM·l <sup>-1</sup> ·sec <sup>-1</sup>	298.8 (273.3; 337.7)	314.9 (296.1; 393.9)	0.01
Catalase, mCat/l	333.7 (199.8; 382.9)	348.3 (302.4; 404.7)	>0.05

## 4. Discussion

The main argument of an increased interest to obesity is its connection with an atherosclerosis and risk of development of cardiovascular diseases. In recent years growth of heart attacks is observed among youth and people of middle age. Believe that the reason this problem is increase among youth of persons with excessive weight which provokes heart complications. The obesity and hyperglycemia correlate with findings of atherosclerotic plaques in coronary arteries and abdomen part of the aorta in young people at the age of 15 - 34 years [10]. It is shown, that the increase in thickness of complex of intima-media which reflects progressing of atherosclerosis and associates with increase in risk of cardiovascular complications, depends on body weight [11]. Does not cause doubts that in pathophysiology of atherosclerosis play crucial role the subclinical chronic inflammation which present already at early stages. Low oxidative stress modulates in endothelia the expression of the gene inducing atherogenous factor that leads to formation of atherosclerotic plaque. Further existence endothelial oxidative stress conducts to continuous accumulation of lipids, strengthens inflammation, destruction of matrix and remodeling of vascular wall in the atherosclerotic plaque [12]. As adipose tissue produces proinflammatory cytokines, therefore, the obesity can be also considered how inflammation. It is proved, that adipose tissue represents the active metabolic and endocrine organ producing adipocytokines (peptide hormones) and adipokines, one of which—leptin is structurally similar to proinflammatory cytokines—interleukin-6 and interleukin-12. There are data, that leptin stimulates secretion of cytokines and strengthens the expression molecules of adhesion in phagocytes, promotes development of oxidative stress [13] [14].

The atherosclerosis is disease with multifactorial etiology, and development of coronary heart disease depends on degree of expressiveness of various factors, which can strengthen each other or act in role of antagonists. Probably that such protective factors as physical activity, antioxidants, and social support can counteract influence of classical risk factors of cardiovascular diseases. It is known, that in regulation of free radical processes in cell play the antioxidative mechanisms, dependent as from antioxidative enzymes, and natural low-molecular formations, which contents in organism of the person is caused by food stuffs. The meta-analysis of numerous researches testifies not only the reduction of antioxidative protection in patients with coronary heart disease, but also the change of its activity depending on smoking, physical activity, and diet [15]. In the present research it is revealed, that men with peripheral obesity less often used of crude vegetables or fruits every day, they had lower physical activity. The use of crude vegetables or fruits every day by obese men was accompanied lower levels of glucose and triglycerides in blood, and also higher antioxidative activity protection at neutrophils. Growth of activity of antioxidative enzymes at neutrophils also was promoted by increase in physical activity in obese men. Results of the research NHANES III, testifying to influence of physical activity on chronic subclinical inflammation, revealed that physical activity back correlates with the quantity of leucocytes and the contents of fibrinogen in blood [16].

## 5. Conclusion

In summary, the use of crude vegetables or fruits every day, and also employment by sports or presence active leisure in men with peripheral obesity promote increase in activity of antioxidative protection at neutrophils, which can reduce the risk of development of oxidative stress.

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