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EFFECTS OF *ARONIA MELANOCARPA* POLYPHENOLS ON OXIDATIVE METABOLISM AND APOPTOSIS OF NEUTROPHILS FROM OBESE AND NON-OBESE INDIVIDUALS*

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Abstract. Reactive oxygen species are postulated to be involved in systemic inflammation and oxidative stress in patients with obesity. Activated polymorphonuclear neutrophils (PMNs) generate extremely high amounts of reactive oxygen species, but these are normally targeted at pathogens inside intracellular phagosomes. The same beneficial antimicrobial functions, if not controlled, contribute to the tissue damaging effects of inflammatory reactions. The evidence from conventional epidemiology strongly implies fruits and vegetables in protection against oxidative stress. In our study, the *in vitro* effects of *Aronia melanocarpa* juice on oxidative metabolism and apoptosis of neutrophils from obese and non-obese individuals has been investigated. We tested 15 obese patients (aged 45 ±9 years, women, BMI = 34 ±4.9 kg/m^2). Nine healthy subjects (BMI = 22.2 ±1.6 kg/m^2) were enrolled as controls. Neutrophils were isolated and oxidant production, in response to phorbol 12-myristate 13-acetate, was characterized by using luminol dependent chemiluminescence (CL) and flow cytometric dichlorofluorescin oxidation assay. Caspase-3 activity, a marker of apoptosis execution, in human neutrophils, measured by a cleavage of the fluorogenic substrate Ac-DEVD-AMC. Additional experiments to assess the direct toxic effect of the aronia polyphenols were also carried out. Neutrophils from obese individuals had a significantly higher H_2O_2 production and CL response compared to controls ($p < 0.05$). The oxidative metabolism of PMNs was decreased by aronia juice treatment in both of groups, obese and non-obese individuals. The caspase-3 activity depended on the time of aronia juice treatment and was markedly increased in phorbol-treated cells incubated with polyphenols for 24 hours. This natural product exert beneficial effects in cells and may, therefore, be useful in the treatment of obesity disorders.

Key words: *Aronia melanocarpa*, obesity, reactive oxygen species, apoptosis, neutrophils

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INTRODUCTION

Obese people are at high risk for developing type 2 diabetes mellitus, dislipidemia, hypertension, and cardiovascular diseases, which lead to an increased risk of mortality [Cope and Allison 2006]. As reported, obesity is associated with increase in systemic oxidative stress, and with elevated systemic inflammation and activation of coagulation cascade [Chan et al. 2002, Morrow 2003]. Some researchers [Higdon and Frei 2003, Keaney et al. 2003] found an increase in oxidants and a decrease in the activities of body's protective antioxidants in obese individuals. It has been hypothesized that [Higdon and Frei 2003, Keaney et al. 2003, Morrow 2003], oxidative stress generated by high loss of body reserves might cause metabolic disorders, tissue injuries, impairment of organ functions, and increased incidence of pathologies.

Leptin, a hormone secreted by adipose tissue, in addition to its activity as a regulator of food intake and energy expenditure can also modulate immune and inflammatory responses through stimulation of macrophages and neutrophils [Zarkesh-Esfahani et al. 2004].

Polymorphonuclear neutrophils (PMNs) are the primary effector cells in host responses to injury and infection. In PMNs recruited to the inflamed tissues phagocytosis triggers a chain of metabolic reactions beginning at membrane receptors for bacteria and other pathogens, followed by protein kinase C activation and subsequent induction of the NADPH oxidase to reduce oxygen to yield superoxide radical, which in cells is then metabolized to other ROS (e.g. hydrogen peroxide, hypochlorous acid) [Nathan 2002, Witko-Sarsat et al. 2000]. These highly reactive products of oxidative pathways are released into phagosomes containing ingested pathogens, or released into the extracellular environment to act upon substances that cannot be phagocytosed [Maianski et al. 2004]. Under homeostatic conditions, PMN antimicrobial functions are beneficial to the host. However if this delicate balance is upset, these same beneficial antimicrobial functions can cause significant local tissue injury and lead to the development of pathologic systemic inflammatory conditions [Maianski et al. 2004].

The antioxidants present in most plant-derived foods are capable of interfering with the processes involved in oxidative stress. The redox properties of plant polyphenols allow them to act as reducing agents, hydrogen-donating antioxidants and singlet oxygen quenchers; in some cases they also chelate transition metal ions [Cotelle 2001, Oszmiański and Wojdyło 2005]. Evidence for the potential role of oxidative stress in various diseases and pathophysiological processes suggests that the dietary intake and the therapeutic use of phytochemicals may have positive health effects [Hertog et al. 1995, Hung et al. 2004].

The fruit of the aronia (*Aronia melanocarpa* Elliot), a shrub of the rosaceous family native to North America and Russia, has a dark purple peel and contains high levels of flavonoids and phenolic acids. Main flavonoid subgroups in chokeberry are proanthocyanins, anthocyanins, flavonols and catechins. Phenolic acids present in chokeberry are hydroxylated derivatives of benzoic acid and cinnamic acid [Oszmiański and Wojdyło 2005]. Recently, it has been reported that the aronia extract has potent antioxidative effects *in vitro* and *in vivo* [Matsumoto et al. 2004].

Thus, the goal of the present study was to investigate the ability of the aronia (*Aronia melanocarpa* Elliot) juice to protect *in vitro* neutrophils from obese and non-obese individuals from oxidative damage.

MATERIALS AND METHODS

Plant material and the mixture obtained after intestinal digestion. Fruits of Aronia melanocarpa Elliot harvested in Poland. The chokeberry juice was made by PTHU ECOAR (Lewin Kłodzki, Poland). The composition of the juice was as follows (mg/100 ml): neochlorogenic acid 49.21, chlorogenic acid 45.50, (-)epicatechin 1.48, p-cumaric acid derivatives 0.4, polymeric procyanidins 293.38, quercetin 3-rutinoside 1.68, quercetin 3-galactoside 2.83, quercetin 3-glucoside 2.25, quercetin 3-wicyanoside 1.15, quercetin 3-robinobioside 1.17, cyanidin 3-galactoside 12.49, cyanidin 3-arabinoside 0.71, cyanidin 3-glucoside 5.12, cyanidin 3-xyloside 0.59, cyanidin 0.22.

For transport experiments, the aronia juice digestion *in vitro* was carried out by sequencing changes of pH value and addition of digestive agents (porcine pepsin, pancreatic extract and bile salts) into a reactor. Afterwards, into the reaction mixture was introduced a faecal bacteria culture in amount of ~10^6 cfu/cm^3. The aronia juice and the mixture obtained after intestinal digestion were centrifuged and sterilized by filtration (0.22 μm).

Study population. Obese patients (aged 45 ±9 years, women, BMI = 34 ±4.9 kg/m^2; n = 15) and healthy subjects (aged 29 ±11 years, women, BMI = 22.2 ±1.6 kg/m^2; n = 9) have been investigated. The inclusion criteria were based on physical examination, body mass index (BMI), waist-hip ratio (WHR), and the body composition examination based on bioimpedance method and biochemical assessment. All patients had a fasting venous plasma glucose less than 110 mg/dL. None of the obese subjects was taking any antioxidant therapy or glucocorticoids.

Isolation of PMNs. Peripheral venous blood, anticoagulated with heparine (10 U/ml blood), was layered on a density gradient medium (Gradisol G, Aqua-Medica, Poland) and centrifuged at 500 × g for 30 min. PMNs were collected and washed in PBS. The obtained population consisted of approx. 96% neutrophils.

Cell viability was determined by the Cell Proliferation Kit I (MTT; Roche Diagnostics GmbH, Germany). PMNs (1 × 10^6 cells/well) were treated with various concentrations of aronia juice (up to a final concentration of 10-100%v/v) for 2 h at 37°C, together with untreated control samples. Cells were subsequently incubated in a 96-well plate with the MTT solution for another 4 h. The water insoluble formazan dye was solubilized overnight at 37°C before measurement of absorbance using an ELISA reader at 570 nm with a reference wavelength of 690 nm. The results of the assay were expressed as percentage of the absorbance value obtained in control PMNs. More than 90% cells were considered to be unaffected by tested compounds, 80-90% as modestly affected, and values of less than 80% viable cells were ascribed to cytotoxic effect of the compounds.

Luminol-dependent chemiluminescence (CL) was measured as described previously [Zielińska-Przyjemska and Wiktorowicz 2006]. Briefly, incubation mixture in total volume of 1 ml contained 1 × 10^6 PMNs pretreated with aronia juice (30 minutes at 37°C), luminol (10 μM), and then challenging with phorbol 12-myristate 13-acetate (1 μM PMA, Sigma Chemical Co., St. Louis, MO, USA.) to induce oxidative stress. CL signals were recorded in the LKB1250 luminometer over 20 minutes and the peak values were expressed in mV per 1 × 10^6 cells.

Neutrophil hydrogen peroxide (H_2O_2) generation was measured by flow cytometry [Bass et al. 1983]. Incubation mixture (final volume 100 μl) contained 50 μl of

heparinized whole blood and aronia juice at the concentration range 0.1 to 50% v/v. Incubation was carried out for 30 minutes at 37°C, then 15 µl of 0.3 mmol/l 2',7'--dichlorofluorescin diacetate (DCFH-DA, Acros Organics, New Jersey, USA) in PBS was added and the incubation was continued for another 30 minutes. After that time 1 µM PMA was added. The erythrocytes were removed by lysis with 1 ml of Orthomune solution. Intracellular DCF fluorescence (λ_{ex} = 488 nm, λ_{em} = 515-548 nm) of PMNs was measured in Cytoron Absolute flow cytometer (Ortho, USA) using the linear amplification of the signal. The fluorescence intensity was expressed as the value of the "mean channel", calculated by ImmunoCount 2 software (Ortho).

PMN culture for the caspase-3 assay. Freshly isolated PMNs (2×10^6 cells) were maintained in RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin, and 5 mM HEPES, Sigma Chemical Co., St. Louis, MO, USA.) in 40 mm culture dishes. Cells were preincubated for 1 h with PBS alone (controls) or various concentrations of aronia juice and then incubated in the presence or absence of PMA (200 nM), at 37°C in humidified atmosphere containing 5% CO_2. After 2 or 24 h of incubation cells with the aronia juice a 1% trypsin solution was used to detach adherent cells.

Caspase-3 activity was measured using a caspase-3 fluorogenic substrate Ac-DEVD-AMC Caspase-3 assay kit (BD Bioscience Pharmingen, San Diego, CA, USA). Briefly, cultured cells were washed with ice-cold PBS (pH 7.4), and lysed in whole cell lysis buffer (10 mM Tris-HCl; 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5); 130 mM NaCl; 1% Triton®-X-100; 10 mM $NaPP_i$ (sodium pyrophosphate)), and protein concentration was determined according to the Lowry [Lowry et al. 1951]. Cell lysates (containing approximately 100 µg of protein) and substrate Ac-DEVD-AMC (20 µM) were combined in a standard reaction buffer (20 mM HEPES pH 7.5; 10% glicerol; 2 mM DTT). After 1 h incubation at 37°C, fluorescence of AMC liberated from Ac-DEVD-AMC was determined in a Hitachi F-2500 spectrofluorimeter (λ_{ex} = 388 nm, λ_{em} = 450 nm). The activity of caspase-3 was expressed in the AMC fluorescence units.

Statistical analysis. Differences between the means of treatment were compared after analysis of variance (ANOVA) by the t-Student's test. Differences were considered significant if $p \leq 0.05$.

RESULTS AND DISCUSSION

Our team has been studying the effects of plant extracts on the oxidative metabolism of phagocytes, with the aim of finding plants rich in antioxidants, determining their action mechanism and evaluating their ability to modulate the activity of native and acquired immunity cells.

It has been demonstrated that flavonoids impair the ROS production and release in neutrophils and macrophages due to the direct scavenging of reactive oxygen intermediates and to the inhibition of enzymes associated to ROS pathways: NADPH oxidase, xanthine oxidase and myeloperoxidase [Tauber et al. 1984, Edwards 1996, Middleton et al. 2000, Cotelle 2001]. Some flavonoids can scavenge hypochlorous acid and highly reactive chlorinated species generated by the MPO-H_2O_2-Cl system. These antioxidant activities are accompanied by the flavonoids potential to suppress

arachidonic acid release from membranes [Middleton 1998], to inhibit cyclooxygenase and lipoxygenase [Robak and Gryglewski 1996, Tordera et al. 1994], to inhibit protein kinases [Middleton 1998] and to diminish the release of hydrolytic enzymes from lysosomes [Tordera et al. 1994].

The antioxidative activity of flavonoids can be evaluated *in vitro* by measuring their capacity to inhibit the production of reactive oxygen species from stimulated PMNs. Chemiluminescence (CL) represents a simple, rapid and sensitive method to study the oxidative metabolism of phagocytes and, indirectly, phagocytosis, which closely correlates with the overall function of PMNs, with killing activity, and with tissue damage at the site of chronic inflammation. This test is also useful to identify compounds with antioxidant and anti-inflammatory activity [Allen 1986].

Using phorbol miristate acetate PMA-activated neutrophils from obese and non-obese individuals, we determine the luminol-dependent CL emission and H_2O_2 production measured in the cytofluorimetric assay in the absence or presence of the aronia juice. Upon PMA activation, non-mitochondrial oxygen uptake is initiated by the PMNs, resulting in the production of ROS. This process, known as respiratory burst, is the result of the assembly of the multi-enzyme NADPH-oxidase system that promotes the one-electron reduction of oxygen to superoxide anion [Babior 2000].

Our results presented in Figures 1-2 show that cells from obese patients give a higher ROS yield in comparison to healthy people ($p < 0.05$).

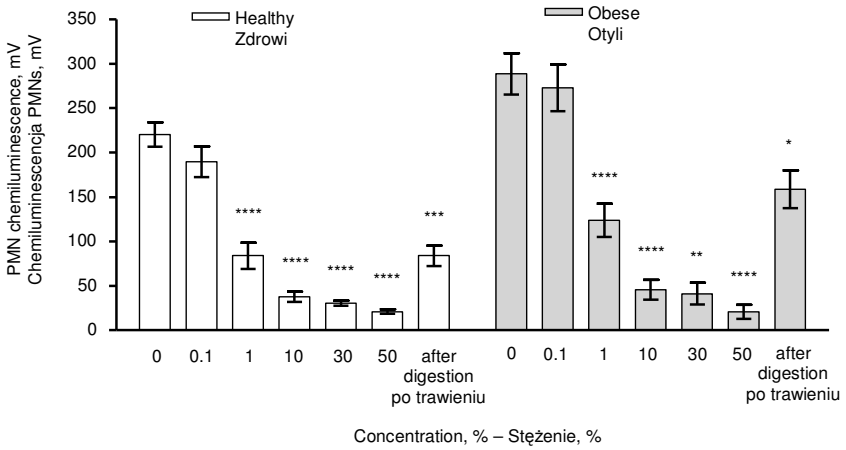

Fig. 1. The effect of aronia juice on the PMA-stimulated chemiluminescence in neutrophils from obese and non-obese individuals. Values are means of 3-15 experiments ± S.E. Differences statistically significant between control and treated cells: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

Rys. 1. Wpływ soku z aronii na intensywność chemiluminescencji stymulowanych PMA neutrofilów osób zdrowych i otyłych. Wyniki wyrażono jako średnie arytmetyczne z uwzględnieniem odchylenia standardowego średniej z 3-15 niezależnych eksperymentów. Różnice statystyczne w porównaniu z kontrolą: ***$p < 0,05$, ****$p < 0,01$, ***$p < 0,001$, ****$p < 0,0001$

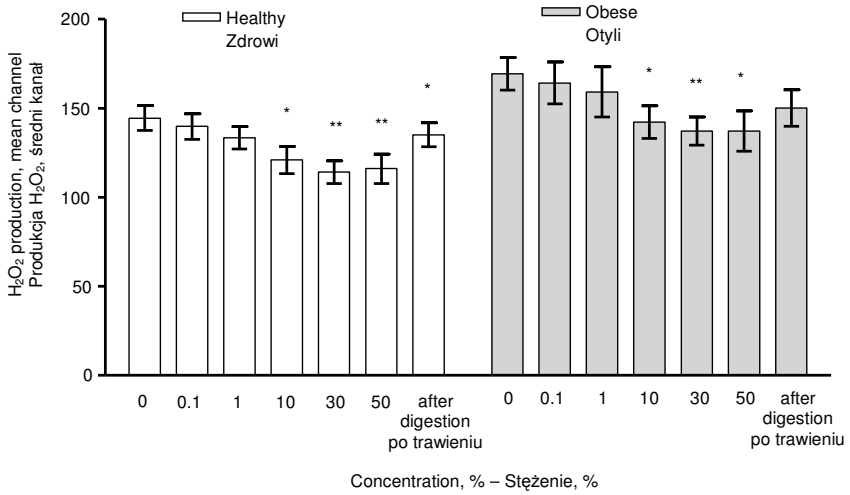

Fig. 2. Hydrogen peroxide production in PMNs from obese and non-obese individuals treated with aronia juice. Values are means of 3-11 experiments ± S.E. Differences statistically significant between control and treated cells: *p < 0.05, **p < 0.01
Rys. 2. Produkcja nadtlenku wodoru przez PMNs osób zdrowych i otyłych pod wpływem soku z aronii. Wyniki wyrażono jako średnie arytmetyczne z uwzględnieniem odchylenia standardowego średniej z 3-11 niezależnych eksperymentów. Różnice statystyczne w porównaniu z kontrolą: *p < 0,05, **p < 0,01

The increase in oxidative damage in obesity may therefore reflect consistent overeating by obese subjects. Dandona et al. [2001] have reported that ROS generation by PMNs falls markedly in association with dietary restriction and weight loss after the institution of a 1000-Cal diet. Furthermore, ROS generation after the cessation of dietary restriction and at 3 months not only reversed, but exceeded, that at baseline for PMNs.

Our results show that the juice of *Aronia melanocarpa* and the active fractions after digestion in artificial food canal significantly decrease the oxidative metabolism of PMNs in healthy and obese subjects. The juice inhibited the H_2O_2 production within the range 10-50%v/v and chemiluminescence's response at concentrations of 1-50%v/v. This activity can be explained either by the scavenger effect of the juice on ROS, or by the functional changes that cells undergo in the presence of the juice. PMA is an oncogenic substance that induces a translocation-activation process by protein kinase C (PKC) [Arroyo et al. 2002]. The inhibition of the respiratory burst by phagocytes could depend on interference of the juice with the PMA-dependent activation of PKC. Impairment by the plant juice of the production of active oxygen intermediates by neutrophils might contribute to the anti-inflammatory activity of these compounds.

The antioxidant activity of *Aronia melanocarpa* could depend on various chemical compounds, above all on flavonoids. The antioxidant properties of anthocyanins and proanthocyanins have been demonstrated by both *in vitro* and *in vivo* experiments [Kahkonen et al. 2001, Lazzè et al. 2004]. Anthocyanins decrease the amount of oxidized LDL and the contents of thiobarbituric acid reactive substances (TBARS), de-

crease the activity of nitric oxide synthase and the level of nitric oxide [Kowalczyk et al. 2003]. Comparison of antioxidative properties of anthocyanins with widely known antioxidants, showed that these compounds had higher antioxidative activity than vitamin E (α-tocopherol), ascorbic acid and β-carotene [Kowalczyk et al. 2003]. Apart from antioxidative activity, anthocyanins and proanthocyanins have anti-inflammatory and anti-bacterial properties as well, for instance they act as cyclooxygenase inhibitors [Howell 2002, Hou et al. 2005, Ohgami et al. 2005]. They inhibit degranulation of mast cells [Middleton et al. 2000] and decrease the level of IL-2, INF-γ, TNF-α [Lin et al. 2002]. It has been suggested that anthocyanins play an important role in the prevention against mutagenesis and carcinogenesis [Omenn 1995]. Investigation of the effect of anthocyanins on systemic sugar balance demonstated their hypoglycemic activity through inhibiting α-glucosidase in intestinal lumen and sensitization of cells to insulin A [Matsui et al. 2002]. The research greatly contributed to the fact that anthocyanins have become not only food products but also therapeutic agents.

Furthermore, in our experimental model we investigated the cytotoxicity of the aronia juice. The performed analyses showed a decrease in MTT (a marker of cell damage) incorporation in the case of these phytochemicals at the concentration higher than 50%v/v (Table 1).

Table 1. The influence of aronia juice on human neutrophil viability
Tabela 1. Wpływ soku z aronii na przeżywalność ludzkich neutrofilów

Aronia juice concentration, %v/v Stężenie soku z aronii, % v/v	Cells viability, % of control Przeżywalność komórek, % kontroli
10	109.2 ± 7.6*
30	94.2 ± 4.6
50	87.5 ± 4.4
100	62.3 ± 4.3
After digestion in artificial food canal Po trawieniu w sztucznym przewodzie pokarmowym	91.9 ± 3.0

*Data are expressed as percentage of value obtained for the control PMNs and represent the means ± S.E. from three independent experiments.

*Wyniki wyrażono jako wartości procentowe w odniesieniu do grupy kontrolnej PMNs przyjętej jako 100% i są one średnimi arytmetycznymi z uwzględnieniem odchylenia standardowego z trzech niezależnych eksperymentów.

Neutrophils are the major participants in the acute inflammatory response in tissues, being recruited from the circulation when local defenses are overwhelmed. Stimulated neutrophils are cleared from the inflamed sites by apoptosis (programmed cell death) and following macrophage phagocytosis, which is the key process in the inflammation resolution [Serhan and Savill 2005]. Caspeses (cysteine proteases, enzymes) play the central role in apoptotic cell death by degradation regulatory and structural proteins essential for cell survival and activate nucleases and caspase-3 is considered the one of the most important apoptosis executors [Vaughan et al. 2002]. Alterations of neutrophil apoptosis are associated with a number of diseases [Maianski et al. 2004].

According to Bruno et al. [2005] human neutrophils express leptin surface receptors under *in vitro* and *in vivo* conditions, and leptin delays apoptosis of mature PMNs *in vitro*. Leptin delayed the cleavage of the pro-apoptotic members: Bid and Bax, the mitochondrial release of cytochrome c and second mitochondria-derived activator of caspase, as well as the activation of both caspase-8 and caspase-3 in these cells. The authors conclude that, leptin is a survival cytokine for human neutrophils, a finding with potential pathologic relevance in inflammatory diseases.

Reactive oxygen species are supposed to be the common ultimate modulators of apoptosis in various experimental models and systems. The mild oxidative stress is an inducer of apoptosis in cells, however prolonged or excessive exposition to the pro-oxidant conditions can prevent caspases from functioning as it is observed in the NADPH oxidase stimulation in PMNs [Fadeelet al 1998, Hampton et al 1998]. Arroyo et al. [2002] reported on the caspase-3 activation in PMNs upon the NADPH oxidase inhibition or ROS suppression. The apoptosis induction *via* caspase-3 activation as a result of ROS decrease and involvement of superoxide dismutase in this process was recently described [Yasui et al 2005].

The proapoptotic role of anthocyanins in various human normal and cancer cell lines was described [Lazzè et al. 2004, Chen et al. 2005, Shih et al. 2005, Seeram et al. 2006]. It has been demonstrated that they are able to induce apoptosis through regulation of antiapoptotic gene Bcl-2 and activation of c-Jun N-terminal kinase cascade in hepatoma cells [Yeh and Yen 2005]. Hou et al. [2003] studied they induction of apoptosis by determining caspase-3 activation in human promyelocytic leukemia cells. Anthocyanins caused appropriate morphological changes in the cells, including chromatin fragmentation and phosphatidylserine exposure. However, these events were observed mainly in HeLa cells, which exhibit also DNA apoptotic laddering [Lazzè et al. 2004]. The implications for cancer treatment are clear from these observations.

In our *in vitro* experiment the juice of *Aronia melanocarpa* has been examined for his ability to activate caspase-3, a marker of apoptosis execution, of stimulated polymorphonuclear neutrophils in healthy and obese women. Caspase-3 activation was assayed by the method of Ac-DEVD-AMC (caspase-3 fluorogenic substrate) cleavage in PMNs cultured up to 24 hours. The caspase-3 activity depended on the time of aronia polyphenols treatment and was markedly increased in PMA-treated cells from healthy and obese subjects incubated with the aronia juice for 24 hours (Fig. 3). The presence of the caspase-3 substrate-specific fluorescence in samples where the PMA-induced oxidative burst was subdued by the juice leads to the indirect conclusion that these plant polyphenols interfere with NADPH oxidase and/or ROS and promote apoptosis. The described result agrees with the suggestions of Fadeel et al. [1998] and Arroyo et al (2002) on the oxidative inhibition of caspase-3 activity in the stimulated PMNs. We also observed pro-apoptotic effects of non-stimulate neutrophils after 24 hours incubation with these phytochemicals (Fig. 3).

The work proposed here is the first step towards setting up a battery of standard cellular and sub-cellular assays to be applied to micronutrients and other phytochemical investigation, individually and in combination, to elucidate their contribution to human health in terms of protection against oxidative damage.

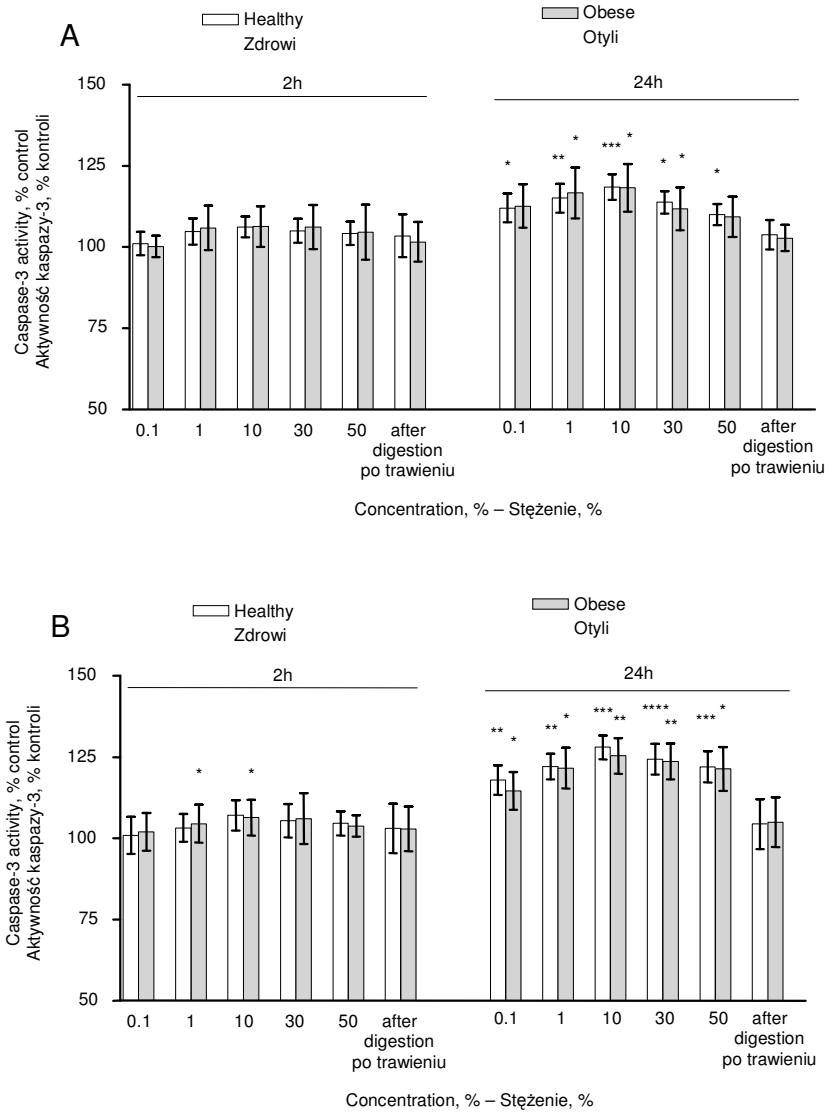

Fig. 3. Effect of aronia juice on caspase-3 activity in nonstimulated (A) and stimulated PMNs (B) from obese and non-obese individuals. Values are means ±S.E. (n = 3-7). Significantly different from the untreated control: *p < 0.05, **p < 0.01, ***p < 0.001
Rys. 3. Oddziaływanie soku aronii na aktywność kaspazy-3 niestymulowanych (A) i stymulowanych PMNs (B) osób zdrowych i otyłych. Wyniki wyrażono jako średnie arytmetyczne z uwzględnieniem odchylenia standardowego (n = 3-7). Znamienność statystyczna w stosunku do kontroli: *p < 0,05, **p < 0,01, ***p < 0,001

CONCLUSIONS

Compelling evidence has led to the conclusion that diet plays a major role in the control of oxidative stress: the juice of *Aronia melanocarpa* decreases oxidative stress, whereas the occidental diet, characteristically rich in fats, induces oxidative stress. The high content of polyphenol antioxidants is probably the main factor responsible for these effects.

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WPŁYW SOKU Z ARONII (*ARONIA MELANOCARPA* ELLIOT) NA METABOLIZM TLENOWY I APOPTOZĘ NEUTROFILÓW OSÓB ZDROWYCH I OTYŁYCH

Streszczenie. Stres oksydacyjny i odczyn zapalny odgrywają istotną rolę w patogenezie otyłości. Jednym z głównych źródeł reaktywnych form tlenu (RFT) w organizmie człowieka są pobudzone neutrofile (PMNs). Zachwianie równowagi pomiędzy produkcją RFT a ich detoksykacją przez ustrojowe systemy antyoksydacyjne prowadzi do stanów zapalnych związanych z infiltracją granulocytów oraz rozprzestrzenienia się reakcji utleniania komórkowych makrocząsteczek. Działania prewencyjne upatruje się między innymi w optymalnym wykorzystaniu potencjału antyoksydacyjnego niektórych nieodżywczych składników diety. Celem pracy była ocena *in vitro* wpływu soku z aronii (*Aronia melanocarpa* Elliot) na metabolizm tlenowy i aktywność kaspazy-3, marker efektorowej fazy apoptozy, neutrofilów osób zdrowych i otyłych. Badaniami objęto 15 pacjentek (w wieku 45 ±9, BMI = 34 ±4,9 kg/m^2) rozpoczynających leczenie z powodu otyłości prostej. Grupę kontrolną tworzyły zdrowe dawczynie krwi (BMI = 22,2 ±1,6 kg/m^2). Oceniano intensywność chemiluminescencji zależnej od luminolu PMNs aktywowanych estrami forbolu (PMA), stężenie nadtlenku wodoru metodą cytometrii przepływowej z zastosowaniem dioctanu 2',7'-dichlorofluorescyny oraz aktywność kaspazy-3 metodą fluorymetryczną rozszczepienia Ac-DEVD-AMC. U pacjentek z otyłością, w porównaniu z osobami zdrowymi, stwierdzono statystycznie znamienne zwiększenie produkcji H_2O_2 i intensywności chemiluminescencji stymulowanych PMNs ($p < 0,05$). Badany preparat w stężeniach 10--50% v/v i poddany trawieniu jelitowemu w tzw. sztucznym przewodzie pokarmowym

wykazywał silne właściwości antyoksydacyjne w grupie osób zdrowych i otyłych. Sok z aronii po 24-godzinnej hodowli indukował spontaniczną i stymulowaną PMA apoptozę neutrofili ($p < 0,05$). Zatem wykorzystanie właściwości antyoksydacyjnych i przeciwzapalnych aronii może przeciwdziałać powikłaniom współistniejącym z otyłością.

Słowa kluczowe: *Aronia melanocarpa*, otyłość, reaktywne formy tlenu, apoptoza, neutrofile

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