Direct contribution of obesity to oxidative damage to macromolecules

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Abstract

BACKGROUND: Obesity constitutes a common modifiable risk factor for certain non-communicable diseases (NCDs) associated with enhanced oxidative stress.
OBJECTIVES AND METHODS: The aim of the study was to examine serum concentrations of malondialdehyde + 4-hydroxyalkenals (MDA+4-HDA), as an index of lipid peroxidation (LPO), and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) concentration in peripheral blood lymphocytes, as an index of nuclear DNA damage, in overweight and obese adult patients. LPO and 8-oxodG, as well as clinical and laboratory parameters, which are frequently affected by obesity, were evaluated in 58 overweight and obese adult patients, and in 20 healthy volunteers.
RESULTS: Both LPO and 8-oxodG levels were increased in overweight and obese patients, with further increase observed with the increasing body mass index (BMI). LPO correlated positively with body mass, BMI, waist circumference, hip circumference, waist:hip ratio, systolic or diastolic blood pressure, glucose, C-reactive protein and ferritin concentrations. 8-oxodG correlated positively with body mass, BMI, hip circumference and triglyceride concentration, whereas it correlated negatively with iron concentration. Expectedly, positive correlation between LPO and 8-oxodG was also found.
CONCLUSIONS: BMI constituted the only independent determinant (predictor) of LPO in overweight and obese patients. Consistently, LPO did constitute the only independent determinant of obesity. Overweight and obesity in adults are directly associated with increased oxidative damage to macromolecules.

Abbreviations:

BMI	- body mass index
CRP	- C-reactive protein
dG	- 2'-deoxyguanosine
HDLC	- high-density lipoprotein cholesterol
LDLC	- low-density lipoprotein cholesterol
LPO	- lipid peroxidation
MDA+4-HDA	- malondialdehyde + 4-hydroxyalkenals
NCDs	- non-communicable diseases
8-oxodG	- 8-oxo-7,8-dihydro-2'-deoxyguanosine
ROS	- reactive oxygen species
RRd	- diastolic blood pressure
RRs	- systolic blood pressure
TC	- total cholesterol
TGs	- triglycerides
WHR	- waist:hip ratio

ORIGINAL ARTICLE

INTRODUCTION

Obesity does constitute one of the most common modifiable risk factors for cancer, such as pancreatic, colon, breast, endometrial, prostate, renal and esophageal cancer (Bianchini *et al.* 2002; Ceschi *et al.* 2007; Flegal *et al.* 2007; Pischon *et al.* 2008; Renehan *et al.* 2008) as well as for other non-communicable diseases (NCDs), mainly cardiovascular disease and diabetes mellitus (Pischon *et al.* 2008; Yan *et al.* 2009). Fat tissue and its hormonal products as well are examined in relation to different types of cancer (Paz-Filho *et al.* 2011). Furthermore, the potential role of various adipokines in pathogenesis of obesity and metabolic diseases has been recently reviewed (Kalisz *et al.* 2012).

The process of carcinogenesis is associated with the enhanced oxidative stress (Evans *et al.* 2004; Dalle-Donne *et al.* 2006; Valko *et al.* 2006). Well recognized are the disturbances of oxidative processes in patients with hypertension, and with diabetes mellitus (Evans *et al.* 2004; Dalle-Donne *et al.* 2006). Lipid peroxidation (LPO), resulting from oxidative damage to membrane lipids, as well as oxidative damage to nuclear DNA, are the most frequently examined processes to evaluate oxidative damage to macromolecules (Karbownik & Reiter 2000; Karbownik *et al.* 2001; Karbownik & Lewinski 2003; Kokoszko *et al.* 2006; Karbownik-Lewinska *et al.* 2008; 2010; Maes *et al.* 2009; Podborska *et al.* 2009; Kokoszko *et al.* 2010; Stasiak *et al.* 2010; Pelclova *et al.* 2011).

The increased level of reactive oxygen species (ROS) were found in patients with obesity (Vincent & Taylor 2006). Additionally, obesity induced by a high-fat diet in rats resulted in enhanced oxidative stress (Dobrian *et al.* 2001; de Assis *et al.* 2009) and insulin resistance (Akamine *et al.* 2010). Increased oxidative damage to DNA (Demirbag *et al.* 2006) or lipids (Cardona *et al.* 2008) and decreased antioxidant concentration (Ford *et al.* 2003; Cardona *et al.* 2008) or total antioxidant capacity (Demirbag *et al.* 2006) were found in patients with metabolic syndrome, of which an essential component is obesity. The overproduction of ROS has been documented in patients with metabolic syndrome (Ando & Fujita 2009) and in a rat model of metabolic syndrome (Roberts *et al.* 2006). The issues related to metabolic syndrome and oxidative stress have been recently reviewed (Ando & Fujita 2009). However, to what extent obesity by itself contributes directly to enhanced oxidative damage to macromolecules has not been defined till now.

The aim of the study was to evaluate to what extent the level of oxidative damage to membrane lipids and nuclear DNA is changed in overweight and obese adult patients, and how these potential changes relate to obesity.

MATERIALS AND METHODS

The procedures, used in the study, were approved by the Ethical Committee of the Medical University of Lodz, and fully informed, written consent was obtained from the patients.

Fifty eight (58) overweight and obese adult patients (body mass index; BMI ≥ 25 kg/m^2) (mean age ± SEM: 54.5 ± 1.9 yrs; 52 females and 6 males) and twenty (20) healthy volunteers (Controls) (BMI < 25 kg/m^2) (mean age ± SEM: 50.7 ± 3.1 yrs; 17 females and 3 males) were enrolled in the study (Table 1). Patients with BMI ≥ 25 kg/m^2 and healthy volunteers were well matched at baseline in terms of sex and age. All of them were patients of the Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, or Outpatient Department of Endocrinology, Polish Mother's Memorial Hospital – Research Institute, or Regional Centre of Menopause and Osteoporosis, University Hospital No 3 in Lodz.

Blood samples were collected after an overnight fast. After collection, blood (15 ml) was used directly to isolate DNA or (1 ml) was centrifuged (3000 × g, 10 min, 4 °C) in order to obtain serum, and stored at –80 °C until assays.

Laboratory parameters

The following measurements were performed in blood serum: glucose, C-reactive protein (CRP), total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), triglycerides (TGs), iron and ferritin concentrations.

LPO assay

The concentrations of malondialdehyde + 4-hydroxyalkenals (MDA+4-HDA), as the index of LPO, were measured in blood serum, using an LPO-586 kit, purchased from Calbiochem (La Jolla, CA). The serum (200 µl) was mixed with 650 µl of a methanol:acetonitrile (1:3, v/v) solution, containing a chromogenic reagent, N-methyl-2-phenylindole, and vortexed. After adding 150 µl of methanesulfonic acid (15.4 M), incubation was carried out at 45 °C for 40 min. The reaction between MDA+4-HDA and N-methyl-2-phenylindole yields a chromophore, which is spectrophotometrically measured at the absorbance of 586 nm, using a solution of 4-hydroxynonenal (10 mM) as the standard. The level of LPO was expressed as the amount of MDA+4-HDA (nmol) per 1 ml of serum.

DNA isolation

Each blood sample was divided into three aliquots by RPMI solution. The blood was carefully applied on top of Histopaque 1077 solution (Sigma Chemical Co., St. Louis, MO) and lymphocytes were isolated by centrifugation according to the procedure laid down by the manufacturer. Briefly, lymphocytes were suspended in 0.5 ml lysis solution (120 mM NaCl, 10 mM Tris, 1 mM

Tab. 1. Mean values (± SEM) of age and BMI (ranges shown in parentheses) in healthy subjects (Controls) and in adult patients with BMI ≥ 25 kg/m^2, subgrouped according to gender.

	Controls			Patients with BMI ≥ 25 kg/m^2		
	Women	Men	All healthy subjects	Women	Men	All patients
n	17	3	20	52	6	58
Age [years]	50.7 ± 3.6 (25–74)	50.7 ± 3.5 (45–57)	50.7 ± 3.1 (25–74)	55.4 ± 1.9 (18–79)	46.3 ± 5.5 (32–63)	54.5 ± 1.9 (18–79)
BMI [kg/m^2]	22.2 ± 0.4 (19.8–24.7)	23.2 ± 0.8 (22.2–24.7)	22.3 ± 1.5 (19.8–24.7)	31.0 ± 0.8* (25.1–44.5)	39.1 ± 4.1** (26.9–55.2)	31.9 ± 0.9*** (25.1–55.2)

Comparison between Controls and adult patients with BMI ≥ 25 kg/m^2 was performed by an unpaired Student's *t*-test; *p<0.05 vs. Control women; **p<0.05 vs. Control men; ***p<0.05 vs. all healthy subjects.

EDTA, 0.5% SDS, pH 8.0) with 20% butylated hydroxytoluene. RNA and protein were digested by incubation with RNase or proteinase K at 55 °C for 30 or 60 min, respectively. After extraction, by successive mixing with saturated phenol, with a mixture of phenol/chloroform/isoamyl-alcohol (25:24:1), and then with a mixture of chloroform/isoamyl alcohol, DNA was precipitated by the addition of five volumes of ethanol (–20 °C). DNA samples were stored at –70 °C.

8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) determination

The isolated DNA was dissolved in 200 µl of 20 mM sodium acetate (pH 5.0), denaturated by heating at 95 °C for 5–10 min and cooled on ice. The DNA samples were digested to nucleotides by incubation with 12 units of nuclease P1 at 37 °C for 30 min. Next, after adding 20 µl of 1 M Tris-HCl (pH 8.0) and 4 units of alkaline phosphatase, the samples were incubated at 37 °C for 1 h. The resulting deoxynucleoside mixture was filtered through a Millipore filter (0.22 µm). 8-oxodG and 2'-deoxyguanosine (dG) in hydrolysates were determined using HPLC with electrochemical detector. The HPLC system consisted of a Smartline Pump 1000, Smartline Autosampler 3800, 250 mm × 4 mm Eurosphere-100 C18 column and electrochemical detector EC3000 with measurement cell model Sputnik; eluent: 10% aqueous methanol containing 12.5 mM citric acid, 25 mM sodium acetate, 30 mM sodium hydroxide and 10 mM acetic acid, at a flow rate of 1 ml/min. The quantities of 8-oxodG and dG were measured using two oxidative potentials (300 mV, 900 mV, respectively). The results are expressed as the ratio of 8-oxodG to dG ×10^5.

Statistical analysis

The data were statistically analysed, using Student's unpaired *t* test or the one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls' test – for continuous variables. The results are presented as means ± SEM. Univariate logistic regression analysis was used to determine which continuous variable might have predicted obesity or increased oxidative damage; in order to adjust for several risk factors, multivariate logistic regression analysis was performed with all the variables, found to be significant at the univariate analysis, entering in a single step. For the evaluation of correlation among particular parameters, Pearson's correlation coefficient was used. Statistical significance was determined at the level of $p<0.05$.

Tab. 2. Correlations between the level of LPO or DNA damage and laboratory parameters in patients with BMI ≥ 25 kg/m^2

	LPO [MDA + 4-HDA (nmol/ml)]		DNA damage [8-oxodG/10^5 dG]	
	r	*p*-value	r	*p*-value
Glucose [mg/dl]	**0.316**	**0.016***	0.439	0.236
CRP [mg/dl]	**0.501**	**<0.001***	0.486	0.184
TC [mg/dl]	0.103	0.444	0.111	0.776
LDLC [mg/dl]	0.114	0.394	–0.353	0.352
HDLC [mg/dl]	–0.036	0.788	–0.375	0.320
TGs [mg/dl]	0.024	0.859	**0.767**	**0.016***
Iron [µg/dl]	–0.102	0.446	**–0.693**	**0.039***
Ferritin [ng/ml]	**0.425**	**0.001***	0.549	0.125

r – Pearson's correlation coefficient, *$p<0.05$.

RESULTS

According to the enrollment criteria, BMI was higher in overweight and obese patients than in healthy subjects (Table 1).

Both LPO and 8-oxodG levels were increased in overweight and obese patients and further increase of both of them was observed with the increasing BMI (Figures 1 and 2).

Positive correlations were found in overweight and obese patients between LPO level and such parameters as body mass, BMI (Figure 3A), waist circumference, hip circumference, waist:hip ratio (Figure 3B), systolic or diastolic blood pressure (Figure 3C), as well as CRP, ferritin and glucose concentrations (Table 2).

In turn, 8-oxodG correlated positively with body mass, BMI and hip circumference (Figure 4), as well as

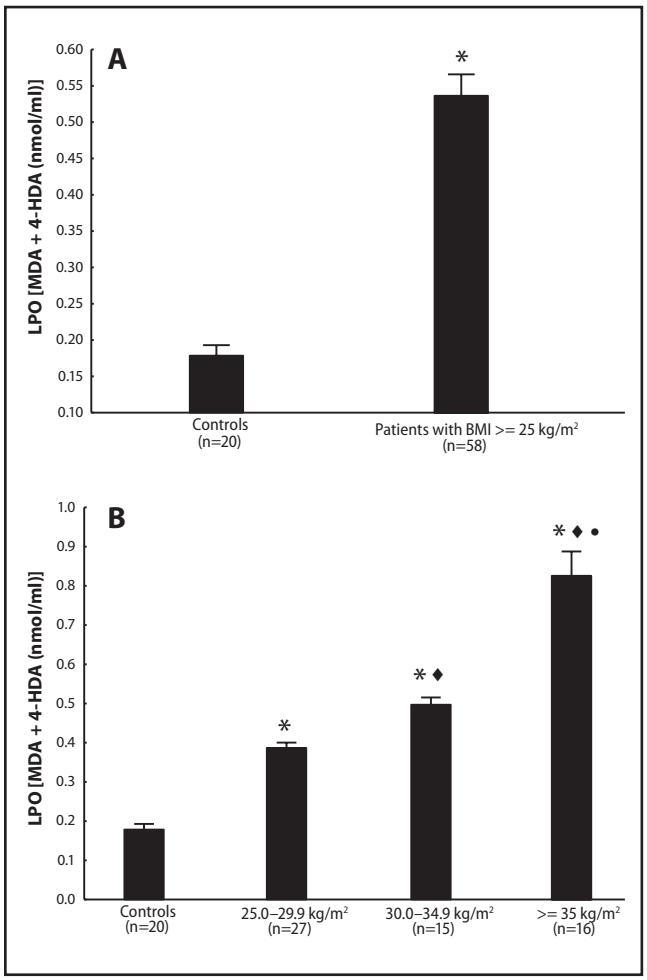


Fig. 1. (A) Mean (± SEM) values of LPO level in Controls and in patients with BMI ≥ 25 kg/m^2. Statistical evaluation was performed by an unpaired Student's t-test. **(B)** Mean (± SEM) values of LPO level in Controls and in patients with BMI ≥ 25 kg/m^2, subgrouped according to the value of BMI. Statistical evaluation was performed by a one-way ANOVA with repeated measures followed by a Student-Newman-Keuls' test. The level of LPO was expressed as the amount of MDA+4-HDA (nmol) per 1 ml of serum. *p<0.05 vs. Controls; ♦p<0.05 vs. subgroup with BMI 25.0–29.9 kg/m^2; •p<0.05 vs. subgroup with BMI 30.0–34.9 kg/m^2.

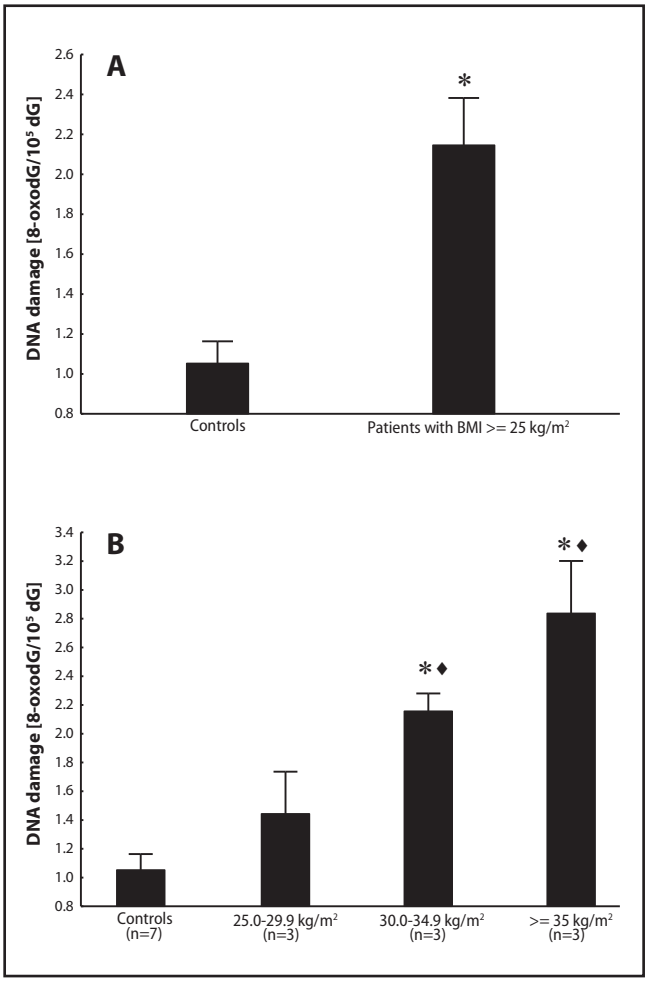


Fig. 2. (A) Mean (± SEM) values of oxidative DNA damage in peripheral blood lymphocytes in Controls and in patients with BMI ≥ 25 kg/m^2. Statistical evaluation was performed by an unpaired Student's t-test. **(B)** Mean (± SEM) values of oxidative DNA damage in peripheral blood lymphocytes in Controls and in patients with BMI ≥ 25 kg/m^2, subgrouped according to the value of BMI. Statistical evaluation was performed by a one-way ANOVA with repeated measures followed by a Student-Newman-Keuls' test. The level of DNA damage was expressed as the concentration of 8-oxodG per 10^5 dG. *p<0.05 vs. Controls; ♦p<0.05 vs. subgroup with BMI 25.0–29.9 kg/m^2.

with TGs concentration, whereas it correlated negatively with iron concentration (Table 2). Expectedly, positive correlation between oxidative damage to membrane lipids and to nuclear DNA was also found (Figure 5).

For the entire study group of subjects (i.e. healthy and obese), variables such as age, body mass, height, BMI, waist circumference, hip circumference, waist:hip ratio (WHR), systolic blood pressure (RRs), diastolic blood pressure (RRd), concentrations of glucose, CRP, TC, LDLC, HDLC, TGs, iron, ferritin and LPO level were submitted to a univariate and a multivariate logistic regression model. The purpose of the model was to determine which of those continuous variables might predict either increased oxidative damage to membrane lipids or obesity. BMI did constitute the only independent predictor of increased oxidative damage to membrane lipids (Table 3). Consistently, LPO did constitute the only independent predictor of obesity (Table 4).

DISCUSSION

It has been shown in the present study that overweight and obesity are associated with increased oxidative damage to membrane lipids and to nuclear DNA. The extent, to which these macromolecules were oxidatively damaged, did depend on the degree of obesity with significant increase in LPO level and 8-oxodG concentration with increasing BMI. Positive correlations between LPO and body mass, BMI, waist circumference, hip circumference and WHR as well as positive correlations

Tab. 3. Univariate and multivariate logistic regression analysis of the univariate oxidative stress (for LPO value > 0.245 nmol/ml, being the lowest LPO value in overweight and obese patients) determinants (variables), performed in all examined subjects (Controls + patients with BMI ≥ 25 kg/m^2) (n=78).

Variable	Univariate regression			Multivariate regression		
	OR	95%CI	*p*-value	OR	95%CI	*p*-value
Age [years]	1.014	0.98–1.05	0.471	–	–	–
Body mass [kg]	**1.15**	**1.06–1.24**	**0.0006***	1.06	0.88–1.27	0.562
Height [cm]	0.987	0.93–1.05	0.662	–	–	–
BMI [kg/m^2]	**3.16**	**1.69–5.92**	**0.0005***	**3.02**	**1.51–6.03**	**0.002***
Waist circumference [cm]	**1.11**	**1.04–1.19**	**0.001***	0.93	0.78–1.11	0.411
Hip circumference [cm]	**1.08**	**1.02–1.14**	**0.009***	0.99	0.89–1.11	0.968
WHR	13.83	0.009–21669.3	0.479	–	–	–
RRs [mmHg]	**1.05**	**1.01–1.10**	**0.027***	1.00	0.88–1.13	0.990
RRd [mmHg]	**1.08**	**1.02–1.14**	**0.01***	1.04	0.87–1.24	0.656
Glucose [mg/dl]	**1.04**	**1.00–1.07**	**0.025***	1.00	0.95–1.05	0.997
TC [mg/dl]	1.01	0.99–1.02	0.209	–	–	–
LDLC [mg/dl]	1.01	0.99–1.03	0.413	–	–	–
HDLC [mg/dl]	**0.96**	**0.93–0.99**	**0.036***	0.99	0.92–1.07	0.906
Iron [µg/dl]	1.00	0.99–1.02	0.815	–	–	–

OR, odds ratio; CI, confidence interval; *p<0.05.

Tab. 4. Univariate and multivariate logistic regression analysis of the univariate obesity (for BMI value ≥ 30 kg/m^2) determinants (variables), performed in all examined subjects (Controls + patients with BMI ≥ 25 kg/m^2) (n=78).

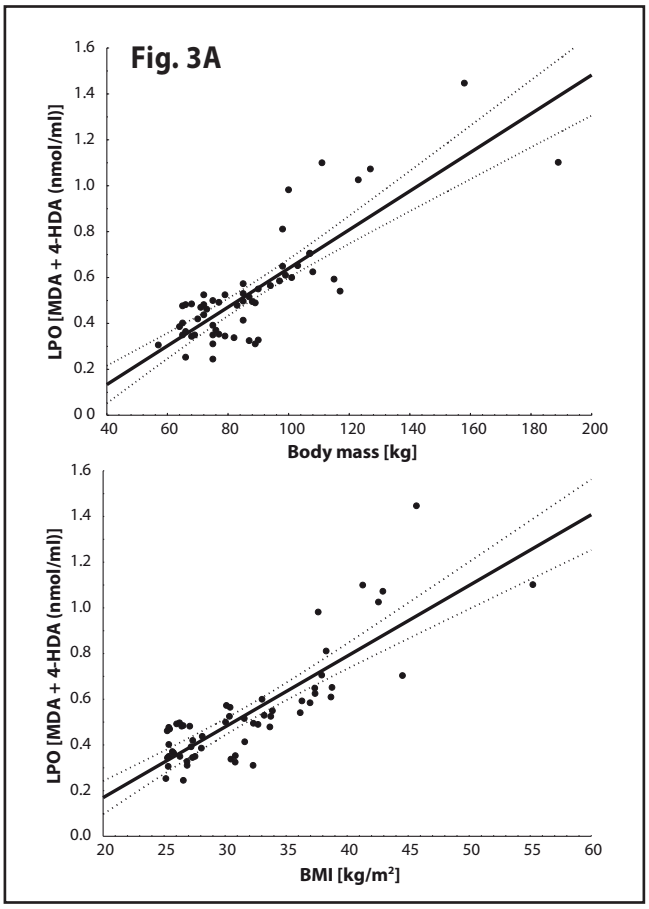
Variable	Univariate regression			Multivariate regression		
	OR	95%CI	*p*-value	OR	95%CI	*p*-value
Age [years]	0.98	0.95–1.02	0.312	–	–	–
RRs [mmHg]	**1.04**	**1.00–1.07**	**0.037***	–	–	–
RRd [mmHg]	**1.07**	**1.02–1.12**	**0.005***	1.01	0.93–1.10	0.774
Glucose [mg/dl]	**1.03**	**1.01–1.05**	**0.003***	1.02	0.98–1.05	0.315
TC [mg/dl]	1.00	0.99–1.04	0.971	–	–	–
LDLC [mg/dl]	1.00	0.99–1.02	0.909	–	–	–
HDLC [mg/dl]	0.98	0.95–1.01	0.175	–	–	–
Iron [µg/dl]	0.99	0.99–1.01	0.564	–	–	–
LPO level [MDA + 4-HDA (nmol/ml)]	**3.96×10^9**	**1.40×10^9–11.16×10^9**	**0.0002***	**4.55×10^9**	**1.61×10^9–12.83×10^9**	**0.0004***

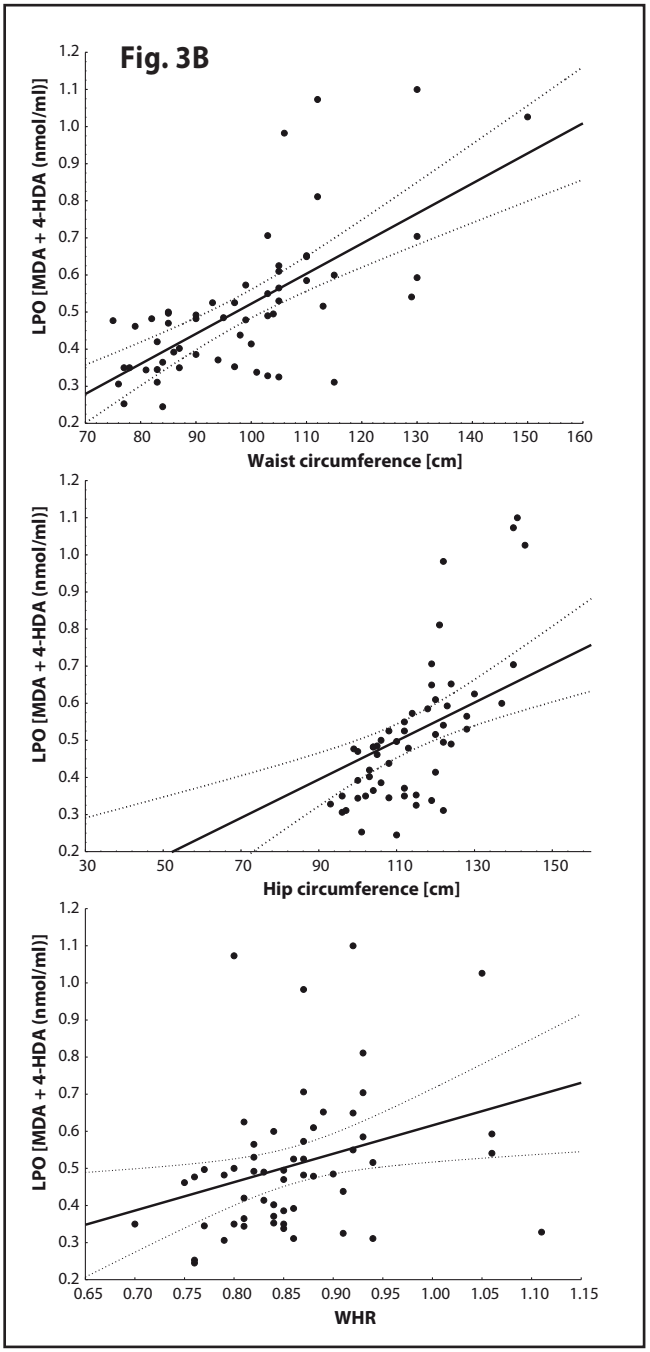
OR, odds ratio; CI, confidence interval; *p<0.05.

between DNA damage and body mass, BMI and hip circumference were expected, as these parameters are related directly or indirectly to obesity definition.

Together with the above, of great importance are two observations from the present study showing that BMI did constitute the only independent determinant (predictor) of increased oxidative damage to membrane lipids, and that, consistently, LPO did constitute the only independent determinant of obesity. These findings suggest that obesity does contribute to increased oxidative damage directly and, in turn, the resulted enhanced oxidative stress may unfavourably affect obesity in terms of further increase of body mass (the mechanism of "vicious circle"). Similarly to the well-known phenomenon of the obesity-induced insulin resistance which contributes further to obesity (Zeyda & Stulnig 2009), also the products of oxidative damage to macromolecules may be involved in a kind of "vicious circle". However, this hypothesis should be proved experimentally and in clinical trials.

Whereas increased oxidative stress, evaluated by measurement of different parameters, was found earlier





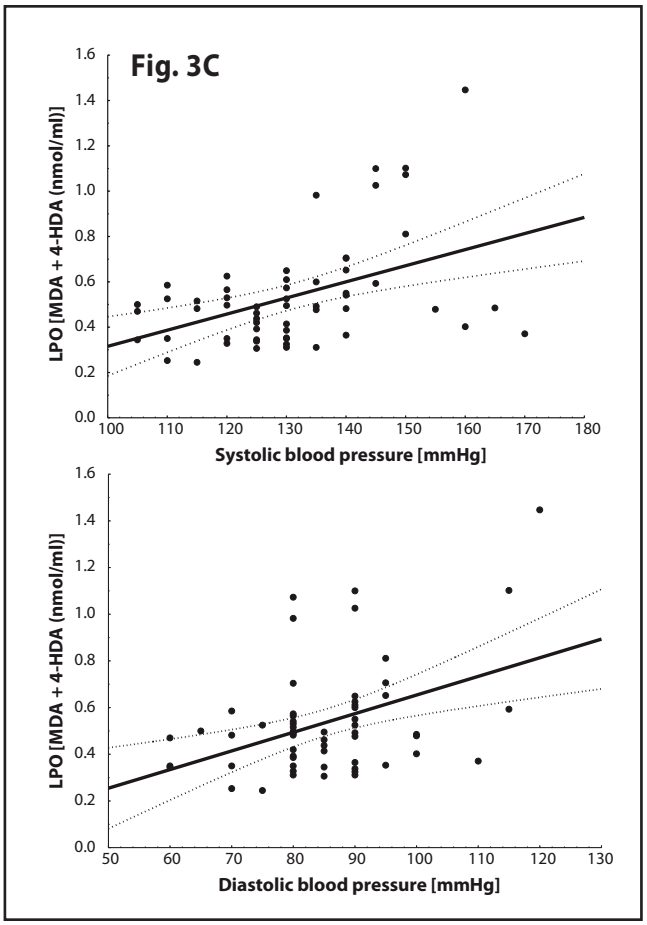
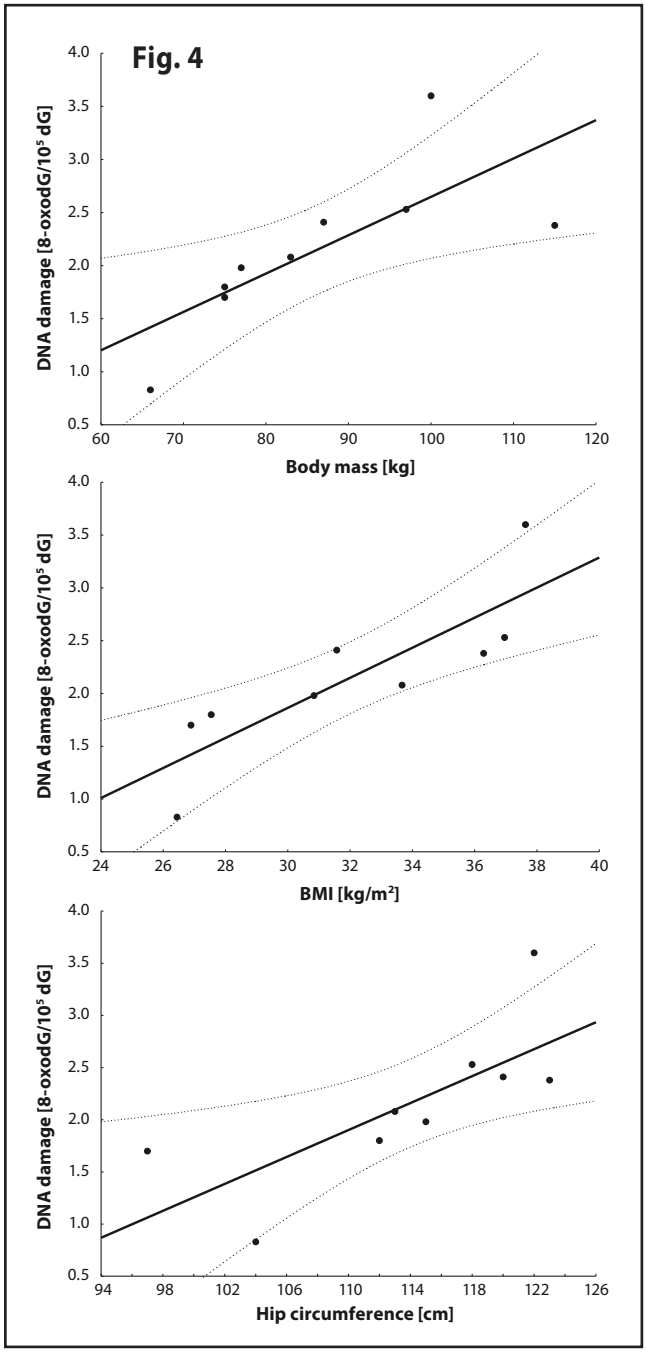
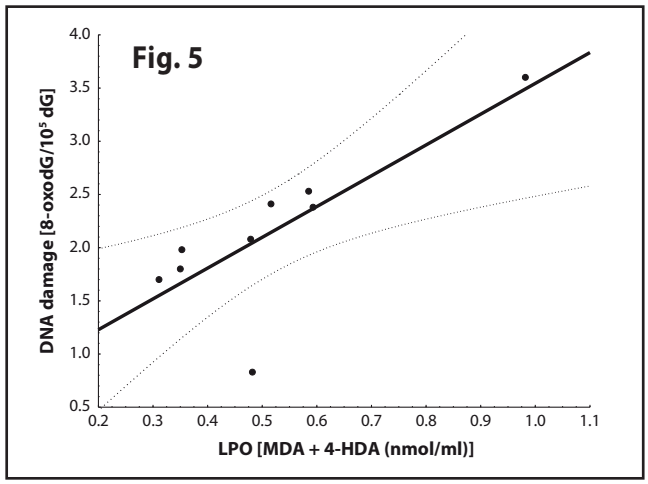


Fig. 3A. Correlations between LPO level and body mass (r=0.82; $p<0.001$), body mass index (BMI) (r=0.84; $p<0.001$) in patients with BMI ≥ 25 kg/m^2.

Fig. 3B. Correlations between LPO level and waist circumference (r=0.68; $p<0.001$), hip circumference (r=0.49; $p<0.001$), waist:hip ratio (WHR) (r=0.32; $p=0.017$) in patients with BMI ≥ 25 kg/m^2.

Fig. 3C. Correlations between LPO level and systolic blood pressure (RRs) (r=0.45; $p<0.001$), diastolic blood pressure (RRd) (r=0.42; $p=0.001$) in patients with BMI ≥ 25 kg/m^2.





in obese patients (Ford *et al.* 2003; Demirbag *et al.* 2006; Vincent & Taylor 2006; Cardona *et al.* 2008; Ando & Fujita 2009; Zeyda & Stulnig 2009; Szosland *et al.* 2010), obesity as an independent determinant (predictor) of increased oxidative damage to macromolecules is documented for the first time in the present study.

In our preliminary study, in which exclusively postmenopausal women were considered, body mass – instead of BMI – appeared to be the only independent predictor for increased oxidative damage to macromolecules (Szosland *et al.* 2010). Nevertheless, when compared to body mass, BMI seems to be more reliable predictor for oxidative damage, as it relates, at least in the present study, to the population represented by both sexes.

The findings on positive correlations between LPO and such parameters as systolic and diastolic blood pressure, as well as concentrations of CRP, ferritin, and glucose were expected and are consistent with previous observations on increased oxidative stress in hypertensive subjects (Harrison & Gongora 2009; Gosmanov *et al.* 2010), as well as on increased CRP, ferritin, and glucose concentration in obesity (Gosmanov *et al.* 2010). Similarly, DNA damage correlated positively with TGs concentration, the lipid fraction being frequently increased in obese subjects, and it correlated negatively with iron concentration, which is frequently diminished in obese patients (Ausk & Ioannou 2008). However, statistical evaluation does not allow to conclude that any disturbances of the above parameters may directly contribute to the increased oxidative damage to macromolecules in overweight or obese patients. In opposite, as it was discussed above, BMI, constituting the basic parameter in obesity definition in adults, appeared to be the only independent determinant (predictor) of the enhanced oxidative damage to membrane lipids.

The results from the present study may constitute the basis to explain the mechanism of increased cancer risk, as well as cardiovascular risk and the risk of other disorders, occurring more frequently in obese subjects. It is well known that red-ox imbalance occurs at each step of carcinogenesis (Evans *et al.* 2004; Dalle-Donne *et al.* 2006; Valko *et al.* 2006; Cortes *et al.* 2011). In turn, ROS by themselves, as well as products of oxidative damage to macromolecules, unfavourably affect endothelium, the well known phenomenon in the pathogenesis of cardiovascular (Evans *et al.* 2004; Dalle-Donne *et al.* 2006; Misra *et al.* 2009) and cerebrovascular (Chrissobolis *et al.* 2011) disorders.

Fig. 4. Correlations between the level of DNA damage and body mass (r=0.74; p=0.022), body mass index (r=0.84; p=0.004), hip circumference (r=0.74; p=0.022) in patients with BMI ≥ 25 kg/m^2.

Fig. 5. Correlation between the level of DNA damage and LPO level in patients with BMI ≥ 25 kg/m^2 (r=0.78; p=0.013).

Summing up, overweight and obesity in adults are directly associated with increased oxidative damage to membrane lipids and to nuclear DNA. BMI does constitute the independent risk factor for oxidative damage to macromolecules. The hypothesis is proposed on the mechanism of "vicious circle", in which obesity directly induces oxidative damage to macromolecules, the products of which, in turn, further enhance obesity. Enhanced oxidative stress in obesity may contribute to increased risk of cancer and other non-communicable diseases.

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Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

1 Akamine EH, Marcal AC, Camporez JP, Hoshida MS, Caperuto LC, Bevilacqua E, et al (2010). Obesity induced by high-fat diet promotes insulin resistance in the ovary. J Endocrinol. **206:** 65–74.
2 Ando K, Fujita T (2009). Metabolic syndrome and oxidative stress. Free Radic Biol Med. **47:** 213–218.
3 de Assis AM, Rieger DK, Longoni A, Battu C, Raymundi S, da Rocha RF, et al (2009). High fat and highly thermolyzed fat diets promote insulin resistance and increase DNA damage in rats. Exp Biol Med. **234:** 1296–1304.
4 Ausk KJ, Ioannou GN (2008). Is obesity associated with anemia of chronic disease? A population-based study. Obesity (Silver Spring). **16:** 2356–2361.
5 Bianchini F, Kaaks R, Vainio H (2002). Overweight, obesity, and cancer risk. Lancet Oncol. **3:** 565–574.
6 Cardona F, Túnez I, Tasset I, Montilla P, Collantes E, Tinahones FJ (2008). Fat overload aggravates oxidative stress in patients with the metabolic syndrome. Eur J Clin Invest. **38:** 510–515.
7 Ceschi M, Gutzwiller F, Moch H, Eichholzer M, Probst-Hensch NM (2007). Epidemiology and pathophysiology of obesity as cause of cancer. Swiss Med Wkly. **137:** 50–56.
8 Chrissobolis S, Miller AA, Drummond GR, Kemp-Harper BK, Sobey CG (2011). Oxidative stress and endothelial dysfunction in cerebrovascular disease. Front Biosci. **16:** 1733–1745.
9 Cortes DF, Sha W, Hower V, Blekherman G, Laubenbacher R, Akman S, et al (2011). Differential gene expression in normal and transformed human mammary epithelial cells in response to oxidative stress. Free Radic Biol Med. **50:** 1565–1574.
10 Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A (2006). Biomarkers of oxidative damage in human disease. Clin Chem. **52:** 601–623.
11 Demirbag R, Yilmaz R, Gur M, Celik H, Guzel S, Selek S, et al (2006). DNA damage in metabolic syndrome and its association with antioxidative and oxidative measurements. Int J Clin Pract. **60:** 1187–1193.
12 Dobrian AD, Davies MJ, Schriver SD, Lauterio TJ, Prewitt RL (2001). Oxidative stress in a rat model of obesity-induced hypertension. Hypertension. **37(2 Part 2):** 554–560.
13 Evans MD, Dizdaroglu M, Cooke MS (2004). Oxidative DNA damage and disease: induction, repair and significance. Mutat Res. **567:** 1–61.
14 Flegal KM, Graubard BI, Williamson DF, Gail MH (2007). Cause-specific excess deaths associated with underweight, overweight, and obesity. JAMA. **298:** 2028–2037.
15 Ford ES, Mokdad AH, Giles WH, Brown DW (2003). The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey. Diabetes. **52:** 2346–2352.
16 Gosmanov AR, Smiley DD, Robalino G, Siquiera J, Khan B, Le NA, et al (2010). Effects of oral and intravenous fat load on blood pressure, endothelial function, sympathetic activity, and oxidative stress in obese healthy subjects. Am J Physiol Endocrinol Metab. **299:** E953–E958.
17 Harrison DG, Gongora MC (2009). Oxidative stress and hypertension. Med Clin North Am. **93:** 621–635.
18 Kalisz M, Baranowska B, Bik W (2012). Do novel adipokines play a causative or only modulating role in the pathogenesis of obesity and metabolic disorders ? Neuroendocrinol Lett. **33:** 11–15.
19 Karbownik M, Lewinski A (2003). Melatonin reduces Fenton reaction-induced lipid peroxidation in porcine thyroid tissue. J Cell Biochem. **90:** 806–811.
20 Karbownik M, Lewinski A, Reiter RJ (2001). Anticarcinogenic actions of melatonin which involve antioxidative processes: comparison with other antioxidants. Int J Biochem Cell Biol. **33:** 735–753.
21 Karbownik M, Reiter RJ (2000). Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc Soc Exp Biol Med. **225:** 9–22.
22 Karbownik-Lewinska M, Kokoszko A, Lewandowski K, Shalet SM, Lewinski A (2008). Growth hormone replacement reduces increased lipid peroxidation in growth hormone-deficient adults. Clin Endocrinol. **68:** 957–964.
23 Karbownik-Lewinska M, Stepniak J, Krawczyk J, Zasada K, Szosland J, Gesing A, Lewinski A (2010). External hydrogen peroxide is not indispensable for experimental induction of lipid peroxidation via Fenton reaction in porcine ovary homogenates. Neuroendocrinol Lett. **31:** 343–347.
24 Kokoszko A, Karbownik M, Lewinski A (2006). Increased lipid peroxidation in growth hormone-deficient adult patients. Neuroendocrinol Lett. **27:** 225–230.
25 Kokoszko A, Dabrowski J, Lewinski A, Karbownik-Lewinska M (2010). Effects of growth hormone and insulin-like growth factor-I on the iron-induced lipid peroxidation in the rat liver and porcine thyroid homogenates. Neuroendocrinol Lett. **31:** 517–523.
26 Maes M, Mihaylova I, Kubera M, Uytterhoeven M, Vrydaqs N, Bosmans E (2009). Increased 8-hydroxy-deoxyguanosine, a marker of oxidative damage to DNA, in major depression and myalgic encephalomyelitis/chronic fatigue syndrome. Neuroendocrinol Lett. **30:** 715–722.
27 Misra MK, Sarwat M, Bhakuni P, Tuteja R, Tuteja N (2009). Oxidative stress and ischemic myocardial syndromes. Med Sci Monit. **15:** RA209–219.
28 Paz-Filho G, Lim EL, Wong ML, Licinio J (2011). Associations between adipokines and obesity-related cancer. Front Biosci. **16:** 1634–1650.
29 Pelclova D, Navratil T, Fenclova Z, Vickova S, Kupka K, Urban P, et al (2011). Increased oxidative/nitrosative stress markers measured non-invasively in patients with high 2,3,7,8-tetrachlorodibenzo-p-dioxin plasma level. Neuroendocrinol Lett. **32 Suppl 1:** 71–76.
30 Pischon T, Nöthlings U, Boeing H (2008). Obesity and cancer. Proc Nutr Soc. **67:** 128–145.
31 Podborska M, Sevcikova A, Trna J, Dite P, Lojek A, Kubala L (2009). Increased markers of oxidative stress in plasma of patients with chronic pancreatitis. Neuroendocrinol Lett. **30 Suppl 1:** 116–120.
32 Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M (2008). Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. **371:** 569–578.
33 Roberts CK, Barnard RJ, Sindhu RK, Jurczak M, Ehdaie A, Vaziri ND (2006). Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in diet-induced metabolic syndrome. Metabolism. **55:** 928–934.

34 Stasiak M, Zasada K, Lewinski A, Karbownik-Lewinska M (2010). Melatonin restores the basal level of lipid peroxidation in rat tissues exposed to potassium bromate in vitro. Neuroendocrinol Lett. **31:** 363–369.
35 Szosland J, Kokoszko A, Zasada K, Stepniak J, Lewinski A, Karbownik-Lewinska M (2010). Obesity as a risk factor for oxidative damage to membrane lipids in postmenopausal women. Menopause Rev. **3:** 159–164.
36 Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. **160:** 1–40.
37 Vincent HK & Taylor AG (2006). Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes. **30:** 400–418.
38 Yan RT, Yan AT, Anderson TJ, Buithieu J, Charbonneau F, Title L, et al (2009). The differential association between various anthropometric indices of obesity and subclinical atherosclerosis. Atherosclerosis. **207:** 232–238.
39 Zeyda M, Stulnig TM (2009). Obesity, inflammation, and insulin resistance – a mini-review. Gerontology. **55:** 379–386.