Bratisl Lek Listy 2009; 110 (3)
152 – 157

CLINICAL STUDY

Oxidative stress and metabolic syndrome in obese adults with and without controlled diet restriction

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Abstract: *Background:* Metabolic syndrome (MetS) represents a collection of markers associated with cardiovascular morbidity and mortality. Due to its high prevalence and steady increase of the occurrence, prevention or management of MetS is of paramount importance. The aim of our study was to evaluate MetS occurrence and extent of oxidative stress by comparing obese adults after diet optimization with untreated controls.
Material and methods: Oxidative stress markers (total amount of free radicals, malondialdehyde, allantoin, α1-antiproteinase, GSSG/GSH ratio), total antioxidant capacity and lipid standardized α-tocopherol were determined in 40 obese people and 48 healthy controls. The obese people were divided into two group A: obese with restricted energy intake with lowered dietary carbohydrates (n=20) and group B: with the same grade of obesity but without following dietary recommendations (n=20).
Results: Group A exhibited lower oxidative stress markers than group B; free radicals (5.18±1.68 vs 8.43±3.66 mmol/l, $p<0.01$), GSSG/GSH ratio (11.74±5.01 vs 15.38±5.93 %, $p<0.05$) and higher antioxidants: lipid standardized α-tocopherol (3.70±0.51 vs 3.35±0.60, $p<0.05$) and ceruloplasmin (0.24±0.08 vs 0.21±0.03 g/l, $p<0.05$), in the course of same grade of obesity. Furthermore MetS occurrence was found significantly lower was in group A.
Conclusion: The energy intake restriction by 2000 kJ, mainly due to carbohydrate limitations, was associated with decreased oxidative stress and simultaneously increased lipid-standardized α-tocopherol and ceruloplasmin in obese people. These changes correlated with diminished MetS occurrence by about 50 % *(Tab. 3, Ref. 32).* Full Text (Free, PDF) *www.bmj.sk.*
Key words: oxidative stress, metabolic syndrome, obesity, energy intake restriction.

Obesity is associated with an increase of circulating markers of oxidative stress and low-grade inflammation (1, 2). Overweight and obesity are associated with an elevated risk of cardiovascular disease and type 2 diabetes. However, negative impacts are caused more by metabolic abnormalities, which often coexist with obesity, than by obesity *per se*. Metabolic abnormalities, which create a cluster of metabolic syndrome (MetS), include impaired fasting glucose, hyperinsulinemia, elevated blood pressure, dyslipidemia (characterized by low level of high-density lipoprotein cholesterol – HDL-C and high plasma triglyceride) and increased systemic inflammation. Specific criteria of MetS were defined by National Cholesterol Education Program Adult Treatment Panel III guidelines (3). Collection of MetS markers is positively associated with cardiovascular morbidity and mortality, independently of other traditional risk factors (4–8). Due to its high prevalence and steady increase in the occurrence, prevention or management of MetS is very important.

The aim of our cross-sectional study was to evaluate the occurrence of MetS and extent of oxidative stress in obese adults. The grade of obesity in all patients was the same and they were divided into two groups: one with diet restriction and the other without (the restriction of energy intake was mainly achieved by reduction of carbohydrates).

In addition to the parameters generally used for assessing the oxidative stress (malondialdehyde – the stable product of oxidative degradation of polyunsaturated fatty acids, ratio of oxidized and reduced glutathione – main intracellular antioxidant, allantoin – oxidative metabolite of uric acid, total antioxidant status, α-tocopherol and ceruloplasmin – antioxidants), we valuated also other parameters: α1-antiproteinase and free radicals. α1-antiproteinase is a predominant component of α1 fraction of human plasma. Reactive oxygen species can inactivate α1-antiproteinase by oxidizing essential methionine of the ac-

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Acknowledgements: This work was supported by the Ministry of Education, Youth and Sports CR grant COST OC124 - COST Action 926 and grant 0021627502. The authors thank to MUDr. R. Krivkova for patients selection and cooperation in this study.

tive site loop to methionine sulphoxide. Because α1-antiproteinase is very sensitive to oxidants, it may be a good biomarker for the evaluation of the oxidative damage in biological systems. Free radical concentration was evaluated by a novel method based on determination of electron acceptance by chlorophyllin developed recently by Votruba (9). This method is suitable for measurement of the total amount all of types of free radicals, both reactive oxygen and reactive nitrogen species (10). The method was validated by comparison with the basic method of electron paramagnetic resonance (9) with an excellent correlation (r=0.997).

Material

This study included 40 obese adults characterized by waist circumference, body mass index (BMI) and age. The persons were divided according to their dietary habits into two groups: those subjected to energy intake reduction and accepted modification of dietary habits (A, n=20), and those who refused to change their dietary habits (B, n=20). The subjects in these groups did not differ significantly in waist circumference, BMI and age. The control group consisted of 48 healthy normal-weight blood donors (C) (for the group characteristics) (Tab. 1).

Both groups, adults with restricted energy intake and those who did not accept dietary recommendations were recruited from trainees of the Healthy Lifestyle Courses, performed by the Health Institute, which provided a guided energy intake reduction under medical supervision. All patients were informed about the recommended dietary guidelines. At the beginning of the course, the energy intake of the patients was calculated on the basis of the list of consumed food (mean value 8314 kJ). The recommended energy intake reduction was 2000 kJ, but the minimum energy input was always above 5000 kJ. Preferably restricted was the carbohydrate and lipid content, namely in a ratio of 3:1. The protein intake was kept at the level of 1 g proteins/kg body weight. Other recommended rules covered especially the increase in liquid intake (2000–3000 ml a day), regular food consumption (5 times a day), and adjustment of the lunchtime energy intake at 40 % of the daily dose. The proportion of fruit, vegetables, fish and legumes was increased at the expense of sweet and fatty dishes, in order to increase the intake of unsaturated fatty acids, antioxidants and fibers (11, 12). The optimum physical activity was chosen (at least 30 min 3 times a week) on the basis of the test of physical condition (step test). Psychosocial load and stress level were evaluated during interview with a supervisor.

All subjects recruited for the study were less than 70 years of age, either abstainers or moderate alcohol consumers (≤20 g/d) and non-smokers. Patients who had any serious health complications were excluded. None of the studied subjects exhibited renal, hepatic, gastrointestinal, pulmonary, endocrine or oncologic disease. Also women taking hormonal contraception or replacement therapy, or nonsteroidal anti-inflammatory drugs were excluded. A written informed consent was obtained from all participants before start and the study was approved by the Hospital Ethical Committee on Human Research.

Biochemical parameters related to metabolic syndrome and to oxidative stress were monitored in all subjects. The occurrence of metabolic syndrome was defined according to National Cholesterol Education Program Adult Treatment Panel III guidelines (3) including the presence of at least three criteria among the following: waist circumference >102 cm in men and >88 cm in women, hypertriglyceridemia ≥150 mg/dl (1.695 mmol/l); low levels of HDL cholesterol <40 mg/dl (1.036 mmol/l) in men and <50 mg/dl (1.295 mmol/l) in women; high blood pressure ≥130/85 mmHg; high fasting blood glucose ≥110 mg/dl (6.1 mmol/l).

Methods

Blood samples

Venous blood was obtained under standard conditions, from 7 to 8 a.m. after fasting for at least 12 h. The serum and plasma samples were kept frozen at -70 °C until assayed. The glutathione analysis was performed from the whole blood. The samples, deproteinized with metaphosphoric acid, were centrifuged and the supernatant was frozen at -70 °C.

Oxidative stress parameters

The concentration of free radicals was determined by a direct spectrophotometric method based on determination of electron acceptance by chlorophyllin (kit Free Radicals, Sevapharma, CZ); the total antioxidant capacity was quantified using the kit Total Antioxidant Status (Randox, UK). Malondialdehyde (MDA) in plasma was measured as MDA-thiobarbituric acid complex. This complex was separated from interfering substances using

Tab. 1. Group characteristics.

	Obese people with intervention (A; n=20)	P (A*vs*B)	Obese people without intervention (B; n=20)	P (B*vs*C)	Control group (C; n=48)	P (A*vs*C)
Age	51.27±11.66	–	48.06±11.04	–	52.12±5.43	–
Male/female	9/11	–	10/10	–	25/23	–
Blood pressure syst. (mm Hg)	130.00±11.80	–	127.35±15.72	–	128.75±12.50	–
Blood pressure diast. (mm Hg)	85.33±5.81	–	85.29±8.74	–	85.02±7.64	–
BMI (kg/m2)	36.52±6.67	–	34.65±5.40	**	27.53±4.15	**
Waist (cm)	107.20±16.10	–	107.76±13.14	**	83.45±14.30	**

The data are expressed as means ± S.D., statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Tab. 2. Effect of dietary habits improvement with carbohydrate restriction on markers for metabolic syndrome.

	Obese people with intervention (A; n=20)	P (A*vs*B)	Obese people without intervention (B; n=20)	P (B*vs*C)	Control group (C; n=48)	P (A*vs*C)
Glucose (mmol/l)	5.63±0.89	–	5.66±0.93	–	5.12±0.70	–
Total cholesterol (mmol/l)	5.05±1.00	–	5.00±0.74	–	5.21±0.60	–
HDL-CH (mmol/l)	1.32±0.29	–	1.20±0.37	**	1.60±0.38	*
LDL-CH (mmol/l)	3.18±1.03	–	2.87±0.97	–	2.92±0.44	*
Triacylglycerols (mmol/l)	1.17±0.57	*	2.15±1.48	**	1.09±0.58	–
Index Athero (Klimov)	2.97±1.00	–	3.48±1.28	**	2.48±0.93	*
Fibrinogen (g/l)	4.29±1.12	–	3.79±0.55	–	3.75±0.83	*
Hs-CRP (mg/l)	2.76±1.55	–	4.03±3.17	**	1.72±1.19	*

The data are expressed as means ± S.D., statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

HPLC (Shimadzu, Japan). For the separation, the reverse-phase column MAC (4x250 mm, Biospher SI 120 PSI C18, particle size 7 μm) was used. The mixture of methanol and 8.3 mmol/l phosphate buffer, pH 7.2, (35:65, v/v) was used as a mobile phase. The analyte was detected at 532 nm (13). The oxidized and reduced forms of glutathione (GSSG, GSH) in the whole blood were analyzed by means of reverse-phase HPLC (Shimadzu, Japan) with fluorescence detection (excitation λ= 350 nm and emission λ= 420 nm). Chromatography of GSH and GSSG after their derivatization with orthophthalaldehyde to form a stable, highly fluorescent tricyclic derivate was accomplished using an isocratic elution on column Discovery C18 (150 mmx4 mm i.d., 5 μm) at 37 °C. The mixture of methanol and 25 mmol/l sodium hydrogenphosphate (15:85, v/v), pH 6.0, was used as a mobile phase (14). Allantoin was determined by means of reverse-phase HPLC (Shimadzu, Japan) with UV/VIS detection at 360 nm. Chromatography of allantoin after its stoichiometric conversion to glyoxylate-2,4-dinitrophenylhydrazone was accomplished using a gradient elution on Discovery C18, 150x4mm i.d., 5 μm analytical column. Two mobile phases were used: A – 5 % acetonitrile in 8.3 mmol/l phosphate buffer (v/v), pH 6.1, and B – 50 % acetonitrile in 8.3 mmol/l phosphate buffer (v/v), pH 6.1 (15). α1-Antiproteinase was measured using the kit Bioxytech α1AP--410 Assay (OxisResearch, USA). The concentration of α-tocopherol was measured in plasma after an extraction into n-hexane by means of reverse-phase HPLC (Shimadzu, Japan) using an isocratic elution on Discovery C18, 250 mm x 4 mm i.d., 5 μm analytical column at 40 °C and UV/VIS detection at 292 nm (16).

MetS parameters

Plasma levels of glucose, total cholesterol, triglycerides, HDL-CH, LDL-CH, fibrinogen, high sensitive CRP (hsCRP), albumin, transferin, ceruloplasmin and uric acid were determined by standard procedures using an automatic biochemistry analyser Dimension (Dade-Behring, USA).

Clinical examination

Blood pressure and physical data (weight, height and waist circumference) were evaluated during a complete clinical examination. A detailed questionnaire concerning dietary habits and lifestyle was filled out by every patients.

Statistical analysis

Inter-group differences in continuous variables were analysed with the use of the Hotelling *T*2-test for independent groups using the software NCSS2000 (Dr. Jerry L. Hintze, Kaysville, Utah 84037, USA). Principal Component Analysis and Analysis of Correlation Matrix were carried out using the software STATISTICA (Statsoft, USA). All the results are expressed as means with the standard deviation given in brackets.

Results

All the patients were recruited from trainees of the Healthy Lifestyle Courses run by Health Institute. Obese patients with an improved dietary regimen (A) visited the courses for about 4.5 months (3–6 months), their energy intake was decreased by ca 2314.3 kJ/day (1000–3700 kJ/day), from about 8314 kJ/day to 6000 kJ/day. The mean value of mass reduction during the courses was 7.8 kg (4.5–14.5 kg), the waist circumference reduction was by about 10.3 cm (5–20 cm). The adoption of recommended dietary guidelines and improvement of dietary habits leading to higher intake of fruits and vegetables and higher physical activity was checked by questionnaires. A higher level of education was found in the group with improved dietary habits.

Patients in both groups exhibited the same level of obesity (according to the waist circumference and BMI) and did not differ significantly in hypertension or age (Tab. 1).

When biochemical parameters were taken into account, the participants who had undergone an energy intake reduction had a significantly lower triglyceride plasma concentration and slightly higher HDL-CH plasma level than those who did not improve their lifestyles. The improvement of dietary habits correlated with a lower hsCRP level, which indicates a lower systemic inflammatory stress condition (Tab. 2).

The improved dietary habits together with a controlled decrease in energy intake were associated with statistically significantly lower free radical levels and GSSG/GSH ratio, which corresponded to the decreased degree of oxidative stress load in comparison with the group exhibiting the same grade of obesity but unchanged habits (Tab. 3). The correlation of the lower level of oxidative stress with a significantly lower occurrence of metabolic syndrome in subgroup with intervention (A) as compared

Tab. 3. Effect of improvement of dietary habits with carbohydrate restriction on markers of oxidative stress.

	Obese people with intervention (A)	P (A*vs*B)	Obese people without intervention(B)	P (B*vs*C)	Control group (C)	P (A*vs*C)
Free radicals (mmol/l)	5.18±1.68	**	8.43±3.66	***	4.71±0.77	–
Total antioxidant capacity (mmol/l)	0.77±0.13	–	0.86±0.13	–	0.81±0.09	–
Malondialdehyde (μmol/l)	2.46±0.52	–	2.64±0.42	***	1.18±0.19	***
α_1-Antiproteinase (μmol/l)	39.25±12.91	–	34.07±11.66	*	26.59±9.60	**
GSSG/GSH (%)	11.74±5.01	*	15.38±5.93	***	6.53±2.65	***
Allantoin (μmol/l)	3.58±2.10	–	3.69±1.28	–	4.01±1.03	–
Vitamin E (μmol/l)	22.63±6.03	–	23.16±4.61	–	23.22±4.80	–
Vitamin E/cholesterol+triacylglycerols	3.70±0.51	*	3.35±0.60	–	3.36±0.51	*
Albumin (g/l)	41.00±3.21	–	41.06±2.90	–	41.15±3.11	–
Transferrin (g/l)	2.37±0.27	–	2.47±0.33	*	2.32±0.58	–
Ceruloplasmin (g/l)	0.24±0.08	*	0.21±0.03	***	0.38±0.11	***
Uric acid (μmol/l)	291.93±53.04	*	334.18±75.81	***	243.75±62.09	**

The data are expressed as means ± S.D., statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

to the subgroup without intervention (B) – 33 % vs 59 % – indicates a high practical importance of dietary habit management.

Discussion

The aim of this study was to evaluate whether an energy restriction and overall improvement of dietary habits may be associated with a decreased oxidative stress and occurrence of MetS, as it is generally accepted that the first line of attack against the MetS should be reduction in body mass. However, the optimum method for attaining the weight loss remains rather unclear. Low fat diets have become a kind of standard, although recent evidence has indicated the value of strategies based on carbohydrate restriction.

The effect of carbohydrate reduction on the MetS symptoms has been recognized. In a classic review, Reaven [17] demonstrated that nutritional approach to MetS reduction might be based on lowering dietary carbohydrates. Volek et al (18) have reviewed many studies, which showed that carbohydrate restriction resulted in significant reductions in postprandial lipemia having beneficial effects on HDL and intravascular processing of lipoproteins (19, 20).

The duration of diet application is a very important aspect, too. It is widely quoted that the low carbohydrate diet should be maintained for 6 months (21). Relatively low differences were found among diets lasting 6–12 months. The obese patients in our studied group visited the courses for about 4.5 months (3–6 months). This relatively short period was sufficient for the improvement of their biochemical parameters in comparison with the group exhibiting the same degree of obesity, but without dietary intervention.

Selvin et al (22) published a comprehensive review of 33 studies and concluded that body weight loss was associated with a decline in CRP level. Rector et al (23) as well as Davi et al (24) or Dandona et al (19) described the decrease in oxidative stress as a consequence of diet-induced and exercise-induced body weight loss in adults with MetS. The members of our group with improved dietary habits and calorie restriction exhibited lower oxidative stress markers (free radical amount and GSSG/GSH ratio), lower hsCRP and uric acid and higher antioxidants (lipid standardized -tocopherol and ceruloplasmin plasma concentrations) as compared to the obese people with the same grade of obesity who did not adhere to dietary recommendations.

Our data on decreased oxidative stress and elevated antioxidant content in the group with an improved diet indicate that an appropriate change of dietary habits may result in reduction of oxidative stress and inflammatory state even when the body weight is not reduced. We consider it a very important fact that the decreased oxidative stress resulting from adoption of diet with carbohydrate restriction correlated with significantly decreased occurrence of MetS. Low level of systemic inflammation in subjects on caloric restriction evidenced by low circulating CRP and TNF alpha, was also described by Holloszy (25).

The significantly higher level of uric acid in the group of patients who did not improve their dietary habits indicates a strong relationship between uric acid content and dietary habits. Uric acid is considered as a risk factor of cardiovascular disease, MetS and diabetes mellitus. It is one of the multiple injurious stimuli to the endothelium of the arterial vessel wall and capillaries. In the pro-oxidative environmental milieu, the original antioxidant properties of uric acid paradoxically become pro-oxidant, thus contributing to the oxidation of lipoproteins within atherosclerotic plaques. Hyperuricemia has been associated with increasing BMI also in recent studies (26, 27).

The dietary regimen modification based on carbohydrate restriction was effective also in ameliorating high plasma triglycerides and lowering the HDL extent (even if this decrease did not reach a statistical significance). Analysis of the GEMS study (28) (Barter, 2007) indicated that overweight people considerably differ at the risk level, with much higher risk being identified in the group with a high plasma triglyceride and low HDL-C level, which indicates a generalized metabolic disorder. Thus improvement of lipid metabolism by dietary regime optimization may contribute to the decrease in the risk of cardiovascular disease and diabetes in obese patients.

The recommended regimen included, apart from the change of dietary habits, also an increase of physical activity, adequate to the abilities of the patients, in order to increase their aerobic fitness. Redman et al (29) found that exercise plays an equivalent role to caloric restriction in terms of energy balance and subsequent body weight loss, as well as it improves aerobic fitness. However, the decrease in metabolic risk factors seems to be caused primarily by the net energy deficit, as regular exercise is only of minor benefit in terms of changes in body composition. Calorie restriction in the diet of obese persons has many positive effects, e.g., a decrease in blood pressure associated with a decrease in plasma norepinephrine and sympathetic activity (30, 31), or increase in insulin sensitivity in peripheral tissues (32).

The above-summarized data suggest that some degree of calorie restriction, based mainly on reduction of carbohydrates, could provide the first line attack against the symptoms of MetS. The principle underlying the positive effects of carbohydrate restriction is the maintenance of low insulin level, when metabolism is biased rather towards lipid oxidation than storage.

In order to evaluate the potential impact of diet restriction on the occurrence of MetS, we chose a cross-sectional study design and compared the obese persons with similar age, gender and grade of obesity, who differed only in the lifestyle and dietary habits. The lower level of oxidative stress found in the group with intervention seems to be promising. Our study represents a sound basis for further long-term follow-up experiments, which would allow to monitore the dynamics of the effect of changed dietary habits as well as the influence of lifestyle behavior in biochemical parameters in the individual obese persons.

Conclusion

An energy intake restriction by 2000 kJ, mainly due to carbohydrate limitations, was associated with a statistically significant lower level of oxidative stress (free radicals, malondialdehyde, GSSG/GSH) and simultaneously with a higher plasma lipid-standardized α-tocopherol and ceruloplasmin in comparison with obese people with the same grade of obesity but without improved dietary regimen. These changes correlated with a decreased occurrence of MetS (by about 50 %) in the group with the improved dietary habits.

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Received August 28, 2008.
Accepted December 18, 2008.