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Short-Term Very Low Calory Diet Reduces Oxidative Stress in Obese Type 2 Diabetic Patients

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Summary

Oxidative stress is higher in obese diabetic than in non-diabetic subjects. This pilot study evaluates oxidative stress during short-term administration of a very low calory diet in obese persons. Nine obese Type 2 diabetic patients (age 55±5 years, BMI 35.9±1.9 kg/m^2) and nine obese non-diabetic control subjects (age 52±6 years, BMI 37.3±2.1 kg/m^2) were treated by a very low calory diet (600 kcal daily) during 8 days stay in the hospital. Serum cholesterol, triglycerides, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (B-HB), ascorbic acid (AA), alpha-tocopherol (AT), plasma malondialdehyde (MDA) and superoxide dismutase (SOD) activity in erythrocytes were measured before and on day 3 and 8 of very low calory diet administration. A decrease of serum cholesterol and triglyceride concentrations on day 8 was associated with a significant increase of NEFA (0.30±0.13 vs. 0.47±0.11 μmol/l, $p<0.001$) and B-HB (0.36±0.13 vs. 2.23±1.00 mmol/l, $p<0.001$) in controls but only of B-HB (1.11±0.72 vs. 3.02±1.95 mmol/l, $p<0.001$) in diabetic patients. A significant decrease of plasma MDA and serum AT together with an increase of SOD activity and AA concentration ($p<0.01$) was observed in control persons, whereas an increase of SOD activity ($p<0.01$) was only found in diabetic patients after one week of the very low calory diet. There was a significant correlation between NEFA or B-HB and SOD activity ($p<0.01$). We conclude that one week of a very low calory diet administration decreases oxidative stress in obese non-diabetic but only partly in diabetic persons. Diabetes mellitus causes a greater resistance to the effects of a low calory diet on oxidative stress.

Key words

Oxidative stress • Very low calory diet • Obesity • Type 2 diabetes mellitus

Introduction

Obesity is associated with insulin resistance in the population with or without diabetes. The presence of arterial hypertension, impaired lipid metabolism, and changes in plasma coagulation creating background of the metabolic syndrome are features occurring in parallel in these individuals. Increased morbidity and mortality due to accelerated atherosclerosis have been repeatedly reported in subjects with the metabolic syndrome. Intensive treatment of the whole cluster of the risk factors involving obesity, arterial hypertension, hyperlipidemia and diabetes was found to be the most effective in prevention of the cardiovascular disease (Gaede *et al.* 2003).

Obese Type 2 diabetic patients with

dyslipidemia have higher oxidative stress, which plays an important role in the pathogenesis of atherosclerosis (Higdon and Frei 2003, Keaney *et al.* 2003). Reduction of oxidative stress may therefore attenuate the risk of the vascular wall changes and improve the patient prognosis. Reduction of body weight in obese diabetic patients has a beneficial effect on insulin sensitivity because of reducing insulin resistance. It further lowers blood glucose and arterial hypertension as well as improves the lipid profile, all factors influencing the vascular pathology.

The aim of this pilot study was to evaluate the effect of a short-term low calory diet on oxidative stress in obese Type 2 diabetic patients and to compare it with the results obtained in non-diabetic subjects. Plasma malondialdehyde concentration was used as a marker of oxidative stress and superoxide dismutase together with ascorbic acid and alpha-tocopherol as indicators of the antioxidant system.

Methods

Ten obese Type 2 diabetic patients (six men and four women) were admitted for one-week hospitalization at our metabolic ward to administer a very low calory diet. Nine patients finished the whole study and their basic characteristics are shown in Table 1. After collecting blood for biochemical parameters on the first day (baseline data), the patients continued their stay on a dietary regimen containing 600 kcal (2500 kJ) daily. Physical activity was limited to a short walk once a day comparable with their previous activity. All patients were previously treated by metformin, but this drug administration was discontinued after the introduction of dietary regimen at the hospital to avoid side effects of metformin by a very low calory diet. Three patients were treated by small doses of insulin still before admission to hospital and the doses were adjusted to their regimen. Antihypertensive drugs (calcium channel blockers in two and ACE inhibitors in five patients) continued without changes. All patients had normal renal and liver biochemical tests and had no evidence of any other active disease. Blood glucose was measured three times daily and blood samples for determination of biochemical variables were collected in the fasting state of the hospital day 3 and 8. Nine patients finished the whole program with blood sampling and their laboratory results were used for evaluation. The control group consisted of nine obese age- and weight-matched non-diabetic subjects. Informed consent was obtained from all persons participated in this study.

Table 1. Basal characteristics of obese Type 2 diabetic patients and control subjects

	Diabetic patients (n=9)	**Control subjects (n=9)**
Age (years)	55 (41-70)	53 (35-69)
Diabetes duration (years)	12±5	–
BMI (kg/m^2)	36.2±1.6	37.7±2.1
Glycated hemoglobin (HbA_1c, %)	10.2±1.6	5.5±0.4

Blood samples were drawn after an overnight fast between 6:00 and 7:00 h. Plasma glucose concentration was measured by the glucose oxidase method on analyzer GLambulance (G. Müller, Freital, Germany). Serum total, HDL and LDL cholesterol, triglycerides, creatinine, uric acid and transaminases were measured by routine methods on Hitachi analyzer. Non-esterified fatty acids (NEFA) were determined photometrically (Duncombe 1964) and β-hydroxy-butyrate (B-HB) fluorometrically (Olsen 1971). Plasma malondialdehyde (MDA) concentration was evaluated by fluorometric method using Perkin Elmer spectrofluorometer (Yagi 1976). Superoxide dismutase (SOD) activity was measured in erythrocyte according to McCord and Fridovich (1969) and expressed in units (Škrha and Hilgertová 1999). Serum ascorbic acid (AA) was determined photometrically (Nakagawa *et al.* 1997) and α-tocopherol (AT) concentration by the HPLC method (Catignani 1986).

Statistical evaluation was done by t-test for paired and unpaired values and analysis of variance was applied to compare the data between and within the groups. Pearson's correlation was used to find the relationships for the differences (day 8 vs. baseline results) of laboratory variables.

Results

Basic biochemical variables and parameters of oxidative stress in obese Type 2 diabetic patients and control subjects before and after dietary intervention are shown in Table 2. Plasma glucose, glycated hemoglobin, serum triglyceride, non-esterified fatty acids, beta-hydroxybutyrate and plasma malondialdehyde concentrations as well as superoxide dismutase activity in erythrocytes were higher in diabetic patients at baseline than in obese control persons. Other parameters were comparable in both groups of subjects.

Table 2. Biochemical variables in diabetic patients and control subjects before and during the administration of a very low calory diet (day 3 and 8).

	Diabetic patients			**Control subjects**		
	Before	**Day 3**	**Day 8**	**Before**	**Day 3**	**Day 8**
FPG (mmol/l)	14.0±4.5$^{###}$	12.6±3.9	9.3±1.9***	5.2±0.5	5.1±0.5	4.8±0.6
T-cholesterol (mmol/l)	5.8±0.5	5.6±0.4	5.0±0.3***	5.8±0.6	5.4±0.6	4.8±0.5**
HDL-cholesterol (mmol/l)	1.45±0.18	1.29±0.16**	1.19±0.14***	1.47±0.17	1.34±0.12**	1.26±0.13***
LDL-cholesterol (mmol/l)	3.44±0.34	3.30±0.36	3.15±0.33*	3.56±0.50	3.34±0.52	2.74±0.52***
Triglycerides (mmol/l)	2.49±0.9$^{##}$	2.29±0.66	1.58±0.42***	1.62±0.36	1.51±0.30	1.36±0.19*
NEFA (μmol/l)	0.39±0.09$^{##}$	0.40±0.06	0.42±0.08	0.30±0.06	0.48±0.08***	0.47±0.11***
B-HB (mmol/l)	1.11±0.72$^{##}$	2.14±1.27***	3.02±1.95***	0.36±0.13	0.98±0.3***	2.23±1.00***
MDA (μmol/l)	2.57±0.36$^{#}$	2.67±0.29	2.43±0.16	2.29±0.30	2.05±0.3	1.96±0.35**
SOD (U)	0.74±0.18$^{##}$	0.83±0.2	0.92±0.15**	0.51±0.14	0.64±0.21	0.86±0.35**
Ascorbic acid (μmol/l)	67±13	57±10	62±14	58±18	66±19	67±13**
Alpha-tocopherol (mg/l)	13.9±2.9	14.1±1.5	12.0±1.2	13.5±1.9	12.9±1.6	10.9±1.3**
AT/(CH+T) ratio	1.62±0.19$^{##}$	1.75±0.1	1.80±0.14*	1.82±0.15	1.88±0.17	1.77±0.18

FPG – fasting plasma glucose, NEFA – non-esterified fatty acids, B-HB – beta-hydroxybutyrate, MDA – malondialdehdyde, SOD – superoxide dismutase, AT – alpha-tocopherol, CH – cholesterol, T – triglycerides. Significant diffrence of baseline data in diabetic patients as compared to control subjects: $^{###}p<0.001$, $^{##}p<0.01$, $^{#}p<0.05$, Significant effects of a very low calory diet vs. baseline data: $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$.

On day 3, the very low calory diet caused a significant decrease of serum total and HDL-cholesterol concentrations in both diabetic and control groups (Table 2). A significant increase of β-hydroxybutyrate concentration was found in both groups, whereas plasma non-esterified fatty acids were increased only in controls. No other significant changes were observed on day 3.

However, a very low calory diet administered for one week caused significant biochemical changes observed on day 8. A significant decrease of BMI (36.2±1.6 vs. 34.6±1.5 kg/m^2 in diabetic patients and 37.7±2.1 vs. 36.4±1.9 kg/m^2 in the controls, both for

p<0.001), serum total, HDL- and LDL-cholesterol and triglycerides concentration was found in Type 2 diabetic patients and obese control subjects, but plasma glucose was significantly decreased in diabetic patients only (Table 2). Administration of the very low calory diet was associated with an increase of β-hydroxybutyrate in both groups, whereas an increase of the non-esterified fatty acids and a decrease of plasma malondialdehyde were significant only in control obese subjects. Superoxide dismutase activity was significantly higher following dietary regimen in both diabetic and control subjects (p<0.01). Serum concentrations of ascorbic acid and alpha-tocopherol were unchanged in diabetic patients but an increase of ascorbic acid and a decrease of alpha-tocopherol was found in control persons (p<0.01). However, when serum α-tocopherol was related to the sum of cholesterol and triglyceride concentrations, an increase of this ratio were found in diabetic patients after 8 days of diet administration (Table 2). Plasma glucose and malondialdehyde concentrations were found to be higher in diabetic patients when compared to controls (p<0.01) on day 8, whereas no differences of other laboratory variables were observed between both groups.

Table 3. Correlation analysis between BMI and biochemical variables of lipid metabolism and oxidative stress

Control subjects

	Δ BMI	**Δ MDA**	**Δ SOD**	**Δ Ascorbic acid**	**Δ α-tocopherol**
Δ BMI	–	0.50 (0.01)	–0.48 (0.01)	0.06	0.17
Δ Cholesterol	–0.16	–0.18	–0.64 (0.01)	–0.28	0.58 (0.01)
Δ Triglycerides	0.77 (0.001)	0.47 (0.01)	–0.27	0.43	0.37
Δ NEFA	–0.61 (0.01)	–0.03	0.81 (0.01)	–0.36	–0.36
Δ B-HB	–0.68 (0.001)	0.25	0.42 (0.05)	0.07	0.18

Diabetic patients

	Δ BMI	**Δ MDA**	**Δ SOD**	**Δ Ascorbic acid**	**Δ α-tocopherol**
Δ BMI	–	0.38	–0.03	–0.03	0.48 (0.05)
Δ Cholesterol	0.74 (0.001)	0.65 (0.01)	–0.39 (0.05)	–0.31	0.62 (0.01)
Δ Triglycerides	0.61 (0.01)	0.50 (0.02)	–0.21	–0.31	0.84 (0.01)
Δ NEFA	–0.11	–0.16	0.31	0.56 (0.01)	–0.15
Δ B-HB	–0.66 (0.01)	–0.36	0.12	0.24	0.01

Statistical significances are expressed in parentheses.

Several relationships between laboratory variables were observed using differences of the values measured on day 8 and baseline (Table 3). Decrease of the body mass index (ΔBMI) influenced both fatty metabolism parameters (ΔNEFA and ΔBHB) and oxidative stress (ΔMDA and ΔSOD) more in control than in diabetic subjects (Table 3, Figs 1 and 2). Positive relationship was observed between non-esterified fatty acids and β-hydroxybutyrate concentrations in both diabetic (r=0.47, p<0.01) and control subjects (r=0.68, p<0.01). Positive correlation was also found between changes of plasma malondialdehyde (ΔMDA) and α-tocopherol (ΔAT) or triglyceride (ΔTG) concentrations (p<0.02 both) as well as between changes of plasma α-tocopherol (ΔAT) and total cholesterol (ΔCH) or triglyceride (ΔTG) concentrations (p<0.01 both). Stronger relationships between the above variables were found in diabetic than in control subjects. On the other hand, significant positive correlation between changes of non-esterified fatty acids (ΔNEFA) and superoxide dismutase activity (ΔSOD) was observed in control but not in diabetic subjects (Fig. 2) and similar was true when β-hydroxybutyrate (ΔBHB) was related to superoxide dismutase (ΔSOD).

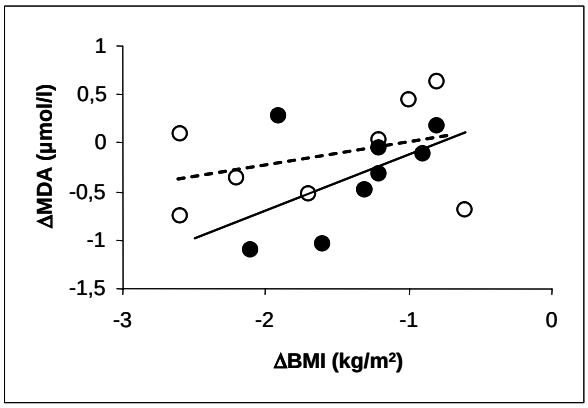

Fig.1. Relationship between changes of the body mass index (ΔBMI) and plasma malondialdehyde concentrations (ΔMDA) on day 8 vs. baseline in controls (full dots, r=0.50, p<0.01) and in diabetic patients (open circles, r=0.38, NS).

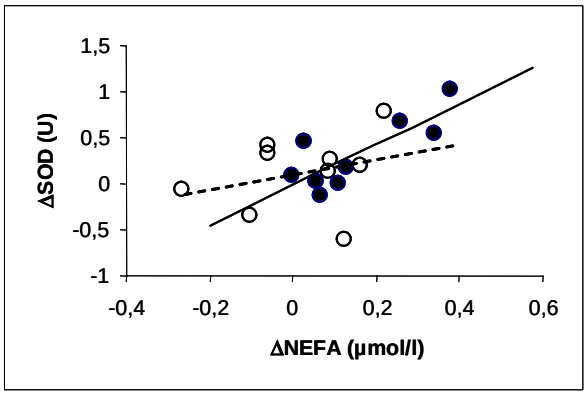

Fig. 2. Relationship between changes of superoxide dismutase (ΔSOD) and non-esterified fatty acid (ΔNEFA) values on day 8 vs. baseline in controls (full dots, r=0.81, p<0.01) and in diabetic patients (open circles, r=0.31, NS).

Discussion

Obesity is associated with accelerated oxidative stress (Prázný *et al.* 1999, Urakawa *et al.* 2003) which aggravates vascular changes and induces early cardiovascular disease (Higdon and Frei 2003). In the present study, we demonstrated that oxidative stress was higher in obese diabetic than in obese non-diabetic subjects. This finding confirmed our previous observation (Škrha *et al.* 2003). The main aim of this study was to ascertain if short-term reduction of energy intake might influence oxidative stress as a key factor in vascular pathology.

We observed that an energy restricting diet reduced oxidative stress already after one week of exposure. This was evident from the reduction of the plasma malondialdehyde levels and increase of superoxide dismutase activity and ascorbic acid concentration as indicators of antioxidant defense. The effect was more pronounced in obese non-diabetic that in obese Type 2 diabetic subjects, although a decrease of BMI was comparable in both groups of subjects. Our results demonstrate that diabetic patients are more resistant to a dietary regimen than non-diabetic subjects. However, plasma glucose was not normalized in diabetic patients and this could contribute to less prominent effect in this group. Low number of investigated patients may also influence the results and their statistical significance. The mechanism of the increase in ascorbic acid concentration following dietary restriction in obese non-diabetic patients remains unclear. A decreased clearance would be proposed. Although using data from a very short-term administration of energy restricting diet our results are in agreement with recent observation that the weight loss significantly reduces oxidative stress markers in obese patients with metabolic syndrome (Hoogeveen *et al.* 2003).

Several questions may be posed if the reduction of oxidative stress would be a direct effect of energy restricting diet or if it would be a consequence of other factors changed by dietary regimen. In both groups we observed significant lowering of serum cholesterol and triglyceride concentrations, which closely correlated with plasma malondialdehyde and serum α-tocopherol. This might contribute to oxidative stress reduction. Dietary regimen administered in obese non-diabetic subjects was associated with a significant increase of non-esterified fatty acids and of β-hydroxybutyrate corresponding to an increase of superoxide dismutase activity in erythrocytes. No significant relationship of these fatty acid variables to plasma malondialdehyde concentration was found.

In diabetic patients, only cholesterol and triglyceride changes were related to plasma malondialdehyde concentrations and BMI decrease was not associated with any changes of the oxidative stress parameters. It further supports the idea of a more complex origin of oxidative stress in obese diabetic patients.

Reduction of body weight in obese subjects improves endothelial dysfunction (Sasaki *et al.* 2002, Mather *et al.* 2003), but lowering of LDL cholesterol was suggested to be a more important contributing factor (Bergholm *et al.* 2003). Long-term exposure to energy restriction with greater physical activity brings significant improvement in endothelial function and reduces insulin resistance (Sciacqua *et al.* 2003). This serves as further

evidence that insulin insensitivity is linked to impaired endothelial function. The oxidative stress may be one of the interrelated factors playing important role in these mechanisms and therefore its reduction by non-pharmacological treatment brings beneficial effects.

We conclude that already very short-term consumption of energy restricting diet may reduce oxidative stress and that this effect is more pronounced in obese non-diabetic than in obese diabetic subjects. It may further positively influence endothelial function with resulting consequences in the vessel wall morphology.

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