Insulin action and fibrinolysis influenced by vitamin E in obese Type 2 diabetes mellitus

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Abstract

Increased oxidative stress, hypofibrinolysis and insulin resistance are present in obese Type 2 diabetic patients. It is supposed that treatment with antioxidant alpha-tocopherol (vitamin E) could not only decrease free radical production, but also ameliorate insulin action. We evaluated the effect of 3 months administration of vitamin E (600 mg daily) on insulin action examined by hyperinsulinemic clamp in 11 obese Type 2 diabetic patients. Oxidative stress and fibrinolysis were also determined. The administration of vitamin E caused a decrease of glucose disposal rate (26.6 ± 9.5 vs 21.3 ± 7.5 μmol/kg/min, P < 0.02) and of metabolic clearance rate of glucose (3.7 ± 1.6 vs 2.9 ± 0.8 ml/kg/min, P < 0.02). A decrease of insulin receptor number was observed on erythrocytes after vitamin E (284 ± 84 vs 171 ± 59 pmol/l, P < 0.01). Significantly higher plasma malondialdehyde (MDA) concentration documented an increased oxidative stress in diabetic patients as compared with healthy persons (3.13 ± 0.68 vs 1.89 ± 0.18 μmol/l, P < 0.001). An inverse relationship was found between MDA concentration and insulin sensitivity expressed by glucose disposal rate (r = −0.73). Vitamin E further worsened the hypofibrinolysis documented by a decrease of tissue plasminogen activator (P < 0.01) without changes in its inhibitor PAI-1. In conclusion, our results demonstrate that higher doses of vitamin E may further deteriorate insulin action and fibrinolysis in obese Type 2 diabetic patients. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Vitamin E; Oxidative stress; Insulin action; Fibrinolysis; Obese Type 2 diabetes mellitus

1. Introduction

Diabetes mellitus is characterised by increased generation of glyoxidation products associated with the advanced oxidative stress [1]. The presence of higher glucose or glycated protein concentration enhances lipid peroxidation [2] and, reversely, lipid peroxides may increase the extent of advanced glycation endproducts [3] participating in the chronic vascular complication develop-
A decrease of oxidative stress in diabetes may diminish not only glycoxidation and lipid peroxidation, but also the risk of cardiovascular disease. Vitamin C and E as antioxidant agents are promising tools for these purposes [5–8].

An improvement of insulin sensitivity and glucose control was previously found in elderly or in Type 2 diabetic patients treated by high doses of vitamin E [9,10]. This effect was not observed in another study, although a reduction of lipid peroxidation was found [5]. The relationship between insulin action and oxidative stress has been proposed [11]. In addition, a modulating effect of oxidative stress on fibrinolysis was also described [12].

The aim of our pilot study was to evaluate the effect of a medium dose (600 mg) of vitamin E on insulin action and fibrinolysis in obese Type 2 diabetic patients with enhanced oxidative stress. This group of patients was characterized by increased risk of cardiovascular disease and, thus, protective therapeutic approaches suppressing oxidative stress are important.

2. Patients and methods

Eleven obese persons with mild Type 2 diabetes mellitus (eight men and three women) were selected for this study. Characteristics of the patients are shown in Table 1. Diagnosis of diabetes was assessed at least 1 year prior to our examination and all patients were without clinical symptoms during the study. Four patients were treated by oral agents (sulphonylurea in two and biguanide in four of them), whereas only dietary regimen was used in the remaining seven subjects. Arterial hypertension (systolic blood pressure above 140 mmHg and diastolic blood pressure above 90 mmHg), manifested in two patients, was treated by nifedipine. All drugs were used more than 6 months prior to enrollment into this study without any changes during vitamin E treatment. Daily dose of 600 mg of alpha-tocopherol (vitamin E®, Slovakofarma), divided into three equal doses, was administered orally during 3 months. The control group consisted of nine healthy non-obese volunteers of appropriate age but not of comparable weight because we could demonstrate the presence of the insulin resistance and of other changes in the investigated variables in obese Type 2 diabetic patients. Informed consent was obtained from all persons. The ethical committee of the Faculty of Medicine approved the protocol prepared in accordance with the Helsinki Declaration.

All laboratory tests were performed prior to vitamin E administration and just at the end of 3 months of treatment. Blood samples were collected after an overnight fast between 7.00 and 8.00 a.m. during cannulation for the clamp procedure.

Insulin sensitivity was determined by isoglycemic hyperinsulinemic clamp on Biostator (GCIIS; Miles, Elkhart, IN, USA), mode 7:1, performed by a previously described procedure [13]. Briefly, a double lumen catheter for continuous blood glucose monitoring in arterialized blood was inserted into an antecubital vein. A second catheter for glucose and insulin infusions was placed into a contralateral cubital vein. Insulin was supplied at a constant rate (1 mU/kg/min) during 90 min after a previous 60 min of the stabilization phase. Blood samples for insulin and C-peptide determination were drawn before the clamp and twice in a 15-min interval at the end of the clamp. Plasma glucose concentration was re-

| Table 1 Clinical characteristics of diabetic patients and healthy controlsa |
|---------------------------------|--------|--------|
| Diabetic patients (n = 11)      | Controls (n = 9) |
|---------------------------------|--------|--------|
| Age (years)                     | 45 (33–58) | 43 (30–55) |
| Duration of diabetes (years)    | 5 ± 2  | –          |
| Treatment (diet/oral agents)     | 7/4    | –          |
| Body-mass index (kg/m²)         | 31.6 ± 3.6a | 25.1 ± 1.3 |
| Blood pressure                  |        |          |
| Systolic (mm Hg)                | 132 ± 14 | 130 ± 11 |
| Diastolic (mm Hg)               | 85 ± 9  | 83 ± 7   |

a Statistical significance as compared with control persons: P < 0.001.
Repeatedly controlled during the clamp by glucose analyzer ESAT 6660.2 (Medingen, Germany). The following variables were evaluated from the clamp. Glucose disposal rate was calculated from glucose maintaining blood glucose concentration at constant level, \( M \) (\( \mu \text{mol/kg/min} \)), metabolic clearance rate of glucose was expressed as a ratio between \( M \) and glucose concentration in the last 20 min of the clamp, \( MCR_G \) (ml/kg/min), and an index of insulin sensitivity calculated as a ratio between glucose disposal rate and insulin concentration evaluated at the end of the clamp, \( M/I \) (\( \mu \text{mol/kg/min per mU/l} \times 100 \)).

Heparinized whole blood was collected for the evaluation of insulin receptor characteristics on erythrocytes [14]. Specific insulin binding, \( B \) (%), insulin binding capacity, \( R_o \) (pmol/l), and receptor affinity, \( K_a \) (10\(^8\) \( 1/\text{mol} \)), were calculated in all patients before and after vitamin E treatment.

Plasma vitamin E concentration was determined by fluorimetric method [15]. Plasma glucose concentration was estimated by the glucose oxidase method, serum fructosamine concentration spectrophotometrically by tetrazolium nitroblue [16] and glycated hemoglobin (HbA\(_{1c}\)) by high performance liquid chromatography [17]. Serum insulin concentration was evaluated by radioimmunoassay technique [18].

Plasma concentration of malondialdehyde was measured by the thiobarbituric acid test of lipid peroxidation [19]. The results were used as an indicator of oxidative stress in diabetes. Cu,Zn-superoxide dismutase (SOD, EC 1.15.1.1) activity in erythrocytes was measured spectrophotometrically by a xanthine–xanthine oxidase system coupled with cytochrome \( c \) as previously described [20]. The enzyme extracts were prepared according to the procedure of Tsuchuhashi [21]. SOD activity was expressed in units (1 unit is equivalent to 250 U SOD/mg hemoglobin standardized by the described method [20]). All chemicals were obtained from Sigma (St. Louis, MO, USA).

Tissue plasminogen activator (tPA) and its inhibitor (PAI-1) concentrations (antigens) in plasma were measured by enzyme-linked immunosorbent assay method using Coailza tPA and PAI-1 kits (Kabi Diagnostics, Sweden). Plasma was stored no longer than 3 months at \(-20^\circ\text{C}\) before testing with a Dynatech MR 700 automatic microplate reader at 405 nm.

Statistical evaluation was performed by analysis of variance and \( t \)-tests were used for the evaluation of differences. Plasma tPA and PAI-1 concentrations were logarithmically transformed before statistical evaluation because of their log–normal distribution. Linear regression analysis and Pearson’s correlation coefficients were used for comparison of laboratory variables. The results were expressed as means ± SD or means with 2SD ranges.

3. Results

Biochemical variables of diabetes control and of insulin action in obese Type 2 diabetic patients are shown in Table 2. Fasting hyperinsulinemia and higher serum C-peptide concentrations were found in all patients. The insulin resistance was documented in diabetic patients as compared with healthy persons by decreased glucose disposal rate (\( P < 0.01 \)), metabolic clearance rate of glucose (\( P < 0.001 \)) or insulin sensitivity index (\( P < 0.001 \)).

No difference in insulin receptor characteristics was observed in diabetic patients at baseline (before treatment) as compared with healthy controls.

Body mass index remained unchanged in patients after 3 months of vitamin E treatment. The administration of 600 mg of vitamin E was accompanied by a slight but significant increase of HbA\(_{1c}\) (\( P < 0.05 \)) and of serum fructosamine concentration (\( P < 0.01 \)), but no changes of fasting plasma glucose (Table 2). A significant decrease of glucose disposal rate and of metabolic clearance rate of glucose (\( P < 0.02 \)) as well as of insulin receptor number on erythrocytes (\( P < 0.01 \)) was found after treatment. A decrease of fasting serum insulin concentration was accompanied by its increased metabolic clearance rate (\( 8.9 \pm 2.0 \) vs \( 10.2 \pm 2.6 \) ml/kg/min, \( P < 0.01 \)). Serum cholesterol and triglycerides did not change significantly.

Selected parameters of oxidative stress and fibrinolysis were estimated. Significantly lower
Parameters of diabetes control and of insulin action in obese Type 2 diabetic patients before and after treatment with 600 mg of vitamin E as compared with the control group of healthy persons

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>fP-G (mmol/l)</td>
<td>8.0 ± 2.6b</td>
<td>7.8 ± 2.0b</td>
</tr>
<tr>
<td>fS-FA (mmol/l)</td>
<td>1.4 ± 0.6c</td>
<td>1.7 ± 0.6b,d</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.7 ± 1.3b</td>
<td>7.0 ± 1.3b,d</td>
</tr>
<tr>
<td>fS-IRI (mU/l)</td>
<td>29 ± 15c</td>
<td>20 ± 8d</td>
</tr>
<tr>
<td>fS-C peptide (ng/ml)</td>
<td>2.3 ± 0.6c</td>
<td>2.5 ± 0.9c</td>
</tr>
<tr>
<td>M (µmol/kg/min)</td>
<td>26.6 ± 9.5c</td>
<td>21.3 ± 7.5c,e</td>
</tr>
<tr>
<td>MCR (ml/kg/min)</td>
<td>3.7 ± 1.6b</td>
<td>2.9 ± 0.8b,e</td>
</tr>
<tr>
<td>IRIC (µmol/l)</td>
<td>121 ± 29b</td>
<td>104 ± 23c,d</td>
</tr>
<tr>
<td>M/I (µmol/kg/min per mU/l×100)</td>
<td>23.4 ± 9.5b</td>
<td>21.7 ± 9.0b</td>
</tr>
<tr>
<td>B (%)</td>
<td>14.4 ± 4.1</td>
<td>14.0 ± 3.0</td>
</tr>
<tr>
<td>R5 (pmol/l)</td>
<td>284 ± 84</td>
<td>171 ± 59b,d</td>
</tr>
<tr>
<td>Kc (10⁸ l/mol)</td>
<td>12.9 ± 6.3</td>
<td>17.5 ± 7.5c</td>
</tr>
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</table>

a Fasting plasma glucose, G; fructosamine, FA; glucose disposal rate, M; clearance rate of glucose, MCRc; insulin sensitivity index, M/I; serum insulin during the clamp, IRIC; insulin binding, B; binding capacity, Rs; receptor affinity, Ke.

b P<0.001 as compared with controls.
c P<0.01 as compared with controls.
d P<0.01 as compared with pretreated values.
e P<0.02 as compared with pretreated values.

plasma vitamin E and higher malondialdehyde (MDA) concentrations were observed in obese Type 2 diabetic patients prior to treatment as compared with those in healthy persons (Table 3). Plasma MDA values positively correlated with fasting serum insulin concentrations \( r = 0.46, P < 0.01 \), whereas an inverse relationship was found between MDA and glucose disposal rate (Fig. 1). No changes of erythrocyte SOD were observed in diabetic patients. Oxidative stress index calculated as a ratio of MDA to SOD (MDA/SOD) was significantly higher in diabetic patients than in healthy controls.

Vitamin E administration caused an increase of its plasma concentrations in all diabetic patients (Table 3). This was accompanied by a borderline decrease of MDA \( P < 0.05 \) but by no significant changes of SOD or MDA/SOD index.

Plasma concentrations of tissue plasminogen activator (tPA) and of its inhibitor (PAI-1) were significantly higher in diabetic patients at baseline conditions than in healthy controls. A significant decrease of tPA antigen with no concomitant changes in PAI-1 antigen were observed after vitamin E administration. A high ratio of PAI-1 and tPA antigens (PAI-1/tPA), expressing an index of hypofibrinolysis, was found at baseline in diabetic patients and it was further increased after vitamin E treatment (Table 3).

An inverse relationship was observed between hypofibrinolytic index PAI-1/tPA and metabolic clearance rate of glucose when the differences of their post- and pre-treated values were used (Fig. 2).

4. Discussion

In the present study, we found further worsening of insulin action and of fibrinolysis in obese Type 2 diabetic patients after the vitamin E treatment. In addition, we demonstrated an inverse relationship between insulin action and oxidative stress or hypofibrinolysis.

Insulin resistance and increased oxidative stress have been observed in obese Type 2 diabetic patients [11,12,22,23]. The relationship between insulin action and oxidative stress was therefore...
Table 3
Indicators of oxidative stress and fibrinolysis in 11 obese Type 2 diabetic patients and in nine healthy controls before and after vitamin E administration

<table>
<thead>
<tr>
<th>Diabetic patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>fP-vitamin E (mg/l)</td>
<td>11.6 ± 4.8(^a)</td>
</tr>
<tr>
<td>fP-MDA (µmol/l)</td>
<td>3.13 ± 0.68(^a)</td>
</tr>
<tr>
<td>SOD (U)</td>
<td>1.53 ± 0.12</td>
</tr>
<tr>
<td>MDA/SOD</td>
<td>2.06 ± 0.54(^b)</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>7.3 (5.7–9.4)(^b)</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>208 (68–638)(^a)</td>
</tr>
<tr>
<td>PAI-1/tPA</td>
<td>30.0 (7.4–123)(^a)</td>
</tr>
</tbody>
</table>

\(^a\) P < 0.001 as compared with healthy persons.
\(^b\) P < 0.01 as compared with healthy persons.
\(^c\) P < 0.01 as compared with pretreated values.
\(^d\) P < 0.05 as compared with pretreated values.

suggested [11,24]. Our finding of an inverse relationship between plasma malondialdehyde concentration and glucose disposal rate during hyperinsulinemic clamp is in agreement with this suggestion. A decrease of oxidative stress could therefore improve insulin action in subjects with insulin resistance. Drugs acting like scavengers of oxygen radicals are promising tools in the treatment of patients with increased oxidative stress.

Alpha-tocopherol (vitamin E) is a potent lipophilic agent forming an important scavenger component of the cell membrane. It may protect integrity of the membrane by reduced production of lipid peroxides. Plasma vitamin E reflects the amount of alpha-tocopherol in the body. Lower plasma vitamin E levels were previously observed in Type 2 diabetic patients [9] and we confirmed this finding.

Our results of decreased insulin action after 600 mg of vitamin E contrast with previous observation of beneficial effect of 900 mg of this drug on insulin action [9]. This discrepancy may be explained by quite different conditions and the patients selected in both studies. We evaluated the effects of vitamin E in obese patients with mild diabetes but advanced oxidative stress. Deteriorated insulin action after vitamin E treatment could be explained as a consequence of vitamin E oxidation to alpha-tocopheroxyl radicals in patients with increased oxidative stress and limited regenerating capacity [25]. The capability of vitamin C to reduce alpha-tocopheroxyl radicals was previously documented [26]. Hyperglycemia may reduce intracellular content of vitamin C [27]. An increased formation of alpha-tocopheroxyl radicals in the presence of advanced oxidative stress may induce an impairment of the cell membrane in patients with insulin resistance syndrome. We did not observe further worsening of plasma ma-

Fig. 1. Relationship of plasma malondialdehyde (MDA) and glucose disposal rate (M) in healthy persons (▪) and obese Type 2 diabetic patients (○) (y = −6.3x + 47, r = −0.73, P < 0.001).
Administration of vitamin E caused significant decrease of tPA antigen in our patients, whereas PAI-1 concentrations remained unchanged. Few data exist on the influence of vitamin E on fibrinolysis in diabetes and more information will therefore be reliable. We suppose that worsening of fibrinolysis may be caused by a similar factor to insulin action because an inverse relationship between PAI-1/tPA ratio changes and metabolic clearance rate of glucose was found.

In conclusion, our data demonstrate that higher doses of vitamin E may further deteriorate insulin action and fibrinolysis in obese Type 2 diabetic patients. These changes may be a consequence of the prooxidative effect of vitamin E in patients with insulin resistance syndrome accompanied by high oxidative stress. Combined therapy by vitamins C and E seems to be more advantageous than by vitamin E alone. More detailed study in this promising field will be necessary.

Acknowledgements

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References


