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Antioxidants modify the relationship between endothelin-1 level and glucose metabolism–associated parameters

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Abstract

Glucose handling impairment and oxidative stress are implicated in the overexpression of endothelin-1 (ET-1). The objective of the study was to assess possible interplay of the 2 systems in relation to ET-1 in clinical setting. In hypertensive outpatients, on top of typical clinical workup, we assessed ET-1 levels, glucose handling parameters (glycated hemoglobin [HbA_{1c}], homeostasis model assessment [HOMA] index, and insulin level), and antioxidative protection (ferric reducing ability of plasma [FRAP], superoxide dismutase [SOD], and vitamin C). Average age of 68 patients (64% women, 50% diabetic, 40% smokers) was 67.7 (10.6) years. Serum ET-1 level averaged 1.09 (0.48) pg/mL and correlated positively with glucose handling–associated parameters (insulin, $r = 0.22$; HOMA, $r = 0.21$; HbA_{1c}, $r = 0.23$; all Ps $< .05$) and negatively with constituents of antioxidative protection system (FRAP, $r = -0.45$; SOD, $r = -0.47$; both Ps $< .0001$; vitamin C, $r = -0.27$; $P \leq .01$). In sex-, age-, blood pressure–, and creatinine-adjusted models, with interchangeable introduction of antioxidative parameters on top of interchangeable introduction of glucose handling–associated parameters, ET-1 levels were each time only significantly associated with FRAP in the context of HbA_{1c}; FRAP, SOD, or vitamin C in the context of HOMA; and FRAP or SOD in the context of insulin concentration. In the stepwise regression with the above parameters offered, only FRAP and vitamin C were associated with ET-1 level. In treated hypertensive patients, impaired glucose handling is associated with higher ET-1 levels. This statistical relation is blunted in the context of parameters of antioxidative protection. The hypothesis that poor antioxidation is mediating the effect of impaired glucose handling on ET-1 levels needs further confirmation.

1. Introduction

A deterioration of glucose metabolism is associated with both oxidative stress [1-4] and increase in endothelin 1 (ET-1) level [5-9]. Plasma ET-1 elevation is well documented in diabetic patients and in insulin-resistant states such as obesity, metabolic syndrome, hypertension, and hyperlipidemia [5,10,11]. It is also accepted that oxidative stress is implicated in the pathogenesis of insulin resistance [1,2], type 2 diabetes mellitus [3,4], and subsequent vascular complications [3,12]. Endothelin-1 synthesis and secretion are stimulated via protein kinase C (PKC) activation by hyperglycemia [6] and by hyperinsulinemia in vitro [13], and in vivo in healthy and in insulin-resistant subjects [13-16]. Increased ET-1 concentration in this pathology is a result of preserved insulin action on vascular smooth muscle cells and ET-1 synthesis mediated through mitogen-activated protein kinase–dependent pathway, whereas metabolic and hemodynamic action of insulin via phosphatidylinositol 3-kinase–dependent signaling cascade is deteriorated [17].

Hyperglycemia results also in the generation of reactive oxygen species (ROS), leading, specially in the absence of an appropriate antioxidant defense observed in diabetes [4,18], to oxidative stress. It in turn stimulates in vascular wall stress-sensitive pathways leading to activation of PKC and nuclear factor κB (NFκB), both implicated in ET-1 transcription, activation of serine/threonine kinases, and formation of advanced glycation end products (AGEs), all of them contributing to insulin resistance and endothelial dysfunction [1,12]. Conversely, treatment of cells with superoxide dismutase (SOD) inhibits ROS production and prevents glucose-induced PKC and NFκB activation [19], as well as abolished inhibitory effect of human glycated hemoglobin on endothelium-dependent vasodilatation [20].

In many experiments, ROS have been shown to increase ET-1 concentration in endothelial and vascular smooth

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muscle cells [21,22]; so it is hypothesized that oxidative stress joins the interaction between hyperglycemia, insulin resistance, ET-1, and diabetic vascular complications.

The aim of the study was to evaluate the influence of antioxidant defense on the relationship between glucose metabolism–associated parameters such as plasma insulin, insulin resistance index (homeostasis model assessment [HOMA]), glycemic control measured with glycated hemoglobin (HbA_{1c}) level, and plasma ET-1 concentration in patients with arterial hypertension.

2. Methods

The study population consisted of consecutive hypertension outpatients (previously hospitalized or followed at hypertension outpatient clinic) who within the scope of their routine follow-up agreed to take part in the extra examination. All patients gave consent to the follow-up at the outpatient clinic with the understanding that part of the data might be used (in a blinded way) for scientific purposes. In all subjects as a part of routine workup, information concerning past medical history and medication status was obtained. The patient was considered diabetic when one of the following was met: current antidiabetic treatment, fasting glucose exceeding 7.0 mmol/L, or HbA_{1c} greater than 7%. For the purpose of our study, all other patients were considered as nondiabetic. The workup also included anthropometric measurements, ambulatory blood pressure monitoring using Spacelab 90207 device (Redmond, WA), and blood test. In analysis, the averages of systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were used. Body mass index was calculated as body mass in kilograms divided by the square of body height in meters.

Venous blood samples were drawn after 12-hour overnight fast. Plasma glucose and uric acid concentrations were assayed using routine methods. Hemoglobin A_{1c} was measured using high-performance liquid chromatography.

Insulin was assayed by electrochemiluminescence immunoassay method in Roche Elecsys 2010 system (Roche Diagnostics, Mannheim, Germany), using 2 types of monoclonal antibodies specific for human insulin.

Insulin resistance was determined using the HOMA calculated as fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter)/22.5.

For determination of ET-1, ferric reducing ability of plasma (FRAP), vitamin C, and SOD activity blood samples were collected in tubes (for ET-1 and SOD, containing K_3 EDTA) and centrifuged at 4°C for 10 minutes. Plasma was separated and kept at −70°C until analysis.

Endothelin-1 concentration was assessed using enzyme-linked immunosorbent assay with Parameter, R&D System (Minneapolis, MN).

Ferric reducing ability of plasma, vitamin C concentration, and SOD activity in erythrocytes were evaluated as an antioxidant protection.

Ferric reducing ability of plasma value (constituted of uric acid, 60% contribution; vitamin C, 15%; α-tocopherol, 5%; bilirubin, 5%; and albumin, 10%) as a method of assessment of total antioxidant capacity of plasma was measured by the spectrophotometric method described by Benzi and Strain [23]. Plasma vitamin C was assayed using high-performance liquid chromatography method.

Erythrocyte SOD activity was determined using adrenaline method according to Misra and Fridovich [24].

Pellet of red blood cells was washed 4 times with 150 mmol/L NaCl. Approximately 0.8 mL of red blood cells pipetted from the bottom of the centrifuged samples was lysed in a total volume of 2 mL double-deionized water by freezing and thawing 3 times. In hemolysates, SOD activity was determined and expressed in units per gram of hemoglobin.

Database management and statistical analysis were carried out using SAS version 9.1 (Cary, NC). Results are presented as means ± SD or percentages. Means were compared using standard normal z test. The relations between variables were assessed with Pearson correlation analysis. Furthermore, using linear regression models (with and without multivariate adjustment for sex, age, blood pressure, and creatinine; both forced and stepwise selection of variables to adjust for), we tested the independence of the relation with ET-1of glucose metabolism–associated parameters and parameters of serum-derived antioxidant system. P values less than .05 were considered to be statistically significant.

3. Results

The mean ± SD age of 68 participants was 67.7 ± 10.6 (range, 42-92) years. The group consisted of 43 postmenopausal women (64%) and 25 men (46%). The characteristic of the group under study is presented in Table 1.

Average ET-1 concentrations (1.09 ± 0.48; range, 0.3-2.62 pg/mL) were similar by sex (men 1.16 ± 0.55 vs women 1.05 ± 0.45, $P = .38$), smoking status (nonsmokers 1.04 ± 0.5 vs smokers 1.15 ± 0.5, $P = .4$), the presence of coronary heart disease (CHD) (without CHD 1.02 ± 0.5 vs with CHD 1.16 ± 0.5, $P = .3$), angiotensin-converting enzyme inhibitor (ACEI) therapy (ACEI [−] 1.14 ± 0.5 vs ACEI [+] 1.08 ± 0.5, $P = .6$), as well as any other used medication (data not shown, available from authors) but were higher in diabetic vs nondiabetic subjects (1.31 ± 0.49 vs 1.08 ± 0.45 pg/mL, $P < .05$). Diabetic patients had lower plasma vitamin C level (2.75 ± 1.97 vs 3.56 ± 1.99 μg/mL, $P = .04$). Although the mean age of men and women differed significantly, the mean values of SOD, FRAP, and vitamin C were similar in both groups (Table 2).

When we divided the group according to the median value of ET-1 (1.13 pg/mL), subjects with lower ET-1 concentration had lower insulin concentration, better insulin sensitivity, better control of diabetes, and higher level of the antioxidants (FRAP, SOD, and vitamin C) (Table 3).

Table 1
Characteristic of the group under study

	All	Women	Men
	N = 68	n = 43	n = 25
Examined subjects	68	43 (64)	25 (36)
Age, y (mean ± SD)	67.7 ± 10.6	69.9 ± 9.9	63.9 ± 10.7*
Current smokers, n (%)	27 (40)	13 (30.2)	14 (56)
Diabetes mellitus, n (%)	34 (50)	19 (44.2)	15 (60)
CHD, n (%)	37 (52.9)	22 (51.2)	15 (60)
Heart failure, n (%)	10 (14)	7 (16.3)	3 (12)
Medication use, n (%)			
ACEIs	50 (73.5)	34 (79.1)	16 (64)
AT_1 inhibitors	1 (1.5)	1 (2.3)	0
Ca-blockers	21 (30.9)	14 (32.6)	7 (28)
β-Blockers	34 (50)	25 (58.1)	9 (36)
Diuretics	37 (54.4)	24 (55.8)	13 (52)
Statins	38 (56)	24 (55.8)	14 (56)
Hormone replacement therapy	0	0	–
Metformin[a]	11 (32.4)	5 (26.3)	6 (40)
Gliclazide[a]	22 (32.4)	11 (57.9)	11 (73.3)
Acarbose[a]	5 (7.4)	2 (10.5)	3 (20)

[a] Percentage calculated for diabetic subjects.
* P equal to .006.

3.1. Relationship between ET-1 and other factors

We found moderate, positive, statistically significant relation between plasma ET-1 concentration and plasma insulin level ($r = 0.22$, $P = .03$), HOMA ($r = 0.21$, $P = .04$), HbA_{1c} ($r = 0.23$, $P = .04$), SBP ($r = 0.24$, $P = .02$), and creatinine concentration ($r = 0.23$, $P = .03$). We also found significant relation between plasma ET-1 level and all examined antioxidants: FRAP ($r = -0.45$, $P < .0001$), vitamin C ($r = -0.27$, $P = .01$), and SOD ($r = -0.47$, $P < .0001$).

To examine the independent influence of selected antioxidants on the relationship between ET-1 and glucose metabolism–associated parameters (insulin, HOMA, HbA_{1c}), we fitted multiple regression models with plasma ET-1 level as dependent variable; insulin, HOMA, or HbA_{1c} concentration forced; and FRAP, SOD, and vitamin C interchangeably added into the subsequent models as independent variables, with additional adjustment for sex, age, SBP, and creatinine concentration. In the models with insulin concentration forced into the model, the relationship between these variables was no longer significant, whereas FRAP ($\beta = -0.668$, $P = .009$) and SOD ($\beta = -0.0002$, $P = .03$) independently influenced plasma ET-1 level. For vitamin C, borderline-significant trend was observed ($\beta = -0.055$, $P = .06$).

When HOMA was forced into the model, also only FRAP ($\beta = -0.664$, $P = .009$), SOD ($\beta = -0.0002$, $P = .02$), and vitamin C ($\beta = -0.057$, $P < .05$) influenced independently plasma ET-1 level. The same result with independent influence of FRAP on ET-1 plasma concentration was observed in the model with HbA_{1c} forced ($\beta = -0.586$, $P = .02$), with trends for relationship between ET-1 and SOD ($\beta = -0.0002$, $P = .06$) and vitamin C ($\beta = -0.050$, $P = .07$).

In the stepwise regression model (all previously used independent variables offered), only FRAP and vitamin C were selected as factors independently influencing plasma ET-1 level (Table 4).

Table 2
Characteristic of the group under study: blood pressure and biochemical evaluation

	All (mean ± SD)	Women (mean ± SD)	Men (mean ± SD)
SBP (mm Hg)	128.1 ± 15.8	128.2 ± 16.7	127.9 ± 14.4
DBP (mm Hg)	74.2 ± 11.4	72.8 ± 11.5	76.8 ± 11.0
Glucose (mmol/L)	6.43 ± 2.12	6.01 ± 1.81	7.14 ± 2.45*
HbA_{1c} (%)	6.86 ± 1.85	6.95 ± 2.04	6.72 ± 1.49
Insulin (μU/mL)	12.09 ± 8.94	12.07 ± 9.95	12.13 ± 7.05
HOMA	3.75 ± 4.05	3.65 ± 4.76	3.93 ± 2.46
ET-1 (pg/mL)	1.09 ± 0.48	1.05 ± 0.45	1.16 ± 0.55
FRAP (mmol/L)	0.484 ± 0.24	0.470 ± 0.23	0.508 ± 0.26
SOD (U/g Hb)	1040.3 ± 587.3	1023.3 ± 629.4	1069.5 ± 517.9
Vitamin C (μg/mL)	3.37 ± 2.07	3.76 ± 2.19	2.70 ± 1.70*

* P less than .05.

Table 3
Glucose metabolism–associated parameters and antioxidants in groups divided according to ET-1 median value

Parameter	ET-1 <1.13 pg/mL	ET-1 ≥1.13 pg/mL	P
Age (y)	65.65 ± 11.8	70.17 ± 9.1	.04
Uric acid (μmol/L)	329.6 ± 110.8	301.1 ± 102.0	.21
HbA_{1c} (%)	6.49 ± 1.4	7.27 ± 2.1	.04
Insulin (μU/mL)	10.7 ± 7.03	15.73 ± 12.9	.02
HOMA	2.91 ± 2.1	4.69 ± 4.9	.03
Vitamin C (μg/mL)	3.66 ± 2.01	2.71 ± 2.03	.03
FRAP (mmol/L)	0.535 ± 0.22	0.295 ± 0.22	<.0001
SOD (U/g Hb)	1133.67 ± 476.3	531.3 ± 650.4	<.0001

4. Discussion

We showed positive relationship between plasma ET-1 and insulin concentration, HOMA as an index of insulin resistance, and HbA_{1c} as an index of long-term glycemic control. These results are consistent with others [5,10,16], although not all authors confirmed these relationships [7-9].

Considering the possibility of interaction between different factors influencing ET-1 concentration, multivariate regression analysis with adjustment for age, sex, SBP, and creatinine concentration was performed, in which mainly parameters of antioxidant protection (FRAP, vitamin C, and erythrocyte SOD) independently influenced ET-1 level. We

Table 4
Multivariate regression analysis with ET-1 as dependent variable: stepwise procedure

Variable	β	SE	F value	P
HbA_{1c}	0.042	0.029	2.03	.16
FRAP	−0.623	0.227	7.57	.008
Vitamin C	−0.054	0.026	4.33	.04

are aware that the part of the antioxidant defense examined in our study and the glucose metabolism–related parameters constitute just a part of the system regulating expression of ET-1. The proportion of variance of ET-1 was influenced in 20% by FRAP, 22% by SOD, and 7% by vitamin C, whereas influence of parameters related to glucose metabolism was considerably lower (insulin, 5%; HOMA, 4%; HbA_{1c}, 5%). From this perspective, our results are different than results of other studies in which insulin [5,16], insulin resistance [16], and HbA_{1c} [5] independently influenced ET-1 level; but these authors did not investigate the role of antioxidants in ET-1 and glucose metabolism relationships. To our knowledge, our study is the first to report such interaction. Although all 3 antioxidants independently influenced only the relationship between ET-1and HOMA, the influence of FRAP was significant for the relationship between ET-1 and all 3 glucose metabolism–related parameters (ET-1–insulin, ET-1–HbA_{1c}, ET-1–HOMA); SOD also influenced significantly relation of ET-1 and insulin (for all remaining interactions, trend for statistical significance was found). Our results can be explained in the context of the findings from the experimental studies, which showed that oxidative stress is implicated in insulin action, insulin resistance, and ET-1 production as activator of stress-sensitive signaling pathway [1,12,25].

4.1. Insulinemia, insulin resistance, and ET-1 level

Insulin resistance is accompanied by compensatory hyperinsulinemia and subsequent increased level of ET-1. Subsequently, ET-1 reciprocally contributes to the maintenance of insulin-resistant state through the inhibitory effect on insulin's phosphatidylinositol 3-kinase activation [26-28] and promotes endothelial dysfunction by increasing superoxide production and competing with nitric oxide.

Large number of reports demonstrated that ROS are implicated in the pathogenesis of insulin resistance [2,25]. Exposure to ROS increases fasting insulin level [29] and decreases insulin sensitivity by inhibition of components of insulin-signaling pathway [30]. Conversely, administration of antioxidants like vitamins C and E, α-lipoic acid, *N*-acetyl-L-cysteine, or glutathione improved insulin sensitivity in insulin-resistant patients [31-33].

In experimental studies, overexpression of SOD in transgenic mice prevents PKC and NFκB activation and AGE formation, all involved in ET-1 transcription, increase in its concentration [1,25], and induction of insulin resistance. Vitamin C also might suppress ROS generation and different stress-activated signaling molecules, and thus indirectly may prevent insulin resistance and ET-1 elevation [34].

Although in this context correlation between ET-1 levels and HOMA (index of insulin resistance) found in different studies [5,10,16] and also in our study, but not in all [35], is reasonable, including antioxidants (FRAP, SOD, and vitamin C) to the analysis revealed their significant influence on insulin resistance and ET-1 interaction.

4.2. Glycemic control and ET-1

Hyperglycemia stimulates both ET-1 expression and activity and ROS generation. Concentration-related relationship between glucose (>16.5 mmol/L) and ET-1 has been shown [6]. In some studies [5,35], but not in all [7-9], the correlation between ET-1 level and HbA_{1c} as an index of long-term glycemic control was found. In our study, such relationship in Pearson correlation and univariate regression analysis was also revealed; but after adjustment for plasma antioxidants (FRAP, vitamin C), but not for intracellular antioxidants (erythrocyte SOD), the correlation was not further significant. This indicates an involvement of ROS and antioxidant defense on HbA_{1c}–ET-1 interaction. The simultaneous influence of FRAP and vitamin C on ET-1 level seems to be reasonable, as the vitamin C is one of the components of FRAP, although its contribution to FRAP activity is estimated to be only about 15%, and FRAP activity is not concentration related [23]. Both FRAP and vitamin C are in plasma containing antioxidants, so their influence on circulating ET-1–HbA_{1c} interaction seems to be more plausible than erythrocyte SOD being an intracellular enzyme. This allows to hypothesize that not only HbA_{1c} is implicated in ET-1 elevation; but hyperglycemia-induced metabolic deteriorations via oxidative stress promote activation of PKC, transcription factors (NFκB, activator protein 1, activator protein 2), JNK/SAPK, and serine/threonine kinases and stimulates formation of AGEs; all of them maintain oxidative stress, increase insulin resistance, and activate proinflammatory signaling contributing to endothelial dysfunction [1,25]. The influence of oxidative stress on glucose control was suggested by Sargeant et al [36] who found negative correlation between HbA_{1c} and vitamin C concentration in the population examined in the European Prospective Investigation Into Cancer–Norfolk Study. It is suggested that vitamin C because of its antioxidant function may protect against impaired glucose regulation and ameliorate insulin resistance [20,36].

The strongest correlation between FRAP and HbA_{1c} as well as FRAP and HOMA and insulin indicates the importance of plasma antioxidants' cooperation in protection against oxidative stress consequences.

Our study needs to be considered in the context of its limitations. First, our sample is highly selected; and the inclusion was at the discretion of caring physician. In addition, we did not collect data on refusal rate. However, the peculiar nature of our study precludes any meaningful randomization of larger samples of individuals of the particular profile. Likewise, the decision to base diagnosis of diabetes on current antidiabetic treatment, fasting glucose exceeding 7.0 mmol/L, or HbA_{1c} greater than 7.0% was arbitrary.

In conclusion, better antioxidant defense prevents increase in ET-1 level in insulin-resistant or diabetic subjects, characterized by hyperinsulinemia, increased HOMA (an index of insulin resistance), or increased HbA_{1c} level as a marker of prolonged glucose load.

In type 2 diabetes mellitus, abated antioxidant defense decreases efficiency of protective mechanisms limiting circulating ET-1 elevation.

References

[1] Kim J, Montagnani M, Kon Koh K, Quon MJ. Reciprocal relationship between insulin resistance and endothelial dysfunction. Molecular and pathophysiological mechanisms. Circulation 2006;113:1888-904.

[2] Paolisso G, Giugliano D. Oxidative stress and insulin action: is there a relationship? Diabetologia 1996;39:357-63.

[3] Laight DW, Carrier MJ, Änggård EE. Antioxidants, diabetes and endothelial dysfunction. Cardiovasc Res 2000;47:457-64.

[4] Ceriello A, Bortolotti N, Crescentini A, et al. Antioxidant defences are reduced during the oral glucose tolerance test in normal and non–insulin dependent diabetic subjects. Eur J Clin Invest 1998;28:329-33.

[5] Piatti PM, Monti LD, Galli L, et al. Relationship between endothelin-1 concentration and metabolic alterations typical of the resistance syndrome. Metabolism 2000;49:748-52.

[6] Park JY, Takahara N, Gabriele A, et al. Induction of endothelin-1 expression by glucose. An effect of protein kinase C activation. Diabetes 2000;49:1239-48.

[7] Kakizawa H, Itoh M, Itoh Y, et al. The relationship between glycemic control and plasma vascular endothelial growth factor and endothelin-1 concentration in diabetic patients. Metabolism 2004;53:550-5.

[8] Bruno CM, Meli S, Marcinno M, et al. Plasma endothelin-1 levels and albumin excretion rate in normotensive, microalbuminuric type 2 diabetic patients. J Biol Regul Homeost Agents 2002;16:114-7.

[9] Bagg W, Ferri C, Desideri G, et al. The influences of obesity and glycemic control on endothelial activation in patients with type 2 diabetes. J Clin Endocrinol Metab 2001;86:5491-7.

[10] Irving RJ, Noon JP, Watt GCM, et al. Activation of the endothelin system in insulin resistance. Q J Med 2001;94:321-6.

[11] Piatti PM, Monti LD, Conti M, et al. Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. Diabetes 1996;45:316-21.

[12] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.

[13] Ferri C, Pittoni V, Piccoli A, et al. Insulin stimulates endothelin-1 secretion from human endothelial cells and modulates its circulating levels in vivo. J Clin Endocrinol Metab 1995;80:829-35.

[14] Wolpert HA, Steen SN, Istfan NW, Simonson DC. Insulin modulates circulating endothelin levels in humans. Metabolism 1993;42:1027-30.

[15] Ferri C, Bellini C, Desideri G, et al. Endogenous insulin modulates circulating endothelin-1 concentration in humans. Diabetes Care 1996;19:504-6.

[16] Andronico G, Mangano M, Ferrara l, et al. In vivo relationship between insulin and endothelin role of insulin-resistance. J Hum Hypertens 1997;11:63-6.

[17] Cusi K, Maezono K, Osman A, et al. Insulin resistance differentially affects the PI-3-kinase– and MAP kinase–mediated signaling in human muscle. J Clin Invest 2000;105:311-20.

[18] Maxwell SRJ, Thomason H, Sandler D, et al. Poor glycaemic control is associated with reduced serum free radical scavenging (antioxidant) activity in NIDDM. Ann Clin Biochem 1997;34:638-44.

[19] Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404:787-90.

[20] Angulo J, Sanchez-Ferrer CF, Peiro C, et al. Impairment of endothelium-dependent relaxation by increasing percentages of glycosylated human hemoglobin. Possible mechanisms involved. Hypertension 1996;28:583-92.

[21] Kähler J, Mendel S, Weckmüller J, et al. Oxidative stress increases synthesis of big endothelin-1 by activation of the endothelin-1 promoter. J Mol Cell Cardiol 2000;32:1429-37.

[22] Kähler J, Ewert A, Weckmüller J, et al. Oxidative stress increases endothelin-1 synthesis in human coronary artery smooth muscle cells. J Cardiovacs Pharmacol 2001;38:49-57.

[23] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70-6.

[24] Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-5.

[25] Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002;23:599-622.

[26] Lteif A, Vaishnava P, Baron AD, Mather KJ. Endothelin limits insulin action in obese/insulin-resistant humans. Diabetes 2007;56:728-34.

[27] Jiang ZY, Zhou QL, Chatterjee A, et al. Endothelin-1 modulates insulin signaling through phosphatidylinositol 3-kinase pathway in vascular smooth muscle cells. Diabetes 1999;48:1120-30.

[28] Strawbridge AB, Elmendorf JS. Endothelin-1 impairs glucose transporter trafficking via a membrane-based mechanism. J Cell Biochem 2006;97:849-56.

[29] Laight DW, Desai KM, Gopaul NK, et al. Pro-oxidant challenge in vivo provokes to onset of NIDDM in the insulin resistant obese Zucker rat. Br J Pharmacol 1999;128:269-71.

[30] Hansen LL, Ykeda Y, Olsen GS, et al. Insulin signaling is inhibited by micromolar concentration of H(2)O(2) in tumor necrosis factor alpha–mediated insulin resistance. J Biol Chem 1999;274:25078-84.

[31] Hirashima O, Kawano H, Motoyama T, et al. Improvement of endothelial function and insulin sensitivity with vitamin C in patients with coronary spastic angina: possible role of reactive oxygen species. J Am Coll Cardiol 2000;35:1860-6.

[32] Cabalerro B. Vitamin E improves the action of insulin. Nutr Rev 1993;51:339-40.

[33] Jacob S, Henriksen EJ, Schiemann AL, et al. Enhancement of glucose disposal in patients with type 2 diabetes by α-lipoic acid. Arznei-mittelforschung 1995;45:872-4.

[34] Ho FM, Liu SH, Liau CS, et al. High-glucose induced apoptosis in human endothelial cells is mediated by sequential activations of c-Jun NH(2) terminal kinase and caspase-3. Circulation 2000;101: 2618-24.

[35] Pontiroli AE, Pizzocri P, Koprivec D, et al. Body weight and glucose metabolism have a different effect on circulating levels of ICAM-1, E-selectin, and endothelin-1 in humans. Eur J Endocrinol 2004;150: 195-200.

[36] Sargeant LA, Wareham NJ, Bingham S, et al. Vitamin C and hyperglycemia in the European Prospective Investigation Into Cancer–Norfolk (EPIC-Norfolk) Study. Diabetes Care 2000;23: 726-32.