

Endothelin-1 infusion inhibits plasma insulin responsiveness in normal men

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Objectives Elevated plasma endothelin (ET)-1 levels have been described in insulin-resistant states such as syndrome X, obesity, non-insulin-dependent diabetes mellitus, and in some studies in essential hypertension. To investigate whether increases in circulating ET-1 to levels observed in insulin-resistant states can modulate insulin levels and/or insulin sensitivity in humans, we assessed these variables during low, non-pressor-dose ET-1 compared with placebo infusion.

Design In a randomized, single blind, crossover design, 10 lean normotensive male subjects received either an intravenous infusion of subpressor doses of ET-1 dissolved in polygeline or a control infusion of polygeline only (placebo). Using dynamic assessment by the minimal model approach with the modified frequent sampling intravenous glucose tolerance test (FSIGT) the following and other parameters were measured: insulin sensitivity; acute insulin response to glucose (AIR_G) calculated as the average of the three peak values between 2 and 5 min after injection of glucose from which the basal insulin levels were subtracted; the initial area under the curve (AUC_{1-19}) from insulin values between time 0 and 19 min and the first-phase insulin secretion (ϕ_1) from insulin kinetics parameters.

Results ET-1 infusion reduced AIR_G (to 34.85 ± 4.27 compared with $49.3 \pm 6.9 \mu\text{U/ml}$ during placebo,

$P = 0.017$) and the acute C-peptide response to glucose (to 2.33 ± 0.41 compared with $3.1 \pm 0.44 \text{ ng/ml}$, $P = 0.018$), decreased plasma insulin levels during the FSIGT compared with placebo (analysis of variance $P < 0.0001$) and decreased the AUC_{1-19} (to 2.1 ± 0.2 compared with $2.9 \pm 0.3 \text{ U/l per 20 min}$, $P < 0.01$) while ϕ_1 tended to be lower. S_I measured during ET-1 infusion was unaltered ($11.11 \pm 1.91 \times 10^{-4}$ versus $10.88 \pm 2.11 \times 10^{-4}/\text{min per mU per l}$, NS).

Conclusions These findings demonstrate that an increase in circulating ET-1 to levels observed in insulin-resistant states acutely diminishes the insulin secretory response but does not significantly modify insulin sensitivity. *J Hypertens* 16:1279–1284 © 1998 Lippincott Williams & Wilkins.

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Introduction

Since the discovery of endothelin by Yanagisawa *et al.* in 1988 [1], its metabolic effects have received much attention. Elevated plasma endothelin (ET)-1 levels have been described in different insulin-resistant states such as syndrome X, obesity and non-insulin-dependent diabetes mellitus (NIDDM), while an association between elevated circulating ET-1 levels and essential hypertension has been noted in several but not in all studies [2–9]. Hyperinsulinemia and hypertriglyceridemia may both induce ET-1 release, while a reduction in insulinemia decreases endothelin-1 levels [2,8,9]. Recently, it was suggested that ET-1 might impair insulin sensitivity [4]. Thus, treatment of lean NIDDM patients with the angiotensin converting enzyme inhibitor captopril improved insulin resistance and lowered ET-1 levels [10].

The influence of ET-1 on insulin secretion is unclear. In the rat, ET-1 infusion dose-dependently increases insulin release [11]. In humans, a pharmacological elevation in plasma ET-1 was accompanied by transient decreases in insulin and glucagon, while a moderate reduction in ET-1 levels has no effect on insulin levels [10,12]. To investigate whether increases in circulating ET-1 to levels observed in insulin-resistant states can modulate plasma insulin levels and/or insulin sensitivity in humans, we assessed these variables during low, non-pressor-dose ET-1 compared with placebo infusion in lean, male, normotensive, healthy subjects.

Methods

Subjects

Ten healthy Caucasian men, aged 25–28 years [26.4 ± 0.3 years (mean \pm SEM)] with a body mass index (BMI) of

$20 \pm 26 \text{ kg/m}^2$ ($22.3 \pm 0.7 \text{ kg/m}^2$) were included in the study. Exclusion criteria were hypertension (resting blood pressure $\geq 140/90 \text{ mmHg}$), diabetes mellitus, a history of cardiovascular or systemic diseases or any other chronic illnesses, and regular intake of medications. Subjects taking any medication during the 2 weeks before the study were excluded.

Study design

The protocol was approved by the ethics committee of the Medical Faculty, University of Bern, Switzerland, and all participants gave their written informed consent. Before inclusion, the subjects came to the clinical research unit after a 10–12 h overnight fast for a baseline medical examination which included measurements of height, weight, blood pressure serum levels of glucose, lipids, electrolytes, creatinine and liver enzymes. They received a standardized diet, containing 10 500 kJ made up of 45% carbohydrates, 40% fat, 15% proteins and 160 mmol sodium daily for 3 days before each test series [13]. None of the subjects engaged in heavy physical activity on the days before the studies. They were not allowed to drink alcohol during the evening before the test day. Caffeine intake and smoking were avoided for at least 12 h before the tests. All subjects were studied at our research unit under the same conditions.

Tests began at 08:00 h at bedrest after the subjects had fasted overnight for at least 10 h. The subjects maintained a supine position throughout the test. Insulin sensitivity was assessed dynamically by the minimal model approach with the use of the modified frequent sampling intravenous glucose test (FSIGT) [14–16]. Intravenous lines (one for sample collection only) were placed in an antecubital vein in both arms and 30 min of rest was allowed for reattainment of basal conditions. Two baseline samples for glucose, insulin and ET-1 were obtained. In a randomized, single blind, crossover fashion the subjects then received either an intravenous priming infusion of ET-1 (Clinalfa, Läufelfingen, Switzerland) at 2 ng/kg per min dissolved in polygeline (Hoechst, Bad Soden, Germany) at a concentration of 400 ng/ml, or a control infusion of polygeline only (placebo) infused at the same rate as the ET-1 infusion over 30 min. After this 30 min priming period, ET-1 infusion was continued at a maintenance rate of 0.5 ng/kg per min throughout the FSIGT, and an injection of glucose was administered intravenously at a dose of 300 mg/kg (given as 50% dextrose) over 1 min, followed 20 min later by 300 mg tolbutamide intravenously over 30 s. Blood samples for measurement of glucose and insulin were obtained at –10, –5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160 and 180 min relative to the start of the glucose injection. Samples for ET-1 determination were taken at –30, –10, –5, 10, 16, 25, 30, 40, 50, 70, 120 and 180 min. The total infusion volume of poly-

geline together with ET-1 or placebo given as a continuous infusion did not exceed 25 ml. The two studies were performed 2 weeks apart.

Analytical methods

Plasma insulin determinations from the two tests in one subject were carried out in the same radioimmunoassay. Measurements of insulin were performed in duplicate, using guinea pig antiporcine insulin antibody (CIS Biointernational, Gif-sur-Yvette, France) and ^{125}I -porcine insulin as a tracer, as described previously from our laboratories [17]. C-peptide was determined by immunoassay (CIS Medipro SA, Geneva, Switzerland). Plasma glucose values were determined by the glucose oxidase technique using a Technicon AAII autoanalyzer (Tarrytown, New York, USA). Potassium was determined using a flame photometer.

For ET-1 determination, blood was immediately transferred into a tube containing ethylenediaminetetraacetic acid and centrifuged at 4°C for 10 min. Plasma was separated at 4°C and kept at –80°C until assay. Extraction was performed on 500 mg SepPak Vac C18 cartridges (Waters, Rapperswil, Switzerland) [18]. Columns were pre-activated by successive washes with 5 ml of 86% ethanol in 4% acetic acid, 5 ml of methanol, 5 ml of sterile distilled water and 5 ml of 4% acetic acid. A 2 ml plasma sample acidified with 6 ml of 4% acetic acid was then applied on the column with the flow rate of 3 ml/min. The columns were then washed with 18 ml sterile distilled water and 18 ml 24% ethanol in 4% acetic acid before endothelin was eluted with 86% ethanol in 4% acetic acid. The eluate was dried under nitrogen at 37°C and redissolved in 230 μl of assay buffer composed of 0.1% phosphate buffer (pH 7.4), 0.05 mol/l NaCl, 0.1% Triton X100 (Merck, Dietikon, Switzerland), 0.02% sodium azide and 0.1% bovine serum albumin (Sigma, Buchs, Switzerland). The radioimmunoassay of plasma endothelin was performed using synthetic human/porcine ET-1 (Sigma), a rabbit antibody against synthetic ET (Peninsula Laboratories, Merseyside, England) and ^{125}I -ET-1 (Amersham, Rahn AG, Zurich, Switzerland). The antibody has 100% cross-reactivity with ET-1, 7% with ET-2 and ET-3, 17% with big ET-1 and no cross-reactivity with other peptides. The anti-ET-1 antibody was reconstituted according to the manufacturer's instructions and then further diluted 1 : 3.5 with the assay buffer before adding 100 μl to the standards or the reconstituted plasma samples (100 μl) analysed in duplicate. After 24 h of incubation, 100 μl of ^{125}I -ET-1 (10 000–12 000 cpm) was added and incubation allowed to continue for an additional 24 h. The separation of bound and free antigen was performed with a second antibody method and pellets were counted by a γ -counter (Canberra Packard, Zurich, Switzerland). Recovery averaged $78 \pm 4\%$ ($n = 8$). The effective range of the standard curve was between 0.16 and 40 pg of

ET-1/tube with a median inhibitory concentration (IC_{50}) of 1.7 pg. Intra- and interassay coefficients of variation averaged 8.6 and 13.6%, respectively ($n = 10$).

Data analysis

Data are presented as means \pm SEM. Basal levels of glucose, insulin, C-peptide and ET-1 were calculated by averaging the values of the pre-injection samples. The acute insulin response (AIR_G) and the acute C-peptide response ($ACPR_G$) were calculated as the average of the three peak values between 2 and 5 min after injection of glucose from which the basal levels were subtracted. Insulin sensitivity and glucose effectiveness were calculated using the MINMOD program (copyright R.N. Bergman, 1986), as described previously [14–17].

The glucose disappearance model accounts for the effect of insulin and glucose disposal after exogenous glucose injection. It provides the insulin sensitivity index, defined as the ability of insulin to increase glucose disappearance, and the parameter glucose effectiveness, which represents insulin-dependent fractional glucose disappearance, as well as their fractional standard deviations. The intravenous glucose tolerance test (K_G) was calculated as the slope of the least-squares regression line of the logarithm of the glucose concentration versus time 10–19 min after glucose injection [19].

The insulin model accounts for the effect of glucose on the insulin concentration and describes the ability of the β cell to secrete insulin in response to glucose. First-phase β -cell responsivity (ϕ_1) was calculated as the total mass of insulin (per unit insulin distribution volume) released per unit increase in glucose during the first phase. The total area under the insulin curve (AUC) was calculated with the use of the trapezoid rule. Similarly, the initial area under the curve (AUC_{1-19}) was calculated from the insulin data measured before tolbutamide administration between time 0 and 19. The adaptation index was calculated by multiplying the insulin sensitivity by ϕ_1 [20].

Differences between the ET-1 and control infusion tests were determined using non-parametric analysis or analysis of variance where appropriate. $P < 0.05$ was considered statistically significant.

Results

First-visit fasting plasma glucose, serum potassium, sodium creatinine, total cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides and liver enzyme levels were consistently normal in each subject. Systolic/diastolic blood pressure was $124 \pm 3/82 \pm 2$ mmHg.

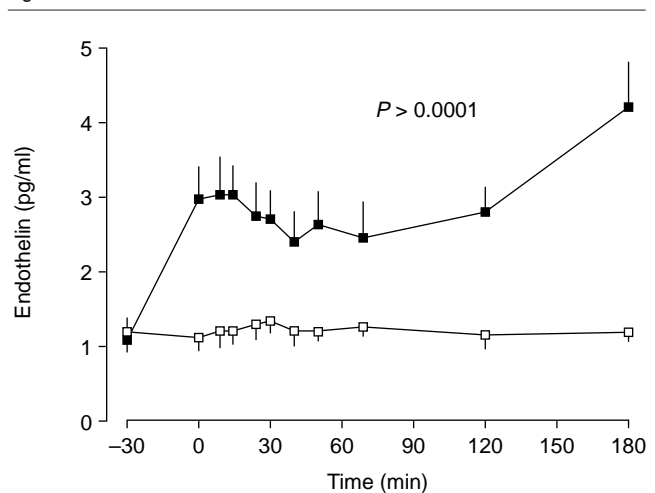
Basal values obtained on the ET-1 and control infusion days did not differ significantly. Body weight averaged 72.7 ± 3.4 and 72.4 ± 3.3 kg, respectively, and blood

pressure $118 \pm 2/81 \pm 2$ and $118 \pm 2/80 \pm 2$ mmHg. In the 24 h urine sample, creatinine level was 14372 ± 872 and 14756 ± 889 μ mol, sodium level 140.8 ± 17.0 and 132.5 ± 14.5 mmol and potassium level 72.9 ± 5.2 and 81.6 ± 5.8 mmol, respectively.

Basal plasma ET-1 levels were 1.14 ± 0.14 and 1.18 ± 0.14 pg/ml, respectively. ET-1 levels were increased 2.3- to 3.7-fold during ET-1 infusion during the FSIGT (Fig. 1). During the control infusion, plasma ET-1 levels were unchanged, except for a slight transient rise from a basal value of 1.12 ± 0.10 pg/ml to 1.30 ± 0.15 and 1.35 ± 0.10 pg/ml at 25 and 30 min ($P = 0.03$ and 0.009), respectively. Blood pressure was unaltered during either the ET-1 or control infusion (Fig. 2).

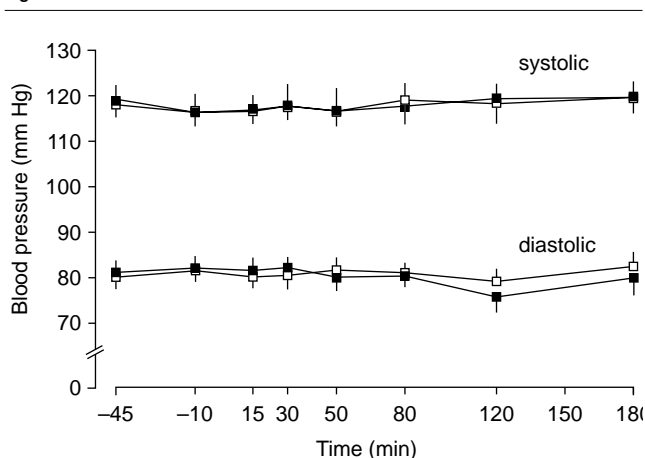
ET-1 infusion significantly reduced AIR_G ($P < 0.02$) and $ACPR_G$ ($P < 0.02$), the acute AUC_{1-19} ($P < 0.01$), the adaptation index insulin sensitivity $\times \phi_1$ and the total AUC_1 ($P < 0.02$, Table 1; $P < 0.0001$ by analysis of variance, Fig. 3), while ϕ_1 tended to be lower (Table 1). Fasting plasma glucose and serum insulin levels, insulin levels, insulin sensitivity and the glucose effectiveness indices, the insulin clearance and post-hepatic insulin delivery did not differ significantly while K_G as a measure of glucose tolerance was reduced during ET-1 infusion compared with placebo infusion (Table 1). During ET-1 and placebo infusion, fractional standard deviations for glucose effectiveness were 12.0 ± 3.1 versus $8.6 \pm 1.0\%$ (NS) and for insulin sensitivity were 2.8 ± 1.0 versus $2.1 \pm 0.5\%$ (NS), respectively. During the FSIGT, glucose levels did not differ between the control and ET-1 infusion tests.

Fig. 1



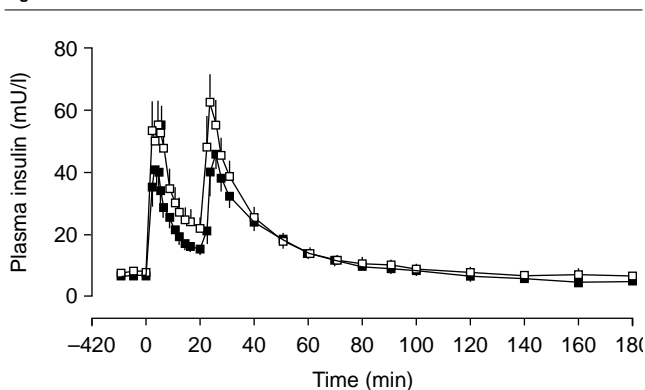
Plasma endothelin (ET)-1 levels achieved by continuous intravenous ET-1 infusion (■), given as a priming infusion over 30 min at 2 ng/kg per min, followed by a maintenance infusion of 0.6 ng/kg per min throughout the frequent sampling intravenous glucose test, and ET-1 levels achieved during a control infusion with polygeline only (□).

Fig. 2



Blood pressure during the frequent sampling intravenous glucose test under continuous endothelin-1 infusion (■) and under a control infusion with polygeline only (□).

Fig. 3



Plasma insulin levels during endothelin-1 infusion (■) and during a control infusion with polygeline only (□; $P < 0.0001$ by analysis of variance). Glucose at 300 mg/kg body weight, was injected at 0 min; 300 mg tolbutamide was injected at 20 min.

Priming ET-1 infusion (2 ng/kg per min over 30 min) did not alter plasma glucose levels (4.5 ± 0.09 mmol/l before versus 4.47 ± 0.08 mmol/l after ET-1 infusion) or basal plasma insulin levels (6.6 ± 0.43 versus 7.55 ± 0.70 μ U/ml).

Discussion

These findings demonstrate that in humans an increase in the plasma ET-1 concentration similar to that observed in insulin-resistant states acutely reduces the plasma insulin response to intravenous glucose and tolbutamide but does not alter insulin sensitivity.

The present findings show that a 2.3- to 3.7-fold rise in circulating endothelin, a degree of hyperendothelinemia found in insulin-resistant states [3,5,6], is capable of reducing the insulin response. The mechanisms involved in this blunting of the insulin response are unclear. Insulin response reportedly increased in rats during ET-1 administration [11] and ET-1 was found to stimulate insulin secretion dose-dependently from isolated islets of Langerhans [21]. However, in humans, pharmacological ET-1 infusion, that raised plasma ET-1 15-fold above basal values during 20 min, reduced splanchnic glucose production and arterial glucose concentration, and was accompanied by a transient decrease in insulin and glucagon levels [12]. ET-1 decreases pancreatic blood flow, suggesting a regulatory role for the peptide in the pancreatic microcirculation [22]. The discrepancy between the findings in rats and in humans could be related to a different distribution of ET-1 or endothelin receptors within the pancreas. ET-1-like immunoreactivity in the pancreas has been localized predominantly to islet cells, and in particular it co-exists with insulin and glucagon as well as with vascular endothelium [23]. Species-specific differences in the ET-1 induced hemodynamic changes could thus counteract, to a different degree, the direct insulin secretory effect of ET-1. However, ET-1 exerts multiple endocrine effects,

Table 1 Fasting levels of glucose, insulin, C-peptide and parameters calculated from the Frequent Sampling Intravenous Glucose Test (FSIGT) for placebo and endothelin-1 infusion in normal subjects

Parameter	Placebo	Endothelin
Fasting plasma glucose (mmol/l)	4.5 ± 0.1	4.5 ± 0.1
Fasting serum insulin (μ U/ml)	8.0 ± 0.9	7.6 ± 0.7
Insulin sensitivity (10^{-4} /min per μ U per ml)	10.9 ± 2.1	11.1 ± 1.9
Glucose effectiveness (min^{-1})	0.022 ± 0.002	0.022 ± 0.003
K_G (%/min)	2.42 ± 0.23	$2.02 \pm 0.33^*$
Insulin clearance (min^{-1})	0.21 ± 0.02	0.26 ± 0.02
AIR_G (μ U/ml)	41.6 ± 7.2	$28.1 \pm 3.2^{**}$
AUC_1 (U/l per 180 min)	2.9 ± 0.3	$2.1 \pm 0.2^{**}$
AUC_{1-19} (U/l per 20 min)	0.67 ± 0.1	$0.46 \pm 0.04^{***}$
ACPR_G (ng/ml)	3.10 ± 1.4	$2.31 \pm 1.3^{**}$
Delivery (U/l per 20 min)	0.21 ± 0.03	0.20 ± 0.03
ϕ_1 (μ U/ml per min)/(mg/dl)	1.71 ± 0.42	0.88 ± 0.12
Insulin sensitivity $\times \phi_1$ (min^{-2} /mg per dl)	15.0 ± 3.2	$8.1 \pm 1.1^*$

Values are means \pm SEM. K_G , glucose tolerance index; AIR_G , acute insulin response to glucose; AUC_1 , total area under the insulin curve; AUC_{1-19} , area under the insulin curve 0–19 min; ACPR_G , acute C-peptide response to glucose; Delivery, post hepatic insulin delivery 0–19 min; ϕ_1 , glucose sensitivity of first-phase insulin secretion; insulin sensitivity $\times \phi_1$, β -cell adaptation index.

* $P = 0.05$, ** $P = 0.02$, *** $P = 0.01$, versus placebo.

including stimulation of aldosterone secretion [24,25], which might be relevant to the changes in insulin response observed in the present study. Nevertheless, ET-1 infusions inducing comparable increases in plasma levels of ET-1 to those of the present study did not significantly modify plasma renin, aldosterone or atrial natriuretic peptide levels in humans [26]. ET-1 infusion also markedly decreased C-peptide secretion, indicating that the decrease in insulin secretion is caused by inhibition of β -cell function and not by concomitant blunting of insulin clearance and/or hepatic extraction.

In the rat, ET-1 infusion dose-dependently decreases glucose [11]. In humans ET-1 infusion in pharmacological doses reportedly lowers blood glucose [12]. Fasting plasma glucose levels were unchanged and glucose levels during the FSIGT decreased at most minimally and not significantly during the ET-1 infusion in the present study.

In previous studies, a negative correlation between total glucose uptake and plasma ET-1 levels during hyperinsulinemic euglycemic clamp studies in lean NIDDM patients led to the suggestion that ET-1 might decrease insulin sensitivity [4]. Furthermore, short-term captopril treatment in NIDDM patients tended to improve total glucose uptake while plasma ET-1 values were decreased [10]. The inverse direction of changes in these studies may reflect either a casual or a causal association. Although insulin sensitivity was not acutely altered by this degree of hyperendothelinemia in the present study, this does not contradict the possibility of a causal interaction, since a pre-existing insulin resistance in NIDDM patients could conceivably react more sensitively to ET-1 variations than the basically normal insulin-mediated glucose uptake mechanisms in healthy subjects. As recently suggested by Kahn *et al.* [19], a hyperbolic relationship may exist between measures of β -cell function and insulin sensitivity in a healthy population, so that changes in insulin secretion may have relatively small effects on insulin sensitivity [19]. Another possible explanation for our finding is that the technique used was insufficiently precise to measure small but relevant differences between the two groups. Furthermore, effects of chronic hyperendothelinemia on the complex mechanisms controlling insulin sensitivity could well differ from acute study conditions. Additionally, we cannot exclude the possibility of a dose-dependent relationship between ET-1 and insulin sensitivity.

It has been suggested that hyperinsulinemia might alter plasma endothelin levels. Some reports have described increased ET-1 values during hyperinsulinemic euglycemic clamp in obese, hypertensive or NIDDM patients, or subjects with syndrome X [2,4,8,27]; during acute pharmacological insulin injection in normal subjects [8]; and acutely after oral glucose loading in normotensive

and hypertensive non-obese subjects [28]. Nevertheless, others have noted that plasma ET-1 levels during acute hyperinsulinemia remained unchanged in men and were decreased in women [29], and that basal ET-values were not elevated in patients with insulinoma [8]. In the present study, which was not primarily designed to evaluate any effect of insulin on endothelin levels, circulating ET-1 during the control infusion was unchanged during the early, glucose-induced, hyperinsulinemia, but increased minimally and transiently during the more pronounced hyperinsulinemic phase following tolbutamide injection. Thus, it is unclear whether a subtle ET-1 stimulation occurred secondary to hyperinsulinemia, hyperglycemia or tolbutamide.

In conclusion, the findings of this study demonstrate that an increase in circulating ET-1 to levels which can be observed in insulin-resistant states acutely diminishes the insulinemic response to intravenous glucose. This is consistent with the possibility that ET-1 can inhibit pancreatic β -cell function.

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