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Decreased levels of asymmetric dimethylarginine during acute hyperinsulinemia

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Abstract

Endothelial dysfunction is reflected by an impaired nitric oxide (NO)–mediated vasodilatation. Insulin resistance may be linked to endothelial dysfunction by several mechanisms, including disturbances in signaling pathways common to both insulin action and NO production. Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, may contribute to endothelial dysfunction, and elevated ADMA levels have been associated with both insulin levels and the degree of insulin resistance. The direct link between insulin and ADMA, however, has not yet been established. In the present study, we aimed to investigate the effects of acute hyperinsulinemia on circulating ADMA and L-arginine levels and on forearm blood flow (FBF). Male volunteers, aged 21 to 24 years, with borderline hypertension were included in the study. The participants underwent a 90-minute hyperinsulinemic isoglycemic glucose clamp with insulin levels at the postprandial levels (n = 20) or a saline infusion (control) (n = 9). Fasting blood samples were drawn at baseline and after 90 minutes. Insulin infusion was accompanied by a reduction in ADMA (0.78 to 0.68 μmol/L, $P < .01$), which was significantly different ($P = .001$) from the increase seen in the saline control group (0.69 to 0.79 μmol/L, $P < .05$). The same profile was obtained for L-arginine with a significantly more pronounced decrease ($P < .001$) in the insulin clamp group (74 to 61 μmol/L, $P < .001$) than in the saline control group (59 to 57 μmol/L, $P = .95$). The FBF level and nitrate/nitrite (NO_x) levels were not affected by any of the clamp procedures. Short-term administration of insulin was accompanied by a decrease in both ADMA and L-arginine levels, with no change in FBF, in our population of young men with borderline hypertension. The possible influence of insulin on ADMA levels in a chronic state of insulin resistance can, however, not be deduced from the present investigation.

1. Introduction

Insulin is an important component of the metabolic syndrome in which the clustering of factors such as obesity, dyslipidemia, hypertension, and insulin resistance leads to a substantial increase in cardiovascular risk [1,2]. There is evidence that insulin per se has important direct effects on the vasculature, exerting both vasodilator and vasoconstrictor effects [3,4]. Several studies suggest the presence of a strong association between insulin resistance and endothelial dysfunction [5-7]. Endothelial dysfunction is reflected by an impaired nitric oxide (NO)–mediated vasodilatation [8]. NO is therefore presumed to be a potential link between atherosclerosis and insulin resistance [5-7], and substances that inhibit NO generation and/or decrease NO bioavailability may be of importance in the development of these metabolic disorders.

The endogenous amino acid asymmetric dimethylarginine (ADMA) inhibits all isoforms of NO synthase, and alterations in the NOS pathway may contribute to endothelial dysfunction and atherosclerosis [9]. Circulating plasma levels of ADMA have been shown increased in patients with chronic renal diseases, hypercholesterolemia, and a variety of disease entities, including essential hypertension [9-13]. Previous findings have demonstrated that ADMA levels are strongly associated with both insulin levels and the degree of insulin resistance [13,14]. The direct link between insulin and ADMA has, however, not yet been established.

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In patients with hypertension and insulin resistance, complications might be caused by abnormal glucose metabolism that augments vascular damage and leads to a poor prognosis [15,16]. The exact mechanisms by which high levels of insulin exert its effect on the vasculature are, however, still not fully understood, and whether hyperinsulinemia per se contributes to endothelial dysfunction in insulin resistance remains debatable.

To further study the relationship between insulin and endothelial dysfunction, using a hyperinsulinemic isoglycemic glucose clamp model, we performed the present study to investigate the effect of acute hyperinsulinemia primarily on circulating ADMA and L-arginine levels and also on forearm blood flow (FBF) in patients with borderline hypertension. Based on the strong correlations previously shown between insulin and ADMA levels, our hypothesis was that ADMA levels would rise during the insulin clamp procedure.

2. Methods

2.1. Participants

We studied young men born in 1977-1978 who had been screened during the military draft procedure in the Oslo area. Recruitment to the study was based on screening blood pressure (BP) values of systolic BP (SBP) of 140 mm Hg or higher and diastolic BP (DBP) of 90 mm Hg or higher. Among the screened individuals, 29 were included in the present investigation, of which 20 successfully completed the main study protocol of insulin infusion and 9 were included for saline infusion as a control group. The participants were 21 to 24 years old at the present examination. All were healthy, as assessed by medical history, physical examination, and routine blood parameter and dipstick urine analyses, and none used regular medication. Each participant gave written informed consent. The study was approved by the regional ethics committee and the procedures were in accordance with the Helsinki Declaration.

All participants refrained from smoking 10 hours before and abstained from alcohol 24 hours before the procedure. The examinations started in fasting condition at 8:00 AM and were conducted in a quiet room at constant room temperature. The participants rested in the supine position at least 30 minutes before the start of the procedure.

2.2. Hyperinsulinemic isoglycemic glucose clamp

The participants underwent a 90-minute hyperinsulinemic isoglycemic glucose clamp (insulin group) or a saline infusion (control group). The clamp procedure has been previously described in detail [17]. In brief, an intravenous catheter was inserted in each forearm for infusion (right) and sampling (left). Blood glucose level was determined as the average of 3 measurements. Insulin infusion (0.86 mU/min per kilogram body weight) was then started and blood glucose was measured every 5 minutes and clamped at the fasting level by glucose infusion (200 mg/mL). The glucose disposable rate (GDR) was calculated as average glucose infusion during the final 20 minutes divided by body weight (milligrams per minute per kilogram), and insulin sensitivity (GDR/I) expressed as GDR adjusted for achieved insulin concentration at 90 minutes × 100 (AU) [18]. All procedures were identical in the saline control group except for substitution of insulin and glucose infusions with matched volumes of 0.9% saline, blinded for the participant.

2.3. Venous occlusion plethysmography

Casual FBF was measured on the left arm, with a mercury-in-Silastic strain-gauge venous occlusion plethysmograph (EC5R Plethysmograph, Bellevue, WA) [19] as described previously [20]. The arm was supported above heart level, and the strain gauge was placed around the proximal part of the forearm. Hand blood flow was arrested by a pediatric-sized cuff placed around the wrist and inflated to suprasystolic pressure 60 seconds before FBF determinations. During flow measurements, a cuff around the upper arm was inflated to 50 mm Hg for brief periods of approximately 5 to 10 seconds and deflated for 5 seconds or more before the next measurement by use of the Hokanson (Issaquah, WA) E-20 rapid cuff inflator. FBF (mL per min per 100 mL) was calculated from the mean vertical deflection per minute on the tracings divided by the electrical calibration signal. FBF was determined as the average of 6 consecutive readings during each measurement period. FBF was measured at baseline and after 60 minutes during both clamp infusion regimens.

2.4. Blood sampling and biochemical measurements

Baseline blood samples were drawn from the indwelling intravenous catheter. Fasting serum glucose, cholesterol, and triglyceride concentrations were measured by conventional methods, and serum insulin with an enzyme-linked two-site immunoassay (DAKO Diagnostics, Cambridgeshire, UK). For the determination of plasma dimethylargi-

Table 1
Baseline characteristics and laboratory variables

	Insulin (n = 20)	Saline (n = 9)	*P*
Age (y) (range)	22 (21-24)	22 (21-24)	NS
Smokers (*n*)	8	3	NS
Resting SBP (mm Hg)	131 (122, 138)	130 (122, 135)	NS
Resting DBP (mm Hg)	80 (75, 86)	80 (74, 86)	NS
Creatinine (μmol/L)	85.5 (78.8, 88.0)	89.0 (75.0, 96.0)	NS
Total cholesterol (mmol/L)	4.2 (3.8, 4.6)	4.2 (4.0, 5.0)	NS
HDL-C (mmol/L)	1.2 (1.0, 1.4)	1.0 (1.0, 1.3)	NS
LDL-C (mmol/L)	2.5 (2.1, 2.8)	2.5 (2.2, 3.4)	NS
Triglycerides (mmol/L)	1.0 (0.6, 1.4)	0.9 (0.8, 1.3)	NS
Waist circumference (cm)	90.0 (83.8, 98.8)	86.5 (81.3, 92.4)	NS
Body mass index (kg/m^2)	24.9 (23.0, 27.9)	23.1 (22.6, 25.6)	NS
Glucose (mmol/L)	5.1 (4.6, 5.3)	5.0 (4.6, 5.3)	NS
Insulin (pmol/L)	39.3 (21.1, 53.8)	39.9 (21.7, 74.5)	NS
HOMA score	1.18 (0.57, 1.62)	1.13 (0.69, 2.14)	NS

Median values (25th, 75th percentiles) or numbers are given. *P* refers to group differences at baseline. NS indicates nonsignificant; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2
Dimethylarginines, NO_x, and GDR in basal state and after insulin/saline clamp

	Insulin (n = 20)		Saline (n = 9)		P_1	P_2	P_D
	Basal	End of clamp	Basal	End of clamp			
ADMA (μmol/L)	0.78 (0.66, 0.99)	0.68 (0.57, 0.83)*	0.69 (0.61, 0.87)	0.79 (0.66, 0.94)**	NS	NS	.001
SDMA (μmol/L)	0.42 (0.40, 0.43)	0.41 (0.40, 0.42)	0.42 (0.41, 0.43)	0.43 (0.41, 0.43)	NS	NS	NS
l-Arginine (μmol/L)	74 (63, 84)	61 (50, 71)***	59 (47, 64)	57 (50, 65)	<.01	NS	<.001
L-Arginine/ADMA ratio	93 (65, 126)	90 (54, 107)	83 (68, 93)	72 (64, 90)	NS	NS	.041
NO_x (μmol/L)	29 (25, 32)	27 (22, 38)	30 (26, 54)	35 (21, 64)	NS	NS	NS
FBF (mL/min per 100 mL)[a]	1.96 (1.79, 2.47)	2.02 (1.88, 2.57)	1.95 (1.50, 2.17)	1.53 (1.14, 2.46)	NS	NS	NS
GDR (mg/kg per minute)		6.6 (3.5, 8.1)					
GDR/I (AU)		1.7 (1.0, 3.1)					

Median values (25th, 75th percentiles) are given.P_1 and P_2 refer to group differences at baseline and after 90-minute insulin or saline clamp, respectively. P_D refers to differences in changes from baseline between the insulin and the saline control groups. NS indicates nonsignificant.

[a] FBF measured at basal state and after 60 minutes of the clamp procedure.

* $P < .01$.

** $P < .05$.

*** $P < .001$.

nines and nitrate/nitrite (NO_x), EDTA blood was drawn at baseline (before infusions) and after 90 minutes of the clamp procedures. Plasma concentrations of L-arginine, ADMA, and symmetric dimethylarginine (SDMA) were measured by high-performance liquid chromatography and precolumn derivatization with *o*-phthaldialdehyde (Sigma, St Louis, MO) as previously described in detail [12]. The recoveries of L-arginine, ADMA, and SDMA with this method were 84%, 91%, and 92%, respectively. Detection limit of the assays were 0.025 μmol/L; the intra- and interassay coefficients of variation, based on pooled plasma samples, were 5% or smaller for all. NO_x was analyzed by using Total Nitric Oxide Assay kit (R&D System Europe, Abingdon, UK) with an interassay coefficients of variation of 5.3%. Blood glucose concentrations during clamp were measured with an Accutrend sensor (Boehringer Mannheim, USA). Insulin resistance was estimated according to a homeostasis model assessment (HOMA) score, calculated with the following formula: (fasting insulin/7.2)/(22.5/fasting glucose), as described by Matthews et al [21].

2.5. Statistical analysis

Because several variables were skewly distributed, data are presented as medians and 25th and 75th percentiles throughout. Correlations between variables were tested by Spearman correlation method. Intragroup changes during the clamp procedures were calculated by Wilcoxon test. Mann-Whitney rank sum test was used for comparison between groups. The level of statistical significance was set at $P < .05$. The SPSS 11.0 (SPSS, Chicago, IL) software package was used for statistical analyses.

3. Results

3.1. Basal state

In the present study, the resting blood pressures were lower than at screening in both groups (Table 1). In the fasting state, there were no differences between the insulin clamp and saline control groups with regard to metabolic variables, blood pressure, and lipids (Table 1). There were

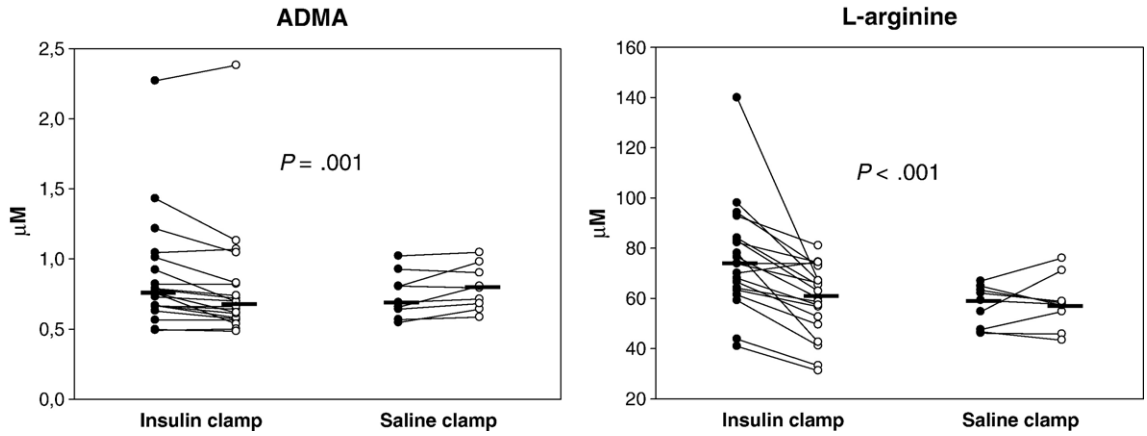

Fig. 1. ADMA and L-arginine levels at baseline (filled circles) and after 90 minutes' (open circles) infusion of insulin (0.86 mU/min per kilogram body weight) or saline. *P* values refer to differences in change between the groups. Median values are marked as a horizontal line.

also no group differences in the levels of ADMA, L-arginine/ADMA ratio, SDMA, NO_x or FBF (Table 2), except significant higher levels of L-arginine in the insulin group than in the saline group. The levels of fasting glucose were also not different between the groups; thus, the glucose levels throughout the procedure were at a similar level.

No significant correlations between ADMA or the L-arginine/ADMA ratio and the variables related to the metabolic syndrome or FBF were obtained in the total population. In the insulin group there was also no correlation with GDR or GDR/I.

3.2. Response to insulin

A significant reduction in ADMA levels was observed in the hyperinsulinemic clamp group ($P < .01$), which was significantly different ($P = .001$) from the increase seen in the saline control group ($P < .05$) (Fig. 1A, Table 2). The same profile was obtained for L-arginine with a significantly more pronounced decrease in the insulin clamp group compared with the saline control group ($P < .001$) (Fig. 1B). The L-arginine/ADMA ratio did not change significantly during the 2 clamp procedures, although there was a significant difference in change between the groups ($P = .041$).

There were no significant changes in NO_x levels or FBF after insulin or saline administration, and no changes in blood pressure were observed during any of the clamp procedures.

4. Discussion

The main finding in this study was that in borderline hypertensive young men short-term administration of insulin for 90 minutes was accompanied by a significant decrease in the plasma levels of ADMA. A parallel finding was that L-arginine followed the same profile and no change in FBF could be demonstrated.

The change in ADMA levels was of limited magnitude, and the duration of insulin infusion might be discussed. Although 90 minutes has been shown sufficient for the evaluation of GDR in this population [22], we cannot conclude if steady state has been attained for ADMA and L-arginine.

The importance of ADMA as an endogenous inhibitor of e-NOS is well established [10,23,24]. Because ADMA inhibits all isoforms of NO synthase, this methylated amino acid may contribute to endothelial dysfunction and atherosclerosis by competing with L-arginine for binding to the catalytic domain of the enzyme [9]. In addition, ADMA has further indirect inhibitory effect on NO production by decreasing the access of L-arginine to the cationic amino acid transporter (y^+ transporter), a common pathway for entering the cell [25].

Insulin-mediated vasodilatation is suggested to be entirely NO dependent [26], although the exact mechanisms are not known. Our observations of decreased levels of both ADMA and L-arginine in hypertensive patients under short-term insulin administration were unexpected. This might be discussed through increased activity of the y^+ transporter by insulin with subsequent increased intracellular concentrations of both ADMA and L-arginine. It is well known that insulin stimulates the uptake of glucose, fatty acids, and amino acids into cells [27]. Although there was no change in SDMA levels, it seems likely that this might be a potential mechanism resulting in the present decreased plasma levels of ADMA and L-arginine after short-term insulin administration. The results are further confirmed by the absence of changes in the L-arginine/ADMA ratio during the insulin infusion. This is supported to some degree by the findings of unchanged levels of the NO metabolites nitrite/nitrate (NO_x) and also in FBF during the insulin clamp. Thus, the production of NO, and thereby the vascular function, seems not to be affected by the short-term reduction in the ADMA levels. However, our observation is somewhat in contrast to that of other studies, which have shown acute hyperinsulinemia to increase endothelium-dependent release of NO and to improve vasodilation in healthy young subjects [28]. It should, however, be emphasized that the measurement of NO_x by the Griess reaction used in our study reflects both NO metabolites as well as bioactive NO; thus, bioactive NO cannot be exactly estimated. Furthermore, it is important for the interpretation of the present data to acknowledge that forearm plethysmography is a dynamic procedure that allows only an indirect estimation of the vascular function.

There is growing evidence that endothelial dysfunction is central in the insulin resistance syndrome and type 2 diabetes mellitus [29,30], in which it has been shown that the release and/or bioavailability of NO are diminished [31,32]. Several cardiovascular risk factors are associated with reduced sensitivity to insulin, and elevated ADMA concentrations have previously been linked to insulin resistance [13,14,33]. Insulin resistance has also been associated with increased oxidative stress in the endothelium [34], and the metabolic enzyme dimethylaminohydrolase (DDAH) has been demonstrated to be inhibited by oxidative stress [35], thus explaining the correlation between insulin resistance and elevated ADMA levels in a chronic disease state. The influence of oxidative stress on DDAH and ADMA during the acute hyperinsulinemic situation in the present study seems, however, more unlikely because of the short-term duration. It seems obvious that there are different mechanisms involved in different phases of hyperinsulinemia. From the present results, the direct influence of insulin on ADMA levels in the chronic disease state cannot be deduced. In chronic insulin resistance, both hyperinsulinemia and hyperglycemia are present. It has recently been demonstrated that hyperglycemia per se elevates ADMA by impairing the activity of DDAH in smooth muscle cells and endothelial cells [35]. Hence, keeping the plasma glucose at fasting low levels throughout the hyperinsulinemic clamp proce-

dure might contribute to our results. In the present investigation, which was performed in young healthy individuals with only borderline hypertension and without severe insulin resistance, we did not find any correlations between ADMA and insulin or insulin resistance. This might have probably influenced our results.

We could not demonstrate any correlation between FBF and plasma ADMA levels. This is somewhat different from the recently demonstrated inverse correlation between ADMA levels and endothelium-dependent vasodilatation in individuals with hypertension [36]. This particular study was, however, performed in an older population with essential hypertension and therefore not quite comparable to our study. It has been shown that increasing age is associated with increased endothelial dysfunction [37] and that endothelial dysfunction in hypertensive persons seems to represent an accelerated form of dysfunction of aging [38].

The relationship between ADMA and essential hypertension has been scarcely explored. In the present study we found that the hypertensive individuals had higher plasma ADMA levels than normotensive healthy subjects (data not shown), which are in agreement with other studies [36,39]. The cause of high plasma ADMA concentration in essential hypertension is presently unknown. Increased shear stress triggers ADMA synthesis, and high ADMA in hypertension may therefore be an epiphenomenon of high blood pressure [40]. Alternatively, high ADMA may result from reduced catabolic rate secondary to DDAH inhibition brought about by oxidative stress, a well-known feature of human hypertension [10] as well as of insulin resistance [34].

The study has some limitations that have to be taken into consideration. Only individuals with borderline hypertension are included; thus, any effects of acute hyperinsulinemia in healthy individuals cannot be ruled out.

The numerical difference in ADMA levels between the groups at baseline was not statistically significant. This might be due to lack of statistical power, thereby influencing the results. In addition, as the significant decrease in ADMA during insulin infusion was of limited magnitude, this potential lack of power might also have influenced the results.

As discussed, the FBF is not quite comparable to the flow-mediated vasodilation methods often used to evaluate vascular function. Thus, the FBF results have to be interpreted with caution in this regard.

As for the NO_x measurements, no dietary restrictions were given to the participants before the start of the study. Therefore, influence of NO sources in the diet or water cannot be excluded, but that would probably be equally distributed between the 2 groups.

In conclusion, in our population of young men with borderline hypertension short-term administration of insulin at a postprandial level was accompanied by a decrease in both ADMA and L-arginine levels, with no changes in the FBF and NO_x levels. The possible influence of insulin on ADMA levels in a chronic state of insulin resistance, however, cannot be deduced from the present investigation.

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