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PRELIMINARY REPORT

Plasma Nitric Oxide Concentrations Are Elevated in Insulin-Resistant Healthy Subjects

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The goal of this study was to compare plasma nitric oxide (NO) concentrations in healthy subjects, defined as either insulin-resistant or insulin-sensitive on the basis of the plasma insulin response to a 75-g oral glucose challenge. For this purpose, 404 healthy subjects were divided into quartiles on the basis of the plasma insulin response to glucose, and 49 individuals were selected from the quartile with the lowest insulin response and 49 from the quartile with the highest insulin response. The two groups of 49 each were selected to be essentially identical in terms of age, gender distribution, body mass index (BMI), and waist to hip ratio (WHR). The quartile with the greatest insulin response also had a significantly higher plasma glucose response to oral glucose, faster heart rate, higher blood pressure, and the combination of higher triglyceride and lower high-density lipoprotein (HDL) cholesterol concentrations. In addition to the latter changes, previously shown to be associated with hyperinsulinemia, NO concentrations were also higher in the hyperinsulinemic group. It is speculated that this increase in the NO concentration in hyperinsulinemic and presumably insulin-resistant, subjects represents a compensatory effort to overcome the untoward effects of insulin resistance and/or hyperinsulinemia.

EVIDENCE HAS RECENTLY been published[1] demonstrating that the ability of insulin-resistant patients with hypertension to avoid a significant salt-induced increase in blood pressure is dependent on their ability to increase the secretion of nitric oxide (NO). In a similar vein, there is evidence that plasma NO concentrations are increased in rats with spontaneous hypertension, and it was suggested that this finding may represent an adaptive effort to combat the hypertensive state by stimulating vasodilation.[2] Since spontaneously hypertensive rats have been shown to be insulin-resistant,[3,4] the results of the two former studies could be interpreted to signify that insulin-resistant individuals with hypertension secrete increased amounts of NO in an effort to compensate for the enhanced degree of peripheral vascular resistance. Type 2 diabetes is another well-recognized state of insulin resistance,[5-7] and there is evidence that NO production is normal and/or increased in patients and/or animal models of this syndrome.[8-11]

On the other hand, there is evidence that insulin resistance in normal subjects is associated with decreased endothelial NO synthesis.[12] This finding provides a possible mechanism to account for the observation that the insulin-mediated vasodilatory response is decreased in obese individuals and patients with type 2 diabetes,[13-14] conditions known to be associated with insulin resistance.[5-7,15] Based on these data, the alternative argument could be made that a decrease in the ability of insulin to increase endothelial NO release prevents insulin-mediated vasodilation, resulting in muscle insulin resistance.

It is apparent from this brief review of published information that there is evidence to support two totally disparate views of the relationship between insulin resistance and NO: ie, insulin resistance leads to a compensatory increase in the NO concentration, versus the notion that the more insulin-sensitive an individual is, the greater the NO secretion will be. At least part of the reason for the two discordant viewpoints as to the relationship between insulin resistance (or insulin sensitivity) and the NO concentration may be that relatively few studies

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Table 1. Demographic Characteristics of the Study Groups

Variable	Insulin-Sensitive	Insulin-Resistant	*P*
Age (yr)*	52 ± 1	52 ± 1	NS
Gender (male/female)	28/21	28/21	NS
BMI (kg/m²)*	26.3 ± 0.3	26.7 ± 0.3	NS
WHR*	0.91 ± 0.01	0.92 ± 0.01	NS

Mean ± SEM.

have been performed in a substantial number of healthy subjects. The current study was initiated to address this limitation and involved a comparison of plasma NO concentrations, as well as other markers of endothelial function, in 98 healthy individuals divided into insulin-sensitive and insulin-resistant groups.

SUBJECTS AND METHODS

We have recently evaluated a variety of risk factors for coronary heart disease (CHD) in 421 apparently healthy subjects.[16] On the basis of the medical history, physical examination, and plasma glucose response to a 75-g oral glucose load, 404 of 421 subjects were disease-free and using no medication that would affect known CHD risk factors. For the purposes of this study, 98 individuals were selected from this healthy cohort to evaluate the effect of insulin resistance, as estimated on the basis of the plasma insulin response to oral glucose, on CHD risk factors known to be associated with hyperinsulinemia, as well as the plasma NO concentration. Forty-nine subjects of the study population were selected from 101 individuals with the lowest total integrated plasma insulin response to oral glucose, whereas the other 49 subjects were recruited from 101 individuals with the highest insulin response to glucose. The two groups of 49 were created to be essentially identical in terms of age, gender distribution, body mass index (BMI), and waist to hip ratio ([WHR] (Table 1). In addition, there were no differences in terms of the family history of obesity (51% *v* 50%), diabetes (20% *v* 18%), dyslipidemia (29% *v* 29%), hypertension (65% *v* 55%), or CHD (69% *v* 65%) between the two groups.

All subjects were instructed to consume 300 g carbohydrate for 3 days preceding the measurements. A complete medical history was obtained and a physical examination performed. Venous blood was drawn after an overnight fast for determination of plasma glucose, insulin, and lipid and lipoprotein concentrations as described previously.[16] In addition, the fasting concentration of NO was measured by determining the end products of its metabolism, ie, nitrate and nitrate levels (NO_1^-/NO_3^-), using enzymatic catalysis coupled with the Griess reaction. Specifically, NO_3^- was reduced to NO_2^- by nitrate reductase 0.1 U, flavin adenine dinucleotide 5×10^{-6} mol/L, and NADPH 250×10^{-6} mol/L. Samples were incubated at 37°C for 3 hours, lactate dehydrogenase 8.8 U and pyruvate 10^{-2} mol/L were added to each well, and the sample was incubated for another 90 minutes at 37°C. Finally, Griess reactives were added to each well and the sample was read at 540 nm.[17]

Table 2. Comparison of Selected Metabolic and Hemodynamic Variables in the Study Groups (mean ± SEM)

Variable	Insulin-Sensitive	Insulin-Resistant	*P*
Systolic blood pressure (mm Hg)	125 ± 1	130 ± 2	<.05
Diastolic blood pressure (mm Hg)	80 ± 1	84 ± 1	<.05
Triglycerides (mg/dL)	91 ± 6	142 ± 10	<.001
LDL cholesterol (mg/dL)	138 ± 6	150 ± 6	NS
HDL cholesterol (mg/dL)	52 ± 2	45 ± 2	<.001
Heart rate (bpm)	67 ± 1	72 ± 1	<.01
NO (mmol/L)	18 ± 1	25 ± 2	<.001

The results are expressed as the mean ± SEM, and values for the two groups were compared by Student's nonpaired *t* test.

RESULTS

Plasma glucose and insulin before and after a 75-g oral glucose challenge are shown in Fig 1. Although all subjects were nondiabetic, plasma glucose was higher in the group with the high insulin response as compared with the low insulin response. Figure 1 also illustrates the relative degree of hyperinsulinemia in the group with a high insulin response.

Table 2 compares variables known to be associated with insulin resistance and compensatory hyperinsulinemia. The high insulin group had a significantly higher plasma triglyceride concentration, heart rate, and blood pressure and lower high-density lipoprotein (HDL) cholesterol compared with the low insulin group. It should also be noted that the low-density lipoprotein cholesterol concentrations were similar in the two groups. In addition, plasma NO concentrations were significantly higher in those with a high insulin response to glucose.

DISCUSSION

The goal of this study was to compare plasma NO concentrations in two groups of healthy subjects who were similar in terms of age, gender distribution, and the degree of obesity but dichotomous as regards the plasma insulin response to a

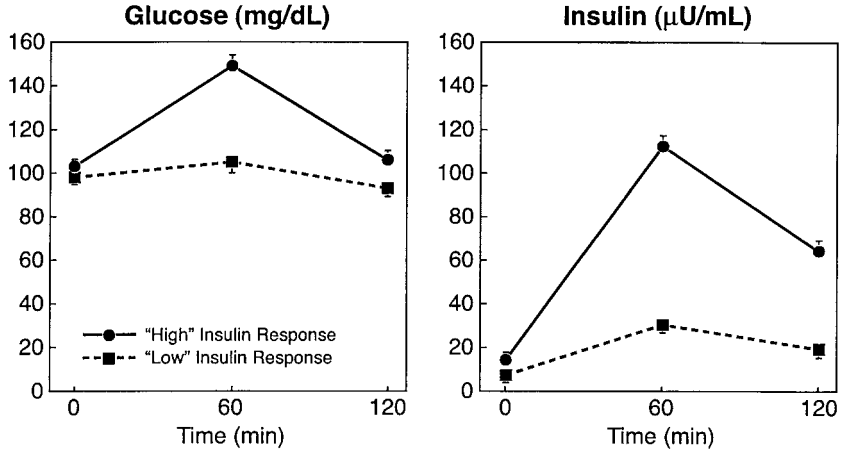

Fig 1. Plasma glucose and insulin before and after a 75-g oral glucose load in the 2 experimental groups.

standard oral glucose challenge. The results show that the hyperinsulinemic group whose plasma insulin values were approximately 4-fold greater also had significantly higher plasma NO values. In addition, the hyperinsulinemic group exhibited the other metabolic and hemodynamic characteristics associated with insulin resistance and compensatory hyperinsulinemia, including a greater plasma glucose response to an oral glucose challenge, higher plasma triglyceride and lower HDL cholesterol concentrations, and significantly higher blood pressure and heart rate.[16,18] These findings suggest that an increase in plasma NO should be added to the cluster of abnormalities initially designated as syndrome X.[18] Given the role of endothelium-derived NO in the regulation of skeletal muscle vasodilation,[19] it can be speculated that the increase in NO in insulin-resistant subjects represents a compensatory effort to enhance muscle insulin sensitivity.

Although the results of the current study are straightforward, disparate views of the relationship between insulin resistance and NO concentrations have been published. However, it is possible that the differences are more apparent than real. For example, the conclusion that endothelial NO synthesis is directly related to the degree of insulin sensitivity was based on the results of a functional estimate of NO synthesis.[12] Similarly, the implicit conclusion that NO synthesis is reduced in insulin-resistant states is based on an impairment of the vasodilatory effects of insulin in obese individuals and patients with type 2 diabetes.[13,14] However, it is possible to interpret these results to indicate resistance to the ability of NO to stimulate vasodilation in these situations. Theoretically, the resistance to NO could result from a defect in the ability of NO to increase the activity of its effector agent, cyclic guanosine monophosphate (GMP),[20] in insulin-resistant subjects. Alternatively, there could be a defect in the vasodilatory response to cyclic GMP associated with insulin resistance.

We believe this is the first study in which plasma NO concentrations have been directly compared in two large groups of normal subjects differing dramatically in the plasma insulin response to glucose and presumably the degree of insulin resistance. However, our finding that plasma NO concentrations were higher in hyperinsulinemic subjects is consistent with results in both insulin-resistant humans[1] and rats[2] with elevated blood pressure. The authors of those studies interpreted their results to signify that the higher NO concentrations represented a compensatory effort to overcome the untoward effects of the physiological events associated with insulin resistance and/or the associated hyperinsulinemia. Given the currently available experimental data, the possibility that plasma NO may be higher rather than lower in healthy subjects who are also insulin-resistant and hyperinsulinemic seems to be a viable possibility. At the least, it is an alternative that seems worthy of further study.

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