Decrease in the Plasma von Willebrand Factor Concentration Following Glucose Ingestion: The Role of Insulin Sensitivity

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Elevated plasma von Willebrand factor (vWF) concentration is thought to be associated with increased prevalence of cardiovascular events in the insulin resistance syndrome. We examined the effects of oral glucose challenge and accompanying metabolic and hemodynamic changes on vWF levels with respect to insulin sensitivity. Forty normotensive and hypertensive subjects (mean age ± SD, 40 ± 5 years) underwent a standard oral glucose tolerance test (OGTT). Plasma vWF antigen, glucose, insulin, catecholamines, and hemodynamics were measured at rest, and at 30, 60, 90, and 120 minutes after glucose intake. Insulin sensitivity was determined by the insulin sensitivity index ($ISI_{0,120}$). Resting plasma vWF concentration was associated with screening systolic blood pressure (BP) ($r = .43$, $P = .005$). There were time effects for all variables of interest. While vWF antigen ($P = .044$), epinephrine ($P = .003$), and diastolic BP ($P = .001$) decreased after glucose challenge, norepinephrine ($P = .009$), systolic BP ($P = .022$), and heart rate ($P < .001$) increased. Decline in vWF (area under the curve) was associated with decrease in epinephrine ($r = .46$, $P = .004$) and with screening systolic BP ($r = .45$, $P = .004$). However, neither resting plasma vWF levels nor vWF decrease following glucose ingestion were significantly associated with the $ISI_{0,120}$. The plasma vWF concentration decreases following glucose ingestion. While mechanisms underlying this phenomenon may relate to sympathetic nervous system function, they seem not related to insulin sensitivity. Endothelial dysfunction such as caused by hypertension rather than metabolic dysregulation per se may underlie the elevated plasma vWF concentration found with insulin resistance.

PROSPECTIVE STUDIES have found a direct association between the plasma von Willebrand factor (vWF) concentration and coronary risk in healthy individuals and in patients with ischemic heart disease.[1,2] The vWF exerts procoagulant function thought to underly atherosclerotic plaque growth by virtue of mediating platelet adhesion to subendothelial structures and platelet aggregation, as well as by increasing the availability of circulating clotting factor VIII.[3] Consequently, elevated levels of plasma vWF are viewed as a marker of endothelial dysfunction and atherosclerosis.[4] Elevated vWF could be related to increased cardiovascular morbidity and mortality observed with low insulin sensitivity[5-7] because vWF is elevated in so many domains clustering with the insulin resistance syndrome such as hypertension,[8] type II diabetes,[9] obesity,[10] dyslipidemia,[11] and high fasting insulin levels.[12]

Several investigators propose that altered activity of the sympathetic nervous system (SNS) underlies the multifaceted syndrome of insulin resistance.[13,14] Subjects who have low insulin sensitivity show a relatively more prominent insulin response to glucose intake as compared to individuals with normal insulin sensitivity.[15] Moreover, insulin plays an important role in the relationship between dietary intake and activity of the SNS,[13] and this relationship can be reliably simulated by the oral glucose tolerance test (OGTT).[16] Given that sympathoadrenal stimuli increase plasma vWF levels,[17,18] changes in SNS activity following dietary intake might be one mechanism regulating plasma vWF. This linkage may be particularly important in the context of insulin resistance when SNS activity is elevated. This study examined the metabolic and sympathetic effects of an oral glucose load on plasma vWF levels in humans with respect to subjects' insulin sensitivity as expressed by the insulin sensitivity index ($ISI_{0,120}$).[19]

MATERIALS AND METHODS

Subjects

All subjects gave written consent to the study protocol approved by the UCSD Human Subjects Committee. The sample included 40 normotensive and hypertensive volunteers recruited by advertisement or word-of-mouth referral from the San Diego community to participate in a study on effects of the SNS on cardiovascular physiology. Table 1 presents demographic and metabolic data. Aside from 15 subjects who had mild screening hypertension (systolic and/or diastolic BP >140/90 mm Hg) based on 3 blood pressure (BP) measurements on each of two occasions 1 week apart, all participants were healthy. They received no medications, including aspirin and nonsteroidal anti-inflammatory drugs. Antihypertensive drugs were tapered at least 3 weeks prior to the study. All subjects had to be in the range of 100% to 150% of ideal body weight as determined by Metropolitan Life Insurance tables.[20]

Protocol

Subjects received a standardized isocaloric diet of 200 mEq sodium/d and 100 mEq potassium/d and were hospitalized in a Clinical Research Center. Upon admission, there was a meal at 5:00 PM, and a bedtime snack at 8:30 PM. On the following morning at 7:00 AM, subjects had an indwelling intravenous catheter inserted. At 8:00 AM, we measured BP and heart rate, drew blood samples, and subjects then drank a solution containing 75 g of glucose. Subsequently, hemodynamic variables were measured, and blood samples for vWF, glucose, insulin, and catecholamines were drawn at 30, 60, 90, and 120 minutes.

As part of the study protocol, subjects' insulin sensitivity was defined by the $ISI_{0,120}$.[19] We chose this measure because of its good correlation ($r = .63$) with the more invasive euglycemic hyperinsu-

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Table 1. Demographic and Metabolic Variables (mean ± SD) of 40 Subjects Studied

Age (yr)	40 (5)
Male/female	30/10
Black/white	22/18
Nonsmoking/smoking	20/20
Systolic blood pressure (mm Hg)	
Normotensive (n = 25)	120 (10)
Hypertensive (n = 15)	148 (9)
Diastolic blood pressure (mm Hg)	
Normotensive	74 (9)
Hypertensive	89 (7)
Insulin sensitivity index ($ISI_{0,120}$)	37 (18)
Body mass index (kg/m^2)	27 (4)
Total blood cholesterol (mg/dL)	194 (34)
Low density cholesterol (mg/dL)	118 (37)
High density cholesterol (mg/dL)	49 (20)
Triglycerides (mg/dL)	126 (88)
Creatinine (μmol/L)	79 (17)
Hematocrit (%)	40.7 (4.3)
Epinephrine (pmol/L)	102 (122)
Norepinephrine (nmol/L)	1.2 (0.5)
von Willebrand factor antigen (%)	148 (52)

linemic clamp, the standard method of measuring insulin resistance.[19] Moreover, the $ISI_{0,120}$ appears to be an equally valid measurement of insulin resistance across different categories of glucose tolerance and obesity.[19] Computation of the $ISI_{0,120}$ uses a simplified formula[19] adapted from that developed by Cederholm and Wibell.[21] The formula considers the 0- and 120-minute glucose and insulin values obtained from the OGTT, and the body weight (kg).[19]

Assays

After discarding the first 2 mL, venous blood for vWF assay was drawn into EDTA sterile tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged within 2 hours at 3,000 × *g* at 4°C for 10 minutes with the plasma obtained immediately stored in plastic tubes at -80°C until further processing. Plasma vWF antigen levels were measured by an enzyme-linked immunosorbent assay (ELISA) following a method by Short et al,[22] with reference to a standard derived from pooled normal human plasma. Rabbit antihuman vWF antibodies, which detect the different vWF multimers with equal sensitivity, were from DAKO (Carpinteria, CA), and peroxidase substrate kit was from Bio-Rad Laboratories (Hercules, CA). The 5 samples obtained from each subject were assayed in duplicates on the same ELISA plate. The interassay and intra-assay coefficients of variation were 6.9% and 2.4%, respectively.

Plasma glucose was measured by a glucose oxidase technique (model 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH), and plasma insulin was determined by a previously described radioimmunoassay.[23] Plasma epinephrine and norepinephrine levels were measured by a radioenzymatic method.[24]

Data Management

Because of occasional assay and technical problems, data from the OGTT were incomplete for glucose and heart rate in 1 subject, for insulin, systolic and diastolic BP in 2 subjects, and for norepinephrine and epinephrine in 3 subjects. The $ISI_{0,120}$ was computable on all 40 subjects who also had complete data for vWF antigen.

The SPSS (9.0) software package (Chicago, IL) was used for statistical analyses. All testing for significance was 2-tailed at the $P \leq .05$ level. Data are presented as means ± 1 SD unless in the figures, where we present the median and interquartile range because some of the values for vWF, glucose, insulin, and catecholamines during the OGTT were skewed towards higher values. These measures were $_{10}$log normalized to approximate a normal distribution; their areas under the curve across the 5 time points of the OGTT were computed on transformed data. We used Student's *t* test, simple correlation analyses, stepwise linear regression, and repeated-measures analyses of variance (ANOVA). Post hoc analyses were by Fisher's least significant difference.

RESULTS

Analyses of Demographic and Resting Metabolic Data

Resting plasma vWF antigen levels correlated positively with screening systolic BP ($r = .432$, $P = .005$), and there was a similar trend for screening diastolic BP ($r = .291$, $P < .07$). In addition, blacks had higher vWF than whites (mean ± SD, 163% ± 50% *v* 129% ± 50%; $P = .040$). In a stepwise linear regression analysis with vWF as the dependent variable, both screening systolic BP and race entered the regression equation to account for a total of 29% of the variance in resting vWF antigen ($R^2 = .294$, $F_{2,37} = 7.7$, $P = .002$). As expected per design, the $ISI_{0,120}$ correlated significantly with resting insulin levels ($r = -.629$, $P < .001$). There were no other significant relationships between vWF antigen or $ISI_{0,120}$ with other variables from Table 1. In particular, resting plasma vWF concentration and $ISI_{0,120}$ were not significantly associated, even when hypertension status, gender, ethnicity, smoking, and impaired glucose tolerance (see below) were separately controlled for.

Oral Glucose Tolerance Test

According to the 1997 American Diabetes Association standards,[25] 13 subjects had impaired glucose tolerance defined as a fasting plasma glucose level greater than 6.1 mmol/L or a 2-hour postchallenge glucose level greater than 7.8 mmol/L.

Following glucose ingestion, ANOVA showed an expected significant quadratic time effect for glucose ($F_{1,38} = 185$, $P < .001$) and for insulin ($F_{1,37} = 78$, $P < .001$). Both variables peaked at 60 minutes and were still significantly elevated at 120 minutes as compared to baseline (all *P*'s < .001; data not shown). There was a significant linear time effect for vWF antigen ($F_{1,39} = 4.3$, $P = .044$). Post hoc analyses showed that the plasma vWF concentration had significantly decreased between 0 minutes and all subsequent time points (all *P*'s < .05; Fig 1).

In addition, there were significant quadratic time effects for both epinephrine ($F_{1,36} = 10.2$, $P = .003$) and norepinephrine ($F_{1,36} = 7.6$, $P = .009$) (Fig 2). In post hoc analyses, epinephrine was decreased and norepinephrine was elevated both at 30 minutes and at 60 minutes as compared to baseline (all *P*'s < .05).

All hemodynamic variables showed a significant time effect, which was quadratic for the systolic BP ($F_{1,37} = 5.7$, $P = .022$) and linear for the diastolic BP ($F_{1,37} = 14.3$, $P = .001$) (Fig 3) and for heart rate ($F_{1,39} = 27.8$, $P < .001$). Post hoc analyses revealed that systolic BP tended to increase between 0 and 60 minutes ($P < .07$) to decrease again between 60 minutes and the two subsequent time points (all *P*'s < .05). Diastolic BP had decreased between rest and all other time points (all *P*'s ≤ .010), while heart rate had increased between resting values and 60, 90, and 120 minutes, respectively (all *P*'s < .001).

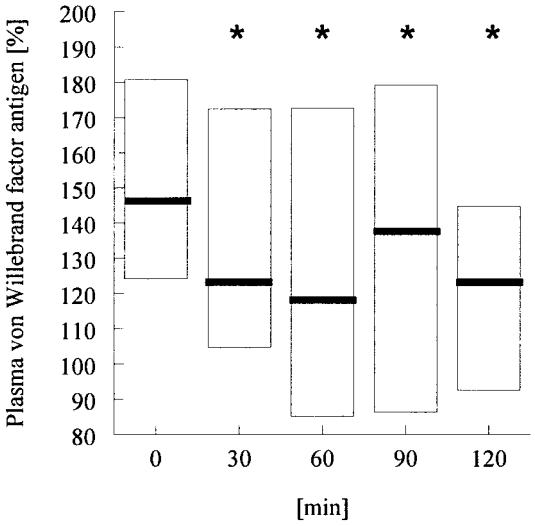

Fig 1. There was a main effect for time on von Willebrand factor ($P = .044$). Values are the median and interquartile range. vWF decreased (*) between 0 and 30 minutes ($P = .017$), between 0 and 60 minutes ($P = .009$), between 0 and 90 minutes ($P = .032$), and between 0 and 120 minutes ($P = .016$).

In a next step, we computed the AUC for vWF, insulin, glucose, epinephrine, norepinephrine, systolic and diastolic BP, and heart rate across the 5 time points of the OGTT. Table 2 shows that there were several significant and positive correlations between AUCs of all variables of interest. vWF_{AUC} correlated only with $epinephrine_{AUC}$ ($r = .462$, $P = .004$), but vWF_{AUC} was also significantly associated with screening systolic BP ($r = .446$, $P = .004$) with a similar trend seen for screening diastolic BP ($r = .299$, $P < .07$), suggesting that the higher the screening BPs were, the less vWF declined to glucose. When $epinephrine_{AUC}$ and screening systolic BP were stepwise-regressed on vWF, $epinephrine_{AUC}$ alone entered the regression equation with the decrease in plasma epinephrine concentration explaining 21% of the variance in the vWF decline ($R^2 = .213$, $F_{1,36} = 8.3$, $P = .004$). In addition, $Glucose_{AUC}$ and $epinephrine_{AUC}$ together explained 42% of the variance in systolic BP_{AUC} ($R^2 = .421$, $F_{2,34} = 11.6$, $P < .001$), while $glucose_{AUC}$ alone accounted for 16% of the variance in diastolic BP_{AUC} ($R^2 = .163$, $F_{1,33} = 6.4$, $P = .016$). $Insulin_{AUC}$ explained 16% of the variance in heart $rate_{AUC}$ ($R^2 = .162$, $F_{1,33} = 6.4$, $P = .016$).

$ISI_{0,120}$ correlated with $insulin_{AUC}$ ($r = -.621$, $P < .001$) and with $glucose_{AUC}$ ($r = -.444$, $P = .005$) as expected per design. Negative correlations between $ISI_{0,120}$ and systolic BP_{AUC} ($r = -.400$, $P = .013$), diastolic BP_{AUC} ($r = -.390$, $P = .015$), and heart $rate_{AUC}$ ($r = -.336$, $P = .037$) implied that subjects with relatively lower insulin sensitivity reacted with more prominent rise in both systolic BP and heart rate and less decrease in diastolic BP as compared to subjects with relatively higher insulin sensitivity. $ISI_{0,120}$ and vWF_{AUC} showed no significant association, even when hypertension status, gender, ethnicity, smoking, and impaired glucose tolerance were separately controlled for.

DISCUSSION

The clustering of several established cardiovascular risk factors accounts for part of the increased cardiovascular morbidity and mortality in the insulin resistance syndrome.[7] Plasma vWF levels are elevated with several of these cardiovascular risk factors,[8-12] and thus, by virtue of exerting procoagulant function,[3] the vWF is believed to have an important role in premature atherosclerosis observed with insulin resistance.[5,6] We wondered whether metabolic changes elicited by the OGTT would influence plasma vWF levels. We further questioned whether such an influence would be related to insulin sensitivity, which we assessed with the $ISI_{0,120}$, which was previously described to correlate well with the euglycemic hyperinsulinemic clamp.[19] Moreover, subjects with insulin resistance have impaired endothelium-dependent vasodilatation because of a failure of insulin to stimulate endothelial secretion of the vasodilator nitric oxide.[7,26] Our observation that subjects with relatively lower insulin sensitivity had relatively higher BP and heart rate responses to glucose suggests that the $ISI_{0,120}$ also was an accurate instrument to tap for vasodilatating function related to insulin sensitivity. The wide range between 16 and 105 $ISI_{0,120}$ index points reflects that a fair number of subjects had clinical features of insulin sensitivity, and may imply that our study population was accurate to test the above hypotheses.

Our results primarily suggest that plasma vWF antigen decreases within 30 minutes after glucose intake with subsequent persistence of diminished plasma vWF levels up to

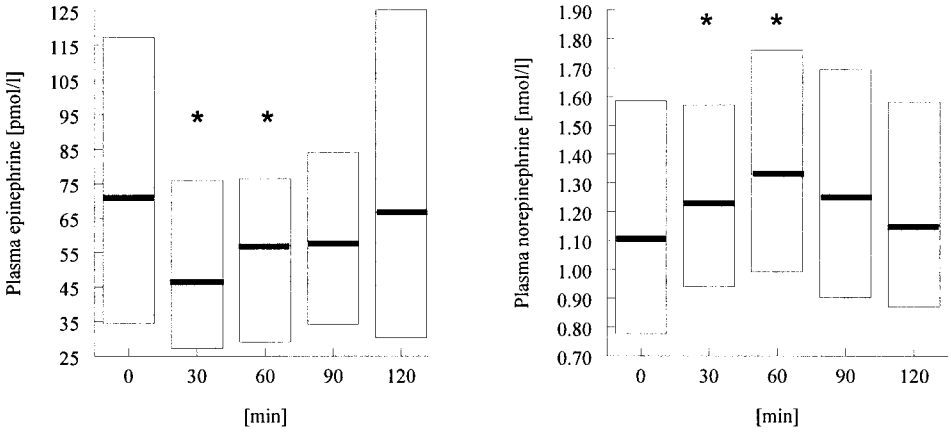

Fig 2. There was a main effect for time on epinephrine ($P = .003$) and for norepinephrine ($P = .009$). Values are the median and interquartile range. Epinephrine decreased (*) between 0 and 30 minutes ($P = .011$) and between 0 and 60 minutes ($P = .002$), while norepinephrine increased (*) between 0 and 30 minutes ($P = .018$) and between 0 and 60 minutes ($P = .008$).

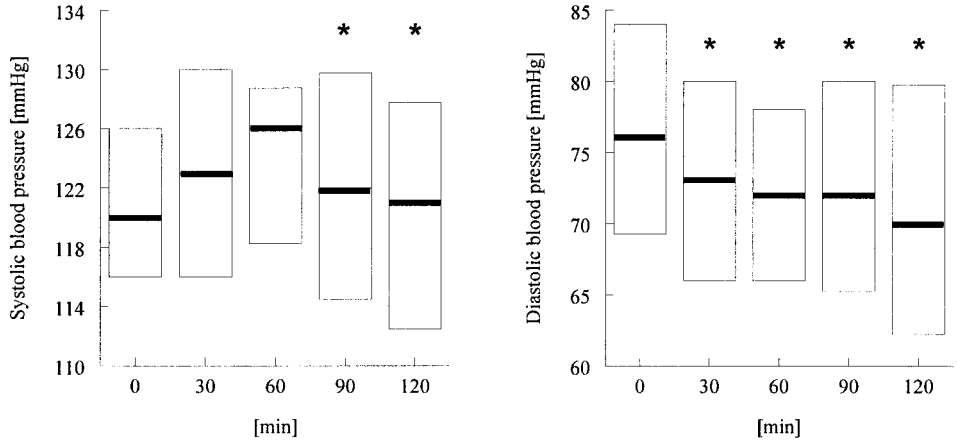

Fig 3. There was a significant main effect for time on systolic ($P = .022$) and on diastolic ($P = .001$) BP. Values are the median and interquartile range. Systolic BP tended to increase between 0 and 60 minutes ($P < .07$) and then to significantly decrease (*) between 60 and 90 minutes ($P = .010$) and between 60 and 120 minutes ($P = .022$). Diastolic BP decreased (*) between 0 and 30 minutes ($P = .010$), between 0 and 60 minutes ($P = .005$), between 0 and 90 minutes ($P = .008$), and between 0 and 120 minutes ($P < .001$). There were further significant decreases in diastolic BP between 30 and 120 minutes ($P = .045$) and between 60 and 120 minutes ($P = .050$).

2 hours. However, insulin sensitivity was neither associated with resting vWF levels nor with observed vWF decline with the OGTT in particular even when demographic variables and hypertension status were separately controlled for. However, we acknowledge that our study population was too small and heterogeneous to probe for reliable differences among the various demographic variables and hypertension status on vWF. It remains to be determined whether the insulin sensitivity mechanism in dietary regulation of plasma vWF might be different in specific homogenous patient groups.

In addition, we found that subjects with relatively higher screening systolic BP had higher basal vWF and less vWF reduction with the OGTT than subjects with relatively lower screening systolic BP (although the relationship with the OGTT did not enter the multiple regression equation). Taken together, our observations support the notion that elevated vWF in the insulin resistance syndrome is due to endothelial dysfunction and damage such as caused by hypertension[8] rather than due to insulin sensitivity per se.

Recent research has added much to the understanding of the mechanisms underlying the clearance of vWF from the circulation in health and disease.[27,28] It is not known, however, how metabolic alterations such as elicited by the OGTT could affect vWF turnover. We found that changes in insulin, glucose, and hemodynamics were not directly associated with decline in plasma vWF levels. Moreover, subjects with impaired glucose tolerance showed no significant difference in both resting vWF levels and vWF decrease with the OGTT as compared to subjects with normal glucose tolerance (data not shown). Our findings are in line with the diabetes literature, in which some investigators question whether glycemic control and insulin are directly involved in the mechanisms of vWF increase.[29] For example, one study reported a similarly significant suppression of vWF ristocetin cofactor activity with the OGTT in patients with type II diabetes and in normoglycemic controls.[30] The findings from that and from our study together suggest that there may be a glucose-induced devrease in both vWF concentration and vWF activity. In addition, in a recent study on healthy subjects undergoing a euglycemic hyperinsulinemic clamp, a 30-fold increase in plasma insulin levels did not affect the plasma vWF concentration.[31] We further do not believe that a vWF decrease reflects a circadian phenomenon because vWF shows no circadian variation in normals and in patients with atherosclerotic disease.[32,33]

Our results suggest that characteristic changes in SNS

Table 2. Bivariate Correlation Coefficients Between Areas Under the Curve of Variables Measured With the Oral Glucose Tolerance Test

	vWF	Glucose	Insulin	EPI	NE	SBP	DBP	HR
vWF								
Glucose								
Insulin								
EPI	.462†		.335*					
NE								
SBP		.552†		.495†				
DBP		.404*	.326*	.338*		.737‡		
HR			.403*		.363*		.535†	

Abbreviations: DBP, diastolic blood pressure; EPI, epinephrine; HR, heart rate; NE, norepinephrine; SBP, systolic blood pressure; vWF, von Willebrand factor.

*$P < .05$; †$P < .01$; ‡$P < .001$.

activity following dietary intake[34] might be involved in regulation of plasma vWF concentration. We found that epinephrine decrease accounted for 21% of the variance in the vWF decline. The parallel decline in vWF and epinephrine is consistent with in vivo studies showing a rise in plasma vWF antigen following epinephrine infusions.[17] Nonetheless, we offer such reasoning tentatively because while epinephrine releases vWF by a β_2-adrenergic mechanism from endothelial cells into the circulation,[17] it remains to be seen whether reduced plasma epinephrine levels are related to diminished vWF release. In addition, epinephrine is an insulin-antagonizing hormone, which decreases in response to hyperglycemia to sustain hyperinsulinemia.[35] The glucose-induced decline in epinephrine and vWF thus might constitute independent phenomena. Norepinephrine could trigger endothelial vWF discharge by virtue of its weak β_2-adrenergic receptor affinity, although this has not been investigated either in vitro or in vivo.[17] However, norepinephrine increased and vWF decreased and their changes were not associated. Therefore, our study does not support an effect of norepinephrine on plasma vWF regulation in vivo with the OGTT.

In conclusion, glucose ingestion results in a decrease in the plasma vWF concentration. While this observation is unlikely related to insulin sensitivity, it might be related to changes in SNS activity following dietary intake.

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