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Valsartan Improves Insulin Sensitivity without Altering Vascular Function in Healthy Overweight Adults without the Metabolic Syndrome

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ABSTRACT

***Background*. We investigated hyperactivity of the renin-angiotensin system (RAS) as a cause of endothelial dysfunction in obese humans.**

***Methods*. Thirty five healthy overweight (BMI = 33.6 ± 6.6 kg m $^{-2}$) adults (33 ± 10 years old) without cardiovascular risk factors received valsartan (160 mg) orally daily or a matching placebo for 6 weeks each.**

***Results*. Baseline flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) were not altered by placebo or valsartan. However, fasting plasma insulin was significantly decreased by valsartan compared to placebo (−4.6 ± 16.0 μUmL^{-1} versus −0.4 ± 11.6 μUmL^{-1}, $P = 0.032$) with no changes in glucose. A secondary analysis in patients with elevated waist to hip ratios (ÿ0.85, n = 18) showed an increase in FMD with valsartan.**

***Conclusions*. Our findings suggest that angiotensin 2 receptor blockade may aid in the prevention of diabetes even at the earliest stages of risk due solely to uncomplicated obesity. The lack of an improvement in FMD does not support a central role of RAS-hyperactivity in the etiology of the vascular dysfunction due solely to obesity. However, it is possible that obese patients with central adiposity may improve FMD with RAS blockade, and future investigation is warranted in this subgroup.**

INTRODUCTION

OBESITY, IN PARTICULAR CENTRAL ADIPOSITY, is associated with insulin resistance and independently predicts the presence of vascular endothelial dysfunction and reduced arterial compliance.[1,2] Even after accounting for other aspects of the metabolic syndrome, excess adiposity per se causes endothelial dysfunction (impaired nitric oxide-dependent vasomotion).[1–3] However, the biological mechanisms underlying this relationship remain incompletely described.

Even modest changes in traditional risk factors within "normal" ranges may negatively affect the vasculature. With insulin resistance, a concomitant impairment in vasodilatory insulin signaling pathways within endothelial cells may also play a more direct role in reducing endothelial-dependent vasodilatation.[2]

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Moreover, fat cells release multiple adipokines capable of affecting systemic vascular function.[2,4] Among these factors, recent evidence suggests that hyperactivity of the renin-angiotensin system (RAS) intrinsic to fat cells may be particularly relevant.[5–8] By contributing to angiotensinogen and angiotensin 2 (AT2) concentrations, either systemically or in a local paracrine fashion from perivascular fat, excess adiposity could directly trigger both insulin resistance and endothelial dysfunction.[2,9] AT2 stimulates oxidative stress within the vasculature via upregulation of NADPH oxidase and thus reduces the bioavailability of nitric oxide.[10]

Support for the physiological relevance of this pathway comes from at least 2 recent studies. Weight loss reduces plasma levels of angiotensinogen, accompanied by a related decrease in blood pressure.[8] AT2 receptor blockade in obese patients with the metabolic syndrome has also been shown to improve brachial artery flow-mediated dilatation (FMD).[7] However, a significant portion of the overweight patients in both studies had accompanying cardiovascular risk factors, such as overt hypertension, and/or other aspects of the metabolic syndrome (impaired fasting glucose, atherogenic dyslipidemia). Therefore, the direct role played solely by a hyperactive RAS in altering systemic vascular function in uncomplicated obese states per se could not be clearly evaluated due to the confounding effects of the associated risk factors on the RAS and on levels of systemic oxidative stress.

The purpose of this study was to investigate the ability of valsartan, a selective AT2 receptor blocker, to improve the vascular dysfunction due to excess adiposity in patients without any other aspects of the metabolic syndrome. It was hypothesized that if upregulation of the RAS within adipocytes is playing a direct etiologic role, blockade of the most active component (AT2) by valsartan would improve the previously described impairment in brachial artery endothelial function (reduced FMD) in otherwise healthy overweight adults.[1] Additionally, we hypothesized that the enhancement of metabolic insulin sensitivity by AT2–blockade would be correlated to the increase in FMD. This would reflect either amelioration of a common underlying causal risk factor such as oxidative stress (common soil hypothesis), or that changes in metabolic insulin sensitivity would subsequently yield an improvement in vascular endothelial function.

METHODS

The study was prospectively performed at the University of Michigan and approved by its Institutional Review Board. Overweight (body mass index [BMI] $> 27\ kg \cdot m^{-2}$) nonsmoking adults without cardiovascular risk factors (defined as blood pressure $< 140/90$ mmHg and no history of hypertension; fasting glucose < 126 mg/dL and no history of diabetes; total cholesterol < 240 mg/dL and no history of hyperlipidemia; triglycerides < 250 mg/dL, and no family history of known premature cardiovascular disease) were invited to participate. No subject met the ATP3 criteria for the metabolic syndrome. All subjects were not taking any medication or substance known to alter endothelial function for 6 weeks prior to and during the study. Subjects signed informed consent, had a physical examination, and gave blood to assure they were healthy and qualified for the study. We tested pregnancy, lipids, basic chemistry, thyroid stimulating hormone, insulin, and glucose. Body height, weight, waist (thinnest circumference), hip (at femoral heads), and waist circumference (at illiac crest), were obtained to determine eligibility and to characterize abdominal obesity. Subjects who planned on initiating an exercise routine during the trial were excluded. Eligible subjects entered into a randomized, double-blind, cross-over study (3 week washout) of valsartan (160 mg orally daily) and placebo for 6 weeks each.

Testing sessions

There were 3 testing sessions involved: baseline, after the first arm, and after the second arm (the cross-over). Subjects fasted for at least 8 hours prior to each testing session. Subjects arrived at approximately the same time of the morning after fasting for more than 8 hours for

all visits, and provided blood samples from an antecubital vein.

C reactive protein (CRP) and lipoprotein subclass levels were measured by LipoScience Incorporated (Raleigh, North Carolina). Lipoproteins were analyzed by nuclear magnetic resonance spectroscopy, and high-sensitivity CRP was analyzed by the Immuline 2000 analyzer (Diagnostic Product Corporation, Los Angeles, California), as described elsewhere.[11] Plasma leptin and adiponectin concentrations were measured with a commercially available enzyme-linked immunosorbent assay kit (Linco Research, St. Louis, Missouri) according to the manufacturer's instructions.

Conduit vessel endothelial function was determined by brachial arterial FMD and nitroglycerin-mediated dilation (NMD) (endothelial-independent vasomotion) using high-resolution vascular ultrasonography by standard methods in our laboratory as previously described.[1] Subjects rested in a supine position in a temperature-controlled room for 15 minutes before each vascular study. Blood pressure was measured immediately prior to the vascular studies and recorded as the average of 3 measurements (Omron 711AC, Omron Healthcare, Inc., Bannockburn, IL). Systemic vascular resistance and large and small artery compliance were determined using computerized arterial pulse waveform analysis of the radial artery by tonometry (Hypertension Diagnostics Incorporated). We planned tonometry for all subjects, but the measurement device was unavailable for some subjects, which explains the different sample size in the results.

Statistical analysis

The treatment effects were determined by calculating the change from baseline for each variable, and changes were compared by a paired t-test. The study was a priori powered to detect a minimum of 1.5% change in FMD following valsartan treatment versus the change after placebo, assuming a standard deviation of ΔFMD in each group = 4.0%. All analyses were performed using SPSS 13.0 with an $\alpha = .05$ level of statistical significance.

RESULTS

Table 1 shows the characteristics of the 35 subjects that completed the study. All participants were overweight, but normotensive and without the metabolic syndrome. Baseline laboratory values and their alterations by treatment limb are shown in Table 2. Mean fasting glucose values were within the normal range, well below even the newly defined impaired fasting glucose value (>100 mgdL^{-1}). Patients were not hyperlipidemic (normal total and low density lipid [LDL]-cholesterol, and triglyceride values). Mean high density lipid cholesterol (HDL-C) level was modestly low; however the more detailed lipoprotein analyses revealed an overall healthy basal profile with a normal LDL particle size and number. As expected due to even uncomplicated obesity, fasting insulin, C-reactive protein, and leptin were mildly elevated, while adiponectin tended to be low. Neither placebo nor valsartan significantly altered any study parameter except for fasting plasma insulin, which was significantly reduced by valsartan versus the effect of placebo. Concomitant with the reduction in insulin, levels of fasting glucose remained statistically unchanged and similar in both groups.

Table 3 demonstrates the vascular and hemodynamic responses to treatments. Baseline arterial diameter did not differ as a result of placebo or valsartan treatment. Baseline pretreatment FMD was low versus the historical normal value of nonobese healthy individuals of similar age in our vascular laboratory (FMD 7% to 10%).[1] However, there were no improvements in conduit artery endothelial func-

TABLE 1. PATIENT CHARACTERISTICS

Characteristics	*Total cohort Mean ± SD (n = 35)*
Age (years)	33 ± 10
Male/Female (n)	6/29
Body Mass Index (kg m^{-2})	33.6 ± 6.6
Body Weight (kg)	97 ± 22
Systolic Blood Pressure (mm Hg)	120 ± 12
Diastolic Blood Pressure (mm Hg)	69 ± 9
Waist (cm)	91 ± 19
Hip (cm)	111 ± 22
Waist circumference (cm)	100 ± 19

Table 2. Laboratory Changes from Baseline with Placebo and Valsartan. Values Represent the Mean = SD

	Baseline (n = 35)	*δ Valsartan*	*δ Placebo*	*n*	*P*
Glucose (mg/dL)	92 ± 10	−1.9 ± 9.5	−2.0 ± 12.8	31	0.927
Insulin (μU/mL)	23.0 ± 22.6	−4.6 ± 16.0	−0.4 ± 11.6	30	0.032*
HDL Cholesterol (mg/dL)	38.7 ± 9.9	−0.64 ± 4.7	0.1 ± 4.4	28	0.516
Triglyceride (mg/dL)	95.9 ± 39.6	6.7 ± 29.3	6.5 ± 31.4	28	0.979
Total LDL Particles (nmol/L)	1072 ± 285	4.5 ± 18.7	4.3 ± 19.3	28	0.952
Small LDL Particles (nmol/L)	593 ± 275	31 ± 211	13 ± 232	28	0.713
LDL Size (nm)	20.6 ± 3.5	0.7 ± 3.9	0.7 ± 3.8	29	0.655
HDL Size (nm)	8.9 ± 0.5	5.7 ± 6.3	6.6 ± 6.4	28	0.717
hs-CRP (mg/L)	7.5 ± 10.6	1.6 ± 7.8	0.4 ± 4.6	28	0.435
Leptin (ng/mL)	31.5 ± 10.1	−0.4 ± 5.6	−1.6 ± 5.4	25	0.126
Adiponectin (μg/mL)	8.7 ± 3.0	−0.2 ± 1.5	−0.4 ± 1.4	25	0.604

P value is for paired t-tests of the delta group differences in outcomes.

tion (FMD), smooth muscle function (NMD), or large and small vessel arterial compliance following valsartan or placebo. We planned tonometry for all subjects, but the measurement device was unavailable for some subjects, which explains the different sample size in the results. There was no difference in variables between subjects who had and did not have tonometry evaluated. Results were not different if we compared mean final FMD results head-to-head via paired t-tests rather than the change in response from baseline.

Based on previous reports,[1] we have shown that subjects with a high abdominal adiposity (waist to hip ratio over 0.85) have a greater impairment in FMD. We therefore performed a secondary analysis among these centrally obese subjects. Results in this specific subgroup (n = 18) showed a significant improvement in FMD with valsartan versus placebo (ΔFMD 1.7 = 8.8% versus −1.9 = 8.7%, $P = 0.044$).

As expected, blood pressure tended to be decreased in the valsartan limb, while systemic vascular resistance was significantly reduced. There were no reported adverse patient events in either study limb or discontinuations due to abnormalities in laboratory safety monitoring (potassium and creatinine).

DISCUSSION

AT2 receptor blockade did not improve the conduit arterial endothelial dysfunction or the impaired vascular compliance that is directly caused by uncomplicated obesity. This does not support the hypothesis that RAS-hyperactivity is a central underlying cause of the vascular dysfunctions due solely to obesity. However, among individuals with central adiposity, RAS-hyperactivity may be directly linked with impaired vascular function. The finding that

Table 3. Vascular Changes from Baseline with Placebo and Valsartan.

	Baseline (n = 35)	*δ Valsartan*	*δ Placebo*	*n*	*P*
Flow-mediated dilatation (%)	5.6 ± 5.7	2.0± 9.5	0.7 ± 8.5	31	0.382
Nitroglycerin-mediated dilation (%)	19.9 ± 6.4	−0.71 ± 0.1	0.97 ± 6.7	28	0.405
Systemic Vascular Resistance (dynes · sec · cm^{-5})	1036 ± 186	−59 ± 25	43 ± 206	15	0.032*
Large Vessel Compliance (10·ml·mm Hg^{-1})	21.1 ± 22.4	−7.5 ± 30.7	−8.2 ± 31.8	15	0.423
Small Vessel Compliance (100·ml·mm Hg^{-1})	10.0 ± 3.7	1.9 ± 2.4	−1.1 ± 3.4	15	0.802
Systolic Blood Pressure (mm Hg)	120 ± 12	−2.8 ± 9.3	0.5 ± 9.8	24	0.108
Diastolic Blood Pressure (mm Hg)	69 ± 9	−1.4 ± 12.1	1.5 ± 10.2	24	0.186

Values represent the mean ± SD

P value is for paired t-tests of the delta group differences in outcomes.

valsartan improved FMD in this subgroup requires confirmation in future studies. Nevertheless, our results confirm and extend previous observations that AT2 blockade improves metabolic insulin sensitivity (as determined by a reduction in fasting plasma insulin concomitant with a stable glucose). [9,12] The novel finding of this study is that this beneficial action occurred even in normoglycemic, normotensive healthy overweight adults without the metabolic syndrome. This suggests that RAS-hyperactivity may play a role in the mechanisms of insulin resistance even in uncomplicated obesity. Furthermore, it can be speculated that valsartan may be beneficial in the prevention of diabetes even at the very earliest stages of risk, such as overweight individuals with normal glucose and blood pressure values.

Obesity directly causes endothelial dysfunction by a variety of mechanisms.[1–3] Our finding of a reduced brachial FMD in the study subjects compared to the usual normal values of healthy non-obese adults in our laboratory (FMD = 7%–10%) confirms this association.[1] Among the variety of biological explanations, we investigated the direct role of RAS-hyperactivity due to obesity. [5–8] It was hypothesized that healthy overweight individuals may have impaired vascular function triggered by higher-than-normal vascular AT2 activity from either a systemic hormonal effect from visceral adipocyte release of angiotensinogen or AT2 and/or from RAS-hyperactivity within local peri-vascular fat. The lack of increase in brachial FMD after effective AT2 blockade with valsartan (demonstrated by reduced blood pressure and vascular resistance) does not support this primary hypothesis. In addition, if subtle defects in insulin signaling (vascular and/or metabolic insulin resistance) secondarily trigger the endothelial dysfunction of uncomplicated obesity, we would have anticipated a parallel increase in both insulin sensitivity and vascular function, which was not found. The negative findings of this study suggest that other mechanisms related to excess adiposity must be promoting the vascular dysfunction in overweight individuals without the metabolic syndrome.

AT2 blockade has been shown to improve metabolic insulin sensitivity by several putative mechanisms and to prevent the onset of diabetes mellitus among hypertensive individuals. [9,12] As far as we are aware, our findings are the first to demonstrate that AT2 blockade can enhance insulin sensitivity, as determined by a reduction in fasting insulin with stable blood glucose, in otherwise healthy normoglycemic overweight adults in the absence of the metabolic syndrome. Weight loss, dietary changes, and exercise may prevent or delay diabetes in at-risk patients. Long term adherence to lifestyle changes is likely to be poor. These findings provide evidence that valsartan, a safe and well-tolerated medication, may be effective for the prevention of diabetes even among healthy overweight adults at the earliest stages of disease risk.

Brachial FMD nonsignificantly trended toward an increase in the valsartan limb. The study may have been underpowered to detect small improvements. We had anticipated a smaller standard deviation (4.0% versus 9.0%) in the FMD treatment change. Post hoc power calculations demonstrate that with this sample size we could determine with 80% power a 4.5% change in FMD. It is possible that smaller, yet physiological meaningful improvements in endothelial function (i.e., ΔFMD of 2.0%) did occur by AT2 blockade (type 2 error). Nevertheless, many studies of similar size and duration have demonstrated improvements in endothelial function when clinically relevant in other disease states. Any change in this study was thus of relatively small magnitude. Larger studies may settle the issue whether inadequate sample size is to blame for the negative finding, or if this truly reflects that 1 or more other biological mechanisms are more primarily responsible for linking uncomplicated obesity with endothelial dysfunction.[3] It is unlikely that the dose and duration of valsartan treatment was inadequate to test this hypothesis, as both the blood pressure and vascular resistance reductions demonstrated a physiological effect of the medication. However, in theory a study of longer duration and/or using a higher dose of valsartan could be required to yield improvements in FMD. We also can not exclude the possibility that differences in the phase of menstrual cycle during testing biased results to the null. In addition, gold standard methods

(e.g., glucose clamp) to measure metabolic insulin sensitivity were not performed. A reduction in fasting plasma insulin (with a stable blood glucose) is an indirect measure of metabolic insulin sensitivity with several limitations and cannot distinguish the biological etiology of the metabolic change (i.e., altered insulin secretion versus improved systemic responsiveness). However, it has been demonstrated that fasting insulin is a valid and useful measures of insulin sensitivity that provides most of the relevant physiological information provided by more invasive techniques. [13–15] Calculated indices such as glucose/insulin or $HOMA_{IR}$ have been shown to not add to the accuracy of fasting insulin concentration to predict metabolic insulin sensitivity in nondiabetic patients with a stable blood glucose[12,13,14] and were not performed. Therefore, we believe the reduction in fasting insulin by valsartan does represent a meaningful improvement in insulin sensitivity for the methodological reasons listed and because it corroborates the many previous studies that have confirmed that RAS-blockade does indeed enhance peripheral insulin utilization along with metabolic insulin sensitivity.

The results from a secondary analysis among patients with an elevated waist to hip ratio are interesting. It is possible that patients with abdominal obesity have an activated RAS that is directly causing systemic vascular function and will therefore show improved FMD with valsartan treatment. Had we included only patients with an increased waist to hip ratio over 0.85 (as per the associations found in our initial study)[1], the results linking FMD with blockade of RAS may have been positive for the entire overall study cohort. However, this was a not a pre-specified analysis and will require further investigation.

Our results do not support the hypothesis that hyperactivity of RAS plays a central role in the vascular dysfunctions due solely to uncomplicated obesity. Further studies are warranted among patients with excess abdominal adiposity. The finding that metabolic insulin sensitivity improves in this healthy group of normoglycemic overweight adults without the metabolic syndrome supports the notion that the RAS may play a role in obesity-related insulin resistance and that valsartan may be an effective preventative modality even at the earliest stages of diabetes risk.

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