Resistance to Acute Insulin Induced Decreases in Large Artery Stiffness Accompanies the Insulin Resistance Syndrome

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Arterial stiffness has recently been recognized as an important cardiovascular risk marker. Physiological concentrations of insulin diminish wave reflection in the aorta in vivo. This decreases central blood pressure augmentation and augmentation divided by pulse pressure (the augmentation index (AgI)), a measure of arterial stiffness. In the present study, we examined whether a defect in this action of insulin is a feature of insulin resistance and how it relates to other acute actions of insulin, including stimulation of glucose uptake, peripheral blood flow, and autonomic control of heart rate variation. These actions of insulin were quantitated in 50 healthy men (age, 34 ± 3 yr; body mass index, 27 ± 1 kg/m²) during 2 sequential insulin infusions, each lasting 120 min (1 and 2 mU/kg/min). Insulin decreased AgI significantly within 30 min, whereas significant increases in peripheral blood flow and normalized low frequency power of heart rate variation, a measure of sympathetic control of heart rate variation, were observed at 150 and 210 min. A blunted decrease in the AgI was significantly associated with a low rate of insulin-stimulated glucose uptake, but not with the other actions of insulin. Insulin action of the AgI was correlated with body mass index and the waist to hip ratio independently of basal AgI, age, and low density lipoprotein cholesterol. We conclude that physiological concentrations of insulin diminish large artery stiffness within 30 min in nondiabetic men. This action precedes insulin action on peripheral vasoconstriction, heart rate, and autonomic control of heart rate variation. It is correlated with insulin stimulation of glucose uptake and is blunted by known causes of insulin resistance, including overall and abdominal obesity. Resistance of large arteries to insulin-induced decrease in their stiffness is therefore another facet of insulin resistance that could contribute to the association between insulin resistance and cardiovascular disease. (J Clin Endocrinol Metab 86: 5262–5268, 2001)

REANALYSIS OF MAJOR epidemiological studies have demonstrated arterial stiffness to be a major cardiovascular risk marker (1, 2). All classic risk factors, including age (3, 4), smoking (5–7), low density lipoprotein (LDL) cholesterol (8, 9), and hypertension (10, 11), are determinants of arterial stiffness. In the Atherosclerosis Risk in Communities Study, the fasting insulin concentration was positively correlated with several indexes of arterial stiffness (12). This relationship was independent of age, cigarette-years, and cholesterol and suggested, assuming fasting hyperinsulinemia to be a marker of insulin resistance, that insulin resistance may contribute to stiffening of large arteries. We have recently shown that insulin acutely (within 30–60 min) markedly decreases large artery stiffness, as determined from changes in aortic augmentation and the augmentation index (AgI) in healthy young male subjects (13, 14). In the present study we wanted to extend these observations to a larger group of subjects, who had various features of insulin resistance. Our aim was, first, to determine which cardiovascular risk factors are associated with insulin-induced changes in arterial stiffness. Second, we wanted to determine how insulin action on the AgI is temporally related to other nonglycemic actions of insulin, including autonomic control of heart rate variation and peripheral blood flow.

Subjects and Methods

Subjects

The characteristics of the 50 men studied are shown in Table 1. The subjects were recruited from occupational health care units of 3 different companies. The inclusion criteria were 1) male gender, 2) age between 18–60 yr, 3) no clinical or laboratory evidence of any disease based on annual examinations performed at the occupational health service, 4) no regular medication, 5) nonsmoker, and 6) able to give informed consent. For 2 d before the study, the subjects consumed a weight-maintaining diet containing at least 200 g carbohydrate/d. Written informed consent was obtained after the purpose, nature, and potential risks had been explained to the subjects. The experimental protocol was approved by the ethical committee of the Department of Medicine, Helsinki University Central Hospital.

Design

Vascular (pulse-wave analysis to determine the AgI, peripheral blood flow), metabolic (whole body glucose uptake), and neural (power spectral analysis of heart rate variability) parameters were determined basally (1 h) and under normoglycemic hyperinsulinemic conditions [insulin infusion rates, 1 (step I) and 2 (step II) mU/kg/min for 120 min each], which were maintained using the euglycemic insulin clamp technique (15). We did not perform a time control study as we have previously demonstrated that both heart rate and blood pressure, augmentation and AgI (13) remain unchanged during a 6-h infusion of saline and sampling of arterialized venous blood from a heated contralateral hand vein in normal subjects.

The study was performed after an overnight fast starting at 0730 h. Two 18-gauge catheters (Venflon, Viggo-Spectramed, Helsingborg, Sweden) were inserted as previously described (16). Insulin and glucose were infused in a catheter inserted in the left antecubital vein. The left hand was kept in a heated chamber (65 °C), and arterialized venous blood

Abbreviations: AgI, Augmentation index; HF, high frequency power; GTN, glyceryltrinitrate; LDL, low density lipoprotein; LF, low frequency power; MSNA, muscle sympathetic neural activation; NO, nitric oxide.
was withdrawn from a heated dorsal hand vein for measurement of plasma glucose and serum insulin concentrations. During the basal and hyperinsulinemic periods, measurements of the AgI (pulse wave analysis), peripheral (forearm) blood flow, and sympathetic nervous system activity were performed at 30-min intervals as detailed below. Whole body glucose uptake was determined from the glucose infusion rate required to maintain normoglycemia corrected for changes in the glucose pool size (M-value) between 30–120 min (step I) and 120–240 min (step II) (15).

**Insulin action on AgI**

The technique of pulse wave analysis was used to determine aortic pressure and AgI (17). All measurements were made by the same observer from the radial artery, with the wrist slightly extended and supported on a pillow, by application tonometry using a Millar tonometer (SPC-301, Millar Instruments, Houston, TX). Data were collected into a desk-top computer and processed with software (SphygmoCor, San Diego, CA). The aortic waveform was then subject to further analysis for calculation of aortic augmentation. The aortic waveform was then subject to further analysis for calculation of aortic augmentation, AgI, and aortic blood flow. The AgI was calculated by dividing augmentation with pulse pressure and provides a measure of global stiffness under conditions where ejection duration and heart rate are unchanged (3, 17, 20).

**Insulin action on peripheral blood flow and vascular resistance**

Peripheral blood flow was measured in the forearm using venous occlusion plethysmography with mercury in SILASTIC rubber strain-gauge apparatus (model EC-4, Hokanson, Bellevue, WA), a rapid cuff inflator (Rapid Cuff Inflator model E20, Hokanson), and computerized analysis of flow curves (MacLab/4e, AD Instruments, Castle Hill, Australia) (16). Peripheral vascular resistance was calculated by dividing mean arterial pressure in the brachial artery by forearm blood flow.

**Insulin action on autonomic control of heart rate variation**

Insulin action on components of heart rate variation was determined using frequency domain analysis (21, 22). R-R intervals were continuously recorded for 5 min every 30 min while the subject’s breathing was paced using a sound signal to denote inspiration and expiration every 2 sec. Frequency domain analysis of R-R interval variability was performed using the CAFTS system (Medikro Oy, Kuopio, Finland). After detrending the R-R interval signal, a least mean square autoregressive model with a model order of 14 was used to obtain the power spectral estimate of R-R interval variability. Total power was determined in the frequency range from 0–0.5 Hz. Low frequency power (LF) was determined in the frequency range from 0.04–0.15 Hz. This component reflects predominantly sympathetic control of heart rate variation (21), when expressed in normalized units. High frequency power (HF) was determined in the frequency range 0.15–0.40 Hz. This component reflects vagal control of heart rate variation (21, 22). The signal powers were calculated as integrals under the respective part of the power spectral density function and were expressed in normalized units [LF or HF divided by total power – very low (<0.04 Hz) power x 100] and as a ratio (LF/HF), which reflects sympathovagal balance (21, 23).

**Other measurements**

Fat-free mass (FFM) and the percent body fat were determined using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model BIA-101A, RJL Systems, Detroit, MI) (24). Serum free insulin concentrations were measured before and at 30-min intervals during the insulin infusion by double antibody RIA (insulin RIA kit, Pharmacia Biotech, Uppsala, Sweden) after precipitation with polyethylene glycol (25). The plasma glucose concentration was measured in duplicate with the glucose oxidase method (26) using a Glucose Analyzer II (Beckman Coulter, Inc., Fullerton, CA). Hemoglobin A1c was measured by HPLC using a fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad Laboratories, Inc., Richmond, CA).

**Statistical analysis**

The actions of insulin at different time points were compared using ANOVA for repeated measures, followed by Bonferroni’s multiple comparison post-hoc test. Simple correlations between normally distributed study variables were calculated using Pearson’s correlation coefficient. All calculations were made using the Systat statistical package (Systat, Evanston, IL). Data are expressed as the mean ± SEM. P < 0.05 was considered statistically significant.

**Results**

**Metabolic and hemodynamic effects of insulin**

Serum insulin concentrations increased from 48 ± 5 basally to 408 ± 10 pmol/liter during the physiological dose (step I, 30–120 min) and to 952 ± 10 pmol/liter during the supraphysiological dose (step II, 120–240 min) insulin infusion. Plasma glucose concentrations averaged 5.6 ± 0.1, 5.3 ± 0.1, and 5.2 ± 0.1 mmol/liter, respectively. The M-values averaged 26 ± 2 and 49 ± 3 μmol/kg·min or, when expressed per FFM, 31 ± 2 and 60 ± 3 μmol/kg·FFM·min during steps I and II, respectively.

**Augmentation and the AgI.** Augmentation, i.e. the pressure difference between the second and first systolic pressure peaks in the ascending aorta, decreased significantly within 30 min from 4.1 ± 0.9 to 2.6 ± 0.9 mm Hg (P < 0.001). Augmentation averaged 2.2 ± 0.8 mm Hg during step I and 1.0 ± 0.9 mm Hg during step II. The AgI decreased significantly within 30 min from 9.2 ± 2.0% basally to 5.5 ± 2.0% at 30 min (P < 0.001; Fig. 1). AgI averaged 4.4 ± 2.1% and 0.9 ± 2.1% during steps I and II.

**Peripheral blood flow and peripheral vascular resistance.** Forearm blood flow increased linearly and within 4 h 1.4-fold from 2.5 ± 0.1 basally to 3.6 ± 0.2 ml/dl·min at 240 min (Fig. 1; P < 0.001). The first significant increase was observed during step II at 210 min (3.5 ± 0.3 ml/dl·min; P < 0.001, 0 vs. 210 min). Peripheral vascular resistance averaged 42 ± 2 mm Hg/
(ml/dl-min) basally, 40 ± 2 at 120 min (P = NS vs. basal), and 28 ± 2 at 240 min (P < 0.001 vs. 120 min).

Other hemodynamic parameters. Heart rate remained unchanged during step I (60 ± 1 vs. 61 ± 1 beats/min; 0 min vs. step I), but increased significantly from 150 min onward (63 ± 1 beats/min at 150 min; P < 0.001 vs. 0 min; Fig. 2). Brachial artery systolic blood pressures remained unchanged during step I (Fig. 3), but increased significantly at 180 min during step II (126 ± 2 vs. 130 ± 2, 0 vs. 180 min; P < 0.001).

Although brachial artery systolic blood pressure increased, aortic systolic blood pressure remained constant during the entire study (114 ± 2 vs. 112 ± 2 mm Hg, 0 vs. 120 min; P = NS; Fig. 3). Brachial diastolic blood pressure was unchanged during step I, but then decreased significantly at 150 min (79 ± 1 vs. 76 ± 2 mm Hg, 0 vs. 150 min; P < 0.001; Fig. 3). Aortic diastolic blood pressure remained unchanged during step I (80 ± 2 vs. 78 ± 2 mm Hg, 0 vs. 120 min), but decreased significantly at 150 min (77 ± 2 mm Hg; P < 0.001 vs. 0 min; Fig. 3).

Insulin action on autonomic control of heart rate variation

There were no significant changes in parameters reflecting autonomic control of heart rate variation during step I (Fig. 2). During step II at 210 min, a significant increase was observed in normalized LF (45 ± 5 vs. 55 ± 4, 0 vs. 210 min; P < 0.05). Normalized HF decreased significantly during step II (49 ± 3 vs. 42 ± 3, 0 vs. 150 min; P < 0.05). The LF/HF ratio increased significantly and averaged 1.19 ± 0.17 basally and 2.21 ± 0.51 at 210 min (P < 0.05). There were no significant relationships between normalized LF or HF or their ratio and the AgI either basally or during hyperinsulinemia or between changes in these parameters by insulin (data not shown).
Factors associated with variation in the Agl

Basal Agl. In simple regression analysis, significant correlates of basal Agl included age (Fig. 4), mean arterial blood pressure, and LDL cholesterol (Table 2). In addition, insulin sensitivity [M-values (expressed in micromoles per kg FFM/min) during steps I and II], waist to hip ratio, and HDL cholesterol, but not weight or body mass index, were significantly correlated with Agl. Height was inversely and hemoglobin A1c was positively correlated with basal Agl. After adjustment for age, none of these associations was significant except for height, which remained marginally significant (Table 2). This was because age was closely correlated with both LDL cholesterol (r = 0.55; P < 0.001), mean arterial blood pressure (r = 0.58; P < 0.001), and features of insulin resistance (waist to hip ratio: r = 0.54; P < 0.001), including insulin sensitivity of glucose uptake (M-value step I: r = −0.47; P < 0.001; step II: r = −0.45; P < 0.001).

Insulin-induced changes in Agl. None of the classic risk factors or other known determinants of arterial stiffness were correlated with changes in the Agl (Table 2), whereas several causes or consequences of insulin resistance were. The latter included insulin action on glucose metabolism, as assessed from the M-value during both steps I and II, weight, body mass index, and the waist to hip ratio (Table 2 and Fig. 5).

Discussion

In the present study we searched for causes of variation in insulin action on arterial stiffness in a group of 50 nondiabetic, nonsmoking men who were characterized by a wide variation in various cardiovascular risk factors, including those associated with insulin resistance. Basal Agl, a measure of arterial stiffness, was correlated with classic cardiovascular risk factors, including age, mean arterial blood pressure, and LDL cholesterol as well as with insulin sensitivity determined from the rate of whole body glucose uptake, HDL cholesterol, and the waist to hip ratio. In contrast to basal Agl, its change induced by insulin was not correlated with age or other classic risk factors, but instead with a classic action of insulin, the rate of whole body glucose uptake and with other features of the insulin resistance syndrome.

We found insulin sensitivity, as quantified directly using the euglycemic insulin clamp technique, to be significantly inversely correlated with both age and stiffness, but the relationship between insulin sensitivity and stiffness was not independent of age. This may be because the age range of our subjects was wide enough (18–60 y) to influence insulin sensitivity (27, 28). Whether aging influences insulin sensitivity via changes in body weight or composition or a decrease in physical fitness or decreases insulin sensitivity independent of these factors is unclear (28–32). Consistent with the idea that insulin sensitivity is related to stiffness, also independent of age, in studies in which subjects have all been of the same age (6, 9) or in which the study population has been very large, as in the ARIC study (12), serum insulin concentrations have been independent determinants of arterial stiffness. In the ARIC study the relationship between serum insulin and various stiffness indexes was not only independent of age, cigarette-years, and total cholesterol, but in white men and women this association was also independent of obesity, lipids, and hypertension (12). In studies where the age range and the number of subjects studied were comparable to that in the present study, an age-independent relationship between serum insulin and stiffness has not been demonstrated (33, 34).

As in a previous study in a small group of men, we found marked temporal dissociation between insulin action on the Agl compared with insulin action on peripheral blood flow. The slow peripheral vasodilating effect is consistent with several previous studies (see Ref. 35 for review). Consistent with the idea that peripheral vasodilatation is the cause of a decrease in diastolic blood pressure and that this increases heart rate via baroreflex mechanism, there were no changes in blood flow, diastolic blood pressure, or heart rate during the first 2 h during which the insulin concentrations were maintained in the physiological range with the 1 mU/kg/min insulin infusion. Under such conditions, the Agl can only change as a consequence of a change in stiffness (17). During the second supraphysiologic insulin dose, the increase in heart rate and the decrease in peripheral vascular resistance could both have contributed to the decrease in the Agl.

In the present study we confirmed our previous finding of acute diminution of the Agl by insulin in a small group of young men (13, 14) in a larger group of nondiabetic middle-aged men. This larger group also allowed us to search for factors associated with insulin action on stiffness. As in previous studies (3, 9–11, 33) using the Agl or other measures of arterial stiffness, age, LDL cholesterol, and blood pressure were closely correlated with basal Agl. Also, components of insulin resistance, including a low M-value or HDL cholesterol and high waist to hip ratio, were significantly related to

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<th>TABLE 2. Simple regression analysis of determinants of the basal augmentation index (Agl) and the change in the Agl by insulin</th>
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<td>Change in peripheral</td>
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<td>blood flow*</td>
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Pearson’s correlation coefficients for the total group are shown (n = 50).

a During the physiological insulin infusion (step I).

b P < 0.001.
c 0.1 > P > 0.05.
d P < 0.05.

e P < 0.01.
basal AgI. However, after adjusting for age, all relationships became nonsignificant, possibly because aging is associated with increase in blood pressure, obesity, and progressive insulin resistance. Study of individuals within a narrow age range might have allowed better identification of other determinants of stiffness than age.

None of the classic cardiovascular risk factors (age, LDL cholesterol, blood pressure) were significantly correlated with arterial stiffness. In contrast, the change in AgI was significantly correlated with features of insulin resistance, including insulin sensitivity (M-value) itself, weight, body mass index, and the waist to hip ratio. As the decrease in AgI occurred at a physiological insulin concentration and within a physiological time frame, these data suggest that resistance of AgI to insulin may stiffen arteries postprandially and perhaps also contribute to a variation in basal stiffness. It is of interest that in insulin-resistant and obese Zucker fatty rats, aortas studied in vitro exhibit resistance to insulin stimulation of phosphatidylinositol 3-kinase activity, which is critical for insulin induced increase in nitric oxide (NO) synthesis in endothelial cells (36). A potent NO donor, sublingual glyceryl trinitrate (GTN), was shown already over 100 yr ago to acutely decrease arterial stiffness (AgI) without changing peripheral blood pressure or heart rate (37, 38). The effect of GTN resembles that of insulin, although GTN acts faster than insulin. It is, however, at present unknown whether insulin diminishes stiffness via an NO-dependent mechanism. In peripheral resistance vessels, which respond to insulin clearly slower than preresistance vessels (35), insulin-induced vasodilatation can be blocked with NO-monomethyl-L-arginine, an inhibitor of NO synthesis (39, 40).

One of the many actions of insulin is to stimulate the autonomic nervous system. This has been documented in various tissues and with different techniques (41). β-Blockade increases wave reflection and the AgI (42). This effect could, however, be explained by inhibition of β-mediated peripheral vasodilation, i.e., an increase in peripheral vascular resistance (42). These data prompted us to establish whether insulin-induced sympathetic activation is temporally associated or correlated with insulin action on stiffness. We chose to monitor insulin-induced changes in components of heart rate variation as determined by frequency domain analysis. The HF component reflects efferent vagal activity and specifically responds to stimuli such as vagotomy, muscarinic receptor blockade, and electrical vagal stimulation (43–45). The LF component has been shown to correlate with muscle sympathetic neural activation (MSNA) during sympathetic stimulation (46), and the normalized LF component is higher in obese than nonobese subjects, as is MSNA under fasting conditions (47, 48). The normalized LF component and MSNA both fail to respond to insulin in obese subjects, suggesting that the autonomic nervous system is insulin resistant (47, 48). The anatomical location where insulin stimulates activity of the sympathetic nervous system is unclear. One study suggested the effect is centrally mediated, as iv, but not intraarterial, infusions of insulin increased the nor-
epinephrine spillover rate (49). At least two mechanisms could increase sympathetic nervous activity under hyperinsulinemic conditions. First, peripheral vasodilatation will diminish venous return and cardiac output as well as arterial blood pressure (50), which will stimulate baroreceptors, resulting in increased sympathetic vasomotor outflow in both veins and arteries (50, 51). The other possibility is that insulin stimulates sympathetic nervous activity independent of any hemodynamic changes (52). In the present study neither heart rate nor normalized LF or HF components changed significantly during the first 120 min of insulin infusion in the entire study group, which implies that changes in components of heart rate variation did not contribute to changes in the AgI and were temporally dissociated from changes in the AgI. Thus, the normalized LF and HF components were apparently less sensitive to insulin than the AgI, as a supraphysiological dose of insulin was required to observe a significant change. The group of 50 men included both insulin-sensitive and -resistant obese subjects, which may explain why it took over 120 min to observe significant changes in LF or HF components. In previous studies including lean young men, insulin has been reported to change LF and HF components after 30 min (47, 48, 53). The present finding of no temporal relationship between changes in stiffness and sympathetic nervous system activity is in keeping with data reported by Sonesson et al. who measured stiffness using an ultrasonic echotracking system in the abdominal aorta during lower body negative pressure induced sympathetic activation, and found no change in aortic wall mechanics (54). Also, in vitro, the influence of sympathetic stimulation on the aorta in humans has been considered insignificant (55, 56).

Several large epidemiological studies examining the relationships between diastolic, systolic and pulse pressures and the risk of cardiovascular disease have recently emphasized the high risk associated with an increase in the pulsatile component of blood pressure, pulse pressure (2, 57, 58). Data from the Framingham Heart Study (1), Physicians’ Health Study (59) and other studies (60) have shown that diastolic blood pressure increases until the age of approximately 60 yr and then decreases, whereas pulse pressure and systolic blood pressure increase steadily. Systolic and pulse pressures also are better predictors of cardiovascular events than diastolic blood pressure in older subjects (59). On the other hand, causes of insulin resistance, such as overall (61) and abdominal obesity (62), and consequences, such as dyslipidemia (high triglycerides and low HDL cholesterol) (63), also are independent risk factors for cardiovascular disease. Data from the present study suggest that one mechanism that may link insulin resistance and cardiovascular disease is the inability of insulin to diminish stiffness in insulin-resistant subjects.

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