Diminished Wave Reflection in the Aorta
A Novel Physiological Action of Insulin on Large Blood Vessels

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Abstract—Epidemiological data suggest that insulin may have direct effects on large-vessel function, but thus far insulin has only been shown, after prolonged infusions, to slowly decrease peripheral vascular resistance by increasing muscle blood flow. We determined whether physiological doses of insulin affect function of large arteries, before any changes in peripheral blood flow, in vivo using pulse wave analysis. Nine normal men were studied on 2 occasions: once during a 6-hour infusion of saline and once under normoglycemic hyperinsulinemic conditions (sequential 2-hour insulin infusions of 1, 2, and 5 mU/kg · min). Central aortic pressure waves were synthesized from those recorded in the periphery with the use of applanation tonometry and a validated reverse transfer function every 30 minutes. This allowed determination of central aortic augmentation (the pressure difference between early and late systolic pressure peaks) and augmentation index (augmentation expressed as a percentage of pulse pressure). Both augmentation and augmentation index decreased significantly within 1 hour after administration of insulin (P<0.001) but not saline. Systolic and diastolic blood pressure and heart rate remained unchanged for the first 2 hours. A significant increase in peripheral (forearm) blood flow was not observed until 2.5 hours after start of the insulin infusion. These data demonstrate that insulin, in normal subjects, rapidly decreases wave reflection in the aorta. This beneficial effect is consistent with increased distensibility or vasodilatation of large arteries. In contrast to the effect of insulin on peripheral blood flow, this action of insulin is observed under conditions in which both the insulin dose and duration of insulin exposure are physiological. Resistance to this action of insulin could provide a mechanism linking insulin resistance and conditions such as hypertension at the level of large arteries. (Hypertension. 1999;33:1118-1122.)

Key Words: blood pressure ■ circulation ■ pulse wave analysis ■ insulin ■ vascular resistance

In addition to its metabolic effects, insulin increases blood flow in skeletal muscle by a mechanism that can be blocked by inhibiting nitric oxide synthesis.1 The ability of insulin to increase peripheral blood flow increases with time and increasing insulin concentrations.2 At physiological insulin concentrations such as those prevailing during a 1 mU/kg · min insulin infusion, however, insulin increases glucose extraction in normal subjects ≈10-fold but produces no or only a trivial increase in limb blood flow within a physiological time frame.3,4 This interpretation is consistent with an extensive analysis of literature demonstrating defects in insulin-stimulated blood flow at supraphysiological insulin concentrations after prolonged insulin infusions in various insulin-resistant conditions.2 At physiological insulin concentrations, defects in glucose extraction appear to be responsible for defects in insulin-stimulated glucose uptake.2

Even if defects in insulin stimulation of peripheral blood flow do not explain insulin resistance of glucose uptake, defects in the vascular actions of insulin could be of relevance in understanding the role of insulin resistance in the pathogenesis of vascular disease. If resistance to the vascular effects of insulin were to contribute to vascular disease, then insulin should regulate vascular function at concentrations lower than those required to increase muscle blood flow. Studies comparing arterial reactivity have shown marked size-dependent variation in the contribution of various endothelium-dependent mediators to vasodilatation.5 In vitro, the contribution of nitric oxide to vasodilatation has been shown to be significantly greater in large arteries than in distal microvessels isolated from human tissues.5

Consistent with heterogeneity among vessels in sensitivity to vasoactive agents in vivo, a low dose of glyceryl trinitrate significantly increases brachial artery diameter but has no effect on systolic or diastolic blood pressure or peripheral resistance.6,7 Similarly, antihypertensive drugs such as angiotensin-converting enzyme inhibitors can decrease the pulsatile pressure load in central arteries, without changing systolic...
or diastolic blood pressure in the brachial artery. It is currently unknown whether insulin affects function of arteries greater than those regulating peripheral vascular resistance.

After the development of pulse wave analysis by O’Rourke and Gallagher, central pressures can now be assessed non-invasively. The system uses the principle of applanation tonometry to accurately record peripheral arterial waveforms. By applying a validated integral transfer function, the central aortic waveform can be derived and analyzed. Pressure waves are reflected back from the periphery and summated with the forward-going wave to produce the characteristic pressure waveform, the contour of which varies along the vascular tree. With vascular stiffening, pulse wave velocity and the amplitude of the reflected wave both increase, such that the reflected wave arrives back earlier. In stiff arteries the amplitude of the reflected wave may exceed that of the first systolic wave. When this happens, the reflected wave adds to (or augments) central systolic pressure. Augmentation is defined as the pressure difference between the first and second systolic peaks (Figure 1) and is determined both by large-artery compliance (diameter and distensibility), and by peripheral resistance. An increase in conduit artery distensibility or diameter or a decrease in peripheral vascular resistance will reduce the amplitude of the reflected wave and decrease augmentation. Augmentation index is obtained by dividing augmentation by pulse pressure and provides a measure of wave reflection when other relevant variables such as heart rate remain constant. In the present study we measured of wave reflection when other relevant variables such as heart rate remain constant. In the present study we determined whether insulin influences wave reflection in vivo in normal subjects by following augmentation and augmentation index during sequential insulin infusions. To define the temporal relationship between the effects of insulin on large-vessel function and peripheral resistance, forearm blood flow was also measured. All subjects participated in a control study, during which saline was infused instead of insulin.

Methods

Subjects

Nine normal men (age, 25±1 years; weight, 73.3±2.0 kg; body mass index, 23.1±0.5 kg/m²; mean±SEM) participated in the study. The subjects were healthy as judged by medical history and physical examination, ECG, and routine laboratory tests. Their fasting plasma glucose4,13 averaged 5.4 (SEM 6) mmol/L and glycosylated hemoglobin A1c (5.1±0.2%; reference range, 4% to 6%) concentrations were in the normal range. The subjects were not taking any medications. For 2 days before the studies, the subjects consumed a weight-maintaining diet containing ≥200 g of carbohydrate per day. 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.

Study Protocol

Each subject participated in a 6-hour sequential dose insulin clamp study and a 6-hour saline infusion control study. The studies were performed in random order within a week. Both studies began at 7:30 AM after an overnight fast.

Sequential Insulin Clamp Study

The study consisted of 3 sequential 2-hour insulin infusions at rates of 1 (step I), 2 (step II), and 5 mL/kg·min (step III) (2 hours each). Normoglycemia was maintained with the use of the euglycemic insulin clamp technique. Before and during the insulin infusions, hemodynamic measurements (forearm blood flow and vascular resistance, heart rate, pulse wave analysis) were performed at 30-minute intervals as detailed below.

Control Study

Each subject participated in a 6-hour control study, during which saline was infused in the left antecubital vein at a rate of 100 (step I), 200 (step II), and 300 mL/h (step III) (2 hours each) to match the volume infused during the clamp. During the control study, fasting plasma glucose averaged 5.4±0.1 and 4.9±0.1 mmol/L at 0 and 6 hours, respectively. Serum-free insulin concentrations (Pharmacia Insulin RIA kit, Pharmacia) averaged 22±4 and 12±3 pmol/L, respectively.

Pulse Wave Analysis

The technique of pulse wave analysis was used to determine central aortic pressure and augmentation index. All measurements were made from the radial artery, with the wrist slightly extended and supported on a pillow, by applanation tonometry with the use of a Millar tonometer (SPC-301; Millar Instruments). Data were collected directly into a desktop computer and processed with recently developed software (SphygmoCor Blood Pressure Analysis System BPAS-1; PWV Medical), which allows continuous online recording of the radial artery pressure waveform. The radial waveform was assessed visually to ensure that artifacts from movement and respiration were minimized. Recordings for pulse wave analysis were made twice basally and every 30 minutes during insulin infusions. The mean of 3 measurements, each consisting of 15 to 20 sequential radial artery waveforms, was used to calculate augmentation and other parameters at the given time point. The integral system software was used to first calculate an average radial artery waveform and then to generate the corresponding central ascending aortic pressure waveform using a previously validated transfer factor. The central aortic waveform was then subject to further analysis for calculation of aortic augmentation, aortic augmentation index, and central systolic and diastolic pressure. Augmentation index was defined as the ratio of the difference between central aortic second and first systolic pressure peaks to pulse pressure (systolic minus diastolic pressure) (Figure 1). As suggested by O’Rourke and Gallagher, the radial pressure was calibrated against the sphygmo-
Sequential Dose-Response Insulin Clamp Study

Three 18-gauge catheters (Venflon; Viggo-Spectramed) were inserted as previously described. 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 was withdrawn from a heated dorsal vein. The third catheter was inserted retrogradely in a median antecubital vein for measurement of glucose concentrations in venous blood–draining forearm muscles.

The euglycemic insulin clamp technique was used to assess tissue sensitivity to insulin as previously described. At each step, insulin (Actrapid Human, Novo Nordisk) was infused in a primed continuous manner at rates of 1 (0 to 120 minutes), 2 (120 to 240 minutes), and 5 (240 to 360 minutes) mU/kg × min. Serum-free insulin concentrations averaged 21 ± 3 (basal), 366 ± 16 (step I), 792 ± 30 (step II), and 2315 ± 193 (step III) pmol/L. Normoglycemia was maintained by adjusting the rate of a 20% glucose infusion on the basis of plasma glucose measurements performed at 5-minute intervals. Plasma glucose averaged 5.3 ± 0.1, 5.2 ± 0.1, and 5.2 ± 0.1 mmol/L during steps I, II, and III, respectively.

Forearm Blood Flow and Peripheral Vascular Resistance

Forearm blood flow was measured every 30 minutes with venous occlusion plethysmography with a mercury-in-Silastic rubber strain-gauge apparatus (model EC-4, Hokanson), a rapid cuff inflator (Rapid Cuff Inflator model E20, Hokanson), and computerized analysis of flow curves (MacLab/4e, AD Instruments). Peripheral vascular resistance was calculated by dividing mean arterial pressure in the brachial artery by blood flow.

Statistical Analysis

Reproducibility of augmentation and the augmentation index cannot be assessed with the coefficient of variation since the mean of both parameters oscillates around zero. We therefore calculated the coefficient of variation, as suggested by Hayward et al, from augmentation values defined as the ratio of the pressure at the second systolic peak to the pressure at the first systolic peak, since this definition gives a continuous positive value. Bland-Altman plots were used to assess the dependence of reproducibility on the mean. Statistical comparisons between saline and insulin studies were made with the use of ANOVA for repeated measures followed by the Bonferroni test. The best fit characterizing the relationship between hemodynamic parameters over time was determined by comparing the goodness of fit of linear and multiple nonlinear equations with the use of GraphPad Prism version 2.01 (GraphPad Software Inc). The results are expressed as mean ± SEM. Probability values <0.05 were considered statistically significant.

Results

Hemodynamic Parameters

In the insulin study, forearm blood flow averaged 2.7 ± 0.2 basally and 3.2 ± 0.3 (P = NS versus basal), 4.5 ± 0.4 (P < 0.01 versus basal and step I), and 5.7 ± 0.6 (P < 0.01 versus basal, step I, and step II) mL/dL × min during steps I, II, and III, respectively. The first significant increase was observed after 150 minutes (3.5 ± 0.3 mL/dL × min; P < 0.05 versus basal). A significant decrease in peripheral vascular resistance was observed at 180 minutes (Figure 2). Blood flow, blood pressure, and peripheral vascular resistance remained unchanged in the control study (Figures 2 and 3).

Pulse wave analysis showed that in the insulin study, central aortic augmentation, ie, the difference between the second and first systolic pressure peaks, decreased significantly by 60 minutes (Figure 2). Mean aortic augmentation averaged −1.1 ± 1.7 mm Hg basally, −2.6 ± 1.6 mm Hg during step I (P < 0.001 versus basal, P < 0.01 for insulin versus saline study), −3.6 ± 1.6 mm Hg during step II (P < 0.001 versus basal, P = NS versus step I, P < 0.01 for insulin versus saline study), and −4.6 ± 0.4 mm Hg during step III (P < 0.001 versus basal, P = NS versus step I, P = NS versus step II, P < 0.01 for insulin versus saline study). This decrease could not be attributed to the decrease in peripheral vascular resistance since aortic augmentation index, ie, the ratio between augmentation and aortic pulse pressure, also was significantly decreased at 60 minutes (Figure 2). Augmentation index averaged −3.2 ± 5.2% basally, −9.0 ± 5.3% during
step I ($P<0.001$ versus basal, $P<0.01$ for insulin versus saline study), $-10.2\pm4.6\%$ during step II ($P<0.001$ versus basal, $P=NS$ versus step I, $P<0.01$ for insulin versus saline study), and $-11.4\pm4.4\%$ during step III ($P<0.001$ versus basal, $P<0.05$ versus step I, $P=NS$ versus step III, $P<0.01$ for insulin versus saline study) (Figure 2). In the control study, both augmentation and augmentation index remained stable over time (Figure 2). The coefficient of variation of augmentation index, defined as described in Methods, averaged $5\pm1\%$. The individual mean augmentations ranged from $-6.3$ to $6.5$ mm Hg and the standard deviations from 0.6 to 1.4 mm Hg. Bland-Altman plots did not reveal any trend for the difference to be dependent on the mean value.

Heart rate remained unchanged for the first 120 minutes (step I). It averaged $55\pm3$, $57\pm3$, $60\pm4$ ($P<0.05$ versus basal), and $61\pm3$ ($P<0.05$ versus basal and step I) bpm basally and during steps I, II, and III, respectively. Diastolic blood pressure, as measured in the brachial artery, remained unchanged for the first 120 minutes and decreased thereafter (Figure 3). It averaged $66\pm3$, $68\pm3$, $62\pm3$ ($P<0.05$ versus basal and step I), and $60\pm3$ ($P<0.05$ versus basal and step I) mm Hg, respectively. Central diastolic blood pressure showed a similar pattern and averaged $67\pm3$ basally, $68\pm2$ during step I, $63\pm2$ during step II ($P<0.05$ versus basal), and $63\pm2$ mm Hg during step III ($P<0.05$ versus basal). Brachial artery systolic blood pressure averaged $114\pm3$ basally, remained unchanged during step I ($117\pm3$), but increased thereafter ($123\pm3$ mm Hg during step II; $P<0.01$ versus basal and step I, $P<0.05$ insulin versus saline study; $124\pm4$ mm Hg during step III; $P<0.01$ versus basal and step I, $P<0.05$ insulin versus saline study) (Figure 3). At the level of the aorta, there was no change in systolic pressure ($98\pm3$ basally, $99\pm1$ during step I, $99\pm2$ during step II, $99\pm2$ mm Hg during step III).

Metabolic Parameters

Whole body glucose uptake rose dose dependently and averaged $39\pm3$ (step I), $69\pm5$ (step II; $P<0.001$ versus step I), and $86\pm4$ (step III; $P<0.001$ versus steps I and II) mmol/kg · min. The arteriovenous difference increased significantly within 30 minutes from $0.2\pm0.1$ to $1.5\pm0.2$ mmol/L ($P<0.001$) and averaged $1.7\pm0.1$, $2.0\pm0.1$, and $1.9\pm0.1$ mmol/L during steps I, II, and III.

Discussion

The present data demonstrate temporal dissociation between the effect of insulin on wave reflection and its effect on peripheral resistance vessels. Within an hour, insulin infusion reduced wave reflection, as assessed by decreases in augmentation and augmentation index. Peripheral systolic or diastolic blood pressure, blood flow, and vascular resistance did not change significantly until 2 to 3 hours after start of the insulin infusion (Figures 2 and 3). Because wave reflection is determined by compliance (arterial diameter and distensibility) and peripheral vascular resistance,9,11 the reduction of wave reflection is consistent with an effect of insulin upstream on the caliber or distensibility of arteries larger than resistance vessels.

The acute effect of insulin on wave reflection is similar to that previously described for nitroglycerin.6,18 which also decreases both augmentation and augmentation index but in low doses has no effect on brachial artery systolic or diastolic pressure or peripheral blood flow.6,18 Nitroglycerin has no effect on aortic compliance but appears to dilate muscular conduit arteries, leading to decreased wave reflection.19 Regarding insulin, a recent study found no effect of combined hyperglycemia and hyperinsulinemia (insulin 672 pmol/L) on carotid compliance in normal subjects.20 This does not, however, exclude the possibility insulin increases compliance in muscular conduit arteries. It would be possible to study this possibility with techniques such as bidimensional dual-crystal pulsed Doppler systems or other comparable devices. On the other hand, the intraobserver and interobserver reproducibility values when brachial artery internal diameter is measured by means of 2-dimensional echocardiography coupled with a Doppler system have been reported to be 14.8% and 11.3%.21 Since the brachial artery is 3 to 4 mm in diameter, such measurement may be subject to a type 2 statistical error. Furthermore, such techniques are limited by the ability of the investigation and thus are only applicable to large accessible arteries. It is therefore an assumption that compliance in the arteries studied accurately reflects compliance of the vasculature as a whole.22 As also found in the present study, pulse wave analysis is reproducible23 and has the advantage of not only reflecting changes in the vasculature as a whole but also allowing noninvasive determination of central aortic pressure, which is the pressure actually observed in the left ventricle.

Augmentation index increases with age and usually becomes positive after the age of 40 years.20 In the present study the mean age of the subjects was 25 years, and, as expected, basal augmentation was negative. Augmentation became even more negative in response to insulin (Figure 1). Because aortic systolic blood pressure is determined by the wave with the highest pressure, the decrease in augmentation had no effect on aortic systolic pressure during the first 2 hours. In contrast, another study measured augmentation after administration of nitroglycerin in older subjects who had positive basal augmentation. In these subjects aortic systolic pressure was raised because the amplitude of the reflected wave was greater than the initial aortic systolic pressure wave.18 After nitroglycerin, augmentation became negative due to a decrease in wave reflection. There was therefore a decrease in aortic systolic pressure, although brachial systolic arterial pressure remained unchanged.18

After 2 hours of the present study, during infusion of a supraphysiological dose of insulin, forearm blood flow increased and was accompanied by a significant decrease in peripheral vascular resistance. These changes are consistent with previously reported slow time course of the vasodilatory effects of insulin on peripheral resistance vessels.3,4 Heart rate and peripheral systolic blood pressure also increased significantly only after 2 hours of insulin infusion. These effects have been observed previously in a number of studies and could reflect either activation of baroreflexes or direct stimulation of the sympathetic nervous system by insulin.24–26 The fact that all these effects of insulin occurred much later than those in augmentation and augmentation index suggests hierarchy in the vascular effects of insulin and suggests that insulin increases the diameter or distensibility of arteries before arterioles (resistance vessels).
At a recent workshop on large-artery structure and function, the classic concept of hypertension as a disease characterized by peripheral vascular resistance with large arteries as simple passive conduits was reevaluated.27 Such a concept ignores the pulsatile component (pulse pressure) of the arterial pressure curve.27 Indeed, increased pulse pressure has been shown to be an independent predictor of cardiovascular mortality, especially for myocardial infarction.27 An explanation for the predictive value of increased pulse pressure is that it better reflects arterial stiffness and central aortic systolic and diastolic pressure than measurements made in the brachial artery. This is especially true in the elderly, in whom wave reflection occurs almost exclusively during systole. Early wave reflection will increase cardiac afterload and lower diastolic pressure, thereby decreasing coronary perfusion.27 It is increasingly clear that measurement of brachial artery systolic and diastolic blood pressure is insufficient to assess the clinical efficacy and mechanisms of action of antihypertensive drugs and other vasoactive agents such as insulin. Pulse wave analysis offers the exciting opportunity to accurately measure wave reflection and central arterial pressure. The present study emphasizes the potential practical importance of pulse wave analysis because the novel effect of insulin on large-artery function would not have been apparent if our study had only used measurements of peripheral vascular resistance.

Many of the physiological actions of insulin can be considered antiatherogenic. Insulin suppresses VLDL triglyceride production from the liver28 and inhibits platelet aggregation.29 Such beneficial actions are impaired in insulin-resistant conditions.30,31 The present study, in demonstrating that physiological concentrations of insulin rapidly decrease wave reflection at the level of the aorta, suggests an additional beneficial effect of insulin. Resistance to this action of insulin could provide a novel mechanism linking insulin resistance and conditions such as hypertension at the level of large arteries.

Acknowledgments

This study was supported by grants from the Sigrid Juselius Foundations (Dr Yki-Järvinen) and from the Academy of Finland (Dr Yki-Järvinen and Vehkavaara).

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_Hypertension_. 1999;33:1118-1122
doi: 10.1161/01.HYP.33.5.1118

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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