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INSULIN ACTION ON LARGE ARTERY STIFFNESS IN NORMAL AND INSULIN RESISTANT SUBJECTS

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ACADEMIC DISSERTATION

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Contents

List of original publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Westerbacka J, Wilkinson I, Cockcroft J, Utriainen T, Vehkavaara S, Yki-Järvinen H. Diminished wave reflection in the aorta. A novel physiological action of insulin on large blood vessels. *Hypertension.* 1999;33:1118-1122.

II Westerbacka J, Vehkavaara S, Bergholm R, Wilkinson I, Cockcroft J, Yki-Järvinen H. Marked resistance of the ability of insulin to decrease arterial stiffness characterizes human obesity. *Diabetes.* 1999;48:821-827.

III Westerbacka J, Uosukainen A, Mäkimattila S, Schlenzka A, Yki-Järvinen H. Insulin-induced decrease in large artery stiffness is impaired in uncomplicated type 1 diabetes mellitus. *Hypertension.* 2000;35:1043-1048.

IV Westerbacka J, Seppälä-Lindroos A, Yki-Järvinen H. Resistance to acute insulin induced decreases in large artery stiffness accompanies the insulin resistance syndrome. *Journal of Clinical Endocrinology and Metabolism.* In press 2001.

Abbreviations

Acyl-CoA	acyl coenzyme A
AgI	augmentation index
ANOVA	analysis of variance
ARIC	Atherosclerosis Risk in Communities
A-ZIP	acidic extension–leucine zipper
AV	arterio-venous
BMI	body mass index
BW	body weight
CAD	coronary artery disease
CV	coefficient of variation
ECG	electrocardiogram
EGIR	European Group for the Study of Insulin Resistance
eNOS	endothelial nitric oxide synthase
Ep	elastic modulus
FFA	free fatty acids
FFM	fat free mass
fP	fasting plasma
fS	fasting serum
GLUT	glucose transporter
G6P	glucose-6-phosphate
GTN	glyceryl trinitrate
HbA_{1c}	glycosylated hemoglobin A_{1c}
HDL	high density lipoprotein
HF	high frequency
HRV	heart rate variation
IMT	intima-media thickness
IRS	insulin receptor substrate
IVGTT	intravenous glucose tolerance test
L-NMMA	N^G-monomethyl-L-arginine
LDL	low density lipoprotein
LF	low frequency
mRNA	messenger ribonucleic acid
MRI	magnetic resonance imaging
MSNA	muscle sympathetic neural activation
N/A	not applicable
NO	nitric oxide
OGTT	oral glucose tolerance test
PC-1	plasma cell differentiation factor-1
PI3	phosphatidylinositol 3
PIUMA	Progetto Ipertensione Umbria Monitoraggio Ambulatoriale
PKC	protein kinase C
PVR	peripheral vascular resistance
PWV	pulse wave velocity
RIA	radioimmunoassay
SEM	standard error of mean
TNF-α	tumor necrosis factor-α
VLDL	very low density lipoprotein

INTRODUCTION

Insulin resistance, defined as the inability of insulin to stimulate glucose uptake, predicts the development of type 2 diabetes (158). Both insulin resistance and type 2 diabetes are associated with a two to seven –fold increased risk of cardiovascular morbidity and mortality (85,216,262). Type 1 diabetic patients are also known to be insulin resistant, mainly due to glucose toxicity caused by hyperglycemia, and also suffer from increased morbidity and mortality from cardiovascular diseases (215). The mechanism(s) underlying the association of insulin resistance and cardiovascular disease are poorly understood.

Of insulin's actions, the ability of insulin to stimulate glucose uptake and inhibit endogenous glucose production has received the greatest attention. Insulin has, however, multiple other effects such as regulation of lipid and lipoprotein metabolism and the activity of the autonomic nervous system. Furthermore, insulin is a slowly-acting weak peripheral vasodilator. These non-classic effects of insulin have been much less examined than those of insulin on glucose metabolism. Study of these effects might, however, be of interest since they potentially provide clues to the mechanisms underlying the association between insulin resistance and cardiovascular disease.

Large arteries serve as a blood buffering vessels between the heart and the peripheral vasculature. Apart from this, they are also subject to regulation by various vasoactive substances, such as NO (121,242,257) and angiotensin II (307). It has been suggested that alterations in the function of large arteries could contribute to the development of macrovascular complications, and serve as a potential risk factor or marker of cardiovascular disease (90). Large artery stiffness, the major determinant of pulse pressure, has recently been shown to be more important than diastolic or mean arterial pressure in predicting the risk of cardiovascular disease (7,23,80,168). Several cardiovascular risk factors, such as hypertension itself, dyslipidemia, smoking, and diabetes, are associated with increased stiffness of large arteries. Since many of the correlates of increased stiffness (hypertension (15), hypertriglyceridemia (188,288), low HDL (141,188), hyperinsulinemia (244)) are also features of insulin resistance, it would be of interest to investigate whether insulin itself regulates arterial stiffness. In the series of studies presented in this thesis, we investigated insulin's acute effects on arterial stiffness *in vivo* in healthy men as well as in insulin resistant obese men and type 1 diabetic patients.

REVIEW OF THE LITERATURE

INSULIN RESISTANCE

Definitions

The term *insulin sensitivity* is commonly used to denote insulin action. The term *insulin resistance* refers to a condition, which is characterized by a blunted response or responses to one or several normal biologic actions of insulin (125).

Normal insulin actions

Glucose metabolism

In the fasting state, skeletal muscle utilizes mainly FFA for energy production (11) and accounts for only 10-20% of whole body glucose uptake (11). Under these conditions, insulin-independent tissues, such as the brain utilize more than half of glucose (54). On the other hand, under basal conditions insulin is critically important in inhibiting endogenous glucose production (151).

Postprandially, glucose and aminoacids stimulate insulin secretion, which inhibits endogenous glucose production and stimulates glucose utilization in skeletal muscle (130). In middle-aged healthy non-obese men, insulin concentrations average 240 pmol/l (40 mU/l) over 2 hrs and 132 pmol/l (22 mU/l) over 5 hrs after a meal (183). Under these conditions, approximately one third of glucose is utilized in skeletal muscle, one third is oxidized in the brain and the remaining third is stored in the liver (130). Under intravenously maintained hyperinsulinemic conditions, skeletal muscle accounts for 70-80% of whole body glucose uptake (56,322). Serum insulin concentrations over 480 pmol/l (80 mU/l) completely suppress hepatic glucose production in normal subjects, while half-maximal suppression is achieved at approximately 102 pmol/l (17 mU/l) (112,318).

In insulin-sensitive tissues such as skeletal muscle, the liver and adipose tissue, insulin binds to the α-subunit of its membrane receptor, which leads to receptor autophosphorylation on several tyrosine residues (128). This leads to phosphorylation of IRS proteins, which in turn activate several other intracellular proteins transmitting the signal downstream. Of IRS proteins, IRS-1 and IRS-2 are considered the most specific for insulin signaling. IRS-1 and IRS-2 bind and activate PI3-kinase, which is essential for stimulation of glucose transport (117) via activation of GLUT-4. It is the

major insulin-sensitive transporter, and is expressed in insulin sensitive tissues, such as skeletal muscle, the heart and in adipose tissue. PI3-kinase activation is also essential for activation of insulin-stimulated glucose phosphorylation by the insulin-sensitive hexokinase II (177,214) and for stimulation of glycogen synthesis (199). In the liver, insulin inhibits hepatic glucose production by inhibiting glycogenolysis and glucogenesis (151) especially postprandially (39). Insulin also stimulates glycogen synthesis after meals (275). Animal studies suggest, that the mechanism by which insulin regulates liver carbohydrate metabolism is mediated via IRS-2, whereas IRS-1 is more important in skeletal muscle (213,234). In IRS-2 deficient mice, both insulin-mediated suppression of hepatic glucose production and glycogen synthesis are decreased *in vivo* (213).

Lipid metabolism

In adipose tissue, insulin inhibits lipolysis and stimulates fractional re-esterification. Both of these insulin's actions have been shown to be PI3-kinase -dependent (59,328). Insulin inhibits hormone sensitive lipase (14) and this leads to a decrease in serum FFA concentrations. This decreases substrate availability for VLDL production in the liver. Acute *in vivo* infusions of insulin decrease both VLDL triglyceride (157,296) and VLDL apolipoprotein B (157,176) production. Insulin specifically suppresses the production of large, triglyceride-rich VLDL1 (176). This occurs not only via decreases in circulating FFA concentrations, but also via direct inhibitory effects in the liver (175).

Vascular function

Large arteries

There are virtually no data on insulin action on large arteries *in vivo*. Lambert et al. studied eleven healthy men using the insulin clamp technique on two occasions (147). Carotid artery distensibility and compliance were measured using an ultrasound device with a non-invasive vessel wall movement detector system. Hyperinsulinemia did not change compliance or distensibility of the carotid artery either under euglycemic (glucose concentration 3.9 mmol/l, insulin concentration 252 pmol/l (42 mU/l)) or hyperglycemic (glucose concentration 14.3 mmol/l, insulin concentration 672 pmol/l (112 mU/l)) conditions (147). However, local arterial diameter increased in both studies (147). Blood pressure values used in the calculation of carotid artery distensibility and compliance were measured in brachial artery, thus ignoring site-dependent differences in blood pressure. The study was uncontrolled, which makes it difficult to make firm conclusions regarding the specificity of the role of insulin in inducing the changes.

In animal studies large arteries have been studied *ex vivo*. In cultured bovine aortic endothelial cells, physiological concentrations of insulin increase eNOS expression (140). In cultured human coronary endothelial cells, insulin increases eNOS protein expression and NO production, and this effect is partly inhibited by hyperglycemia

(63). These data support the idea that endothelial NO could contribute to insulin-induced vasodilatation in large arteries in humans. Flow-dependent vasodilatation in the radial artery has been shown to be NO-dependent in normal subjects (121). In isolated human arteries, it has been shown that NO contributes to arterial vasodilatation in arteries greater than distal microvessels (282).

Resistance arteries

Insulin is a slow vasodilator of peripheral resistance arteries in skeletal muscle (321). With a few exceptions, a significant increase in limb blood flow has only been found in studies in which high, supraphysiological doses of insulin have been infused for prolonged time periods. Yki-Järvinen et al. (322) and Bonadonna et al. (30) measured forearm glucose uptake on separate days using different doses of insulin. At the highest concentrations (1600-1800 mU/l), blood flow increased by 15% during 120 min in the former (322) and 25% during 130 min in the latter (30) study. In normal subjects, infusion of insulin using a physiological dose (1 mU/kg·min) increases peripheral blood slowly. Significant 20% (range 10-90%) increases in blood flow are usually observed after approximately 2 hours in normal subjects while a 10-fold increase in glucose extraction is already detectable after 30 min (143,284,321). The importance of the duration and dose of insulin infusion was documented in an extensive analysis of studies, where effects of insulin (75 studies with intravenous and 23 with an intra-arterial infusion) on limb blood flow were measured under euglycemic hyperinsulinemic conditions. The analysis demonstrated marked variability in blood flow responses to insulin (321). When the increase in limb blood flow was plotted against an insulin exposure index (a product of the insulin dose times the duration of the infusion) a significant correlation was found suggesting that the dose and/or duration of insulin infusion contribute to the discrepant findings regarding insulin's effect on peripheral blood flow. Other factors which contribute to interindividual variation in blood flow responses to insulin include limb muscularity (284), the number of capillaries surrounding muscle fibers (283) and possibly endothelial function (285). The ability of insulin to induce peripheral vasodilatation can be abolished by co-infusion of L-NMMA (vide infra). Although these data would suggest that insulin is an endothelium-dependent vasodilatator, the time course for insulin action on peripheral blood flow is markedly slower than that of classic endothelium-dependent vasodilatators such as acetylcholine, which increases blood flow 5-fold within a minute in the human forearm (170). The reason for the slow vasodilatory effect of insulin on peripheral resistance vessels is unknown. One possibility is that insulin rapidly activates the sympathetic nervous system (see the chapter Autonomic nervous tone), and that this counteracts the vasodilator effects of insulin.

Regarding the mechanism responsible for insulin-induced vasodilatation of resistance vessels *in vivo*, stimulation of endothelial NO synthesis by insulin seems to be of importance. Both Scherrer et al. (246) and Steinberg et al. (264) demonstrated that the insulin-induced increase in blood flow can be abolished by inhibiting NO-dependent vasodilatation with L-NMMA, but not by other vasoconstrictors such as norepinephrine (246). *In vitro* studies support these observations. Insulin increases NO production in

human vascular endothelial cells *in vitro* (326), and both removal of the endothelium and inhibition of eNOS using L-NMMA abolishes insulin-induced vasodilatation in isolated rat skeletal muscle arterioles (38). Insulin induces NO-mediated endothelium-dependent vasodilatation in arterioles from red and white gastrocnemius muscles (249). After removal of functional endothelium in these arteries, insulin paradoxically evokes vasoconstriction. Insulin also increases eNOS gene expression in microvessels in lean but not insulin-resistant obese hyperglycemic rats (140).

Capillaries

Insulin seems to increase capillary blood volume in skeletal muscle, as determined from insulin induced increases in the rate of 1-methylxanthine (a substrate for capillary endothelial xanthine oxidase) endothelial metabolism in anesthetized rats (221). Studies using laser Doppler flowmetry on a surface of a perfused rat hindlimb have suggested that insulin induces capillary recruitment (41). In healthy humans, Raitakari et al. showed insulin to increase muscle blood volume (218). Recently, physiological insulin concentrations were shown to increase microvascular blood volume in the forearm when measured using micro-bubbles and contrast enhanced ultrasound method (91).

Veins

Insulin action on venous tone has been studied by infusing insulin locally into dorsal hand veins *in vivo* and by following changes in venous pressure using a tonometer or changes in diameter with ultrasonography. Since veins are normally dilated at resting state they need to be preconstricted before effects of vasoactive substances can be detected (96,230). In studies in normal subjects, insulin (8-24 μU/min) caused a dose-dependent venodilation of preconstricted veins (73,96,268). In one study, in which a much higher insulin concentrations were infused (1-100 mU/min), only a small venodilatory effect could be demonstrated during infusion of the highest insulin dose (50).

Autonomic nervous tone

Under normoglycemic conditions, physiological concentrations of insulin increase the activity of sympathetic nervous system, as determined from increases in plasma norepinephrine but not epinephrine concentrations (9,219). Physiological insulin concentrations, lower than those needed for peripheral vasodilatation, also increase muscle sympathetic nerve activity, as measured directly in the peroneal nerve with microneurography (9,299). In studies, which used power spectral analysis of HRV, insulin increased the low frequency (LF) component of HRV, a measure of predominantly sympathetic nervous system activity, in lean insulin sensitive subjects in all (24,186,203), except one (146) study. Insulin also acutely decreased the high frequency (HF) component of HRV, which reflects vagal control of HRV (24,186,203).

Platelet function

Human platelets have insulin receptors, which participate in the regulation of platelet functions (72). *In vitro* and *in vivo* studies have demonstrated that insulin inhibits platelet aggregation in healthy, non-obese subjects (104,278,280) under euglycemic hyperinsulinemic conditions. Insulin's antiaggregatory effects have been suggested to be NO-dependent, since insulin stimulates intraplatelet NO synthesis, and through increases in NO intraplatelet cGMP and cAMP concentrations (279).

Causes of insulin resistance

Obesity

Since the studies performed over 35 years ago by Rabinowitz et al. (217), it has been clear that one target of insulin resistance in obesity is skeletal muscle. In obese subjects, the ability of insulin to suppress endogenous glucose production is also blunted as is the sensitivity of antilipolysis to insulin (14). The mechanisms via which obesity causes insulin resistance of glucose or FFA metabolism are still incompletely understood. It has been suggested that in obese subjects the excess release of FFA from adipose tissue inhibits glucose uptake in peripheral tissues and stimulates hepatic glucose production (95). Another possibility is that accumulation of adipose tissue in organs such as the liver and/or skeletal muscle underlies insulin resistance in obese subjects. Consistent with this possibility, in A-ZIP/F-1 transgenic fatless mice with virtually no white fat tissue, triglyceride content in the liver and skeletal muscle are greatly increased and associated with insulin resistance of glucose metabolism in these tissues (88,135). This insulin resistance is reversed by subcutaneous fat transplantation (88). Counterregulatory hormones e.g. TNF-α and resistin secreted from adipose tissue (see Chapter Hormones, cytokines and FFA) could also hypothetically cause insulin resistance via direct actions in insulin sensitive tissues such as skeletal muscle. Regarding the cellular mechanisms underlying insulin resistance in obesity, obese subjects have a decrease in insulin-stimulated tyrosine kinase activity of the insulin receptor in skeletal muscle (36) and adipocytes (198), the activity of which is restored concomitant with insulin sensitivity by weight loss (83). Additionally, IRS-1 -associated PI3-kinase activity has been found to be decreased in obesity (93).

Non-classic insulin actions appear also to be blunted in obesity. Although basal sympathetic activity is increased in obese subjects (298), *in vivo* insulin stimulation of autonomic nervous system is resistant in obese subjects (186,203). This resistance to insulin does not appear to be a consequence of an increase in basal sympathetic tone. This is because resistance to insulin stimulation of sympathetic nervous system activity and to insulin inhibition of vagal control of HRV can also be demonstrated in groups which differ with respect to insulin sensitivity but not body weight or basal activity of the autonomic nervous system (24). The effect of weight loss on autonomic nervous system function was studied by Karason et al. (127) in 28 obese patients

referred for weight reducing gastroplasty operation and in 24 obese, who received dietary recommendations to lose weight. The surgically treated patients lost an average of 32 kg (28%), whereas those on diet did not lose weight. At baseline the obese patients had higher sympathetic activity as measured by HRV. After surgery, the obese subjects who had lost weight showed a decrease in sympathetic activity (127) suggesting reversibility of sympathetic overactivity in obesity.

In obese subjects the platelet antiaggregating effect of *in vitro* insulin has been shown to be blunted (280). The impaired antiaggregatory effect has been attributed to obesity *per se*, since in lean type 2 diabetic patients, the effect of insulin to antagonize platelet aggregation is preserved (12,280). These data suggest that insulin resistance also involves platelets, but it is currently unknown whether resistance of platelets to insulin occurs *in vivo* and whether insulin also regulates adhesion and activation of platelets on subendothelial matrix proteins, such as collagen, which are exposed after intravascular injury. Regarding vascular defects in obesity, insulin-induced vasodilatation of peripheral resistance arteries is blunted in obesity (143). This defect has not been observed at physiological insulin concentrations but has been observed using prolonged supraphysiological doses of insulin. It is unknown whether this defect is specific to insulin and whether it is due to a defect in the function of the endothelium or vascular smooth muscle, or to overactivity of the sympathetic nervous system, which could counteract insulin induced vasodilatation in obesity (298). There are no data on insulin action on large arteries in obese subjects.

Physical inactivity

Physical inactivity is associated with glucose intolerance and hyperinsulinemia (160,161), while aerobic physical training increases insulin stimulated glucose uptake (150,259,315).

Data are limited regarding effects of physical training on insulin actions other than those on glucose metabolism (315). Physical training increases the ability of insulin to stimulate blood flow (98) and lowers serum triglycerides (92). Physical training may enhance insulin action since the activity of oxidative enzymes, glycogen synthase activity (223,309), capillary density (159,301) and the proportion of insulin sensitive fibres in skeletal muscles are increased (159). Physical training is also associated with less adiposity, which may contribute to enhanced insulin action on glucose metabolism (315).

Type 1 diabetes and chronic hyperglycemia

In type 1 diabetic patients, poor glycemic control is associated with insulin resistance independent of other factors (316). Since peripheral insulin concentrations are usually normal in type 1 diabetic patients, peripheral insulin deficiency cannot explain peripheral insulin resistance in these patients. Acute induction of hyperglycemia in the face of unchanged insulin concentrations by a glucose infusion for 24 hrs decreases

insulin sensitivity significantly in type 1 diabetic patients (314). This observation is in line with animal data (233), and suggests that hyperglycemia or 'glucose toxicity' *per se* causes insulin resistance (312). The insulin resistance in type 1 diabetes is due to reduced insulin-stimulated glucose extraction rather than to defects in insulin-induced peripheral vasodilatation (173,300,320). The defects in glucose AV-difference have been shown to be responsible for insulin resistance at both physiological (320) and supraphysiological (173) concentrations of insulin (320), as well as during hyperglycemia-induced insulin resistance (300), independent of changes in peripheral blood flow. Insulin resistance in type 1 diabetes has been localized to skeletal muscle but not to other muscle types such as the heart muscle (192). In skeletal muscle of type 1 diabetic patients, the defect in glucose disposal is associated with a defect in muscle glycogen synthesis (320). Regarding the sensitivity of endogenous glucose production to insulin in type 1 diabetes, it is similarly suppressed by insulin in type 1 diabetic patients and in healthy subjects (55,239), although endogenous glucose production is increased in the fasting state in these patients (55). Insulin's antilipolytic effect has also been found to be impaired in type 1 diabetes (166,206). Overactivity of the hexosamine pathway has been suggested to be the critical signal which leads to glucose induced insulin resistance (313) and defects in insulin signaling (294).

As discussed above, most investigators have found that the ability of insulin to increase peripheral blood flow is normal in patients with type 1 diabetes (173,320) compared to healthy subjects using plethysmography to measure limb blood flow. Contradictory findings, i.e. that insulin-induced peripheral vasodilatation is blunted in type 1 diabetes, have also been reported in a study of 5 poorly controlled type 1 diabetic patients using the thermodilution technique to measure blood flow (22). Insulin's effects on hemodynamics in type 1 diabetes include provocation or enhancement of postural hypotension (202,211,250,271), which seems to be caused by impaired hemodynamic compensatory mechanisms due to autonomic neuropathy (102). Compared to healthy subjects, insulin-induced increase in heart rate is blunted (171). Mäkimattila et al. studied 28 type 1 diabetic patients and 7 control subjects under euglycemic hyperinsulinemic conditions and assessed various parameters of autonomic function using HRV measurements (171). In this study, type 1 diabetic patients were characterized by various defects in autonomic functions. Insulin-induced changes in heart rate were associated with predominantly parasympathetic autonomic neuropathy, whereas changes in PVR were associated with disturbances in sympathetic nervous function (171).

Hormones, cytokines and FFA

Catecholamines. Epinephrine is a hormone secreted from the adrenal medulla in stress conditions, such as exercise, hypoglycemia or sepsis. It has insulin antagonistic effects in various tissues including skeletal muscle, pancreas (229), liver (240) and adipose tissue (42). After an overnight fast, acute administration of epinephrine impairs glucose utilization in humans (226,241). This effect is sustained, in contrast to it's transient effect to increase endogenous glucose production (227,240,241). The decrease in

glucose uptake induced by epinephrine can be reversed by propranolol suggesting that this effect is mediated via β-receptors. Epinephrine also impairs insulin stimulated glucose uptake in peripheral tissues. When infused at a rate of 0.05 μg/kg·min for 2 hrs under euglycemic hyperinsulinemic conditions, epinephrine decreases whole body glucose uptake by 50 % (222). A similar decrease was observed by Bessey et al. (27) with an epinephrine infusion rate of 0.025-0.030 μg/kg·min. In the latter study, forearm blood flow doubled documenting that epinephrine induces insulin resistance via effects on cellular glucose extraction rather than via decreases in blood flow. Furthermore, patients with pheocromocytoma are also insulin resistant (281).

Norepinephrine is a neurotransmitter secreted into the synaptic cleft. It is difficult to study physiological effects of norepinephrine since systemic administration of norepinephrine does not necessarily alter it's synaptic concentration. Increases in plasma norepinephrine were without effect (255) (105) or decreased glucose uptake (44,154). Lembo et al. assessed effects of stimulation of the sympathetic nervous system via lower body negative pressure on glucose metabolism in humans (154). The maneuver increased plasma norepinephrine concentrations 2-fold above normal. Insulin, glucagon and glucose concentrations remained unchanged. However, insulin stimulated forearm glucose uptake decreased by 30 % (154).

Glucagon. Glucagon is the most important counterregulatory hormone against hypoglycemia in normal humans (47). It stimulates hepatic glycogenolysis and gluconeogenesis (267), but has no extrahepatic effects in humans (17,119).

Cortisol. Cortisol is a stress hormone secreted from the adrenal medulla. An acute infusion of cortisol increases hepatic glucose production and impairs insulin-induced suppression of hepatic glucose production after an overnight fast in normal subjects (232). Cortisol also decreases peripheral glucose uptake in normal subjects in the fasting state (256) and stimulates protein catabolism and lipolysis in contrast to insulin (6,64,256).

Growth hormone. Growth hormone secretion is stimulated by physical exercise, stress, decreases in FFA, hypoglycemia and certain hormones (glucagon, ACTH, estrogens) (32). Growth hormone increases endogenous glucose production, increases blood glucose concentrations and decreases glucose utilization (32,167,228). As with cortisol, the above actions of growth hormone require prolonged exposure of approximately 2-3 hrs of tissues to the hormone (78,144).

TNF-α is a cytokine secreted from activated macrophages in response to e.g. infection or injury. TNF-α mRNA has been detected also in adipocytes (111). TNF-α has hemodynamic and tumoricidal effects, and effects on glucose metabolism (see (107) for review). TNF-α appears to impair insulin signaling by increasing serine phosporylation of IRS-1 (110), which leads to inhibition of insulin receptor tyrosine kinase activity and finally impairment of downstream signaling (110). This implies that TNF-α is also an insulin antagonistic hormone (207) (109,110,134,138).

TNF-α has been suggested to contribute to insulin resistance in variety of catabolic states, including cancer, sepsis and trauma (13,148). Adipocytes of obese animals and humans overexpress TNF-α and this expression is positively correlated with obesity (108,111). Weight loss decreases TNF-α expression (108,207). Although local release of TNF-α has little effect on systemic concentrations, local concentrations of free and membrane-bound TNF-α are likely to be increased in obesity and possibly induce insulin resistance in adipose tissue via paracrine effects.

Leptin is a peptide hormone secreted from adipose tissue (327). Leptin mRNA expression is higher in subcutaneous than visceral adipose tissue in humans (178). In cross-sectional studies, serum leptin concentrations correlate closely with obesity, (expressed as percentage body fat (45) or BMI (169)) and also with insulin resistance (49,67). There are, however, presently no data which would classify leptin as an insulin-antagonist or agonist in humans.

Resistin is a recently identified hormone secreted exclusively from adipose tissue (266). In mice, circulating resistin concentrations are increased in both diet-induced and genetic forms of obesity. Treatment with anti-resistin antibody decreases blood glucose concentrations and improves insulin action on glucose metabolism (266). Additionally, resistin treatment in normal mice impairs glucose tolerance and insulin action on glucose metabolism. These data suggest that resistin may link obesity with insulin resistance. Human data regarding resistin are so far lacking.

FFA. An increase in circulating FFA concentrations induced by infusion of triglyceride-rich fat emulsion and heparin have been shown to decrease insulin-stimulated glucose utilization in humans (74). This decrease in glucose utilization occurs in both skeletal muscle and the heart (193). Various cellular mechanisms have suggested to be involved in FFA induced insulin resistance. These include activation of the hexosamine pathway (100), classic competition between glucose and FFA as proposed by Randle et al. (220), inhibition of PI3-kinase activity by FFA (69), which leads to a decrease in glucose transport and a decrease in G6P concentrations (231), activation of PKC by diacylglycerol or long-chain acyl-CoA, which also results in impaired insulin signaling (248).

Other

Ethanol acutely impairs insulin sensitivity in healthy men (29,251,319). This effect is not caused by increases in acetate, a metabolite of ethanol, concentrations, since infusion of acetate alone does not affect glucose uptake (317). Other causes of insulin resistance include electrolyte disturbances such as hypercalcemia (20,212), hypokalemia (10), hypomagnesemia (204) and hypophosphatemia (57,212). Drugs including corticosteroids (33), diuretics (208), non-selective beta-blockers (162), cyclosporin (200), and protease inhibitors (292) cause insulin resistance. Androgens explain at least part of insulin resistance in women with polycystic ovary syndrome (71). In men, high-dose testosterone appears to decrease insulin sensitivity, whereas

dehydroepiandrosterone has no effect (225). Insulin resistance also characterizes several disease states including infections (293,311), conditions caused by excess secretion of counterregulatory hormones (144), uremia (52) and acidosis (53).

Heritability of type 2 diabetes estimated from monozygotic twin studies ranges from 60 to 90% (21,189) suggesting that genetic factors are important in the pathogenesis of the disease. Rare mutations of the insulin receptor cause severe insulin resistance (124,323), but insulin receptor mutations do not seem contribute to insulin resistance in type 2 diabetes (139). The genetic defects that predispose to obesity and type 2 diabetes are largely unknown. Several candidate genes, defined as a gene the products of which influence glucose and fat metabolism, have been suggested to predispose to insulin resistance and type 2 diabetes. These include e.g. genes for IRS-1 (8), PC-1 (70), glycogen synthase (94,291) and the beta-3 adrenergic receptor (304).

Insulin resistance and cardiovascular disease

Insulin resistance is associated with an increased risk of cardiovascular disease (216). Several large prospective studies have shown that at least in univariate analysis hyperinsulinemia, which is a marker of insulin resistance in non-diabetic subjects (142), is a predictor of CAD (77,216,303). Insulin resistance or hyperinsulinemia associate also with markers of atherosclerosis, such as carotid artery intima-media thickness (2,113,269) and carotid artery stiffness (244).

In addition to CAD patients, hypertensive patients, both untreated and treated, have been found to be insulin resistant compared to normotensive subjects as measured with the insulin clamp technique (75,209), the IVGTT (252) or OGTT (76). An increased concentration of insulin in hypertensive patients was first reported over 30 years ago (302). The association between hyperinsulinemia and hypertension has been reported in cross-sectional epidemiological studies (76,184), although this relationship is not found in all populations (68).

ARTERIAL STIFFNESS

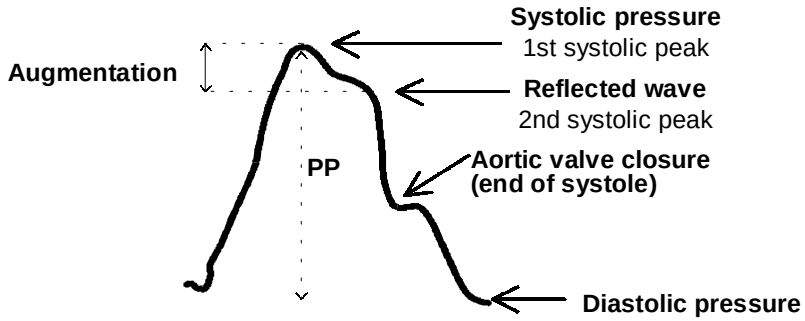

Fig. 1. Schematic illustration of pulse wave during one cardiac cycle. The AgI is defined as the ratio between augmentation and pulse pressure (PP).

Physiology of arterial function. An arterial pressure wave is generated during each cardiac cycle (**Fig. 1**). In early systole, contraction of the left ventricle causes the first systolic pressure peak seen in the pulse wave (**Fig. 1**). When blood travels along the arteries, part of the pressure or flow wave is reflected back from the periphery, e.g. from arterial walls, branching points and arteriolal terminations (190). The reflected wave travels back to the heart and causes a second systolic peak to the arterial pulse waveform (**Fig. 1**). The faster the wave travels, the earlier it is reflected back and the higher is the second systolic peak.

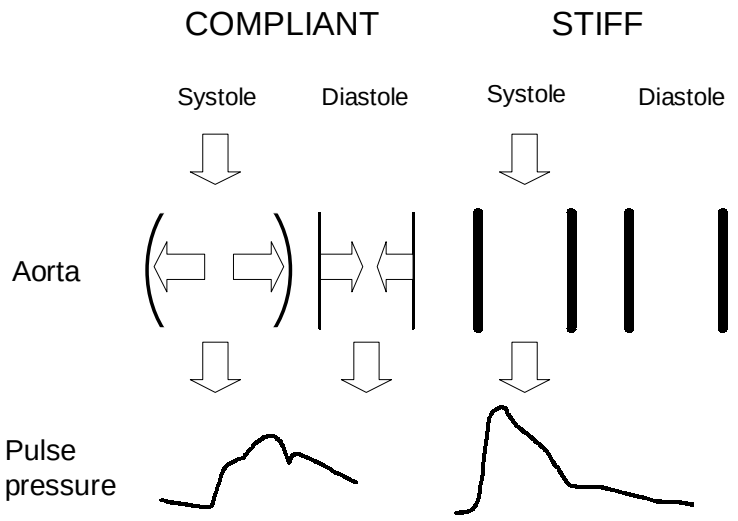

Fig. 2. Examples of pulse waveforms caused by compliant (on the left) and stiff aortas (on the right) at constant stroke volume.

Arterial stiffening impairs the normal buffering function of arteries, leading to earlier wave reflection from periphery back towards the heart. This increases the height of the reflected wave in systole and decreases the height of the diastolic pressure wave (**Fig. 2**). If the pressure of the reflected wave exceeds that caused by left ventricular ejection, as occurs during aging, central systolic pressure increases and also pulse pressure increases. These changes have potentially harmful consequences as they increase left ventricular afterload and impair coronary filling during diastole (163,190).

Definitions

Arterial stiffness is a term which characterizes the artery's ability to expand and contract with cardiac pulsation and relaxation (66,190). Several parameters have been developed to quantitate stiffness. Definitions of the most commonly used indeces of arterial stiffness have been listed in **Table 1**.

Table 1. Indeces of arterial stiffness. Modified from (87,190,197).

Index	Definition
Pulse pressure	Difference between systolic and diastolic pressure
Augmentation index	Ratio of pressure augmentation caused by wave reflection to local pulse pressure
Arterial distensibility	Relative diameter (or area) change for a pressure increment, 1/elastic modulus
Arterial compliance	Absolute diameter (or area or volume) change for a given pressure step at fixed vessel length
Pulse wave velocity	Speed of travel of the pulse along an arterial segment
Elastic modulus (Ep)	Pressure step required for (theoretical) 100% stretch from resting diameter at fixed vessel length
Volume elastic modulus	Pressure step required for (theoretical) 100% increase in volume
Young's modulus	Elastic modulus per unit area; the pressure step per square centimetre required for (theoretical) 100% stretch from resting length
Characteristic impedance	Relationship between pressure change and flow velocity in the absence of wave reflections
Stiffness index β	Ratio of logarithm (systolic/diastolic pressures) to relative change in diameter

Methods for determing arterial stiffness

Measurement of arterial stiffness of a single artery. Methods to determine arterial stiffness *in vivo* can be divided into those measuring stiffness in a single artery and those measuring stiffness of the entire vascular tree. Ultrasound techniques allow visualization of *in vivo* wall thickness and vessel diameter (16,197). Some systems allow for unprocessed ultrasound echoes to track electronically diameter changes during the cardiac cycle (16). Measurements are performed upon a certain segment of the artery. Depending on method and stiffness index to be calculated, the changes in either

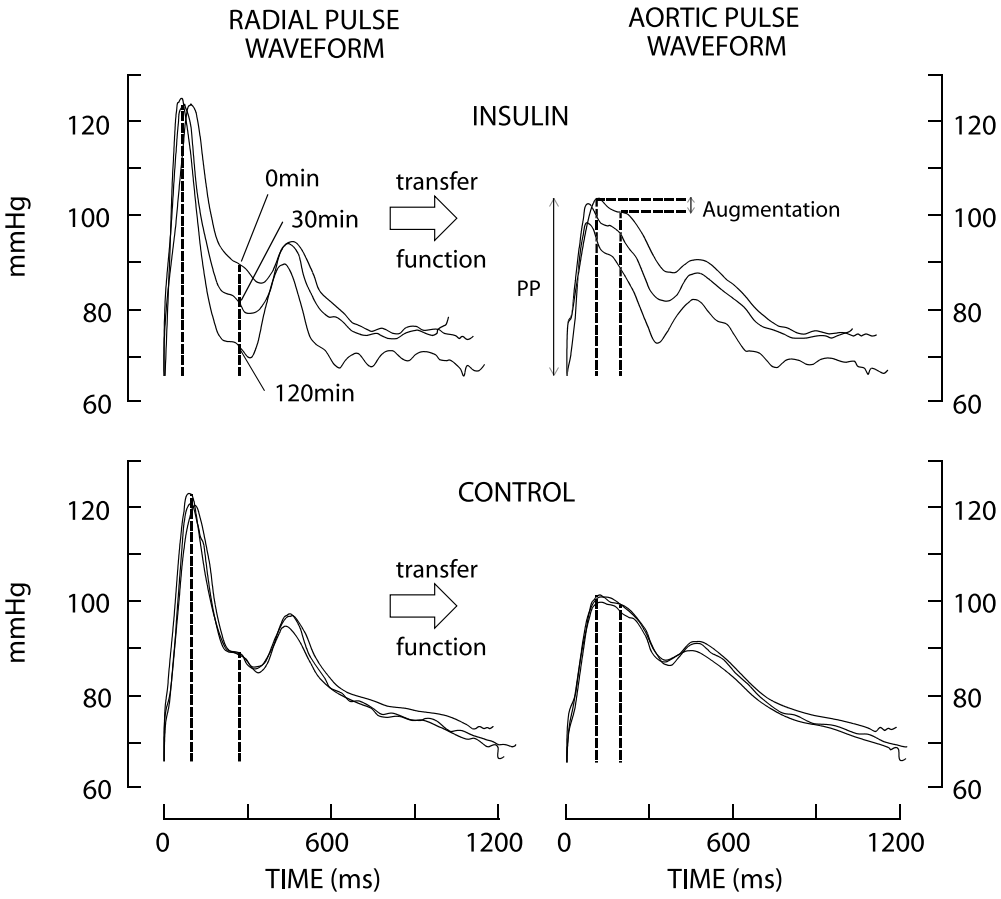

Fig. 3. Examples of radial and aortic waveforms during the euglycemic insulin clamp (INSULIN) at various time points and in the saline control (CONTROL) study. For pulse wave analysis, all measurements were made from the radial artery. The average radial artery waveform was calculated and the corresponding aortic pressure waveform was generated using a validated transfer factor. Augmentation is determined by the difference in pressure between the second and first systolic peaks (shown by the hatched lines). The AgI is defined as the ratio between augmentation and pulse pressure (PP).

vessel diameter, area or calculated volume are related to simultaneous changes in blood pressure. Since blood pressure is different along the arterial tree, and blood pressure is usually determined from brachial artery when ultrasound techniques are used, it is a source of error when local stiffness is measured (190,197). Properties of one artery may also not be identical to those of another (18,195,197).

Indirect measurement of arterial stiffness. When arteries stiffen, the pulse wave propagates faster and increases *pulse wave velocity (PWV)*, which can also be used to measure arterial stiffness between two arterial sites (133). *Vascular impedance* is a measure of the opposition force to flow from the arterial system. Concomitant measurement of both blood pressure and flow (190) estimates impedance during oscillating conditions in the artery. The dependence of these measurements on blood pressure makes the results difficult to interpret because of blood pressure alterations at various central and peripheral sites.

Measurement of global arterial stiffness. Methods attempting to characterize properties of the entire arterial tree have recently been developed. Cohn et al. have developed a technique (43), which measures exponential decay of pressure during diastole as a marker of large artery compliance (43). Another approach, developed by Michael O'Rourke (131,194), measures augmentation of the central arterial pressure wave, measured directly using applanation tonometer on the carotid artery or from the aortic pressure wave synthesized from the radial or carotid pressure wave using a validated transfer function (194) (**Figs. 1 and 3**). Augmentation is a result of pressure wave reflections along the arterial tree back to the heart (**Fig. 1**), and can be expressed in absolute values in mmHg or as a ratio between augmentation and pulse pressure (the augmentation index, AgI). Augmentation increases as a consequence of arterial stiffening, since when aorta and other large arteries stiffen, the pulse wave propagates faster resulting in earlier return of the reflected wave and an increase in central pressure augmentation (194). AgI can be interpreted as a measure of large artery stiffness, when ejection duration, heart rate and PVR remain constant since changes in these parameters also alter the timing (ejection duration and heart rate) and site (PVR) of wave reflections (190,191,306).

Correlates of arterial stiffness

Age

Stiffening of large arteries seems to be a consequence of the aging process (18). Arterial stiffness increases both systolic and pulse pressure (243). Kelly et al. determined arterial stiffness from the carotid, femoral and radial arteries using an applanation tonometer, and analyzed arterial pressure waveforms in 1005 normal subjects aged 2 to 91 years (131). Aging was associated with an increase in pulse amplitude, steepening of the diastolic decay and a decrease in the pressure of the diastolic wave (131). Stiffening thus explains why diastolic pressure normally decreases and pulse pressure

increases during aging (**Fig. 4**). A decrease in diastolic pressure is observed from the age 50-59 years onwards (35,80). In a recent analysis of the Framingham cohort, 75 % of all hypertensives (over 160/90 mmHg) were older than 50 years (79). This information has several important prognostic and therapeutic implications (vide infra). That aging stiffens arteries has also been demonstrated also in several other studies using techniques such as ultrasound (129,260), tonometry (131,181,286), magnetic resonance imaging (185), arterial catheterization (181), photoplethysmography (31,272) and PWV (18,31,286). To what extent aging stiffens arteries independent of other factors (vide infra) is unclear. However, stiffening does occur even in the absence of atherosclerosis (18).

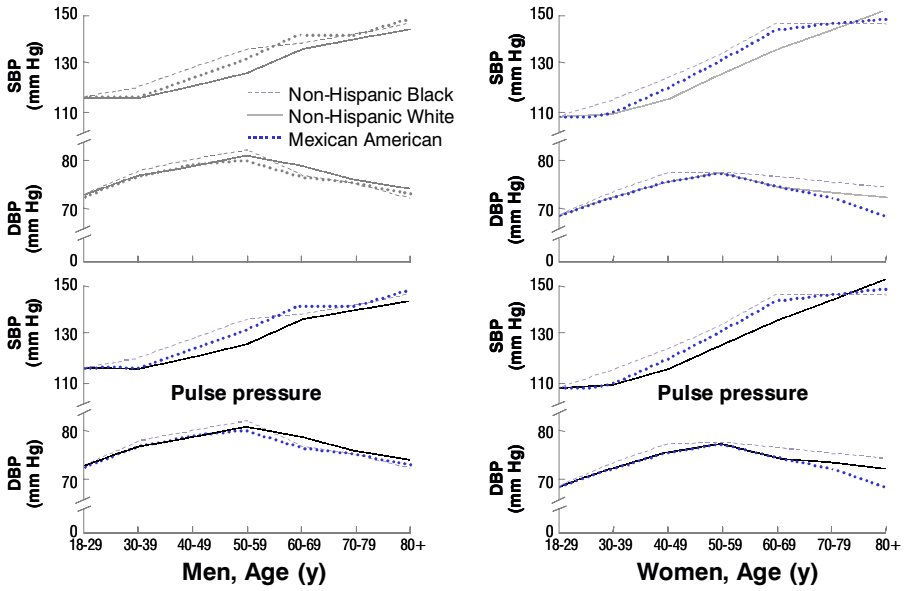

Fig. 4. Effect of aging on systolic, diastolic and pulse pressure in men and women. Adapted from (35).

Dyslipidemia

Data regarding the association between lipid abnormalities and arterial stiffness are few and controversial. In a study of 62 normotensive and 201 uncomplicated hypertensive subjects with a wide range of total cholesterol concentrations, arterial stiffness was measured with ultrasound and by applanation tonometry (238). Serum cholesterol was not correlated with either the carotid stiffness index β or the AgI (238). In contrast, Dart et al. found a significant age-independent relationship between serum cholesterol and arterial stiffness in 54 healthy subjects using the ultrasound technique (48). Toikka et al. measured compliance of the aorta with MRI in 25 healthy men aged 29 to 39 years and in 10 age-matched subjects with familial hyper-

cholesterolemia but no cardiovascular disease (277). Aortic compliance was similar between the groups, and compliance was not related to standard lipid variables, but was inversely correlated with oxidized LDL cholesterol (277).

Smoking

Smoking may have both short- and long-term effects on arterial stiffness. Levenson et al. (156) studied the relationship between smoking and stiffness in 33 normotensive and 80 hypertensive subjects. In both groups, smokers had increased arterial stiffness (156). In a group of 248 healthy subjects, all 50-years of age, stiffness of the common carotid artery was measured using ultrasound (122). In multivariate analysis, smoking measured as pack-years, was independently associated with arterial stiffness (122). In a study by Taniwaki et al., arterial stiffness was measured using aortic PWV measurements in 271 diabetic patients and 285 healthy subjects (mean age 50 years) (273). In multivariate analysis, smoking (along with diabetes, hypertension and age) was independently associated with increased arterial stiffness (273). Even short-term passive smoking appears to acutely increase arterial stiffness (136,263).

Hyperglycemia and hyperinsulinemia

The ARIC study was the first to implement measurements of carotid artery stiffness with the use of ultrasound in a large population survey comprising of 4701 white and black (19% black) subjects (244). Of these subjects 5% had type 2 diabetes. In the entire study group, arterial stiffness increased with increasing concentrations of fasting glucose, independent of race or gender. The relationship between glucose and insulin and stiffness remained highly significant also after adjustment for age, smoking and total cholesterol. In all non-diabetic patients, fasting serum insulin was associated with arterial stiffness, again even after adjustment for age, smoking and total cholesterol. After further adjustment for BMI, triglycerides, HDL cholesterol, and hypertension status (49% of the black and 25% of the white subjects were hypertensive), glucose was significantly associated with stiffness in white and black female and insulin in white female and male participants. This cross-sectional study also found that hyperinsulinemia and hyperglycemia synergistically contributed to arterial stiffness, independent of artery wall thickness, in both men and women (244).

Increased arterial stiffness has been a consistent finding in type 2 diabetic patients in several studies (90,153,182). In the Strong Heart Study, 1810 diabetic and 944 normal American Indians with a mean age of 60 years were studied using an ultrasound technique (62). Diabetic patients had significantly increased arterial stiffness as measured from pulse pressure to stroke volume ratio. In multivariate analysis, diabetic status was independently associated with stiffness even after adjustment for age, gender, height, BMI, systolic blood pressure and use of antihypertensive medication (62). In the study by Taniwaki et al. in Japanese subjects, arterial stiffness (aortic PWV) was measured in 271 diabetic patients and 285 healthy age-matched control subjects (273). Stiffness was significantly increased in diabetic patients compared to control subjects. In multiple

regression analysis in diabetic patients, age and duration of diabetes were independently associated with arterial stiffness (273).

Type 1 diabetic patients have also been shown to have stiffer large arteries in many (26,40,89,114,201,237), although not all (137,152) studies. Giannattasio et al. measured arterial stiffness in the abdominal aorta and in radial and common carotid artery using an arterial wall echo-tracking technique in 133 type 1 diabetic patients (mean age 35 years) and in 70 age-matched control subjects (89). Diabetic patients were considered free of macrovascular disease, but 59% had microvascular complications. In the diabetic patients, regardless of the presence of complications, arterial stiffness was increased at all arterial sites when compared to control subjects (89). Brooks et al. measured AgI with the use of applanation tonometry and pulse wave analysis in 89 type 1 diabetic patients (age 34 years) and in 95 control subjects (34). Although there was no significant difference in the AgI between diabetic patients and control subjects, diabetes was (together with age, height and heart rate) an independent determinant of AgI. The lack of a significant difference in AgI might have been missed because heart rate was 10 beats/min higher in the diabetic patients than in the normal subjects (34). Wilkinson et al. also determined arterial stiffness using applanation tonometry and pulse wave analysis in 35 type 1 diabetic patients and 35 matched control subjects (308). In this study, diabetic patients had a significantly higher AgI and PWV than the normal subjects (308). Intensive insulin therapy has been shown to slow arterial stiffening in type 1 diabetic patients (118). Glycemic control as measured with HbA_{1c} has not been reported to be correlated with arterial stiffness in non-diabetic subjects. At least theoretically, increases in blood glucose concentrations within the non-diabetic range could damage arterial wall because of increased glycosylation of matrix proteins as in diabetic patients (3). In non-diabetic subjects, HbA_{1c} has been reported to be correlated with thickening of arterial intima media (295) and endothelium dependent vasodilatation (289) suggesting that even small increases in blood glucose may be harmful to vascular function or may serve as markers of altered vascular function.

Arterial stiffness and cardiovascular disease

Pulse pressure, a surrogate indirect measure of arterial stiffness, has been shown to be a strong predictor of coronary heart disease independent of systolic, diastolic or mean arterial pressure (7,23,80,168). In the Framingham Heart study, the hazard ratio for CAD increased as a function of pulse pressure regardless of systolic pressure (81) (**Fig. 5**). The importance of pulse pressure for CAD risk at different ages was also studied in 3060 men and 3479 women in the same Framingham population (82). Age was found to strongly influence the predictive value of various components of blood pressure. Pulse pressure was the strongest predictor in subjects over 60 years (82) suggesting that age-related stiffening of the arteries is an important risk factor for CAD. In contrast, in individuals less than 50 years of age, diastolic blood pressure was the strongest predictor of CAD risk, whereas in individuals aged 50 to 59 years, all (systolic, diastolic and pulse pressure) components of blood pressure were comparable (82). The effects of pulse pressure vs. mean arterial pressure in predicting

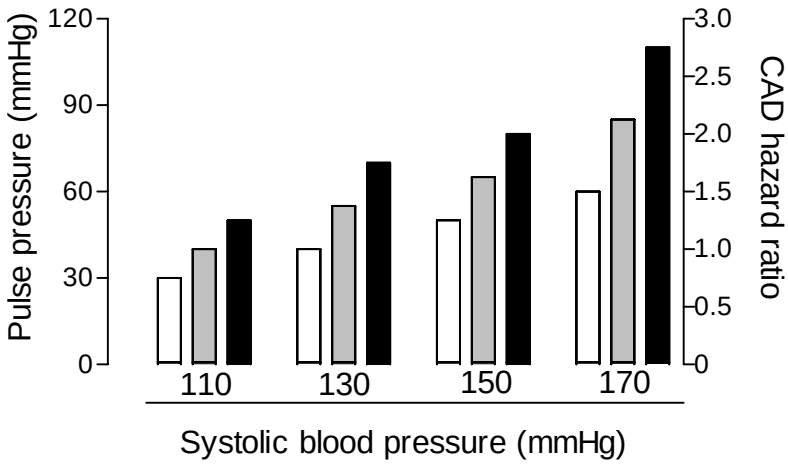

Fig. 5. Independent influence of pulse pressure on CAD risk at different levels of systolic blood pressure. Adapted from (81).

risk of CAD and cerebrovascular events in 2311 hypertensive subjects (mean age 51 years, 53% men) was studied using 24-hour ambulatory blood pressure measurements in the PIUMA study (290). Over a mean follow-up period of 4.7 years, 132 cardiac and 105 cerebrovascular events occured. Pulse pressure, but not mean arterial pressure, was a major predictor of cardiac events after adjustement for age, sex, diabetes, serum cholesterol, and cigarette smoking (290). In contrast, mean arterial pressure was the major independent predictor of cerebrovascular events, whereas pulse pressure did not yield significance (290). These data demonstrate that an increase in the dynamic component of blood pressure i.e. pulse pressure or stiffness is indeed harmful for cardiac function, as might be predicted from the increase in afterload and decrease in diastolic filling of coronary arteries that accompany an increase in wave reflection.

Direct measurements of arterial stiffness. Results from several small studies have suggested that subjects with cardiovascular disease have increased arterial stiffness compared with healthy subjects (48,87,103). Gatzka et al. (87) measured aortic stiffness by echocardiography in 55 subjects with previously unknown CAD and 55 control subjects from a cohort of 50000 people (87). Aortic stiffness, as determined from pulse pressure, Ep and stiffness index β, and left ventricular mass were increased in the CAD patients (87) compared to controls subjects matched for gender, age and serum cholesterol concentration. Mean arterial pressures were similar between the groups. In a cohort of 1980 hypertensive patients who were followed for 9 years, high carotid-femoral PWV at baseline significantly predicted all-cause and cardiovascular mortality, independent of previous cardiovascular disease, age or diabetes (149). The association between arterial stiffness and atherosclerosis was studied in a cross-sectional study by van Popele et al. (287) in 3481 subjects aged 60 to 101 years. Aortic stiffness was assessed by carotid-femoral PWV, carotid stiffness by measuring

distensibility with ultrasound and an arterial wall echo-tracking system, and atherosclerosis was determined by means of common carotid artery IMT, plaque index in carotid artery and aorta as well as presence of peripheral arterial disease (287). Both aortic and carotid stiffness were strongly associated with common carotid artery IMT and plaque index in carotid artery and aorta, also after adjustment for various other cardiovascular risk factors (287).

AIMS OF THE STUDY

The present studies were undertaken to answer the following questions:

1) Does insulin have acute effects on large artery stiffness, in addition to, or independent of its effects on PVR in normal subjects (I)?

2) Does the time course of insulin's effects on large arteries and resistance arteries differ (I)?

3) Is the effect of insulin on large arteries altered in conditions characterized by insulin resistance, such as obesity (II) or type 1 diabetes (III)?

4) Does insulin resistance, its causes or consequences correlate with large artery stiffness, and is this relationship independent of other known correlates of arterial stiffening, such as age and LDL cholesterol (IV)?

5) Which are the factors associated with insulin induced changes in large artery stiffness (IV)?

SUBJECTS AND STUDY DESIGNS

Baseline characteristics of the subjects are shown in **Table 2**. All subjects were male, non-smokers, and did not use any regular medications. Written informed consent was obtained from all subjects. The aims and study designs are described below. The study protocols were approved by the ethics committee of Helsinki University Central Hospital. All studies were performed after an overnight fast starting at 7.30-8.00 a.m.

Study I

Aims: To determine whether insulin acutely changes large artery stiffness, in addition to, or independent of its effects on PVR in normal subjects. Does the time course of insulin's effects on large arteries and resistance arteries differ?

Design: Nine healthy men participated in a 6 h sequential dose insulin clamp study and a 6 h saline infusion control study. The studies were performed in random order within a week. The sequential insulin clamp study consisted of 3 sequential 2 h insulin infusions at rates of 1 (step I), 2 (step II) and 5 mU/kg·min (step III). Normoglycemia was maintained using the euglycemic insulin clamp technique (58). Before and during the insulin infusions, hemodynamic measurements (forearm blood flow and PVR, heart rate, pulse wave analysis) were performed at 30 min intervals as detailed in Methods. Each subject also participated in a 6 h control study, during which saline was infused in the left antecubital vein at a rate of 100 ml/h (step I), 200 ml/h (step II) and 300 ml/h (step III) (2 h each) to match the volume infused during the clamp.

Study II

Aim: Is the effect of insulin on large arteries altered in obesity?

Design: A total of 23 men were studied. Of these, eight non-obese and eight obese men participated in studies addressing insulin's vascular effects, while another group of seven non-obese men participated in a saline control study (vide infra). Insulin's actions on glucose uptake and vascular function were determined under normoglycemic hyperinsulinemic conditions, which were created using the insulin clamp technique (58). Each study consisted of 2 sequential 2 hr insulin infusions at rates of 1 (step I) and 2 (step II) mU/kg·min as illustrated in **Fig. 6**. Before and during the insulin infusions, metabolic and hemodynamic measurements (pulse wave analysis, heart rate, forearm glucose extraction, blood flow and PVR) were performed at 30 min intervals as detailed in Methods. A saline control study was performed in 7 normal men (age 25±1 years, BMI 23.1±0.5 kg/m^2), in whom pulse wave analysis and measurements of forearm blood flow, heart rate and blood pressure were performed for 4 hours during infusion of saline instead of glucose and insulin.

Table 2. Characteristics of the study subjects.

	STUDY I	STUDY II		STUDY III		STUDY IV	
Variable		Non-obese	Obese	Normal	Type 1 diabetic patients		range
Number of subjects	9	8	8	9	9	50	
Age (years)	25 ± 1	25 ± 1	27 ± 2	26 ± 1	28 ± 2	34 ± 4	(18-60)
Height (cm)	179 ± 2	178 ± 2	179 ± 2	178 ± 2	181 ± 2	178 ± 1	(170-191)
BMI (kg/m^2)	23.1 ± 0.5	22.7 ± 0.4	30.6 ± 0.9***	22.3 ± 0.7	23.9 ± 1.0	27.4 ± 0.9	(18.9-45.1)
Body fat (%)	14 ± 1	12 ± 1	27 ± 1***	13 ± 1	15 ± 2	21 ± 1	(8-39)
Heart rate (beats/min)	55 ± 3	53 ± 3	63 ± 3*	55 ± 2	64 ± 4	60 ± 1	(39-75)
Systolic BP (mmHg)	114 ± 3	113 ± 3	127 ± 3*	114 ± 4	126 ± 3*	127 ± 2	(96-160)
Diastolic BP (mmHg)	66 ± 3	67 ± 3	81 ± 2*	70 ± 2	75 ± 2	79 ± 1	(52-100)
fP-glucose (mmol/l)	5.3 ± 0.1	5.3 ± 0.1	5.6 ± 0.1	5.3 ± 0.1	7.4 ± 0.8*	5.6 ± 0.1	(4.9-6.7)
fS- insulin (pmol/l)	21 ± 4	24 ± 6	60 ± 12*	18 ± 4	48 ± 6***	48 ± 5	(6-156)
HbA_{1c} (%)	5.1 ± 0.2	5.0 ± 0.2	5.2 ± 0.2	5.1 ± 0.2	7.6 ± 0.3***	5.4 ± 0.1	(3.7-6.2)
S-cholesterol (mmol/l)	4.4 ± 0.5	4.3 ± 0.3	5.2 ± 0.5	4.3 ± 0.3	4.4 ± 0.2	4.9 ± 0.2	(2.9-8.1)
S-HDL cholesterol (mmol/l)	1.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	(0.7-2.2)
S-LDL cholesterol (mmol/l)	2.6 ±0.2	2.4 ± 0.2	3.1 ± 0.4	2.5 ± 0.2	2.5 ± 0.2	3.0 ± 0.2	(1.1-6.5)
S-triglycerides (mmol/l)	0.8 ± 0.1	0.7 ± 0.1	1.5 ± 0.2**	0.7 ± 0.1	0.9 ± 0.2	1.4 ± 0.1	(0.3-3.8)

Data are shown as mean ± SEM, *p < 0.05, ** p< 0.01, *** p<0.001 non-obese vs. obese or normal vs. type 1 diabetic patients

Study III

Aim: To determine whether the ability of insulin to decrease arterial stiffness is altered in uncomplicated type 1 diabetes.

Design: Nine type 1 diabetic men and 9 matched normal men were studied under normoglycemic hyperinsulinemic conditions (sequential 2 h insulin infusions of 1 (step I) and 2 (step II) mU/kg·min, **Fig. 6**). Before and during the insulin infusions, metabolic and hemodynamic measurements (pulse wave analysis, heart rate, forearm glucose extraction, blood flow and PVR) were performed at 30 min intervals as detailed in Methods. The diabetic patients were recruited from the diabetic outpatient clinic by the following criteria: age at onset of disease less than 30 years; 2) an undetectable serum C-peptide concentration; 3) no clinical or chemical evidence of thyroid, liver, or heart disease or hypertension; 4) normoalbuminuria (albumin excretion rate <30 μg/min); 5) normal retinal photographs; and 6) no symptoms or signs of autonomic neuropathy. The diabetic patients did not use any medications except for insulin. The insulin treatment regimen consisted of three (n=4 subjects) or four (n=3) injections of a combination of intermediate and short acting insulin injections per day, or of continuous subcutaneous insulin infusion therapy (n=2).

Study IV

Aim: To determine, which factors are associated with basal arterial stiffness and its change by insulin?

Design: 50 normal men were studied. Vascular (AgI, peripheral blood flow), metabolic (whole body glucose uptake) and neural (power spectral analysis of HRV) parameters were determined basally (1 hr) and every 30 min under normoglycemic hyperinsulinemic conditions (insulin infusion rates 1 (step I) and 2 (step II) mU/kg·min for 2 hrs each, **Fig. 6**), which were maintained using the euglycemic insulin clamp technique (58).

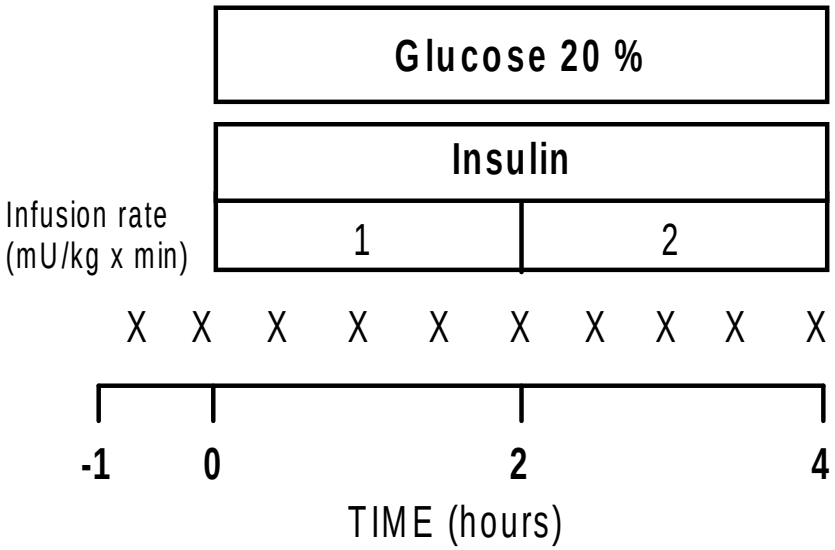

Fig. 6. Design of the euglycemic hyperinsulinemic clamp in studies II-IV. Before and during insulin infusion, (rates of 1 (step I) and 2 (step II) mU/kg·min for 2 hrs each), PVR (mean arterial pressure divided by forearm blood flow measured using venous occlusion plethysmography), the AgI (pulse wave analysis) and spectral power analysis of HRV in study IV were determined every 30 min (denoted with X). In study I, an additional 2-hr insulin infusion (infusion rate 5 mU/kg·min, step III) was performed.

METHODS

WHOLE BODY GLUCOSE UPTAKE

Insulin action on whole body glucose uptake was quantitated using the euglycemic hyperinsulinemic clamp technique (58). The studies were begun after a 10-12 hrs overnight fast. Insulin and glucose were infused into a 18 G catheter (Venflon, Viggo-Spectremed, Helsingborg, Sweden) inserted in the left antecubital vein. Another 18 G catheter was inserted retrogradely in a heated dorsal hand vein. This hand was kept in a heated chamber (65° C) to arterialize venous blood. At each step insulin (Actrapid Human, Novo Nordisk, Copenhagen, Denmark) was infused in a primed-continuous manner at rates of 1 (0-120 min), 2 (120-240 min) and in study I also at a rate of 5 (240-360 min) mU/kg·min. After start of the insulin infusion, plasma glucose was allowed reach normoglycemia in the diabetic patients. Normoglycemia was thereafter maintained by adjusting the rate of a 20% glucose insulin based on plasma glucose measurements, which were performed at 5 min intervals. Whole body glucose uptake was calculated from the glucose infusion rate corrected for changes in the glucose pool size at 30 min intervals (56). Blood samples were drawn at 30 min intervals for measurement of serum free insulin concentrations.

PULSE WAVE ANALYSIS

Acquisition of peripheral pressure waveform

All measurements were made from the radial artery, with the wrist slightly extended and supported on a pillow, by applanation tonometry using a Millar tonometer (SPC-301; Millar Instruments, Houston, TX). Data were collected directly into a desk top computer and processed with recently developed software (SphygmoCor Blood Pressure Analysis System BPAS-1; PWV Medical, Sydney, Australia), which allows continuous on-line recording of the radial artery pressure waveform. The radial waveform was assessed visually to ensure that artifacts from movement and respiration were minimized. Pulse wave analysis measurements were made twice at 30 min intervals at baseline and every 30 min during the insulin infusions. The mean of three measurements, each consisting of 15-20 sequentially recorded radial artery waveforms, was used to calculate augmentation (the difference between the second and first systolic pressure peaks, **Fig. 1**) and other parameters at a given timepoint. As suggested by O'Rourke et al., radial blood pressure was calibrated against the sphygmomanometrically determined brachial blood pressure, thus ignoring the small amplification between the brachial and radial sites (194).

Processing the aortic pressure waveform

The integral system software was used to calculate an average radial artery waveform, and generate the corresponding ascending aortic pressure waveform using a previously validated transfer factor (**Fig. 3**) (37,126,194). The aortic waveform was then subject to further analysis for calculation of augmentation, the AgI, central blood pressure and ejection duration. The AgI was calculated by dividing augmentation with pulse pressure (**Fig. 1**) (131,194,196). Ejection duration was determined as a time period from the start of the pulse wave until the closure of the aortic valve detected as an incisura in the aortic pressure wave (**Fig. 1**).

FOREARM BLOOD FLOW AND PVR

The deep branch of the right medial cubital vein draining forearm muscles was cannulated retrogradely to obtain blood samples from venous blood draining forearm muscle tissue. Forearm glucose uptake (320) was calculated by multiplying forearm blood flow by the glucose concentration difference between arterialized venous and deep venous plasma (AV-difference) (179,329). Forearm blood flow was measured at baseline and every 30 min during the euglycemic hyperinsulinemic clamp with venous occlusion plethysmography using a mercury in silastic rubber strain-gauge apparatus (Model EC-4, Hokanson, Bellevue, WA). The gauge was attached around the widest, most muscular segment of the forearm. Before blood sampling and flow measurements, circulation to the hand was interrupted by rapidly inflating a pediatric blood pressure cuff around the wrist to above the systolic blood pressure. Venous return was the occluded by a rapid cuff inflator (Rapid Cuff Inflator model E20, Hokanson) by increasing pressure in a sphygmomanometer cuff around the upper arm to 50 mmHg. Several blood flow curves were recorded with the use of an analog-to-digital converter (MacLab / 4e, AD Instruments, Castle Hill, Australia) connected to a personal computer. At least five flow curves were recorded for each flow measurement. Arterial inflow was determined with the use of computerized analysis of flow curves by drawing a tangential line for the first few pulses following cuff inflation. The slope of this line reflects the volume change per unit time. Calibration was performed with the use of use the built-in electronic calibration signal for a 1 per cent volume change, the height of which is used for blood flow calculations. PVR was calculated by dividing mean arterial pressure in the brachial artery by blood flow.

AUTONOMIC CONTROL OF HRV

Insulin regulation of autonomic function in study IV was determined using frequency domain analysis, which provides measures both parasympathetic and sympathetic control

of HRV. R-R intervals were continuously recorded for 5 minutes every 30 min while the subject's breathing was paced using a sound signal to denote inspiration and expiration every 2 seconds. Frequency domain analysis of R-R interval variability was performed using the Cardiovascular Autonomic Function 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 to 0.5 times the heart rate in Hz. LF power was determined in the frequency range from 0.04-0.15 Hz. This component reflects predominantly sympathetic control of HRV (274), when expressed in normalized units. HF power was determined in the frequency range 0.15-0.40 Hz. This component reflects vagal control of HRV (4,274). 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 · 100], and as a ratio (LF/HF), which reflects sympathovagal balance (86,274).

BODY COMPOSITION

Fat free mass and the percent body fat were determined using a single frequency bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model #BIA-101A; RJL Systems, Detroit, MI) (165).

The equation used for estimating fat free mass (FFM, kg) was:

$$FFM = 13.74 + 0.34 \cdot (h^2 / R) + 0.33 \cdot W - 0.14 \cdot age + 6.18 \cdot sex$$

Where subject's height (h) is expressed in centimeters, resistance (R) in ohms, weight (W) in kilograms, and age in years; a value for sex variable is 1 for males and 0 for females (224). This equation has been validated against body fat measured with underwater weighing in Pima Indians (224).

LABORATORY ANALYSES

Plasma glucose concentrations were measured in duplicate with the glucose oxidase method (123) using Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Serum free insulin concentrations were measured by double antibody RIA (Pharmacia Insulin RIA kit; Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol (61). HbA_{1c} was measured by high performance liquid chromatography using a fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, CA). Serum concentrations of cholesterol, triglycerides, and HDL cholesterol

were determined by enzymatic colorimetric assays with an automated Cobas Mira analyzer (Hoffmann-La Roche, Basel, Switzerland). The concentration of LDL cholesterol was calculated by the formula of Friedewald (84).

STATISTICAL METHODS

Reproducibility of augmentation and the AgI cannot be assessed using 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 (101), from augmentation values defined as the ratio of the pressure at the second systolic peak to the pressure at the first systolic peak, as this definition gives a continuous positive value (101). Bland-Altman plots were used to assess dependence of reproducibility on the mean (28).

Analysis of group, time and group x time effects between saline and insulin infusion (study I), between nonobese and obese subjects (study II) and between normal subjects and type 1 diabetic patients (study III) were made using ANOVA for repeated measures as described by Ludbrook et al. (164). Correlation analyses were performed using Spearman's non-parametric correlation coefficient in studies I-III. Simple correlations between normally distributed study variables were calculated using Pearson's correlation coefficient (study IV). All calculations were made using the Systat statistical package (Systat, Evanston, IL).

The best fit characterizing the relationship between hemodynamic parameters over time was determined by comparing the goodness of fit of linear and multiple non-linear equations using GraphPad Prism v. 2.01 (GraphPad Software Inc., San Diego CA). The results are expressed as means±SEM. All P values are 2-tailed. P values < 0.05 were considered statistically significant.

RESULTS

EFFECTS OF INSULIN ON METABOLIC AND HEMODYNAMIC PARAMETERS IN NORMAL SUBJECTS (Study I)

Metabolic effects

Serum insulin and plasma glucose concentrations and rates of whole body glucose uptake during the three insulin infusion steps are shown in **Table 3**. Forearm glucose AV-difference increased significantly within 30 min over 7-fold from 0.2±0.1 basally to 1.5±0.2 mmol/l at 30 min ($p<0.001$) and averaged 1.7±0.1, 2.0±0.1 and 1.9±0.1 mmol/l during steps I, II and III. In the saline control study, plasma glucose and serum insulin concentrations remained unchanged.

Hemodynamic effects

Aortic pressure augmentation and the AgI. Insulin significantly decreased central aortic augmentation, i.e. the pressure difference between the second and first systolic pressure peaks by 60 min (**Fig. 3**). Mean aortic augmentation averaged -1.1±1.7 mmHg basally, -2.6±1.6 mmHg during step I ($p<0.001$ vs basal, $p<0.01$ for insulin vs saline study), -3.6±1.6 mmHg during step II ($p<0.001$ vs basal, NS vs step I, $p<0.01$ for insulin vs saline study) and -4.6±0.4 mmHg during step III ($p<0.001$ vs basal, NS vs step I and NS vs step II, $p<0.01$ for insulin vs saline study). This decrease could not be attributed to a decrease in PVR since the aortic AgI, i.e. the ratio between augmentation and aortic pulse pressure also was significantly decreased at 60 min (**Fig. 3**). AgI averaged -3.2±5.2 % basally, -9.0±5.3 % during step I ($p<0.001$ vs basal, $p<0.01$ for insulin vs saline study), -10.2±4.6 % during step II ($p<0.001$ vs basal, NS vs step I, $p<0.01$ for insulin vs saline study) and -11.4±4.4 % during step III ($p<0.001$ vs basal, $p<0.05$ vs step I and NS vs step III, $p<0.01$ for insulin vs saline study, **Fig. 7**). In the saline study, both augmentation and the AgI remained stable over time (**Fig. 7**). The coefficient of variation of the AgI averaged 5±1 %. The individual mean augmentations ranged from -6.3 to 6.5 mmHg and the standard deviations between 0.6 and 1.4 mmHg. Bland-Altman plots did not reveal any trend for the difference to be dependent upon the mean value (data not shown).

Forearm blood flow and PVR. In the insulin study, forearm blood flow averaged 2.7±0.2 basally and 3.2±0.3 (NS vs. basal), 4.5±0.4 ($p<0.01$ vs. basal and step I) and 5.7±0.6 ($p<0.01$ vs. basal, step I and II) ml/dl·min during steps I, II and III. The first significant increase was observed after 150 min (3.5±0.3 ml/dl·min, $p<0.05$ vs. basal) during step II. A significant decrease in PVR was observed at 180 min (**Fig. 7**). Blood flow, blood pressure and PVR remained unchanged in the saline study (**Figs. 7 and 8**).

Heart rate, brachial and aortic blood pressure. Heart rate remained unchanged for the first 120 min (step I). It averaged 55±3, 57±3, 60±4 ($p<0.05$ vs basal) and 61±3 ($p<0.05$ vs basal and step I) beats/min basally and during steps I, II and III. Diastolic blood pressure, as measured in the brachial artery, remained unchanged for the first 120 min

Table 3. Serum insulin and plasma glucose concentrations, and rates of whole body glucose uptake during the euglycemic hyperinsulinemic clamp studies.

Original publication		Insulin infusion (mU/kg·min)	Serum insulin (pmol/l)	Plasma glucose (mmol/l)	Whole body glucose uptake (μmol/kgBW·min)
I		0	21±3	5.3±0.1	N/A
		1	366±16	5.3±0.1	39±3
		2	792±30	5.2±0.1	69±5***
		5	2315±193	5.2±0.1	86±4***
II	**Non-obese**	0	24±3	5.3±0.1	N/A
		1	372±9	5.2±0.1	40±3++
		2	804±20	5.2±0.1	69±6***++
	Obese	0	60±10	5.6±0.1	N/A
		1	414±30	5.1±0.1	21±3
		2	942±78	5.0±0.1	41±5***
III	**Normal**	0	18±6	5.3±0.1	N/A
		1	366±18	5.2±0.1	39±3++
		2	792±30	5.2±0.1	70±5***++
	Type 1 diabetic patients	0	48±6	7.4±0.8	N/A
		1	378±30	5.3±0.1	22±2
		2	834±54	5.1±0.1	44±3***
IV		0	48±5	5.6±0.1	N/A
		1	408±10	5.3±0.1	26±2
		2	952±10	5.2±0.1	49±3***

For whole body glucose uptake:

within group comparison *** $p<0.001$ vs. 1 mU/kg·min insulin infusion

between groups comparisons ++ $p<0.01$

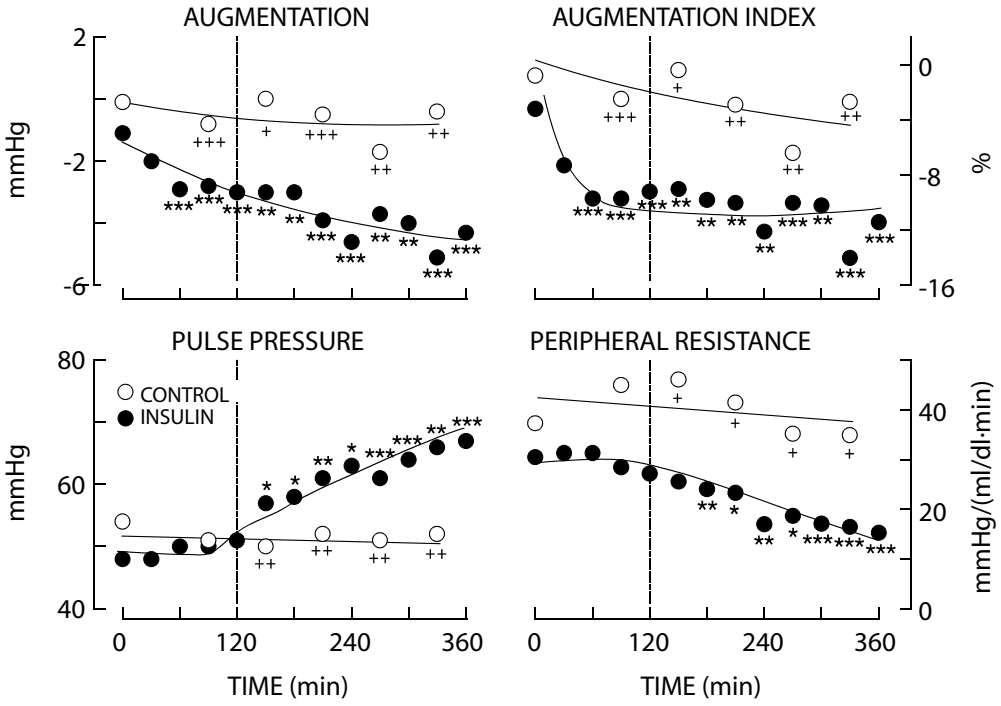

Fig. 7. Central aortic augmentation, pulse pressure, the AgI and PVR plotted as a function of time during the euglycemic insulin clamp (INSULIN) and the saline control (CONTROL) studies in study I. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ for change in parameter at a given time point vs 0 min. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ for difference at a given time point during the euglycemic insulin clamp (INSULIN) vs the saline control (CONTROL) study. Vertical hatched lines at 120 min indicate end of step I (insulin infusion at rate of 1mU/kg·min, physiological dose of insulin).

and decreased thereafter (**Fig. 8**). It averaged 66±3, 68±3, 62±3 ($p<0.05$ vs basal and step I) and 60±3 ($p<0.05$ vs basal and step I) mmHg, respectively. Central diastolic pressure showed a similar pattern and averaged 67±3 basally, 68±2 during step I, 63±2 during step II ($p<0.05$ vs basal) and 63±2 mmHg during step III ($p<0.05$ vs basal). Brachial artery systolic blood pressure averaged 114±3 basally, remained unchanged during step I (117±3) but increased thereafter (123±3 during step II, $p<0.01$ vs basal and step I, $p<0.05$ insulin vs saline study; 124±4 mmHg during step III, $p<0.01$ vs basal and step I, $p<0.05$ insulin vs saline study) (**Fig. 8**). At the level of the aorta, there was no change in systolic pressure (98±3 basally, 99±1 during step I, 99±2 step II, 99±2 mmHg step III).

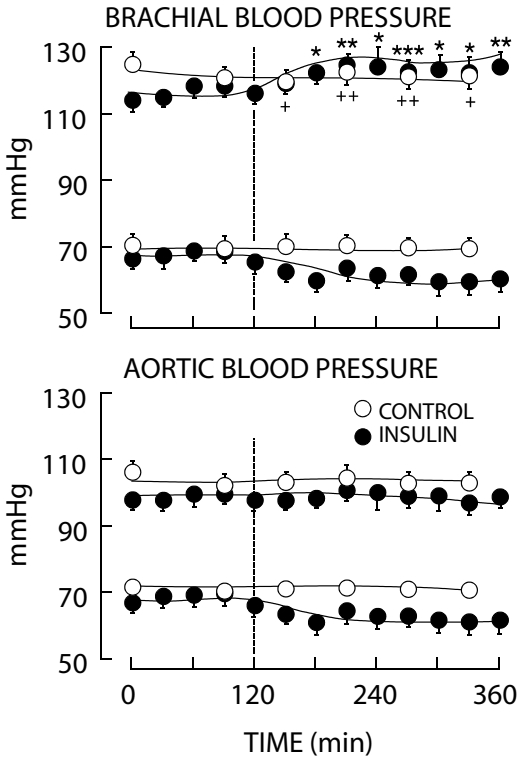

Fig. 8. Brachial and central aortic systolic and diastolic blood pressure plotted as a function of time during the euglycemic insulin clamp (INSULIN) and the saline control (CONTROL) studies in study I. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ for change in parameter at a given time point vs 0 min. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ for difference at a given time point during the euglycemic insulin clamp (INSULIN) vs the saline control (CONTROL) study. Vertical hatched lines at 120 min indicate end of step I (insulin infusion at rate of 1mU/kg·min, physiological dose of insulin).

EFFECTS OF INSULIN ON METABOLIC AND HEMODYNAMIC PARAMETERS IN OBESE SUBJECTS (Study II)

Metabolic effects

Serum insulin and plasma glucose concentrations and rates of whole body glucose uptake during the three insulin infusion steps are shown in **Table 3**. Whole body glucose uptake was 48 % lower during step I (21±3 vs. 40±3 μmol/kg·min, obese vs. non-obese $p<0.01$) and 41 % lower during step II (41±5 vs. 69±6, $p<0.01$, respectively) in the obese than the non-obese subjects. This decrease in whole body glucose uptake was paralleled by a comparable and temporarily similar defect in forearm glucose uptake

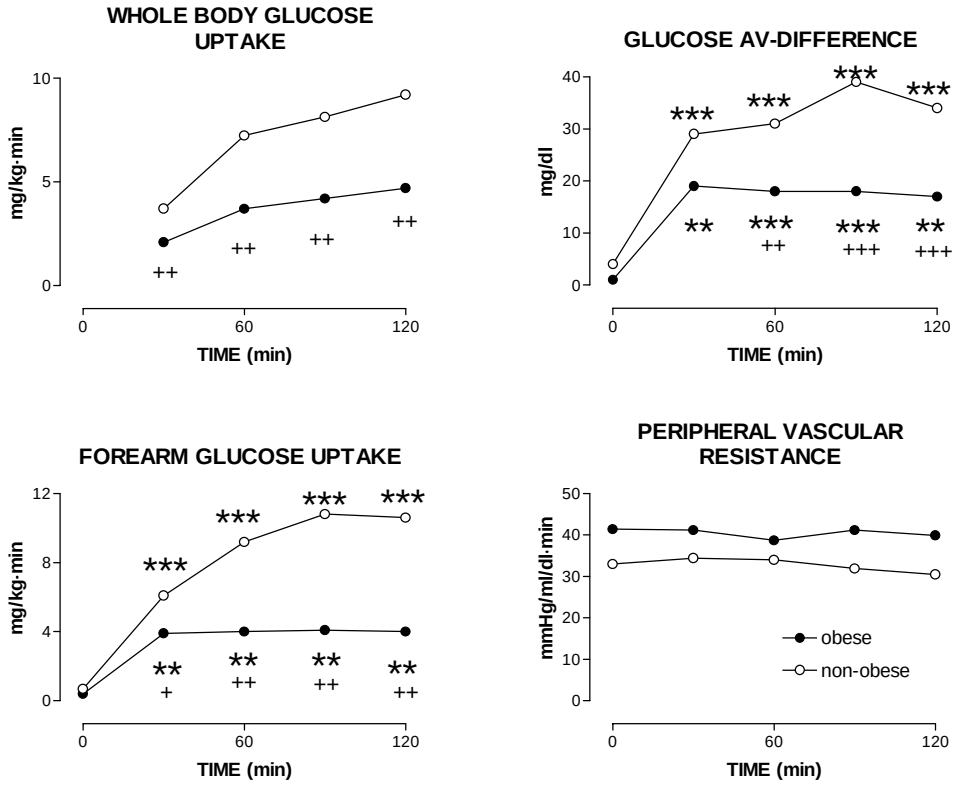

Fig. 9. Whole body glucose uptake, glucose AV-difference, forearm glucose uptake and PVR in the non-obese and obese subjects plotted as a function of time during step I (insulin infusion rate 1 mU/kg·min) of euglycemic hyperinsulinemia in study II. For whole body glucose uptake the open and filled circles denote mean glucose uptake during the preceding 30 min for non-obese and obese subjects. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ for change vs 0 min. $^{+}p<0.05$,$^{++}p<0.01$,$^{+++}p<0.001$ non-obese vs. obese subjects.

(**Fig. 9**). During the first step, i.e. during the 1 mU/kg·min insulin infusion, the decrease in insulin-stimulated glucose uptake could be attributed to a decrease in glucose extraction (AV-difference, **Fig. 9**), since during the first 2 hours forearm blood flow (**Fig. 10**) and PVR (**Fig. 9**) were comparable between the groups.

Hemodynamic effects

Aortic pressure augmentation and the AgI. Pulse wave analysis showed that augmentation decreased significantly already during the first hour (**Fig. 10**) in the non-obese subjects. Mean augmentation averaged -0.5±1.2 mmHg basally, -3.3±1.2 mmHg during step I ($p<0.001$ vs. basal) and -4.8±1.2 mmHg during step II ($p<0.001$ vs. basal, NS vs. step I) in the non-obese subjects. This decrease during step I could not be attributed to a decrease in PVR since both forearm blood flow (**Fig. 10**), mean arterial pressure and PVR remained unchanged (**Table 4**). AgI was significantly decreased already at 1 hour (**Fig. 10**) and

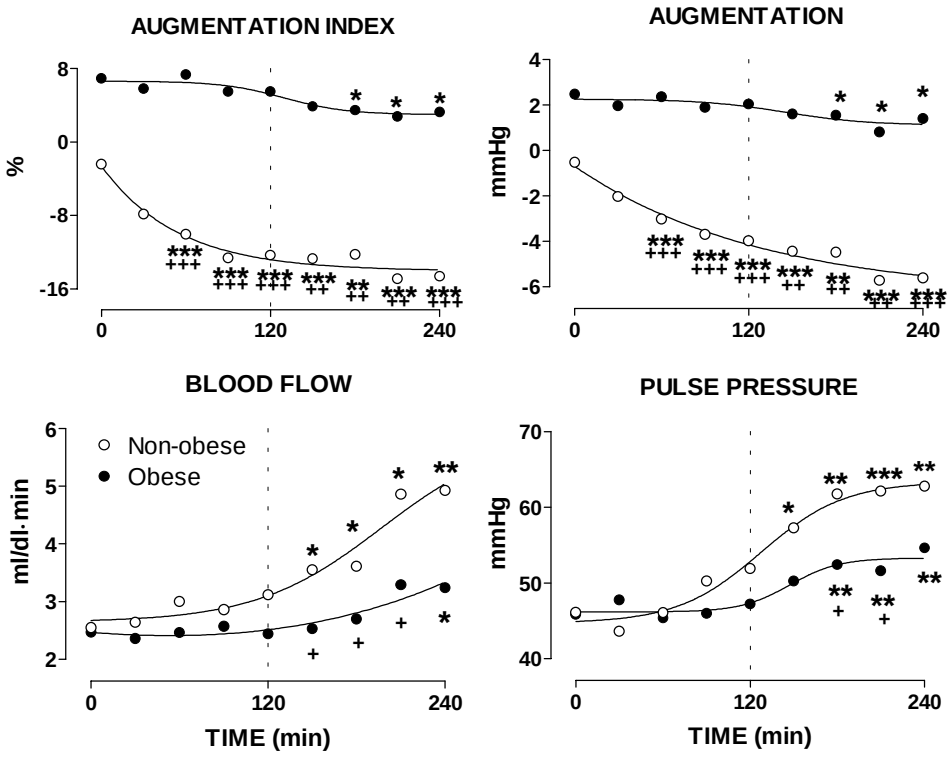

Fig. 10. The AgI, augmentation, forearm blood flow and pulse pressure plotted as a function of time during sequential insulin infusions of 1 (0-120 min) and 2 (120-240 min) mU/kg·min in study II. Normoglycemia was maintained using the euglycemic insulin clamp technique. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ for change in parameter at a given time point vs. 0 min. $^{+}p<0.05$,$^{++}p<0.01$,$^{+++}p<0.001$ for difference between non-obese and obese subjects.

averaged -2.4±3.9 % basally, -11.1±4.3 % during step I ($p<0.001$ vs. basal) and -13.1±3.4 % during step II ($p<0.001$ vs. basal, NS vs. step I) (**Fig. 10**).

In the obese subjects, in contrast to the non-obese subjects, augmentation did not decrease by insulin during the first hour (**Fig. 10**). A significant decrease in augmentation was not observed until after 3 hours (**Fig. 10**). As in the non-obese subjects, blood flow, pulse pressure (**Fig. 10**), mean arterial pressure and PVR (**Table 4**) remained unchanged during step I. Mean augmentation averaged 2.5±0.8 mmHg basally, 2.1±0.7 mmHg during step I (NS vs. basal in the obese subjects) and 1.5±1.0 mmHg during step II ($p<0.05$ vs. basal, NS vs. step I). AgI averaged 7.1±1.8 % basally, 6.1±2.1 % during step I (NS vs. basal) and 3.4±2.6 % during step II ($p<0.05$ vs. basal, NS vs. step I). The first significant decrease in the AgI was detected after 3 hrs (3.5±2.4 %, respectively, $p<0.05$ vs. basal) in the obese (**Fig. 10**). The changes in augmentation and the AgI were significantly

Table 4. Hemodynamic characteristic of the subjects of study II basally and during insulin infusions of 1(Step I) and 2 (Step II) mU/kg·min.

	Basal		**Step I**		**Step II**	
	Non-obese	Obese	Non-obese	Obese	Non-obese	Obese
Heart rate (beats/min)	53 ± 3	63 ± 3[a]	56 ± 3	65 ± 3	59 ± 4*	64 ± 3
Brachial systolic blood pressure (mmHg)	113 ± 3	127 ± 3[a]	115 ± 2	126 ± 3[a]	121 ± 2**[++]	131 ± 3[a]*[+]
Brachial diastolic blood pressure (mmHg)	67 ± 3	81 ± 2[b]	67 ± 3	79 ± 3[a]	60 ± 3**[++]	78 ± 3[b]
Aortic systolic blood pressure (mmHg)	98 ± 2	111 ± 3[b]	97 ± 2	110 ± 4[b]	98 ± 2	112 ± 3[b]
Aortic diastolic blood pressure (mmHg)	69 ± 3	80 ± 3[c]	68 ± 3	80 ± 3[b]	61 ± 3**[++]	80 ± 3[b]
Pulse pressure (mmHg)	46 ± 3	46 ± 2	48 ± 3	47 ± 1	61 ± 3***[+++]	52 ± 2[a]**[++]
Mean arterial pressure (mmHg)	83 ± 3	96 ± 3[b]	83 ± 2	95 ± 3[b]	81 ± 2*[+]	96 ± 3[b]
Blood flow (ml/dl·min)	2.6 ± 0.2	2.5 ± 0.2	3.0 ± 0.3	2.5 ± 0.2	4.0 ± 0.5**[++]	2.9 ± 0.2[a]
PVR (mmHg/ml/dl·min)	33 ± 3	41 ± 3	33 ± 3	40 ± 2	25 ± 3**[++]	35 ± 2[a]**[++]

[a]p<0.05, [b]p<0.01, [c]p<0.001 non-obese vs. obese, *p<0.05, **p<0.01, ***p<0.001 vs. basal, [+]p<0.05, [++]p<0.01, [+++]p<0.001 vs. step I

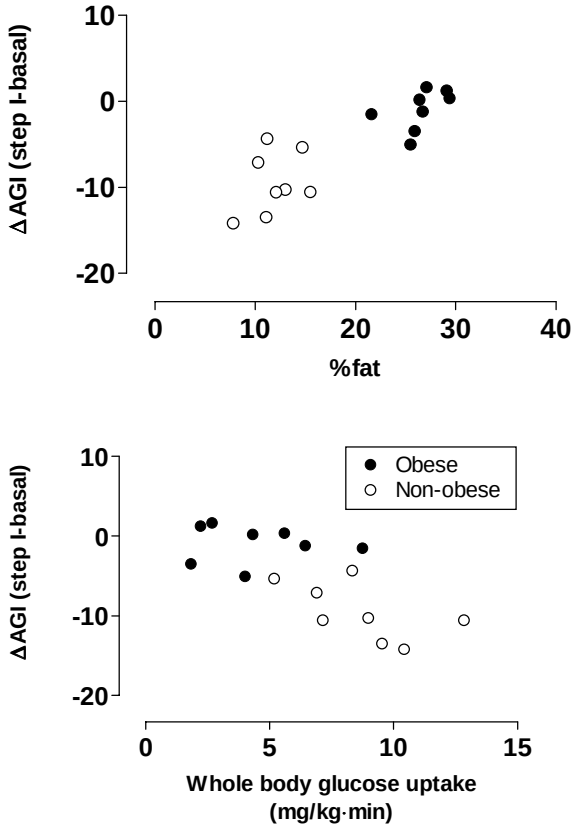

Fig. 11. Relationship between the %fat (upper panel) and whole body glucose uptake (lower panel) and the change in the AgI (ΔAGI) during the 1 mU/kg·min insulin infusion in study II.

different between the non-obese and obese subjects from 1 hour onwards (**Fig. 10**). The % fat ($r = 0.86$, $p < 0.0001$ for pooled data) and whole body glucose uptake ($r = -0.72$, $p = 0.0015$ for pooled data) were significantly correlated with the change in AgI by insulin during step I (**Fig. 11**). Both augmentation and the AgI remained unchanged during the saline control study (data not shown).

Peripheral blood flow. Forearm blood flows were comparable between the obese and non-obese subjects basally and during the first step i.e. during the first 2 hours of hyperinsulinemia (**Fig. 10, Table 4**). The first significant increase in blood flow in the non-obese was observed after 2.5 hrs (**Fig. 10**). In the obese subjects, the first significant increase was observed after 4 hrs (**Fig. 10**). Both flow during the highest insulin dose (step II) and the increase in forearm blood flow above basal were significantly lower in the obese than in the non-obese subjects from 2.5 hrs onwards (**Fig. 10**).

*Heart rate, brachial and aortic blood pressures (**Table 4**).* Peripheral and central diastolic blood pressure decreased significantly in the non-obese subjects after 3 hours. In the obese subjects, neither peripheral nor central blood pressure was significantly decreased by insulin (**Table 4**). Peripheral systolic blood pressure increased significantly during step II, while central systolic blood pressure remained unchanged in both groups (**Table 4**). Pulse pressure increased and PVR decreased significantly in both groups during the step II (**Fig. 10, Table 4**) The increase in pulse pressure and the decrease in PVR were significantly blunted in the obese compared to the non-obese during step II (**Fig. 10, Table 4**). Heart rate remained unchanged for the first 1.5 hrs in the non-obese and until 4 hrs in the obese. The increase in heart rate during step II was significantly lower in the obese than in the non-obese (**Table 4**). Both heart rate and blood pressure remained unchanged during the saline control study (data not shown).

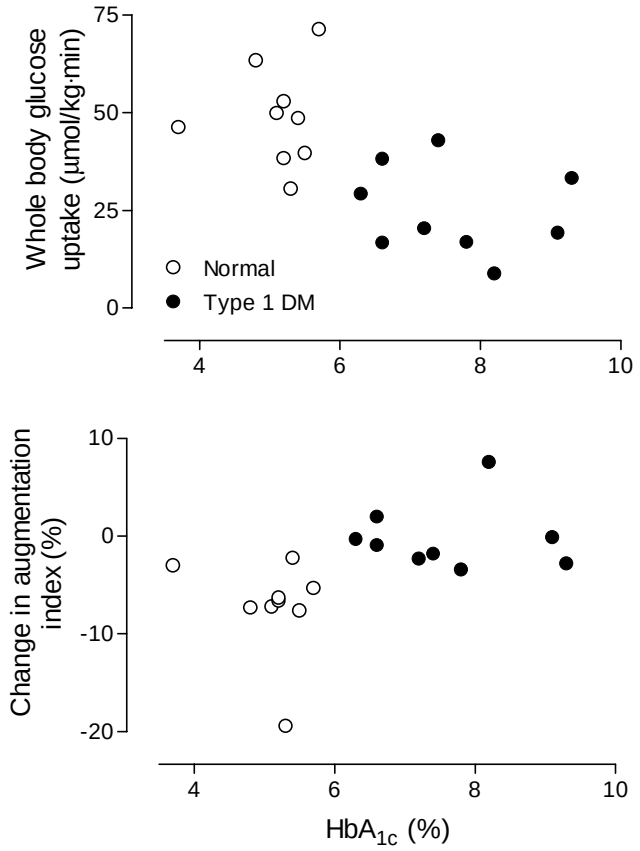

Fig. 12. Relationship between the concentration of HbA_{1c} and whole body glucose uptake (upper panel) (r=-0.69, p<0.01 for pooled data) and between the concentration of HbA_{1c} and change in the AgI (lower panel) (r=0.65, p<0.01 for pooled data) during the 1 mU/kg·min insulin infusion in study III.

EFFECTS OF INSULIN ON METABOLIC AND HEMODYNAMIC PARAMETERS IN TYPE 1 DIABETIC PATIENTS (Study III)

Metabolic effects

Serum insulin and plasma glucose concentrations and rates of whole body glucose uptake during the two insulin infusion steps are shown in **Table 3**. Whole body glucose uptake was 44% lower during step I (22.2±2.2 vs. 39.4±3.3 μmol/kg·min, patients with type 1 diabetes vs. normal subjects, $p<0.001$) and 37% lower during step II (44.4±2.2 vs. 70.0±5.0, $p<0.001$, respectively) in the patients with type 1 diabetes than the normal subjects. Forearm glucose uptake was 42% lower during step I (28.3±6.1 vs. 48.9±7.2 μmol/kg forearm·min, patients with type 1 diabetes vs. normal subjects, $p<0.05$) and 40% lower during step II (45.0±9.4 vs. 75.6±0.6, $p<0.05$, respectively). In correlation analysis for pooled data, whole body glucose uptake was significantly correlated with forearm glucose uptake during step I ($r=0.82$, $p<0.0001$) and step II ($r=0.60$, $p<0.02$). The concentration of HbA_{1c} was inversely correlated with whole body glucose uptake ($r=-0.69$, $p<0.01$, **Fig. 12**).

Hemodynamic effects

Aortic pressure augmentation and the AgI. Augmentation decreased significantly already during the first hour in the normal subjects by -2.6±1.5 mmHg ($p<0.01$ vs. basal). Mean augmentation averaged -0.5±1.5 mmHg basally, -2.2±1.3 mmHg during step I ($p<0.001$ vs. basal) and -3.0±1.3 mmHg during step II ($p<0.01$ vs. basal, NS vs. step I) in the normal subjects. This decrease during step I could not be attributed to a decrease in PVR since both forearm blood flow, mean arterial pressure and PVR remained unchanged

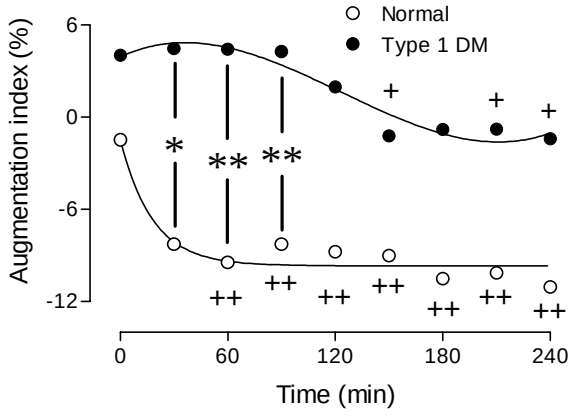

Fig. 13. The AgI during insulin infusions of 1 (0-120 min) and 2 (120-240 min) mU/kg·min in study III. Euglycemia was maintained with the use of insulin clamp technique. *$p<0.05$, **$p<0.01$ for difference between the changes in the AgI in the normal subjects and type 1 diabetic patients. $^{+}p<0.05$, $^{++}p<0.01$ for change in the AgI at a given time point vs 0 min.

Table 5. Hemodynamic characteristics of the subjects basally and during insulin infusions of 1 (*Step I*) and 2 (*Step II*) mU/kg·min.

	Normal subjects (n=9)			**Type 1 diabetic subjects (n=9)**		
Variable	Basal	Step I	Step II	Basal	Step I	Step II
Heart rate (beats/min)	55±2	58±3	61±3$^{\#\#}$	64±4	64±4	65±4
Ejection duration (ms)	349±4	341±4	334±4$^{\#\#}$	334±7	320±6	318±7$^{\#\#}$
Brachial systolic blood pressure (mmHg)	114±3**	117±2$^{\#}$	123±3$^{\#\#}$	126±3	121±3	123±3
Brachial diastolic blood pressure (mmHg)	70±2	71±2	66±2*$^{\#\#}$	75±2	75±2	72±2
Aortic systolic blood pressure (mmHg)	100±2*	100±2	101±2	109±3	105±3	105±3
Aortic diastolic blood pressure (mmHg)	70±2	72±2	67±2*	76±2	76±2	73±2
Pulse pressure (mmHg)	44±2*	46±2	58±2*$^{\#\#}$	51±3	46±2	51±2
Mean arterial pressure (mmHg)	84±2*	86±2	85±2	92±2	90±2	89±2
Forearm blood flow (ml/dl·min)	2.6±0.2	3.1±0.3	4.3±0.5$^{\#\#}$	3.2±0.4	2.8±0.4	3.3±0.5
PVR (mmHg/ml/dl·min)	34±3	31±2	24±3$^{\#}$	33±4	38±5	34±5

*p<0.05, **p<0.02 for comparison of type 1 diabetic patients vs normal subjects basally, during step I or step II. $^{\#}$p<0.05, $^{\#\#}$p<0.02 for change in parameter step I or step II vs basal.

(**Table 5**). The AgI decreased significantly already at 30 min (-8.3±4.6 %, p<0.05 vs. basal) (**Fig. 13**). The AgI averaged -1.5±4.5 % basally, -8.7±4.5 % during step I (p<0.01 vs. basal) and -10.2±4.0 % during step II (p<0.01 vs. basal, NS vs. step I) (**Fig. 13**).

Basally, before the insulin infusion, augmentation and the AgI were comparable between type 1 diabetic patients and normal subjects. In contrast to the normal subjects, however, neither augmentation nor the AgI decreased by insulin in the diabetic patients during the first hour. Mean augmentation averaged 1.6±1.3 mmHg basally, 1.3±1.2 mmHg during step I (NS vs. basal in the patients with type 1 diabetes) and 0.1±1.5 mmHg during step II (NS vs. basal and step I). AgI averaged 3.7±3.8 % basally, 3.5±4.0 % during step I (NS vs. basal) and -0.9±4.5 % during step II (NS vs. basal, p<0.05 vs. step I). The first significant decrease in AgI occurred at 150 min (0.9±4.0 %, p<0.05 vs. basal) in the patients with type 1 diabetes (**Fig. 13**). The changes in the AgI were significantly different between the patients with type 1 diabetes and normal subjects from 30 until 90 min (**Fig. 13**). The rate of whole body glucose uptake was significantly inversely correlated with the change in the AgI by insulin during step I (r=-0.61, p<0.01 for pooled data **Fig 14**). The concentration of HbA_{1c} was positively correlated with the change in the AgI during step I (r=0.65, p<0.01for pooled data **Fig. 14**).

Other hemodynamic parameters. The data on heart rate, ejection duration, peripheral and central blood pressures, pulse pressure, mean arterial pressure, forearm blood flow and PVR basally and during steps I and II are shown in **Table 5**. Basal heart rates, ejection duration and PVR were comparable at baseline. Heart rate remained unchanged in both groups during step I as did forearm blood flow and PVR (**Table 5**). Brachial and aortic systolic and pulse pressures were slightly higher in the type 1 diabetic patients basally than in the normal subjects. Diastolic blood pressures were not significantly different between the groups.

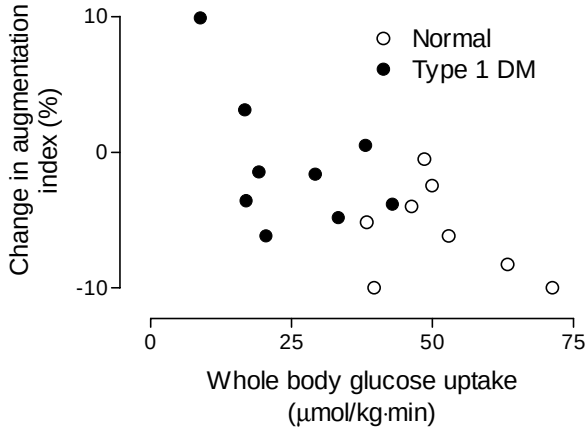

Fig. 14. Relationship between whole body glucose uptake and the change in AgI during the 1 mU/kg·min insulin infusion in study III. Spearman's non-parametric correlation coefficient was -0.61, p<0.01.

DETERMINANTS OF BASAL ARTERIAL STIFFNESS AND OF ACTION OF INSULIN ON LARGE ARTERY STIFFNESS *IN VIVO* (Study IV)

Metabolic and hemodynamic effects of insulin

Serum insulin and plasma glucose concentrations and rates of whole body glucose uptake (M-value) during the two insulin infusion steps are shown in **Table 3**. The M-values averaged 26±2 and 49±3 mmol/kg·min during steps I and II. The values, calculated as in the EGIR study (glucose infusion rate between 80 and 120 min), during step I have been superimposed on data showing the relationship between BMI (range 18 to 50 kg/m^2, 26±1 kg/m^2, mean±SEM) and the M-value in 752 European men (age range 18 to 60 yrs, 38±1 yrs) (**Fig. 15**) (115). Therefore the 50 men in study IV can be considered to represent average European men with respect to their insulin sensitivity.

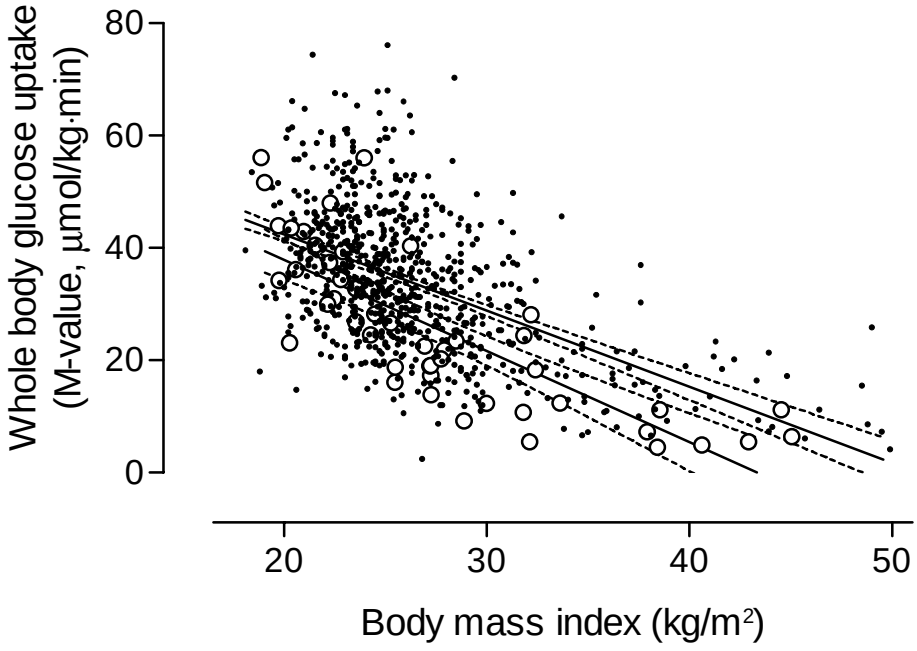

Fig. 15. The relationship (Spearman's nonparametric correlation coefficient) between BMI and whole body glucose uptake in the subjects of study IV (open circles, r=-0.86, p<0.001, whole body glucose uptake (µmol/kg·min)=70.43 - 1.63 × BMI (kg/m^2)) and in 752 European men whose data are included in the EGIR database (closed circles, r=-0.49, p<0.001, whole body glucose uptake=69.24 - 1.34 × BMI).

Aortic pressure augmentation and the AgI. Augmentation decreased significantly within 30 min from 4.1±0.9 to 2.6±0.9 mmHg ($p<0.001$). Augmentation averaged 2.2±0.8 mmHg during step I and 1.0±0.9 mmHg 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. 16**). AgI averaged 4.4±2.1 and 0.9±2.1 % during the steps I and II.

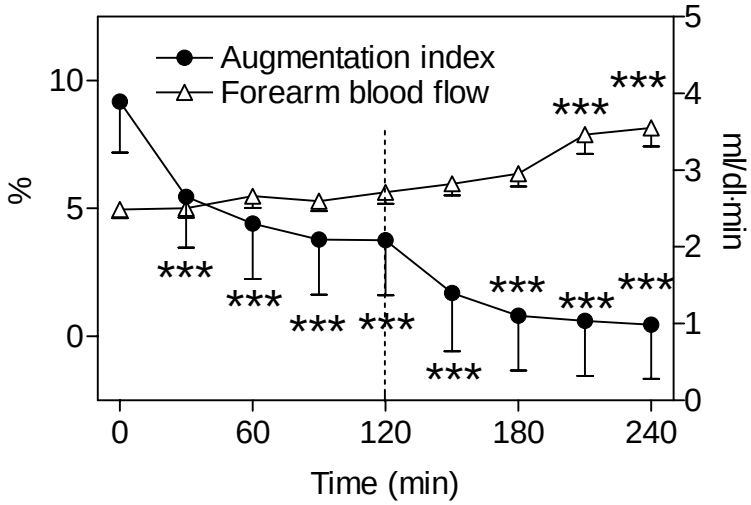

Fig. 16. The AgI and forearm blood flow under normoglycemic hyperinsulinemic conditions (insulin infusion of 1 and 2 mU/kg·min, steps separated by the hatched line) in study IV. *** $p<0.001$ for change in parameter at a given time point vs 0 min.

Peripheral blood flow. Forearm blood flow increased linearly and within 4 hours 1.4-fold from 2.5±0.1 basally to 3.6±0.2 ml/dl·min at 240 min (**Fig. 16**, $p<0.001$). 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).

Other hemodynamic parameters. Heart rate remained unchanged during the step I (60±1 vs. 61±1 beats/min 0 vs step I), but increased significantly from 150 min onwards (63±1 beats/min at 150 min, $p<0.001$ vs 0 min, **Fig. 17**). Brachial artery systolic blood pressure remained unchanged during step I (**Fig. 18**) 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 mmHg, 0 vs 120 min, NS, **Fig. 18**). Brachial diastolic blood pressure was unchanged during step I but then decreased significantly at 150 min (79±1 vs 76±2 mmHg, 0 vs 150 min, $p<0.001$, **Fig. 18**). Aortic diastolic blood pressure remained unchanged during step I (80±2 vs 78±2 mmHg, 0 vs 120 min), but decreased significantly at 150 min (77±2 mmHg, $p<0.001$ vs 0 min, **Fig. 18**).

Insulin action on autonomic control of HRV

There were no significant changes in parameters reflecting autonomic control of HRV during step I (**Fig. 17**). During step II at 210 min, a significant increase was observed in normalized LF (45±5 vs. 55±4, 0 vs 210 min, $p<0.05$) (**Fig. 17**). 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).

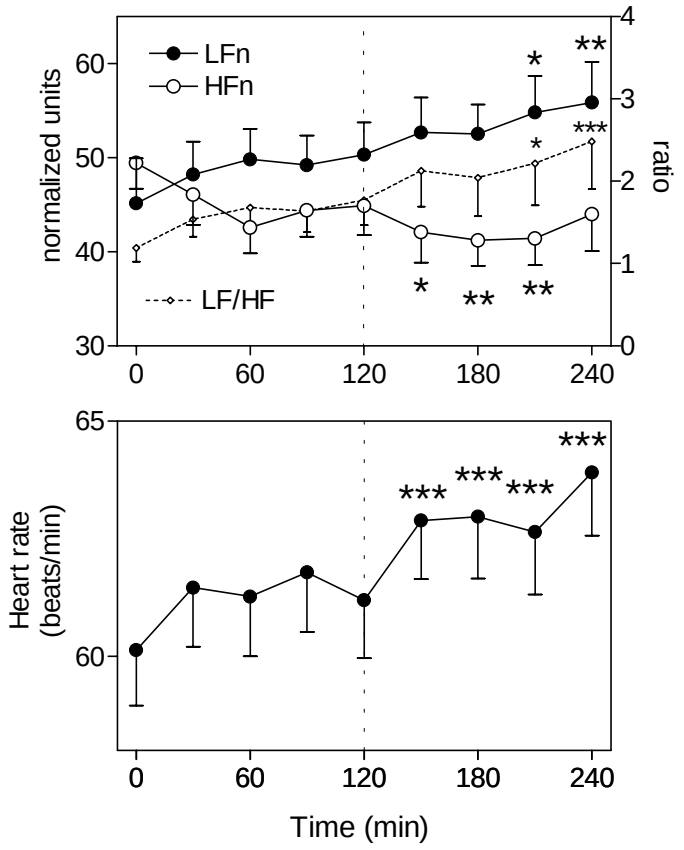

Fig. 17. Normalized low frequency (LFn), high frequency (HFn) and low/high frequency ratio (upper panel) and heart rate (lower panel) under normoglycemic hyperinsulinemic conditions (insulin infusion of 1 and 2 mU/kg·min, steps separated by the hatched line) in study IV. *p<0.05, **p<0.01, *** p<0.001 for change in parameter at a given time point vs 0 min.

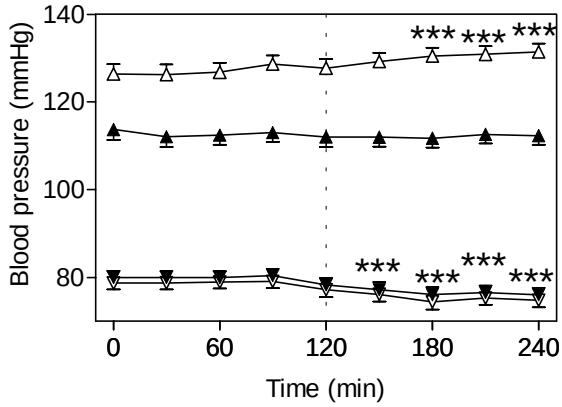

Fig. 18. Aortic (black triangles) and brachial (open triangles) systolic and diastolic blood pressures under normoglycemic hyperinsulinemic conditions in study IV. *** p<0.001 for change in parameter at a given time point vs 0 min.

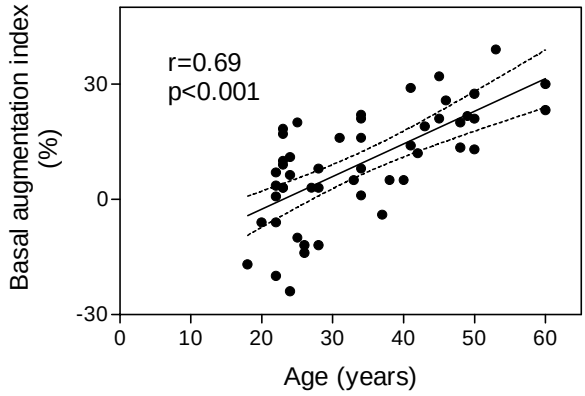

Fig. 19. The relationship (Spearman's nonparametric correlation coefficient) between age and basal AgI in study IV.

Factors associated with variation in the AgI

Basal AgI. In simple regression analysis, significant correlates of basal AgI included age (**Fig. 19**), mean arterial blood pressure and LDL cholesterol (**Table 6**). In addition, insulin sensitivity (M-values during steps I and II), the waist to hip ratio, HDL cholesterol but not weight or BMI were significantly correlated with the AgI. Height was inversely and HbA_{1c} positively correlated with basal AgI. After adjustment for age, none of these associations were significant except for height, which remained marginally significant (**Table 6**). 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 the AgI. None of the classic risk factors or other known determinants of arterial stiffness were correlated with changes in the AgI induced by insulin (**Table 6**), while several causes or consequences of insulin resistance were. The latter included insulin action itself, as assessed from the M-value during both steps I and II, weight, BMI and the waist to hip ratio (**Table 6**).

Table 6. Simple regression analysis of determinants of the basal AgI and the change in the AgI by insulin. Pearson's correlation coefficients for the total group (n=50).

	Basal AgI	**Age adjusted**	**Change in AgI[#]**
Classic risk factors			
Age	0.69***	-	0.16
Mean arterial pressure	0.49***	0.16	0.16
Serum LDL cholesterol	0.45***	0.12	-0.01
Components of the insulin resistance syndrome			
M-value, step I	-0.24(*)	0.03	-0.32*
M-value, step II	-0.30*	0.06	-0.31*
Serum HDL cholesterol	-0.31*	-0.01	0.01
Waist to hip ratio	0.30*	-0.11	0.37**
Serum triglycerides	0.25(*)	-0.01	0.23
BMI	0.15		0.36**
Weight	0.09		0.37**
Other factors			
Basal AgI	-		-0.11
Systolic blood pressure	0.53***	0.17	0.13
HbA_{1c} (%)	0.40**		0.10
Change in LFn[#]			0.04
Height	-0.26(*)	-0.17	0.08
Change in heart rate[#]	-0.11		
Change in peripheral blood flow[#]			-0.01

(*)0.05<p<0.1, *p<0.05, **p<0.01, p<0.001, [#] during the physiological insulin infusion (step I)

DISCUSSION

Insulin resistance is associated with an increased risk of cardiovascular disease (77,303) independent of conventional risk factors (216). Insulin resistance is also associated with hypertension (75,209,252) and markers of atherosclerosis, such as arterial IMT (2,113,269) and stiffness (244). The mechanisms underlying the association between insulin resistance and cardiovascular disease are currently incompletely understood. The present studies were undertaken to explore the existence of a novel action of insulin to regulate arterial stiffness in normal subjects. Within a physiological time-frame and at physiological concentrations, insulin was found to decrease large artery stiffness in healthy subjects. This action was temporarily clearly distinct from insulin action on peripheral blood flow. In insulin resistant obese subjects and in uncomplicated type 1 diabetic patients, the ability of insulin to decrease stiffness, as determined from a decrease in the AgI, was blunted. In a group of 50 healthy men, action of insulin to diminish stiffness was found to be associated with various components of insulin resistance but not with classic cardiovascular risk factors. *In vivo* insulin regulation of large arteries and its impairment in insulin resistance conditions could provide a novel mechanistic link between insulin resistance and macrovascular disease.

METHODS

Pulse wave analysis

We performed pulse wave analysis using applanation tonometry from the radial artery to examine insulin action on large vessel function (194). This method has the advantage of not reflecting changes in an isolated artery but in the entire vascular tree and that it also allows non-invasive determination of central aortic pressure, which is the pressure actually seen by the left ventricle. Because of pulse wave amplification along the arterial tree, accurate estimation of aortic pressures simply from peripheral arterial pressures is not possible. Although both diastolic and mean arterial pressures between the radial artery and ascending aorta are significantly different when recorded intra-arterially, the greatest difference is between systolic aortic and peripheral pressures (205). Radial artery pressure waveforms recorded with tonometry have been shown to equal those measured intra-arterially in a large group of normal subjects (133). Although changes in the aortic pressure waveform can be inferred from changes in the contour of the radial pressure wave, accurate assessment of the aortic pressure waveform necessitates either direct measurements in the aorta, or use of a transfer function to calculate aortic pressure based on measurements in the radial artery. Several studies have now demonstrated that a single generalized transfer function can be used to accurately determine central from peripheral pressure in normal subjects and in patients

with a variety of diseases (37,126,194). 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 opportunity to accurately measure wave reflection and central aortic pressure. The data generated in studies I-IV emphasize the importance of analyzing the entire arterial waveform and recording of central pressure. In these studies, the ability of insulin to diminish wave reflection and the AgI would not have been observed if only brachial artery blood pressure and peripheral blood flow would have been recorded. We also found pulse wave analysis to be highly reproducible consistent with other reports (254,305). However, the limitations of pulse wave analysis should not be forgotten. First, use of the transfer function gives only an estimate of the central waveform, although mathematically derived waveforms have been comparable to invasively measured waveforms in catheterization studies (37,126). Second, the AgI is influenced not only PWV (arterial stiffness), but also by ventricular ejection and arterial reflecting sites (190). However, in our studies, the decrease in the AgI occurred when ejection duration, heart rate and PVR remained unchanged suggesting that these factors were not responsible for the change in the AgI but rather a change in stiffness. Furthermore, a global approach to measure properties of the whole arterial tree does not allow identification of the exact arterial segment(s) which caused the change in the AgI.

Assessment of autonomic function

We chose to monitor insulin induced changes in components of HRV 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 (5,174,210). The LF component has been shown to correlate with MSNA during sympathetic stimulation (25), and the normalized LF component is higher in obese than non-obese subjects as is MSNA under fasting conditions (186,203). The normalized LF component and MSNA both fail to respond to insulin in obese subjects suggesting that the autonomic nervous system may be insulin resistant (186,203).

EFFECT OF INSULIN ON WHOLE BODY AND FOREARM GLUCOSE UPTAKE

In the obese subjects in study II, glucose extraction, as determined from the glucose AV-difference across the forearm, was resistant to stimulation by insulin, in keeping with previous data (143,145,217). The glucose extraction defect cannot be ameliorated by increasing blood flow (145) and is therefore a defect which is distinct from defects in insulin's vascular actions. The degree of insulin resistance in type 1 diabetic patients

in study III was comparable to what has been reported previously (316). Since whole body and forearm glucose uptake were significantly correlated, and forearm blood flows were similar between the two groups during the first 2 hour insulin infusion in the studies II-III, the defect in insulin stimulation of glucose uptake could be attributed to a cellular rather than vascular defect in peripheral tissues also in this group (172). As in previous studies including larger numbers of type 1 diabetic patients, the defect in peripheral glucose uptake in study III correlated with the degree of chronic hyperglycemia as measured by HbA_{1c} (316).

EFFECT OF INSULIN ON HEMODYNAMIC PARAMETERS

Effect of insulin on large artery stiffness

A consistent and novel finding in studies I-IV was the finding that insulin decreased central pressure augmentation and the AgI. This effect was observed within 30 to 60 minutes at physiological insulin concentrations in normal lean men. In obese men and in type 1 diabetic patients, the decrease in the AgI required supraphysiological insulin concentrations and was only observed after 1.5 to 2.5 hours of insulin infusion. Changes in the AgI provide a measure of changes in stiffness provided both heart rate and PVR remain unchanged (190,194). This was true in studies I-IV during the 1 mU/kg·min insulin infusion. During the higher dose insulin infusions (2 mU/kg·min in studies I-IV, and 5 mU/kg·min in study I) in the normal, lean subjects, heart rate increased and ejection duration shortened. This will increase the fraction of wave reflection which occurs in the diastole and decrease the AgI independent of any change in stiffness. This did not, however, influence interpretation of the data since the AgI decreased maximally in the normal, lean subjects in studies I-III already during the first step when ejection duration and heart rate remained unchanged. Also in study IV, a significant decrease in the AgI was detected already within 30 min. Similarly, the lack of change in the AgI in the obese subjects in study II or type 1 diabetic patients in study III could not be attributed to heart rate or ejection duration as these remained unchanged.

The acute effect of insulin on wave reflection is similar to that previously described for GTN (132,310), which also decreases both augmentation and the AgI but in low doses has no effect on brachial artery systolic or diastolic pressure or peripheral blood flow (132,310). A decrease in the AgI reflects diminished stiffness but does not necessarily imply a change in stiffness at the level of the aorta (310). For example, GTN does not alter aortic compliance but clearly decreases wave reflection without changing PVR (310). This effect of GTN reflects decreased stiffness at the level of muscular conduit arteries rather than the aorta (258,310). Of note, most ultrasound techniques and magnetic resonance imaging techniques measure compliance of the aorta, which is unlikely to change acutely. These techniques may therefore miss changes which occur in conduit arteries (90). Regarding insulin, a recent study found no effect of combined hyperglycemia

and hyperinsulinemia (serum insulin 112 mU/l) on carotid compliance in normal subjects (147). This does not, however, exclude the possibility that insulin increases compliance in muscular conduit arteries. It would be possible to study this possibility using techniques such as bidimensional dual-crystal pulsed Doppler systems or other comparable devices. On the other hand, the intra- and inter-observer reproducibilities when measuring brachial artery internal diameter using two-dimensional echocardiography coupled with a Doppler system have been reported to be 14.8 and 11.3% (60). Since the brachial artery is 3-4 mm in diameter, such measurement may be subject to a type 2 statistical error. Even if aortic compliance remains unchanged, diminished wave reflection at the level of the aorta has several beneficial hemodynamic effects. The delay in the return of the reflected wave will increase diastolic pressure and augment left ventricular filling and thereby coronary blood flow (90).

In study II, the ability of insulin to decrease aortic wave reflection, as determined from augmentation and the AgI, was severely blunted in the obese subjects. Thus, in contrast to the non-obese subjects, in which insulin, within 1 hour and at a concentration of 64 mU/l significantly decreased augmentation, in the obese a decrease was not detected until after 3 hrs at a serum insulin concentration of 155 mU/l. Since this defect was observed under conditions where PVR was unchanged, the failure of insulin to decrease the AgI is consistent with diminished arterial stiffness in obese subjects. The defect in stiffness was not observed in the basal state but became evident during insulin stimulation suggesting that the defect may be a consequence of insulin resistance. On the other hand, both obese human subjects (145,265) and obese animals (180) are characterized by a defect in endothelium-dependent vasodilatation in peripheral resistance vessels. Although it is currently unknown whether endothelial dysfunction characterizes large arteries in obese humans, large arteries are more dependent on NO than microvessels (282). It is therefore possible that the defect was not specific to insulin but that insulin merely acted to unmask a defect in large artery endothelial function. Regarding the location of insulin's effect, it was localized to arteries larger than those controlling PVR i.e. conduit or large arteries.

Effect of insulin on peripheral blood flow

After 2 hours in the studies I-III during infusion of a supraphysiologic dose of insulin, forearm blood flow increased and was accompanied by a significant decrease in PVR in lean, normal subjects. These changes are consistent with previously reported slow time course of insulin's vasodilatory effects on peripheral resistance vessels (143,284). Also consistent with previous studies (143,265), insulin stimulation of blood flow was blunted in the obese subjects in study II. In the obese, an increase in blood flow was not observed until after 4 hrs of insulin infusion. Heart rate and peripheral systolic blood pressure also increased significantly only after 2 hours of insulin infusion in normal lean subjects in studies I-III and in the group of 50 men in the study IV. 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 (25,297,299). The fact that all of these effects of insulin occurred much later than those

in augmentation and the AgI, suggests temporal hierarchy in insulin's vascular effects, and that insulin increases the diameter or distensibility of arteries before arterioles (resistance vessels). Such hierarchy could have multiple causes, which cannot be determined from the present study but could involve variation in the sensitivity of different size vessels to insulin, or heterogeneity of insulin signalling pathways amongst vessels. There are to our knowledge no data comparing insulin's effects on arteries of various sizes but there are reasons to believe such differences do exist. For example, the contribution of NO to endothelium-dependent vasodilatation depends on the size of the artery (282). This has even been shown for human vessels in which the contribution of NO to endothelium-dependent vasorelaxation is significantly larger in arteries such as the gastroepiploic artery than in distal microvessels (282).

Effect of insulin on heart rate

Basally heart rate was significantly higher in the obese than the non-obese subjects in the study II. This finding is consistent with data by Vollenweider et al. who showed muscle sympathetic nervous activity to be increased, possibly as a consequence of hyperinsulinemia *per se*, in obese as compared to non-obese subjects (298). In study II, insulin failed to increase heart rate in the obese as it did in the non-obese, a defect which could reflect lack of insulin induced peripheral vasodilatation and reflex sympathetic activation. Alternatively, as insulin is known to activate the sympathetic nervous system even in the absence of changes in PVR (9,99,299), it is also possible that the lack of a change in heart rate was a consequence of impaired direct insulin stimulation of the sympathetic nervous system.

Effect of insulin on peripheral and central blood pressure

The AgI increases with age and usually becomes positive after the age of 40 years (131). In studies I-III, the mean age of the normal lean subjects was 25 years and as expected basal augmentation and the AgI were negative. Augmentation and the AgI became even more negative in response to insulin. Since 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 GTN in older subjects who had positive basal augmentation. In these subjects, aortic systolic pressure was increased because the amplitude of the reflected wave was greater than the initial aortic systolic pressure wave (310). Following GTN, 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 (310). Both in the obese subjects (study II) and type 1 diabetic patients (study III), the AgI decreased significantly after 3 hours and when the supraphysiological dose of insulin was used. However, this decrease was not sufficient enough to be reflected as a decrease in aortic systolic blood pressure, although there was a clear trend in diabetic patients, which also had more pronounced decrease in the AgI.

EFFECT OF INSULIN ON AUTONOMIC NERVOUS TONE

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 (247). In skeletal muscle, low physiological concentrations of insulin increase sympathetic nervous activity as determined by microneurography within a time frame similar to that observed in the present study for insulin action on the AgI (247). This action of insulin is temporally dissociated from insulin-induced peripheral vasodilatation (99). β-blockade increases wave reflection and the AgI (276). This effect could, however, be explained by inhibition of β-mediated peripheral vasodilatation i.e. an increase in PVR (276). Based on these data, and because insulin enhances β-adrenergic vasodilatation via an NO-synthesis dependent mechanism (155), we determined whether insulin induced sympathetic activation is temporally associated or correlated with insulin action on stiffness in the study IV. The anatomical location where insulin stimulates activity of the sympathetic nervous tone is unclear. One study suggested the effect is centrally mediated since intravenous but not intra-arterial infusions of insulin increased the norepinephrine spillover rate (235). 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 (1), which will stimulate baroreceptors resulting in increased symphatetic vasomotor outflow in both veins and arteries (1,97). The other possibility is that insulin stimulates sympathetic nervous activity independent of any hemodynamic changes (99).

In study IV, neither the 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 HRV were temporally dissociated from changes in the AgI. The normalized LF and HF components were apparently less sensitive to insulin than the AgI since a higher 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 our own insulin has been reported to change LF and HF components already after 30 min (24,186,203). The changes in LF and HF during the higher dose insulin infusion are difficult to interpret because of simultaneous peripheral vasodilatation and an increase in heart rate, which themselves might have increased sympathetic and inhibited vagal control of HRV. The result of study IV regarding absence of temporal relationship between changes in stiffness and sympathetic nervous system activity is in keeping with data of 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 (261). Also, *in vitro*, the ability of the sympathetic stimulation to change elastic properties of aorta has been considered insignificant (19,65).

CORRELATES OF INSULIN'S HEMODYNAMIC EFFECTS

In study IV, 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 to 60 years) to influence insulin sensitivity (51,115). Whether aging influences insulin sensitivity via changes in body weight or composition, lipids or a decrease in physical fitness, or decreases insulin sensitivity independent of these factors is unclear (46,115,116,236,253). Consistent with the idea that insulin sensitivity is related to stiffness also independent of age, in studies where subjects have all been of the same age (122,141) or in which the study population has been very large as in the ARIC study (244), serum insulin concentrations have been independent determinants of arterial stiffness. In the ARIC study, the relationship between serum insulin and various stiffness indeces 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 (244). In studies where the age range and the number of subjects studied has been comparable to that in the present study, an age-independent relationship between serum insulin and stiffness has not been demonstrated (106,277).

Surprisingly, although the subjects of study IV had normal HbA_{1c} concentrations, a highly significant correlation between basal AgI and HbA_{1c} was found. Increased arterial stiffness has been a consistent finding in diabetic patients (for review see (90)), but glycemic exposure measured with HbA_{1c} has not previously been reported to be correlated with arterial stiffness in non-diabetic subjects. At least theoretically, increases in blood glucose concentrations within the non-diabetic range could damage arterial wall because of increased glycosylation of matrix proteins as in diabetic patients (3). In non-diabetic subjects, HbA_{1c} has been reported to be positively correlated with thickening of arterial intima media (295) and endothelium-dependent vasodilatation (289) suggesting that even small increases in blood glucose may be harmful to vascular function.

In contrast to previous studies, where various relationships between cardiovascular risk factors and stiffness have been analyzed, we also wished to clarify mechanisms linking insulin action to arterial stiffness. We confirmed the finding of acute diminution of the AgI by insulin in a small group of young normal men in the studies I-III in a larger group of non-diabetic middle-aged men in the study IV. This larger group also allowed us to search for factors associated with insulin action on stiffness. None of the most important correlates of basal AgI (age, LDL cholesterol, blood pressure) were significantly associated with insulin action on the AgI, but the change in the AgI was significantly correlated with several features of insulin resistance including insulin sensitivity ('M-value') itself, weight, BMI and the waist to hip ratio. Since the decrease in the AgI occurred at a physiological insulin concentration and within a physiological time frame, these data suggest that resistance of the AgI to insulin may stiffen arteries postprandially and perhaps also contribute to variation in basal stiffness.

MECHANISM OF INSULIN ACTION ON ARTERIAL STIFFNESS

Regarding the site where insulin acts to diminish wave reflection, it is clear that this effect cannot be explained by changes in PVR, which are dominated by the caliber of the arterioles (190). Even low concentrations of insulin, which have no other hemodynamic effects, increase sympathetic nerve activity in muscle (9). This action of insulin is thought to produce vasoconstriction at the level of arterioles and has been suggested to counteract insulin induced peripheral vasodilatation at physiological insulin concentrations such as those induced by the 1 mU/kg·min insulin infusion during the first step of the studies I-IV (245). Small increases in systolic blood pressure, also observed in the studies I-IV may reflect these direct sympathetic effects of insulin (245). Regarding pre-resistance arteries (arteries greater than arterioles), it is presently unclear where insulin acts and what is the site of abnormal action of insulin on central pressure augmentation in obese subjects or patients with type 1 diabetes. This could theoretically be sorted out using a single vessel rather than global approach to study arterial stiffness. It is, however, uncertain whether insulin would change the diameter of a single artery of any size measurably under conditions where very small if any changes are observed in systemic hemodynamic parameters. This is because even in a 20 kg dog, there are approximately 40 arteries with a mean diameter of 4 mm (the size of a human brachial artery) and 500 arteries with a diameter of 1.3 mm (190). An anatomical localization of insulin's effects would, however, be important to establish whether the defect in insulin action might be localized at sites later predisposed to arteriosclerosis. The latter include the usual type of arteriosclerosis characterized by intimal calcifications, especially in central large arteries, and medial artery calcification (Mönckeberg's arteriosclerosis). Diabetic patients are particularly prone to develop the latter type, which is characterized by uniform arterial narrowing and is most commonly found in muscular arteries, especially those in thigh and those affected by neuropathy (187,324).

The cellular mechanism underlying resistance to insulin's vascular effects are poorly understood. Recent data have, however, demonstrated that both the aorta and smaller arteries contain all the necessary signaling molecules to respond to insulin directly and that these tissues can be resistant to insulin action (120) In obese Zucker (fa/fa) rats, insulin induced tyrosine phosphorylation of IRS-1 and IRS-2 and their protein levels were decreased in the aorta (120). In contrast, the mitogen-activated protein kinase pathway was intact (120). It is also of interest that in insulin resistant and obese Zucker fatty rats, aortas studied *in vitro* exhibit resistance to insulin stimulation of PI3-kinase activity which is critical for insulin induced increase in NO synthesis in endothelial cells (325). These studies thus documented selective insulin resistance in vascular tissues in obesity. Whether similar alterations characterize humans with obesity or hyperglycemia induced insulin resistance, as in type 1 diabetes, remains to be investigated.

SUMMARY

The present series of studies examined whether insulin acutely affects wave reflection and the AgI, a measure of large artery stiffness, in normal subjects and in insulin resistant subjects *in vivo*. In addition, we determined which cardiovascular risk markers or factors are associated with basal AgI and its change by insulin.

A novel insulin action on large arteries

In study I, a novel action of insulin was discovered. Acute infusion of physiological dose of insulin to normal subjects, during maintenance of normoglycemia, decreased, within one hour, wave reflection, and AgI, a measure of large artery stiffness. Peripheral systolic or diastolic blood pressure, blood flow and vascular resistance did not change significantly until 2-3 hours after start of the insulin infusion. Since wave reflection is determined by compliance (arterial diameter and distensibility), and PVR, the decrease in wave reflection in the face of unchanged PVR implies that insulin had an effect on the caliber or distensibility of arteries larger than resistance vessels.

Insulin action on large arteries is insulin resistant in obese subjects and in patients with type1 diabetes

In study II, we examined whether the ability of insulin to diminish wave reflection is blunted in obesity. In the obese subjects, three defects in insulin action were observed. First, glucose extraction was resistant to stimulation by a physiological concentration of insulin. Second, supraphysiological insulin concentrations did not normally stimulate peripheral blood flow or decrease PVR. In addition, physiological insulin concentrations failed to normally decrease augmentation in the aorta and the AgI in the obese. The magnitude of the this defect was closely correlated with whole body glucose uptake implying that insulin resistance in obesity involves not only resistance of glucose uptake but also a defect in the ability of insulin to diminish arterial stiffness.

We also studied effects of insulin on wave reflection in another group characterized by insulin resistance, in patients with type 1 diabetes. We found the type 1 diabetic patients to be resistant not only to the action of insulin to stimulate glucose uptake but also to its ability to decrease central aortic pressure augmentation and the AgI. Furthermore, the rate of insulin stimulated glucose uptake was inversely correlated with the change in the AgI by insulin, implying that the greater insulin's effect was to stimulate glucose uptake, the more it diminished the AgI.

Correlates of insulin action on arterial stiffness

In study IV, we searched for determinants in arterial stiffness in a group of 50 non-diabetic, non-smoking men who were characterized by wide variation in various cardiovascular risk factors including those associated with insulin resistance. The AgI was correlated with classic cardiovascular risk factors including age, mean arterial blood pressure and LDL cholesterol, as well as with whole body glucose uptake, HDL cholesterol and the waist to hip ratio. These correlations were, however, strongly influenced by age. In contrast to basal AgI, it's change by insulin was not correlated with age or other classic risk factors, but with insulin sensitivity as assessed by the rate of whole body glucose uptake and various other features of insulin resistance.

CONCLUSIONS

In conclusion, physiological concentrations of insulin rapidly diminish large artery stiffness, as judged from a decrease in the AgI. This action of insulin is blunted in at least two insulin resistant conditions, obesity and type 1 diabetes. It correlates with several other features of insulin resistance. These data, given that arterial stiffness is increased in individuals with insulin resistance (244) and in patients with type 2 diabetes and that the increase in stiffness cannot be attributed to classic cardiovascular risk factors, suggest that arterial stiffening may be one facet of insulin resistance and be a mechanism which may link insulin resistance to cardiovascular disease.

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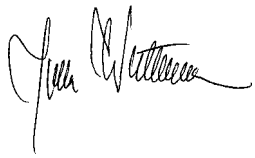

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