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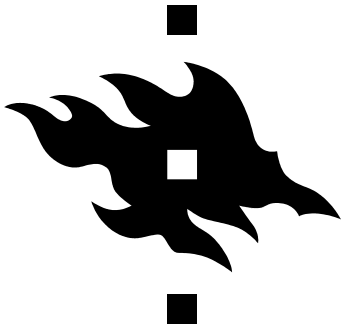

UNIVERSITY OF HELSINKI

VASCULAR AND PLATELET FUNCTION IN INSULIN RESISTANCE

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1 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, Tamminen M, Cockcroft J, Yki-Järvinen H: Comparison of in vivo effects of nitroglycerin and insulin on aortic pressure waveform. Eur J Clin Invest, 34:1-8, 2004

II Tamminen M, Westerbacka J, Vehkavaara S, Yki-Järvinen H: Insulin-induced decreases in aortic wave reflection and central systolic pressure are impaired in type 2 diabetes. Diabetes Care 25: 2314-9, 2002

III Tamminen M, Westerbacka J, Vehkavaara S, Yki-Järvinen H: Insulin therapy improves impaired insulin actions on glucose metabolism, aortic wave reflection and central systolic blood pressure in type 2 diabetes. Eur J Clin Invest 33:855-60, 2003

IV Tamminen M, Lassila R, Westerbacka J, Vehkavaara S, Yki-Järvinen H: Obesity is associated with impaired platelet-inhibitory effect of acetylsalicylic acid in non-diabetic subjects. Int J Obes Relat Metab Disord 27: 907-11, 2003

2 ABBREVIATIONS

AA	Arachidonic acid
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
AgI	Augmentation index
ALT	Alanine aminotransferase
AMPK	Adenosine monophosphate kinase
ANOVA	Analysis of variance
ASA	Acetylsalicylic acid
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BMI	Body mass index
C1	Large artery compliance
C2	Peripheral artery compliance
cAMP	Cyclic adenosine monophosphate
CETP	Cholesterol ester transfer protein
cGMP	Cyclic guanosine monophosphate
CHD	Coronary heart disease
COX	Cyclo-oxygenase
CRP	C-reactive protein
DIGAMI	The Diabetes Insulin-Glucose in Acute Myocardial Infarction study
ECG	Electrocardiogram
GGT	Gamma glutamyl transferase
eNOS	Endothelial nitric oxide synthase
FFA	Free fatty acids
FFM	Fat free mass
GP	Glycoprotein
GTN	Glyceryl trinitrate
HbA_{1C}	Glycosylated hemoglobin A_{1C}
HDL	High density lipoprotein

iNOS	Inducible nitric oxide synthase
LDL	Low density lipoprotein
L-NMMA	N^G-monomethyl-L-arginine
MAP	Mean arterial pressure
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NO	Nitric oxide
OHA	Oral hypoglycaemic agent
PAI-1	Plasminogen activator inhibitor-1
PCI	Percutaneous coronary intervention
PGI_2	Prostacyclin
PI3-kinase	Phosphatidylinositol 3-kinase
PP	Pulse pressure
PPP	Platelet poor plasma
PRP	Platelet rich plasma
PWV	Pulse wave velocity
RGD	Arginyl-glycyl- alpha-aspartyl sequence
TXA_2	Thromboxane
UKPDS	United Kingdom Prospective Diabetes Study
VCAM-1	Vascular cell adhesion molecyle-1
VLDL	Very low density lipoprotein
vWF	von Willebrand factor

3 ABSTRACT

Background: Obesity and type 2 diabetes are associated with increased arterial stiffness and abnormalities in platelet function. In normal subjects, insulin decreases central arterial wave reflection and the augmentation index (AgI), a measure of arterial stiffness. This action of insulin is impaired in obese, insulin resistant subjects. Insulin also reduces adenosine diphosphate (ADP) induced platelet aggregation *in vitro*, and this action of insulin is impaired in obese and type 2 diabetic subjects.

Aims: The present studies were undertaken to investigate the following questions: I) Is interindividual variation in insulin's ability to acutely decrease the AgI specific to insulin or can similar variation be observed in response to glyceryl trinitrate (GNT)? II) Does insulin resistance in type 2 diabetic patients involve a defect in the action of insulin to diminish the AgI and central blood pressure? III) Does insulin treatment enhance the action of insulin to acutely lower the AgI in type 2 diabetic patients? IV) Is insulin resistance related to the antiaggregating effect of acetylsalicylic acid (ASA)?

Subjects: The study populations consisted of seven healthy young men and 20 middle-aged subjects (Study I), 16 type 2 diabetic patients and 19 matched normal subjects (Study II), 13 type 2 diabetic patients before and after six-month insulin therapy (Study III), and 11 healthy obese and 10 non-obese subjects (Study IV).

Methods: Whole body insulin sensitivity was determined in all studies by euglycemic insulin clamp technique. The basal AgI and insulin and GTN induced changes in the AgI were measured non-invasively from radial artery using applanation tonometry and pulse wave analysis. Platelet aggregation responses to ADP and arachidonic acid (AA) were assessed in platelet-rich plasma before and 1 hour after ingestion of 50 mg ASA using optical aggregometry.

Results: Insulin and GTN both decreased the AgI in a dose-dependent manner and the haemodynamic effects of insulin and GTN were closely correlated. Type 2 diabetic patients were resistant not only to the action of insulin to stimulate glucose uptake, but also to its ability to decrease the AgI and central systolic blood pressure. Six-month combination therapy with glimepiride and bedtime insulin improved the AgI measured basally and during hyperinsulinemia. The effect of ASA to inhibit platelet aggregation induced by AA and ADP was significantly

blunted in a group of obese insulin-resistant subjects compared to age- and gender matched normal-weight subjects.

Conclusions: These data suggest that the action of insulin on large arteries is not specific to insulin and supports the idea that insulin may act in part via the NO pathway. The defects in insulin-mediated decreases in central blood pressure might predispose insulin resistant subjects to systolic hypertension and insulin therapy has beneficial effects on vascular function. The blunted platelet-inhibitory effect of ASA may contribute to the increased risk of atherothrombosis in insulin-resistant individuals.

4 INTRODUCTION

Insulin resistance and type 2 diabetes are major causes of morbidity and mortality in the industrialized world. It has been estimated that the prevalence of type 2 diabetes will increase from the present 160 million to 215 million in 2010.[16] Type 2 diabetes increases the risk of cardiovascular disease 2 to 4 fold as compared to non-diabetic subjects.[168; 197] Of the patients diagnosed with myocardial infarction about 20% have previously known type 2 diabetes.[234; 349] The association between insulin resistance and cardiovascular disease cannot be explained by classic risk factors such as smoking, cholesterol and hypertension.[169]

Large arteries serve as the main buffering vessels for the systolic pressure load. Arteries stiffen by ageing, which increases pulse pressure.[76] After the age of 50-60 years pulse pressure is a better predictor of coronary heart disease (CHD) than systolic or diastolic blood pressure.[119] Smoking,[228; 246; 399] hypertension,[101; 118] hypercholesterolemia.[8; 286; 399] and diabetes[99; 211; 245; 310] are known causes of both CHD and arterial stiffness, but measures of arterial stiffness also predict cardiovascular morbidity and mortality independent of classic risk factors.[51; 74; 219] When large arteries are stiff they have an impaired ability to buffer the systolic pressure load, which leads to early return of the reflected pressure wave from the periphery (aortic bifurcation) and augmentation of systolic blood pressure at the level of the ascending aorta. Early wave reflection increases left ventricular afterload, decreases diastolic blood pressure and thereby decreases coronary blood flow.[266]

With the use of pulse wave analysis, it is possible to non-invasively estimate central aortic blood pressure. Stiffening of arteries can be observed before clinical signs of atherosclerosis develop. Obese children and young adults have increased arterial stiffness, even in the face of normal peripheral blood pressure.[354; 394] It has been previously shown that insulin acutely decreases central aortic pressure and the augmentation index (AgI),[390] which is a measure of arterial stiffness derived from pulse wave analysis.[258] Insulin induced vasodilatation of peripheral resistance vessels is nitric oxide (NO) dependent.[314; 333] Insulin resistance can impair the ability of insulin to cause vasodilatation.[196; 198; 415] It has also been shown previously that the ability of insulin to acutely decrease AgI is blunted in obese and type 1 diabetic subjects.[11; 390-392] It is unknown whether this defect in insulin action is specific to insulin or whether it is also observed in response to other agents known to acutely decrease the AgI such as glyceryl trinitrate (GTN). In the present series of studies we used the technique of pulse wave analysis to compare the effects of two vasodilatating substances, insulin and NO donor GTN on large arteries. We also investigated

whether type 2 diabetic patients have increased arterial stiffness and impaired insulin-induced decrease in the AgI. Since insulin therapy is known to increase insulin sensitivity of glucose metabolism, we studied if insulin therapy can decrease stiffness and restore insulin's vasodilatory actions back to normal.

Platelets play an important role in atherosclerosis and thrombus formation. Platelets have insulin receptors and insulin can inhibit platelet aggregation.[106; 357] Platelets from obese subjects and type 2 diabetic patients are resistant to the ability of insulin to counteract platelet aggregation.[145; 359; 388] ASA is largely used as an anti-platelet drug and recommended for virtually all type 2 diabetic patients to prevent cardiovascular events.[4] The platelet response to ASA is blunted in a significant proportion of ASA users.[142; 174; 367] The reason for such ASA resistance is unknown. We therefore studied whether obesity and insulin resistance impair the ability of ASA to prevent platelet aggregation.

5 REVIEW OF THE LITERATURE

5.1 Normal insulin action

5.1.1 Glucose metabolism

Insulin is a peptide hormone produced by the β-cells of the islets of Langerhans. Binding of insulin to its receptor leads to autophosphorylation of several tyrosine residues.[173] This leads to phosphorylation of insulin receptor substrate proteins and thereafter activation of several intracellular proteins transmitting the signal downstream. Activation of PI3-kinase is an essential step for stimulation of glucose transport to the cell.[159]

The main target organs for insulin action are skeletal muscle, liver and adipose tissue. In the skeletal muscle free fatty acids (FFAs) are the main source of energy in the fasting state and under these conditions skeletal muscle accounts for only 10 % of whole body glucose uptake.[20] Under fasting conditions insulin-independent tissues, such as the brain, account for more than half of whole body glucose uptake.[91] Glucose and amino acids of food stimulate insulin secretion, which in turn stimulates glucose utilization in skeletal muscle.[177] Under postprandial conditions skeletal muscle, brain and liver each utilize approximately one third of whole body glucose uptake.[177] Under intravenously maintained hyperinsulinemic conditions, the proportion of glucose used by skeletal muscle accounts for 70-80% of whole body glucose uptake.[93; 416]

In the liver insulin inhibits endogenous glucose production.[208] In the postprandial state insulin suppresses glycogenolysis[90] and diverts gluconeogenetic flux from glucose release into glycogen synthesis.[63; 102] At insulin concentrations over 80 mU/l, hepatic glucose production is completely suppressed in normal subjects and half maximal suppression is achieved at 17 mU/l.[149; 414] Fasting blood glucose is mainly determined by endogenous glucose production[92] and both suppression of endogenous glucose production and peripheral glucose disposal influence blood glucose levels at postprandial state.[109]

5.1.2 Vascular effects of insulin

Insulin has a vasodilatatory effect on resistance vessels in skeletal muscles.[415] This effect is slow and in most studies only observed at supraphysiological insulin concentrations.[49; 196; 364; 415; 416] During the first 2 hours of insulin infusion at submaximal, high physiological rate of 1 mU/kg·min, forearm blood flow increases by approximately 20%.[415] This vasodilatatory effect is trivial when

compared to for instance a classic endothelium-dependent vasodilatator such as acetylcholine (ACh), which increases blood flow 500% above basal within a minute at a submaximal concentration.[257] The ability of insulin to induce peripheral vasodilatation can be abolished by inhibiting nitric oxide synthase enzyme with L-NMMA.[314; 333] Norepinephrine and other vasoconstrictors cannot abolish the effect of insulin on peripheral arteries, suggesting that insulin-induced vasodilatation is endothelium dependent (**Fig. 1**).[314] Vasodilatator action of insulin is blunted in subjects with insulin resistance and endothelial dysfunction.[334] Insulin-infusion acutely potentiates endothelium-dependent vasodilatation[294] and long term insulin therapy has been shown to improve endothelial function in type 2 diabetic patients.[294; 373]

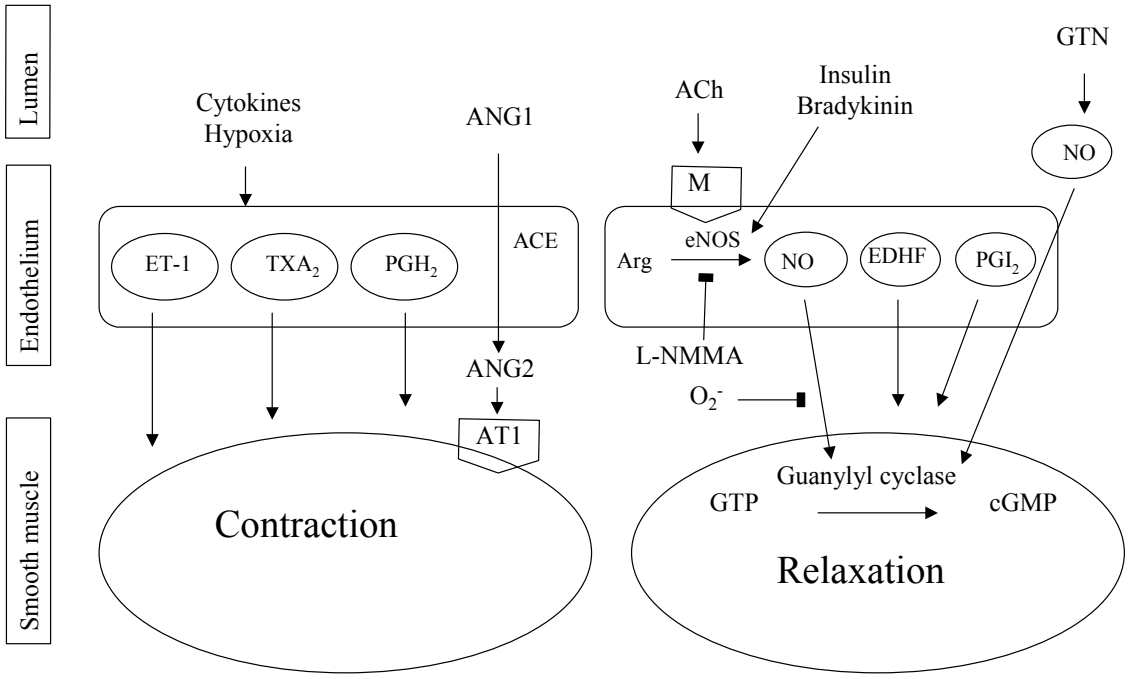

Figure 1. Endothelium-dependent contracting and relaxing systems are represented in this figure. Acetylcholine (ACh) binds to muscarinic receptor (M) and activates nitric oxide (NO) synthesis via the L-arginine (Arg) pathway. NO causes relaxation of the smooth muscle cells by increasing the synthesis of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). Glyceryl trinitrate (GTN) is an exogenous NO-donor. The action of insulin on vessels is at least partially mediated by endothelium-derived NO. Relaxation of smooth muscle cells is induced by NO, endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (PGI_2). Contracting factors are endothelin-1 (ET-1), Thromboxane A_2 (TX A_2) and prostaglandin (PGH_2) and angiotensin II (ANG II). N^G-monomethyl-L-arginine (L-NMMA) competitively blocks endothelial nitric oxide synthase (eNOS). O^-_2 =superoxide, AT1= type 1 angiotensin 2 receptor, ACE= angiotensin converting enzyme.

Insulin dilatates also large pre-resistance arteries. Unlike at the level of resistance arteries, insulin action on large arteries can be demonstrated at physiological insulin concentrations within 30-60 minutes.[25; 199 391-393] Carotid and femoral artery diameters, measured with ultrasound method, have been shown to increase during insulin infusion.[25; 199] Insulin acutely decreases the AgI, a measure of systemic arterial stiffness.[258; 391; 392] The ability of insulin to decrease aortic augmentation and the AgI is blunted in insulin resistant obese and type 1 diabetic subjects.[390; 391] It is unknown whether this defect in insulin action is specific to insulin or whether it is also observed in response to other agents known to acutely decrease the AgI such as GTN.

5.1.3 Effects of insulin on platelet function

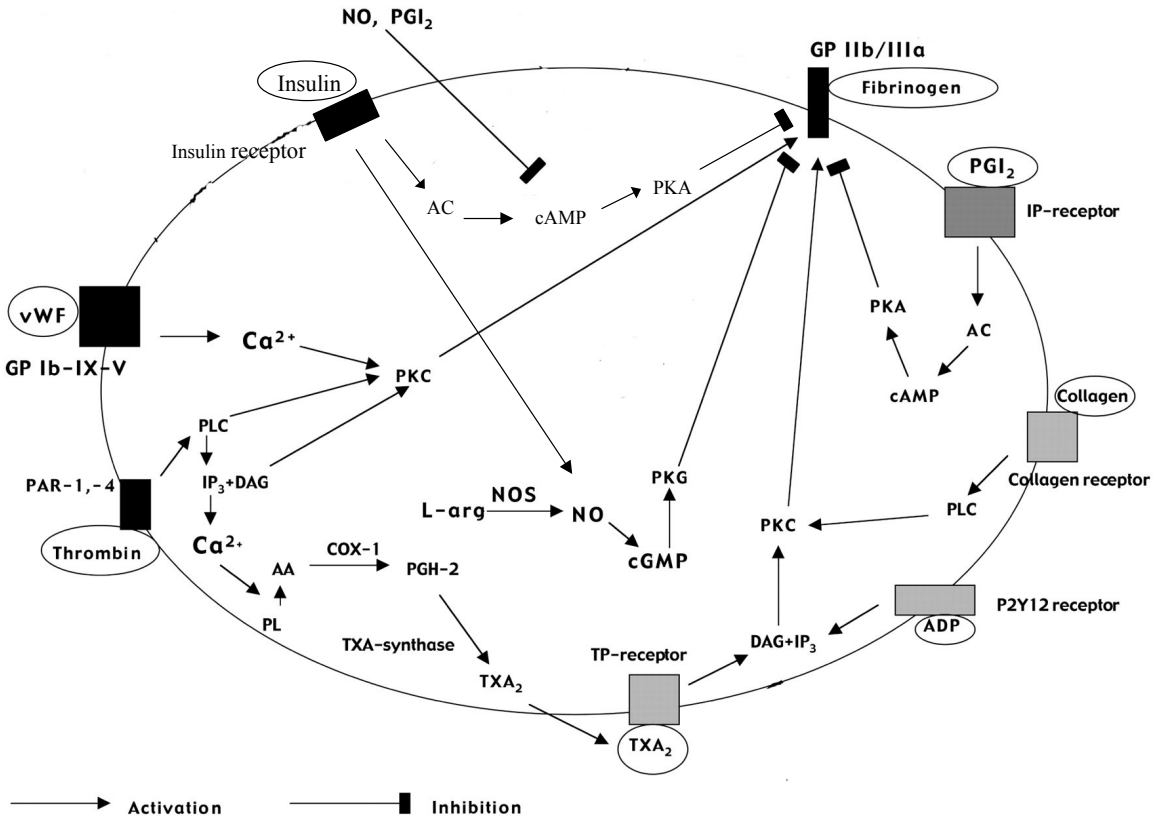

Figure 2. Overview of major platelet reactions and signalling pathways. Receptors are boxed and ligands are circled. ADP indicates adenosine diphosphate; AA, arachidonic acid; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; COX, cyclooxygenase; DAG, diacyl glycerol; GP, glycoprotein; IP3, inositol trisphosphate; PL, membrane phospholipids; NO, nitric oxide; NOS, nitric oxide synthase; PLC, phospholipase C; PGI2, prostacyclin; IP-receptor, prostacyclin receptor; PAR, proteinase-activated receptor; PKA, protein kinase A; PKG, protein kinase G; TXA2, thromboxane A2; TP-receptor, thromboxane receptor; and vWF, von Willebrand factor. [110; 162; 358]

Insulin has both direct and indirect effects on platelets. Platelets have functional insulin receptors but the effects of insulin may also be mediated through vascular endothelium or changes in platelet agonists in plasma. Insulin receptors on platelets are capable of binding insulin and undergo autophosphorylation.[106] Insulin counteracts platelet responses to agonists such as ADP, collagen, thrombin, arachidonate and platelet activating factor (**Fig. 2**).[357] Insulin inhibits platelet aggregation both *in vitro*[303; 357; 359] and *in vivo.*[145; 388] This action of insulin is associated with increases in platelet cyclic GMP and AMP and can be overcome by inhibiting nitric oxide (NO) synthesis with L-NMMA.[358] The PI3-kinase pathway may mediate the insulin-induced NO-dependent increase in cyclic nucleotides.[303; 420] It has been suggested that insulin attenuates NO dependent increase in cyclic nucleotides.[303; 420] It has also been suggested that insulin attenuates aggregation by inhibiting cAMP suppression through IRS-1 and G_i, the inhibitory G-protein of adenylyl cyclase.[110] An increase in cAMP and cAMP-dependent protein kinase A interferes with the elevation of intracellular calcium, which is a key step in platelet activation.[110] In *in vitro* studies*,* insulin has been shown to dose-dependently inhibit platelet aggregation to ADP in healthy subjects and lean type 2 diabetic patients.[21; 357] This *in vitro* effect of insulin has been suggested to be impaired in obese subjects and in obese type 2 diabetic patients compared to lean subjects.[359] There is however also conflicting evidence from other studies, which suggest that insulin may even enhance platelet aggregation.[152]

Platelet hyperaggregability could contribute to the increased risk of atherothrombotic vascular disease in type 2 diabetes.[135; 169] A defect in insulin-induced inhibition of platelet aggregation is only one of several platelet abnormalities described in type 2 diabetes and insulin resistance. Other abnormalities include increased arachidonic acid metabolism which increases platelet thromboxane production in type 2 diabetes and obesity,[79] subnormal threshold for platelet activation by agonists such as ADP[309] and collagen, which release arachidonic acid (AA) from membrane phospholipids in type 2 diabetes and insulin resistant subjects,[79; 97; 157; 309] increased platelet size,[317] thrombin generation,[24; 69]shortened life span in circulation,[72] an increase in advanced glycosylation end-products in platelets[402] and decreased membrane fluidity.[401] Vascular synthesis of prostacyclin (PGI_2) and platelet sensitivity to PGI_2 may be decreased in diabetes.[10; 136] The activity of nitric oxide synthase may be decreased in type 1 and 2 diabetes.[289; 290] Increased PAI-1 release[161] and shear-induced adhesion and aggregation to subendothelium[188] have been described in type 2 diabetes. Increased number or function of platelet adhesion proteins GP IIb/IIIa and P-selectin[129; 176; 192; 243; 260; 361] have been described in both obesity and type 2 diabetes. Calcium concentrations are increased in type 2 diabetes, hypertension and insulin

resistance[34; 156; 213; 355; 362] and intracellular magnesium concentrations are decreased in obesity and type 2 diabetes.[342] In addition increased oxidative stress associated with hyperglycemia[405] and decreased plasma antioxidant capacity[312; 360] may cause platelet hyperaggregability perhaps through increased isoprostane formation.[80] Increased isoprostane production, which characterizes humans with type 2 diabetes and obesity, could activate platelets through thromboxane receptor, even when thromboxane synthesis is inhibited by ASA.[81] Hyperglycemia as such may be a cause of abnormal platelet function[129; 176; 405] and treatment of hyperglycemia has been shown to reduce ADP induced platelet aggregation and to reduce thromboxane and isoprostane formation in some[78; 80] but not all studies.[157; 391; 392] Increased microparticle formation has also been described in type 2 diabetes. Microparticles are released when platelets become activated and they may also enhance thrombus formation.[307; 392; 417]

5.1.4 FFA and lipid metabolism

Hypertriglyceridemia, low HDL cholesterol and small dense LDL cholesterol are metabolic abnormalities that characterize insulin resistant obese subjects and type 2 diabetic patients.[346] In adipose tissue insulin inhibits lipolysis and increases re-esterification of intracellular FFAs into triglycerides.[421] In the liver insulin decreases VLDL production by decreasing availability of FFA for VLDL synthesis and by directly decreasing VLDL triglyceride[214; 379] and VLDL1 Apo B synthesis.[214; 236; 237] Insulin also stimulates the removal of VLDL by increasing the activity of lipoprotein lipase, the rate-limiting enzyme of intravascular triglyceride hydrolysis.[346]

An increase in plasma VLDL, especially VLDL 1 concentration is the most important determinant of LDL size.[244; 346] When fasting serum triglyceride levels are below 1.5 to 1.7 mmol/l, LDL particles are typically large and buoyant.[271] Cholesterol ester transfer protein (CETP) exchanges lipids between triglyceride rich particles (VLDL and chylomicrons) and LDL and HDL, and thereby enriches LDL and HDL with triglycerides and depletes them from cholesterol esters.[407] Hepatic lipase hydrolyses triglycerides and phospholipids, which renders LDL and HDL particles small and dense.[172; 343] The concentration of LDL cholesterol in insulin resistant subjects is usually normal, but the clearance rate of the small dense HDL is increased and therefore the HDL cholesterol concentration is decreased.[293] Small dense LDL is atherogenic because it is easily oxidized and binds readily to arterial wall proteoglycans.[69]

5.1.5 Other effects of insulin

Insulin lowers serum potassium concentrations by stimulating potassium uptake into skeletal muscle and the splachnic bed.[89] It also inhibits sodium, potassium and phosphate excretion by the kidney.[88] The hypokalaemic and antinatriuretic effects of insulin are preserved in patients with insulin resistance and type 2 diabetes.[256; 322] Insulin attenuates agonist-induced intracellular calcium increase in skin fiberoblasts, vascular smooth muscle cells and platelets.[60; 155; 164] In insulin resistant subjects, high uric acid levels and low renal uric acid clearance rates cluster with insulin resistance and dyslipidemia.[256] Insulin acutely reduces renal uric acid clearance and this effect of insulin is preserved in insulin resistant subjects, thus providing a link between insulin resistance and hyperuricemia.[256; 382] Insulin also stimulates sympathetic nervous system centrally and increases plasma norepinephrine concentrations.[18] Insulin regulates the autonomic control of heart rate, decreasing vagal tone and increasing sympathetic drive. In insulin resistant subjects, basal sympathetic tone is increased and the subsequent response to insulin is blunted.[38; 380]

5.2 Insulin resistance

5.2.1 Definitions

Insulin is a polypeptide hormone that has many normal biological actions in different tissues. Insulin resistance is a state where the normal insulin action is blunted. Insulin resistance may affect insulin action in many tissues such as muscle, liver and adipose tissue.[39] In addition, insulin has effects in blood vessels, platelets, the kidney, and the autonomic nervous tissue, which at least in theory could be affected by insulin resistance.

Insulin resistance may be said to exist whenever normal concentrations of insulin produce a less than normal biologic response. Hormone resistant states may be divided into those due to decreased sensitivity to a hormone (i.e., a shift in the dose-response curve to the right), those due to a decrease in the maximal response to the hormone, and those that are combinations of decreased sensitivity and decreased responsiveness.[165] The term insulin sensitivity is, however, commonly used merely to denote insulin action.

5.2.2 Causes of insulin resistance

Obesity is perhaps the main cause of insulin resistance. There is an inverse relationship between body mass index (BMI) and insulin sensitivity, although insulin sensitivity may vary greatly at any

given BMI. Upper-body obesity is more closely associated with insulin resistance than lower-body obesity, which is considered a fairly harmless state.[120] There are regional differences in adipocyte function. The lipolytic hormones (i.e. catecholamines) are more active in visceral than abdominal subcutaneous fat.[26] The "portal theory" suggests that FFAs from visceral fat are carried to the liver, where they induce hepatic insulin resistance.[26] This theory has not been directly proven. Another correlate of insulin resistance in obesity is the amount of fat that is stored in the liver and skeletal muscle. Ectopic fat accumulation in fatless mice models leads to insulin resistance that can be corrected with subcutaneous fat transplantation, thus proving that having fat in the wrong place indeed causes insulin resistance.[124; 337]

Other causes of insulin resistance include physical inactivity. Physical training and increased muscle mass improve insulin sensitivity. [207; 412] Men with low physical fitness have a 3.7 fold risk of type 2 diabetes as compared to those in the high-fitness category, after adjusting for age, smoking, alcohol consumption and parental diabetes. Even after eliminating the influences of BMI, HDL-cholesterol, triglycerides and hypertension, the low-fitness group still has a 2.6 fold risk of diabetes.[385] Genetic factors contribute to insulin resistance. In white population the lifetime risk for developing type 2 diabetes was 20-40% for the first-degree relatives of type 2 diabetic patients but only 6% for age and weight matched subjects with no family history of type 2 diabetes.[410] Chronic hyperglycemia,[411] hypoglycemia,[31] high FFA concentrations[108] and acidosis[87] also decrease insulin sensitivity. Many diseases also impair insulin sensitivity. Among them are conditions associated with excessive secretion of insulin's counter-regulatory hormones such as acromegaly,[255] Cushing's disease[261] and phaeochromocytoma.[46] Hypo- and hyperthyroidism[284; 291] both decrease insulin sensitivity as well as uremia,[85] infections,[409] growth-hormone deficiency[13] and non-alcoholic fatty liver.[311]

5.2.3 Insulin resistance and cardiovascular disease

Coronary heart disease is the major cause of excess mortality in type 2 diabetes. About 70% of deaths in type 2 diabetes are caused by CHD. Mortality from cardiovascular disease and the incidence of non-fatal coronary heart disease is 2 to 4 times higher in patients with type 2 diabetes than in non-diabetic subjects even after adjusting for the classic risk factors[135; 168; 287] and the difference between diabetic and non-diabetic women may be as high as 8 fold.[197] The risk of myocardial infarction in type 2 diabetic patients is equally high as in non-diabetic subjects with previous myocardial infarction.[135] The risk of coronary heart disease is already increased in non-

diabetic subjects with hyperinsulinemia.[288; 386] The prognosis of myocardial infarction is also worse in diabetic than in non-diabetic subjects.[7 248]

5.3 Measurement of arterial stiffness

5.3.1 Definitions

Pulse pressure: Difference between systolic and diastolic blood pressure

Augmentation: The rise in central systolic pressure caused by wave reflection

Augmentation index: Augmentation divided by aortic pulse pressure

Pulse wave velocity: The speed of travel of the pulse along an arterial segment

Stiffness index β: The ratio of natural logarithm (systolic/diastolic pressures) to the relative change in vessel diameter, ln(systolic BP/diastolic Bp)/[(systolic diameter-diastolic diameter)/diastolic diameter].

Compliance: A measure of the capacity of volume containing structure to accommodate further increases in volume (Δvolume /Δpressure)

Distensibility: The relative change in vessel diameter (or area) for given change in pressure, Δdiameter /(Δpressure·diameter).

Elastic modulus: The pressure change required for (theoretical) 100% stretch from resting diameter. Elastic modulus is the inverse of distensibility, (Δpressure·diameter)/Δdiameter

5.3.2 Pulse pressure

Pulse pressure is a surrogate marker of arterial stiffness. In the Framingham cohort diastolic blood pressure was the best predictor of CHD in patients < 50 years of age whereas in patients 50-59 years of age systolic and diastolic blood pressure as well as pulse pressure predicted CHD comparably. From 60 years on diastolic blood pressure was negatively correlated with CHD risk and pulse pressure was superior to systolic blood pressure in predicting CHD.[119] In the Systolic Hypertension in the Elderly Program (SHEP) both stroke and total mortality were related to pulse pressure independent of mean arterial pressure.[101] Pulse pressure can be calculated by dividing stroke volume by arterial compliance. In the young pulse pressure and systolic blood pressure is determined by stroke volume while the increase in pulse pressure with ageing is caused by a decrease in arterial compliance.[76] The relationship between atherosclerosis and pulse pressure may be bi-directional. Not only do ageing and hypertension decrease arterial wall elasticity and predispose to CHD, but arteriosclerosis also decreases elasticity and increases pulse pressure.[76]

The rise in pulse pressure is therefore not only a surrogate marker for arterial stiffness but it may also be a feature of atherosclerosis.

Cardiac afterload is mainly determined by peak systolic blood pressure.[119] In young subjects peripheral amplification of systolic blood pressure masks the rise in central systolic blood pressure. The diastolic blood pressure measured peripherally reflects the rise in central diastolic blood pressure more accurately. Both central and peripheral diastolic and systolic blood pressures rise in parallel until diastolic blood pressure begins to decrease after the age of 50-59 years.[54 258] With age-dependent increase in large artery stiffness, there is a narrowing of differences between central and peripheral systolic blood pressures as a result of both diminished peripheral amplification and early wave refection (**Fig. 3**).[119 258]

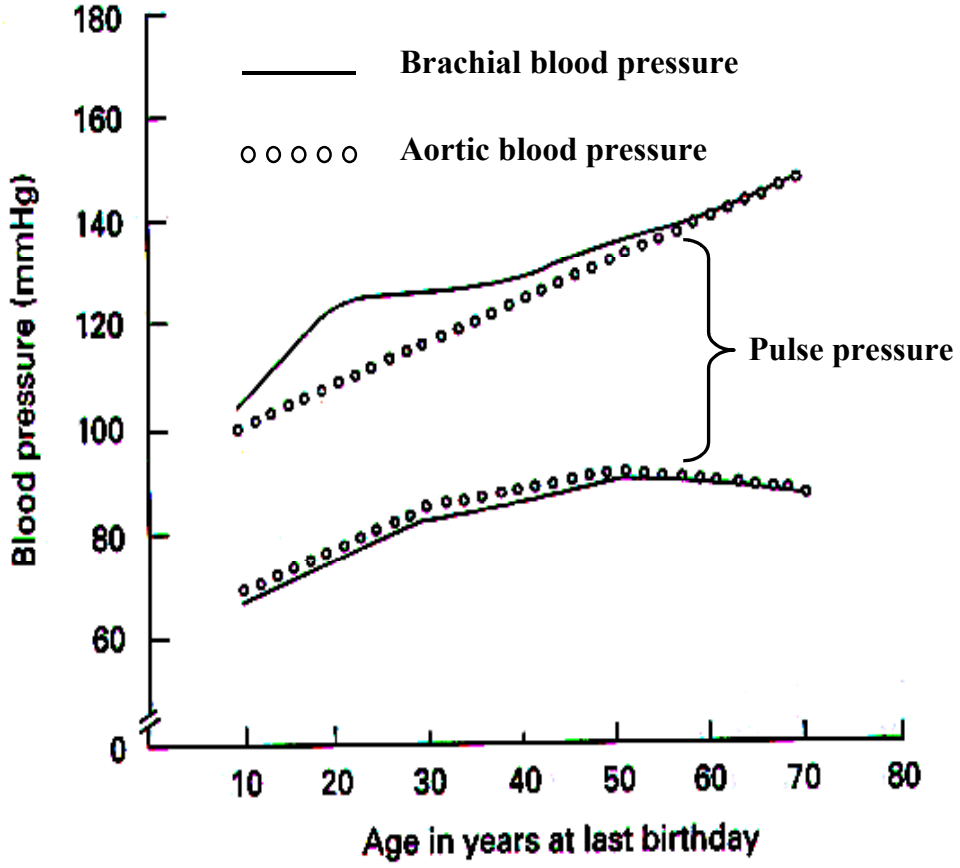

Figure 3. The effect of aging on aortic and brachial systolic and diastolic blood pressures and pulse pressure.[54]

5.3.3 Pulse wave velocity

Pulse wave velocity (PWV), the velocity of arterial wave propagation between two arterial sites, can be measured invasively or non-invasively and used as an index of regional vascular stiffness. The speed at which the pulse wave travels through an arterial segment increases with increasing stiffness.[269] Arterial pulse waves can be detected by pressure-sensitive transducers,[29] Doppler ultrasonography,[338] applanation tonometry,[395] photopletysmography[224] or MRI.[254] The time delay between the proximal and distal arterial sites can be measured simultaneously or by gating to the peak of the R-wave of the ECG. The distance travelled by the pulse is measured over the body surface and calculated as distance/time (m/s).[269] Pulse wave velocity can be measured between any arterial segments, but it is usually used to measure the wave travel between common carotid and femoral or carotid and radial arteries. The distance within arterial tree and body surface is to some extent dependent on body habitus and with increasing age the abdominal aorta becomes more tortuous[387] and can cause underestimation of PWV. The advantage or MRI is the accurate determination of path length but its use is limited by cost and time.

PWV has been shown to predict all-cause and cardiovascular mortality independent of blood pressure in patients with end-stage renal failure[219] and cardiovascular events in hypertensive patients independent of classic cardiovascular risk factors.[51; 206] An increase of 1 m/s in PWV is associated with significant increase in mortality.[132] Although measurements of PWV are considered to have a good repeatability[216; 395] the day to day variation in PWV has also been reported to be as high as 16%.[66] An average PWV increases from 5 to 12 m/s between 20 and 75 years of age.[267] An aortic PWV > 13 m/s is a strong predictor of mortality in hypertension.[42]

5.3.4 Augmentation index

The AgI derived from systolic pulse wave analysis can be used as a measure of arterial stiffness. AgI is the proportion of central pulse pressure that results from arterial wave reflection (**Fig. 4**). It can be measured invasively or non-invasively from superficial peripheral arteries by using applanation tonometry. The measurements can be made from radial, carotid or femoral arteries. Central blood pressure and the AgI can be calculated from the aortic pressure waveform derived using a generalized transfer function or measured directly from carotid artery.[250; 269] It correlates with PWV ($r=0.29$, $p<0.005$)[406] but is actually a composite measure of left ventricular outflow (influenced by myocardial damage, gender, heart rate, aortic stenosis), anatomy (gender, height) and aortic stiffness (age, blood pressure and presence and severity of cardiovascular disease).[183]

Vasoactive drugs influence the AgI independently of PWV,[181] suggesting that the AgI is influenced by the intensity of wave reflection, which is determined by the diameter, muscular tone and elasticity of small arterioles and arteries.[269]

The AgI increases with age[138; 178] and is higher in patients with type 1 [397] and type 2 [53] diabetes and hypercholesterolemia[399] than in controls with similar peripheral blood pressure. The AgI measured from radial artery also correlates with carotid artery intima-media thickness in diabetic and non-diabetic subjects and predicts coronary artery disease independent of other risk factors.[295; 384] Because of peripheral pulse wave amplification in subjects <50 years of age, systolic blood pressure and pulse pressure measured in periphery may not accurately reflect blood pressure in ascending aorta (**Fig. 3**). The AgI and aortic blood pressures derived from pulse wave analysis might therefore in the future help in differentiating systolic hypertension from pseudohypertension in younger subjects.[269] So far there is no evidence that the AgI measured from radial artery has prognostic value,[269] but a high carotid AgI has been shown to be an independent predictor for ischemic threshold in patients with coronary heart disease and of all-cause and cardiovascular mortality in patients with end-stage renal failure.[184; 219]

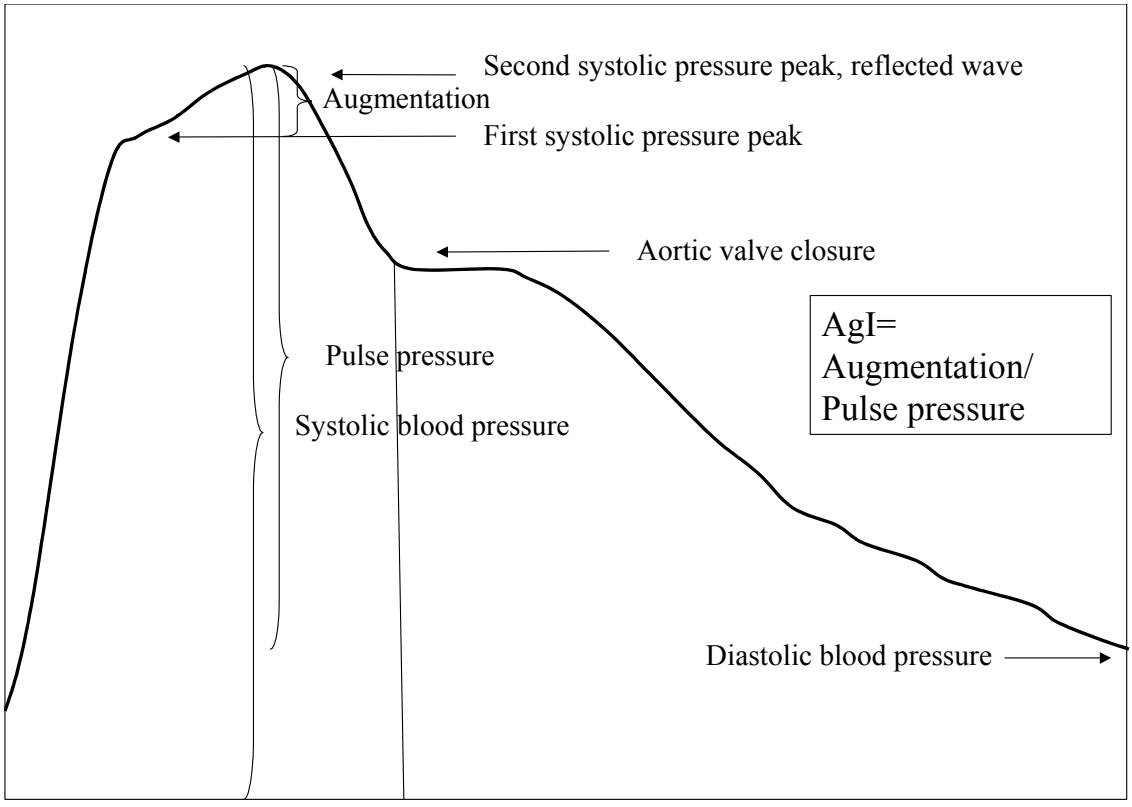

Figure 4. Aortic pulse waveform from a 50-year-old subject derived from pulse wave analysis.

Several studies have shown a strong correlation between invasively measured AgI and the AgI measured from the radial artery using a transfer function.[61; 170; 281] The use of generalized transfer function is possible because wave transmission properties of the upper limb change little with age, disease or drug therapy in adults.[258] In these studies however, the pulse wave was calibrated against intra-aortic blood pressures. The validity of generalized transfer function has been questioned in some recent studies. When calibrated against a sphygmomanometer, central blood pressures derived from radial measurements are accurate but the AgI does not correlate well with the AgI measured invasively[147] or from carotid artery.[250] Therefore some authors consider direct measurement of the carotid artery AgI[250; 269] or even AgI measured at radial artery without use of a transfer function[147] as a preferable method.

5.3.5 Diastolic pulse contour analysis

The diastolic part of the pulse wave can be recorded by applanation tonometry and calibrated using a sphygmomanometer. Information can be received from both large (capacitative, C1) and peripheral (C2) artery compliance. Compliance calculated from invasive and non-invasive measurements correlate significantly.[70] Reduced C2 is considered to be an early sign of impaired pulsatile function. C2 is decreased in hypertensive patients,[70] type 2 diabetic patients,[245] postmenopausal women with CHD,[70] and smokers.[246] Longitudinal studies for diastolic pulse contour analysis have not been performed.[269] There is no significant correlation between measurements performed at radial and posterior tibial arteries. Local arterial properties are likely to influence measurement of C1 and C2[238] and therefore these measures do not necessarily provide measures of overall global stiffness. C2 but not C1 correlates negatively with the AgI measured from radial artery ($r=-0.36$, $p<0.001$), but in the latter study the AgI was considered a better measure of arterial stiffness than C1 or C2 because the AgI changed more by nitroglycerin and has a simpler computational model.

5.3.6 Vascular ultrasonography

The change in the diameter of an artery at a given distending pressure provides a measure of arterial stiffness.[269] Ultrasound and MRI can both be used for measurement of the change in diameter. Parameters such as compliance, distensibility, elastic modulus and stiffness index (β) can be calculated if pulse pressure is known at the site of the measurement. Usually the brachial artery pulse pressure is used for measurements performed at the carotid artery although pulse wave amplification influences pulse pressure at brachial site. Mean brachial arterial blood pressure can

be combined with applanation tonometry at the site of the carotid artery to measure carotid pulse pressure more accurately.[269] The measurements can be made at several different sites such as brachial, radial and femoral arteries or aorta. Carotid artery stiffness has been shown to predict mortality in end-stage renal failure[44] and cardiovascular events after renal transplantation.[35] Reduced aortic distensibility has been associated with increased age,[327] CHD,[329] hypertension,[327; 328] hypercholesterolemia[327] and acute smoking.[331] Stiffness indices are also increased in type 2 diabetic patients. [103; 310]

5.3.7 Digital volume pulse

Digital volume pulse can be measured noninvasively by using photopletysmography. Both digital and radial pulse waveforms can be predicted from digital volume pulse through a generalized transfer function in healthy subjects, in hypertensives and after GTN.[249] Endothelium-dependent vasodilatation in type 2 diabetic subjects is blunted when measured with digital volume pulse.[67] The method is inexpensive and easy to use, but further studies are needed to validate this method.[269]

5.4 Arterial stiffness and vascular risk

5.4.1 Age

Arteries become less elastic by ageing. This is caused by an increase in arterial wall thickness secondary to hyperplasia of the intima and by loss of elastin in the media and its replacement by collagen.[82] Pulse pressure, a surrogate marker of arterial stiffness, increases with age. In the Framingham cohort pulse pressure averaged 42 mmHg before the age of 50 years, 50 mmHg between ages of 50 and 59 years and 62 mmHg after the age of 60 years.[119] In *cross-sectional* studies age correlates closely with arterial stiffness when measured with any technique such as ultrasound,[148; 175; 325] MRI with cine velocity mapping,[254] PWV[32; 264] and the AgI.[180; 258]

5.4.2 Height

Height is inversely related to the risk of cardiovascular disease even after adjusting for known CHD risk factors.[73; 140; 166; 273; 298] An increased risk of CHD has been associated with short stature.[298] An increased risk has been observer in both men and women in *cross-sectional*[273] and *follow-up*[73; 140; 166; 298] studies. Regarding the reasons underlying this association, short stature has been associated with reduced pulmonary function,[73] genetic factors,[14; 73] poor childhood

nutrition[273] and small diameter of coronary arteries.[112] Short stature and arterial length increases the carotid AgI independent of mean arterial pressure.[324] Short stature also correlates with carotid artery compliance, heart rate and age.[324] Increased wave reflection in systole and increased ventricular load may therefore increase the risk of CHD in short individuals.

5.4.3 Gender

Cross-sectional studies. Women have a higher AgI than men in all age groups and the difference increases with age.[138] In a group of elderly hypertensive patients matched for age, height and mean arterial pressure, there was no difference in systolic blood pressure between men and women, but diastolic blood pressure was lower and pulse pressure and the AgI higher in women. Women had a 5% smaller aortic arch and 11% smaller outflow area. Aortic stiffness indices β and E_p were also higher in women. There were no differences in heart rate or stroke volume between men and women.[123; 406] In an urban Chinese population aged 17 to 85 years there was an increase in aortic, arm or leg pulse wave velocities with age but there were no gender differences.[32] In another study, the aortic and carotid artery compliances were greater in young women than men and decreased rapidly with age so that after middle-age women had lower compliance than men.[203]

In a 3-year *follow-up study* of hypertensive women who went through menopause during the follow-up, aortic root compliance decreased significantly faster than in the control groups of hypertensive men and premenopausal women.[171] Hormone replacement therapy did not, however, lower blood pressure or the AgI values in a *follow-up study* of post-menopausal diabetic women.[139] In women the systolic outflow time was longer and diastole was shorter than in men.[123; 138] The greater aortic stiffness after menopause and the increase in the AgI may contribute to the greater age-associated increase in left ventricular mass and excess symptomatic heart failure in women than in men that are not explained by differences in brachial blood pressure.[138 240; 320]

5.4.4 Heart rate

An increased heart rate[167] and low heart rate variability[95; 96; 217] are associated with an increased risk of cardiovascular mortality and morbidity in *follow-up studies*. Increased heart rate in patients with type 2 diabetes is associated with impaired autonomic control of heart rate variation.[377] In a *cross-sectional study*, an increased heart rate was also associated with features of insulin resistance in non-diabetic subjects.[272] The AgI correlates inversely and linearly with heart rate. When heart rate was increased with a pacemaker by 10-beats/minute, the AgI decreased 5.6%.[398] Ejection

duration also shortened and peripheral systolic and diastolic blood pressures increased, but central systolic blood pressure measured non-invasively from brachial artery remained unchanged.[396] The AgI decreases because an increase in heart rate decreases the duration of systole and shifts the reflected wave to diastole. The lack of rise in central systolic blood pressure is explained by the decrease in wave pressure augmentation.[396] The dependency of the AgI on heart rate can be considered as a limitation of the method.[82] PWV has increased with heart rate in some[201] but not in all[406] *cross-sectional studies* and the results are conflicting also in studies where pacemakers were used to regulate heart rate.[202; 398; 406] The increace in PWV may be due to the shortened time available for recoil, which results in vessel stiffening.[202]

5.4.5 Obesity and insulin resistance

An association between arterial stiffness and obesity or components of insulin resistance syndrome has been shown in several studies. In the Atherosclerosis Risk in Communities (ARIC) study, arterial stiffness measured with the use of ultrasound was positively correlated with the fasting insulin concentration in a cohort of 4701 subjects after adjusting for age, smoking and total cholesterol.[310] Other *cross-sectional studies* have confirmed an association between arterial stiffness and obesity with methods using ultrasound,[127; 354; 371] PWV, [226; 339; 394] the AgI [128; 390] and by measuring aortic elasticity with MRI.[75] There is also an association between measures of arterial stiffness and insulin sensitivity measured with the euglycemic clamp technique[127; 390] or by using fasting serum insulin concentration.[195; 226; 339; 371]

5.4.6 Type 2 diabetes

Increased arterial stiffness measured with ultrasound,[103; 310] the AgI,[53; 247; 295] PWV,[9; 57; 182; 210; 263; 344] diastolic pulse contour analysis,[245] and by assessing the ratio of pulse pressure to stroke volume[99] has been a consistent finding in type 2 diabetes in several *cross-sectional studies*. Arterial stiffness in type 2 diabetes has been associated with increased left ventricular mass and wall thickness,[99] intima-media thickness,[263; 295; 344] and cardiovascular and all-cause mortality in end-stage renal failure.[319] Arterial stiffness in type 2 diabetes has been positively correlated with hyperglycemia,[143; 247; 295; 310; 369] hyperinsulinemia,[143; 310] insulin resistance measured by clamp study,[103; 368] duration of diabetes, [103; 344; 369] dyslipidemia[263; 344] and advanced glycosylation end products in tissue specimens.[9]

5.4.7 Hypertension

Blood pressure is a major risk factor for cerebrovascular events and coronary heart disease.[116; 375] Traditionally blood pressure has been defined as a systolic and diastolic blood pressure. Recent studies have suggested that blood pressure should be envisioned as consisting of a static component, mean arterial pressure, and a dynamic component, pulse pressure.[258] Mean arterial pressure, the product of cardiac output multiplied by total peripheral resistance, is the pressure for the steady flow of blood to peripheral tissues and organs. Pulse pressure is the consequence of intermittent ventricular ejections. Pulse pressure is influenced by several cardiac and vascular factors, but it is the role of large conduit arteries, mainly the aorta, to minimize pulsatility.[258] In addition to the pattern of left ventricular ejection, the determinants of pulse pressure include the cushioning capacity of arteries and the timing and intensity of wave reflections.[258] The cushioning capacity is influenced by arterial stiffness, usually expressed using the terms of compliance and distensibility.[258]

Increased pulse pressure is a predictor of cardiovascular risk in subjects with essential hypertension and after myocardial infarction in *follow-up studies*.[101 118; 253] Two haemodynamic components of pulse pressure have been shown to independently predict cardiovascular risk: aortic stiffness measured from aortic PWV,[45; 205] and early return of reflected waves to the heart evaluated from the AgI.[219] On the other hand acute changes in blood pressure at the time of measurement influence measures of arterial stiffness without changing vessel structure.[122] PWV has been shown to increase faster in hypertensive than normotensive subjects during a six-year follow-up.[36] Well-controlled hypertensive patients had similar increase in PWV as normotensive subjects.[36] In the latter study increased serum creatinine, uncontrolled hypertension and increased heart rate were significantly correlated with the progression of PWV.[36]

5.4.8 Smoking

Acute smoking increases blood pressure, heart rate, the AgI and PWV in *intervention studies*.[105; 228] Smokers have stiffer arteries than non-smokers measured using the AgI,[107; 128; 228; 399] PWV,[212] diastolic pulse contour analysis[246] and ultrasound[105] in *cross-sectional studies*.[191; 215; 330] The vascular effects of smoking and hypertension may however be different. Both smoking and hypertension are associated with increased vascular stiffness indices and intima-media thickness,[150] but only hypertension is associated with increased central pulse pressure and lumen-to-wall ratio of carotid artery.[215]

5.4.9 Hypercholesterolemia

Children and adolescents with heterozygous familial[8; 286; 378] or sporadic[153] hypercholesterolemia have been found to have stiffer arteries than normocholesterolemic subjects in *cross-sectional studies*. Arterial stiffness is increased in hypercholesterolemia when measured with different ultrasound methods.[8; 153; 286; 378] In adults the data are more conflicting. The AgI and central pulse pressure have been found to be higher in middle-aged, hypercholesterolemic patients than control subjects matched for peripheral blood pressure, height, age, smoking, weight and fasting glucose.[399] In young men oxidized but not total low density lipoprotein concentration was associated with increased stiffness measured with ultrasound and MRI.[352] In other studies no associations were found between LDL or HDL cholesterol and arterial stiffness.[11; 305]

5.4.10 Statins

Despite conflicting data in cross-sectional studies regarding the association of hypercholesterolemia and stiffness, statins have consistently been found to decrease arterial stiffness in non-diabetic[111; 189; 323; 353] and type 2 diabetic patients[154] in *treatment studies*. Statin treatment has not improved pulse wave velocity[292] or arterial distensibility and compliance[190] in short-term studies in contrast to studies where treatment has lasted longer.[111; 154; 189; 323; 353] This suggests that statins may induce structural changes in the vessel wall.[269]

5.4.11 Antihypertensive drugs

The effect of antihypertensive drugs on arterial stiffness needs to be differentiated from that on blood pressure. GTN, at low doses, decreases the AgI, central systolic blood pressure and pulse pressure, with little effect on peripheral vascular resistance, peripheral blood pressure or PWV.[179; 404] In larger doses nitrates lower peripheral systolic blood pressure effectively, but the blood pressure returns to baseline within a week of continuous nitrate treatment.[276] In the V-HeFT I and II studies (Veterans affairs vasodilator-heart failure trials) the combination of hydralazine and isosorbide dinitrate had a beneficial effect on the prognosis and symptoms of heart failure. Enalapril, however, had a more favourable effect on 2-year survival than the combination of hydralazine and isosorbide dinitrate.[58] Nitrates might have clinical use in the treatment of systolic hypertension if the problem with nitrate tolerance could be solved.

In *treatment studies* ACE inhibitors,[30; 62; 77; 132; 222] angiotensin II inhibitors[28; 187] and calcium channel blockers[133] appear to have beneficial effects on arterial stiffness. Omepatrilat, an agent

that inhibits both angiotensin converting enzyme and neutral endopeptidase has also been shown to decrease the AgI[242; 252] and carotid-femoral PWV.[252] In comparative follow-up studies angiotensin converting enzyme inhibitors have been shown to reduce PWV similarly[221] or more[318] than calcium channel blockers. ACE inhibitors,[30; 62; 77] angiotensin II receptor[187] and calcium channel blockers[133] reduce PWV[133] and the AgI[62] more than diuretics and beta-receptor blocking agents.[30; 77; 133; 187]

5.4.12 Arterial stiffness as a predictor of vascular disease

In *follow-up studies* pulse pressure has been shown to predict cardiovascular disease independent of mean arterial pressure.[37; 101; 117; 227] This has also been shown in type 2 diabetic patients.[68] In the Framingham cohort pulse pressure was the best predictor in subjects over 60 years suggesting that age-related stiffening of arteries is an important risk factor for coronary heart disease.[119] The relation of pulse pressure and mean arterial pressure may be different in cerebrovascular disease. In the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale (PIUMA) study of 2311 hypertensive subjects, pulse pressure was an independent predictor of cardiac events, while mean arterial but not pulse pressure predicted cerebrovascular events.[375]

Recently the association between stiffness and cardiovascular disease has also been studied using direct measures of arterial stiffness. *In cross-sectional studies* PWV correlated with cardiovascular risk factors such as age, gender, systolic blood pressure, diabetes, heart rate and apolipoprotein B,[15; 43] carotid plaques and intima-media thickness.[422] The AgI derived from non-invasive radial artery tonometry predicts the presence and severity of coronary artery lesions independent of smoking, total and LDL cholesterol, age, hypertension and diabetes.[384] Increased AgI is associated with the cardiovascular risk scores ESC (The European society of cardiology), SMART (The second manifestations of arterial disease) and EPOZ (The epidemiological prevention study of Zoetermeer).[262] Increased AgI is also associated with cardiovascular risk factors such as age, blood pressure, smoking, cholesterol, body mass index, height, heart rate and gender[262] and with left ventricular mass and carotid wall thickness.[306] Both the AgI and PWV predicted ischemic thresholds in patients with coronary heart disease,[184] and the AgI measured invasively from the ascending aorta with a fluid-filled system is associated with angiographic changes in coronary arteries.[137] In diastolic pulse contour analysis, especially peripheral arterial compliance (C2) correlated with the extent of angiographic changes in coronary arteries as well as various risk factors (cholesterol, blood pressure, age and gender).[340] In over 3000 elderly subjects of the Rotterdam study, aortic stiffness measured by carotid artery distensibility and carotid-femoral

PWV measured by ultrasonography were associated with severity of carotid and aortic atherosclerotic plaques and the presence of peripheral arterial disease.[370]

In follow-up studies PWV has predicted cardiovascular and all cause mortality independent of other risk factors (age, pre-existing cardiovascular disease, blood pressure, anaemia and left ventricular hypertrophy) in end-stage renal disease.[45; 220; 319] In end-stage renal disease, the AgI has been shown to be even better predictor of overall and cardiovascular mortality than PWV. In the latter study every 10 % increase in the AgI increased the risk of cardiovascular death 1.5 fold.[219] After renal transplantation carotid artery distensibility has predicted occurrence of cardiovascular disease independent of sex, age, smoking habits, blood pressure, heart rate, serum creatinine, cholesterol and haemoglobin levels.[35] In hypertensive patients without pre-existing cardiovascular disease, PWV has been found to predict coronary events independent of Framingham score or classic risk factors[51] and cardiovascular, stroke and all-cause mortality independent of previous cardiovascular disease, age or diabetes.[205; 206] PWV has also been shown to be an independent predictor of cardiovascular and all-cause mortality in both diabetic and control subjects.[74]

5.4.13 Acute regulation of the augmentation index

In 1879 Murrel showed that GTN acutely decreases the second pressure peak of the pulse wave (the AgI) and suggested that this might be the mechanism by which GTN relieves symptoms of CHD.[265] Since then, relatively few studies have tried to identify factors, which acutely influence the AgI.

Nitric oxide is synthesized from L-arginine in endothelial cells, when muscarinic receptors are stimulated by acetylcholine (**Fig. 1**).[274] The reaction is catalyzed by nitric oxide synthase (NOS) and can be specifically and competitively blocked by L-NMMA.[296] Several isoforms of NOS have been identified, but only two have been found in the endothelium; inducible NOS (iNOS) and endothelial NOS (eNOS).[241] eNOS is stimulated by agonists such as ACh, bradykinin, substance P, thrombin and ADP.[23] The iNOS enzyme is activated in cells by inflammation.[372] Nitric oxide, released from endothelial cells, binds to guanylate cyclase and increases the concentration of intracellular cGMP in the smooth muscle cells, which causes their relaxation and vasodilatation.[27] GTN is a NO donor that causes endothelium independent relaxation of vascular smooth muscle cells (**Fig. 1**).[48]

As discussed above, it has been recently shown that insulin, at physiological doses acutely decreases the AgI (**Fig. 5**)[391-393] and that action of insulin is blunted in insulin resistant subjects.[390-392] The mechanism underlying this effect is, however, unknown. Theoretically this effect could be endothelium dependent or –independent. The blunting of insulin action could be either specific to insulin or a consequence of e.g. impaired vasorelaxation of vascular smooth muscle cells to agents such as GTN.

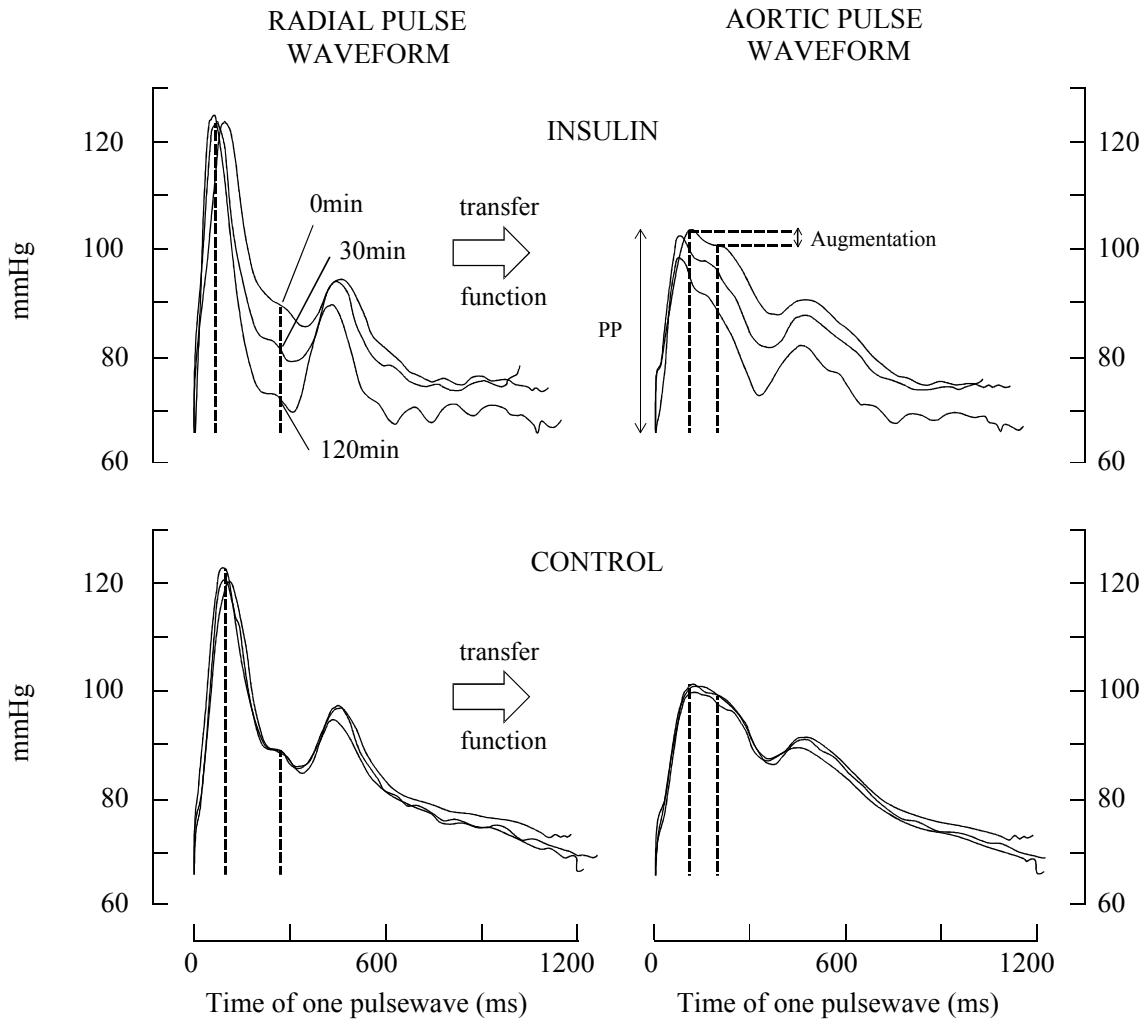

Figure 5. Examples of the effect of insulin on radial and aortic pressure waveforms in nonobese and obese subjects at various time points of insulin infusion, as measured using applanation tonometry and pulse wave analysis.[392]

Low doses of GTN decrease the AgI and central systolic blood pressure by decreasing the amplitude of the reflected wave without changing heart rate and brachial artery blood pressure or peripheral artery resistance. This effect of GTN occurs in the absence of changes in aortic compliance or pulse wave velocity, and reflects vasorelaxation of vascular smooth muscle in pre-resistance

arteries.[113; 179; 404] At intermediate doses GTN induces also venous dilatation and large doses cause arteriolar vasodilatation.[160]

5.5 Platelet function

5.5.1 Normal platelet function

Platelets play an important role in thrombosis and haemostasis. Platelets start thrombus formation by adhesion to damaged endothelium through von Willebrand factor (vWF) and glycoproteins (GP) Ib and IIb/IIIa, and to collagen through GP Ia/IIa.[321] Aggregation with other platelets is mediated by platelet membrane receptor GP IIb/IIIa through vWF and fibrinogen/fibrin. Platelets activate the coagulation cascade and secrete vasoactive substances such as serotonin and thromboxane A_2 that cause vasoconstriction. In addition platelets have receptors to soluble agonists such as thrombin, ADP, adrenalin, platelet activating factor, serotonin and thromboxane A_2, which enhance platelet aggregation by activating GP IIb/IIIa (**Fig. 2**). To prevent uncontrolled thrombus formation antiaggregatory mechanisms need to be present. The most important ones are NO, PGI_2 and ecto-ADPase secreted by the endothelial cells.[204] NO is synthesized also in platelets and NO production can be stimulated by insulin and β-adrenoreceptors.[21; 289; 358] Platelets have both eNOS and iNOS enzymes, but iNOS is only present in small quantities.[383] NO produced from L-arginine by NOS activates guanylate cyclase enzyme. The increase in cGMP and cAMP concentrations in platelets leads to decrease in calcium fluxes and inhibition of aggregation.[356]

5.5.2 Nitrates and platelets

Direct NO donors nitroglycerin and sodium nitroprusside decrease platelet aggregation. 300 µg GTN sublingually decreases ADP induced aggregation significantly. *In vitro* the concentrations of GTN required to inhibit aggregation are larger than the concentrations used in nitrate therapy.[65] The inhibition of ADP induced aggregation by GTN *in vitro* is impaired in obesity, obese type 2 diabetic patients and in patients with stable angina pectoris.[21; 64] Both decreased activity of nitric oxide synthase [289; 290] and decreased action of NO on guanylate cyclase[22] have been described in type 2 diabetes.

5.5.3 ASA

ASA is the recommended first choice for antiplatelet therapy for primary and secondary prevention of vascular disease in diabetic patients. ASA at doses exceeding 30 mg inhibits platelet

function by permanent acetylation of platelet cyclo-oxygenase (COX-1) at the functionally important amino acid serine 529. This prevents the access of AA to the catalytic site of COX-1 at tyrosine 385 and irreversibly inhibits platelet-dependent thromboxane formation (**Fig. 6**).[315] This occurs in the portal circulation, which is why 95 % of platelet COX-1 is inhibited although very little drug is detected systemically and why blood levels of ASA do not correlate with its antiplatelet effect.[283] The recommended dose of ASA based on large primary and secondary prevention trials is 75-325 mg/day.[4; 282] Therapeutic benefit has been demonstrated with doses ranging from 30 to 1500 mg/day.[33] Higher doses may cause more bleeding and gastrointestinal side effects and at doses large than 325 mg/day ASA may have a paradoxical effect, because it also inhibits prostacyclin synthesis in endothelial cells, which might favour thrombus formation.[2; 33; 351] ASA is 170-fold more potent in inhibiting COX-1 than COX-2 enzyme. COX-1 is a constitutive enzyme while COX-2 is induced in cells in response to inflammation.[194] Theoretically it is possible that induction of COX-2 in the endothelial cells might impair the action of ASA on platelets. Normally ASA inhibits COX-1 in endothelial cells when platelets adhere to endothelium, but if COX-2 is induced, retrograde transfer of prostaglandin precursors from endothelial cells to platelets may occur.[315]

5.5.4 Other antiplatelet agents

ADP receptor inhibitors clopidogrel and ticlodipine are thienopyridines. They block ADP receptors on platelets and subsequently decrease activation of GP IIb/IIIa (**Fig. 7**). In comparison to ticlodipine clopidogrel has lower toxicity, lower rate of neutropenia and thrombocytopenia and when a loading dose is used, faster inhibition of aggregation.[348] In Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events study (CAPRIE) approximately 20% of patients had diabetes. Clopidogrel 75 mg was 19.2% more effective than ASA 325 mg in preventing myocardial infarction.[3] There was also a significant risk reduction in the diabetic subgroup.[40] In Clopidogrel in Unstable Angina to Prevent Recurrent Events study (CURE) clopidogrel and ASA in combination was 20% more effective than ASA alone to prevent composite end-point of MI, stroke and CV death. In subgroup analysis of diabetic patients (22 % of patients had diabetes), there was a trend favouring the use of clopidogrel, but the difference was not statistically significant. [419] Clopidogrel is now recommended for treatment of unstable angina pectoris and non-Q myocardial infarction, after percutaneous coronary intervention (PCI) and for those intolerant to ASA.[52; 71]

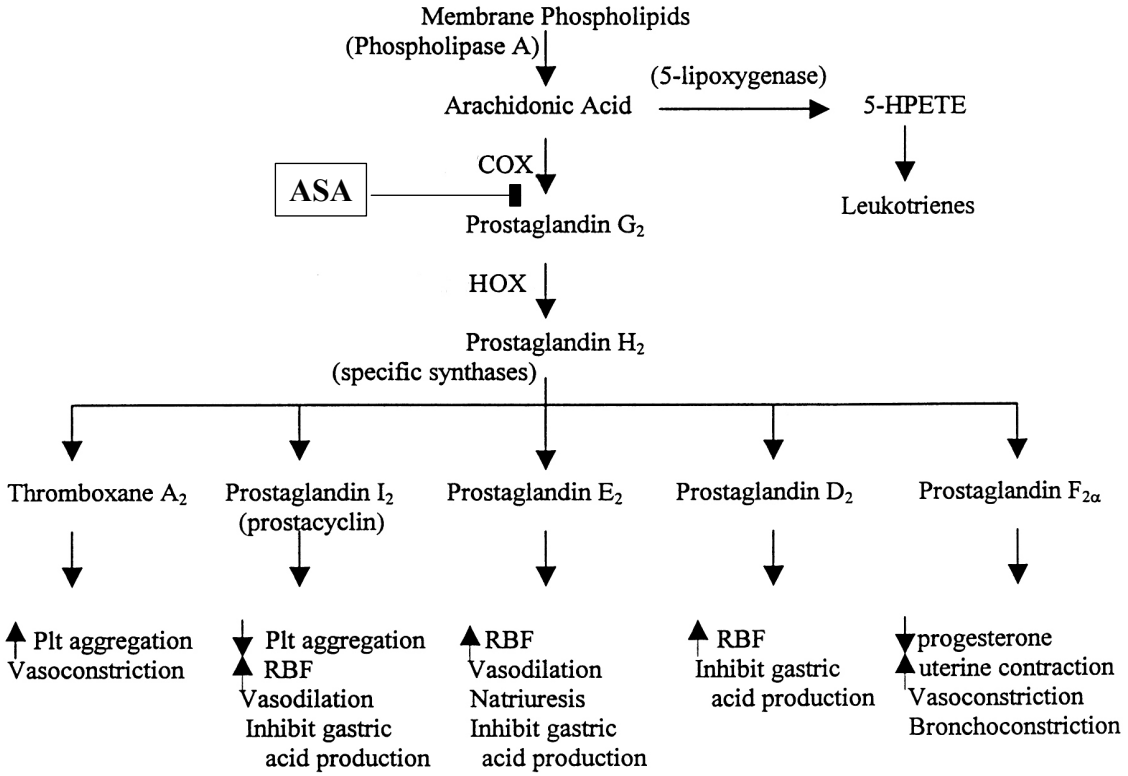

Figure 6. The production of prostaglandins from arachidonic acid and their physiological effects. HPETE indicates hydroperoxyeicosatetraenoic acid; COX, cyclo-oxygenase; HOX, hydroperoxidase; PG, prostaglandin; Plt, platelet; and RBF, renal blood flow.[33]

Dipyridamole is a drug with vasodilator and antiplatelet properties. It inhibits phosphodiesterase and increases cAMP concentration in platelets (**Fig. 7**). Other mechanisms of action have also been suggested. It has been proved beneficial in prevention of stroke alone and in combination with ASA.[280]

GP IIb/IIIa receptor antagonists are a group of intravenous drugs for short-term use for patients undergoing PCI or in acute coronary syndrome (ACS). In case of vascular injury, platelet adhesion to damaged endothelium starts clot formation. GP IIb/IIIa receptors participate in platelet adhesion to vascular wall collagen through von Willebrand factor.[121] Binding of vWF and fibrinogen to GP IIb/IIIa is mediated by the RGD sequence. GP IIb/IIIa is also the final step for all pathways of platelet activation (**Fig. 7**). When any agonist activates a platelet, GP IIb/IIIa goes through a change in conformation. GP IIb/IIIa then binds to vWF, and vWF mediates binding to other platelets and causes platelet aggregation. The change in conformation also increases fibrinogen binding and coagulation.[285; 316]

GP IIb/IIIa inhibitors that are in clinical use in Finland are abciximab, Fab-fragment of a monoclonal antibody against GP IIb/IIIa receptor, and competitive inhibitors tirofiban and epitifibatide that mimic RGD. GP IIb/IIIa inhibitors should always be used in combination with aspirin and/or thienopyridines that reduce the number of activated GP IIb/IIIa receptors.[316] A meta-analysis of six trials of intravenous GP IIb/IIIa receptor antagonists in ACS included 6458 diabetic patients and more than 23072 other patients. In this meta-analysis 30-day mortality was reduced from 4 to 1.2 % in diabetic patients. Non-diabetic patients had no survival benefit.[300] In addition in another meta-analysis of Abciximab use in PCI studies, either with elective or unstable angina patients, 1462 diabetic and 5072 non-diabetic patients were analysed. Use of abciximab reduced mortality in diabetic patients significantly from 4.5% to 2.5 %. In non-diabetic patients the reduction was statistically non-significant. [41] Trials with oral GP IIb/IIIa agonists have not shown benefit in any patient group, but they have increased bleeding complications.[348]

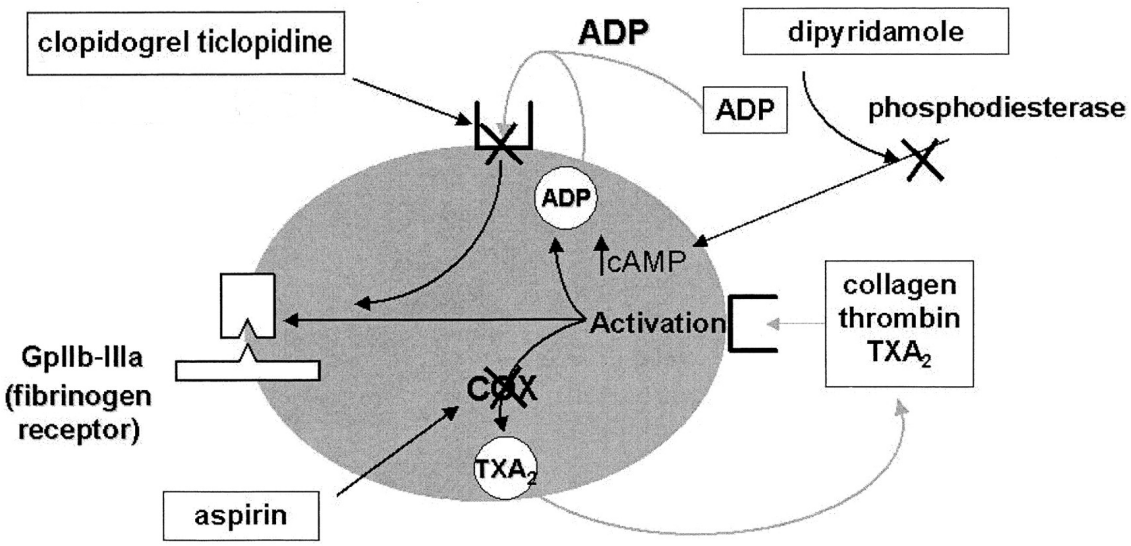

Figure 7. Antiplatelet agents and their mechanisms of action.[71]

5.5.5 ASA resistance

Since 1997, the American Diabetes Association has recommended ASA for all adults with diabetes who have cardiovascular disease or its risk factors.[4] The term 'ASA resistance' has been used in several contexts to describe either individuals who experience vascular events despite

using ASA,[297] or who fail to prolong bleeding time,[174] or produce appropriate responses to platelet inhibition tests[17; 131; 301] and to inhibit aggregation responses to various agonists.[134; 142; 367] At the moment there are no uniform definition or diagnostic criteria for ASA resistance.[151] The causes of interindividual variation in ASA responsiveness are poorly understood and could involve in clinical trials factors such as non-compliance or drug-interactions.[297] Use of non-steroidal anti-inflammatory drugs (NSAID) may be one cause of ASA resistance. Unlike ASA, NSAIDs are reversible inhibitors of COX. They impede the access of ASA to its target in COX-1 enzyme, but don't inhibit COX-1 enzyme permanently. They may prevent cardiovascular events when used alone but weaken the cardioprotective effect of ASA when used simultaneously with ASA. Ibuprofein interferes with ASA action more than diclofenac and COX-2 inhibitors.[114]

The antiplatelet effect of a fixed dose of ASA is known not to be constant in all subjects over time.[142] In the Primary Prevention Project ASA 100 mg/day was given to 4784 subjects with one or more cardiovascular risk factors. 1031 subjects had diabetes. Diabetic patients were more obese than the non-diabetic subjects, BMI 29 vs 27.3 kg/m^2 respectively. While ASA reduced vascular events by 41% in non-diabetic subjects, the risk reduction was non-significant (10%) in the diabetic patients.[308] In a meta-analysis serious vascular events in diabetic subgroup were found to be reduced only by 7%, while the risk reduction was about 25% in the whole study population.[6] The reason for the blunted effect of ASA in diabetic patients is unclear. Any of the previously mentioned defects in platelet function might be involved. Isoprostanes for instance could activate thromboxane receptors despite inhibition of thromboxane production by ASA.[59; 80] High circulating levels of P-selectin have been associated with ASA resistance.[301] Given that insulin normally counteracts agonist-stimulated platelet aggregation[357] and inhibits platelet recruitment to collagen in a whole blood perfusion system,[388] it is possible that insulin resistance may impair the ability of ASA to inhibit platelet aggregation.[21; 359] It is however unclear whether insulin resistant obese subjects are also resistant to the antiplatelet effect of ASA.

5.6 Insulin and sulphonylurea treatment

5.6.1 Insulin treatment

Insulin treatment in type 2 diabetes is mainly targeted to treat chronic hyperglycemia when OHA have failed, but it also has other metabolic effects. Insulin therapy improves whole body glucose disposal by decreasing hepatic glucose output and enhancing peripheral glucose uptake.[144] Insulin treatment decreases FFA levels by inhibiting lipolysis and stimulating FFA re-esterification in the

adipose tissue.[200; 421] Insulin stimulates lipoprotein lipase on the surface of endothelial cells to hydrolyse triglyceride-rich lipoproteins.[346] Intensive insulin treatment also reduces total serum triglyceride, VLDL triglyceride, total cholesterol phospholipids, LDL, apolipoprotein B and HDL3 subfractions and increases HDL2 subfraction.[345]

Insulin may have some effects on components of *coagulation and fibrinolysis*. Increased concentrations of PAI-1, vWF and fibrinogen are associated with insulin resistance and type 2 diabetes.[104; 129; 161; 209; 239; 251] Insulin has in most[158; 223; 275] but not all[381] studies decreased PAI-1 activity in type 2 diabetic patients. In the study of Yudkin et al CRP decreased with 16-week insulin treatment, but parameters of endothelial function and cytokines (vWF, cellular fibronectin, thrombomodulin, tissue plasminogen activator antigen, tumor necrosis factor-α, interleukin-6, fibrinogen) did not change.[418] In Rotterdam study plasma fibrinogen was higher in insulin treated type 2 patients than those treated with oral hypoglycaemic agents or diet only, but this was considered to reflect poor metabolic control in insulin treated subjects.[251] Markers of *endothelial activation* E-selectin and VCAM are increased in type 2 diabetes.[12; 55; 260] E-selectin[304] and VCAM concentration have decreased in some[12] but not in all studies with insulin treatment.[418]

Insulin treatment causes *weight gain*. With poor glycemic control, people usually lose weight. When blood glucose is lowered (with insulin or sulphonylurea) below level of 10-12 mmol/l, glucosuria discontinues and this leads to weight gain if eating and exercise habits stay unchanged. In average a 1% unit improvement in HbA_{1C} causes 2 kg increase in weight.[230]

Microalbuminuria is a strong predictor of cardiovascular morbidity and mortality. In addition to progression of renal failure, microalbuminuria has been related to increased transcapillary albumin leakage, suggesting that microalbuminuria represents a surrogate measure of endothelial dysfunction.[332] Presence of microalbuminuria increases mortality 2 fold in both type 2 diabetic and non-diabetic subjects.[100; 126] Insulin or any effective treatment of hyperglycemia decreases urinary albumin excretion rate and prevents progression of diabetic nephropathy.[5; 268]

In DIGAMI I study insulin-glucose infusion was started immediately after myocardial infarction and it was followed by long-term subcutaneous insulin treatment. In this study, in patients without prior insulin therapy, one-year mortality was reduced by 52% when compared to conventionally treated patients.[235] At supraphysiological doses insulin has a sluggish vasodilatatory effect in peripheral resistance arteries.[415] Insulin therapy improves endothelial function in type 2 diabetic

patients,[294; 373] but insulin therapy has not been shown to decrease blood pressure in type 2 diabetes except in one study where insulin pump but not injected insulin decreased blood pressure.[313] Insulin, on the other hand, has been shown to acutely decrease the AgI at physiological doses. In these studies, there were no changes in brachial or aortic blood pressures during the time period when the changes in the AgI occurred. The subjects were in average 25-28 years old and the first systolic peak determined their aortic systolic blood pressure.[391-393]

5.6.2 Sulphonylureas

Improving glycemic control with strategies including metformin and sulphonylureas has been shown to reduce both macro- and microvascular complications in the UKPDS study.[363] The effect of HbA_{1c} is much greater for retinopathy than for myocardial infarction.[186; 336; 363] A 1% unit reduction in HbA_{1c} caused 14% reduction in myocardial infarction.[336] Lowering of blood glucose with oral sulphonylureas or metformin will also secondarily cause small decrease in plasma triglycerides and LDL cholesterol.[86; 347]

Sulphonylureas stimulate insulin production in pancreatic β-cells by closing the ATP dependent K^+-channels ($K\text{-}_{ATP}$).[232] Glibenclamide, glipizide and glimepiride are the most frequently used sulphonylureas in Finland. Glimepiride is long acting 3rd generation sulphonylurea, which can be taken once per day and has less hypoglycaemic side effect than older sulphonylureas.[302] Sulphonylureas not only block $K\text{-}_{ATP}$ channels in pancreatic β-cells, but also in heart and vessels.[130] They may interfere with vasodilatation and prevent ischemic preconditioning.[130; 270] Glimepiride is a weaker inhibitor of cardiac $K\text{-}_{ATP}$ channels than glibenclamide and might therefore be less harmful for ischemic preconditioning.[125] FFAs can activate $K\text{-}_{ATP}$ channels.[115] High FFA concentrations interfere with both NO-dependent and independent vasodilatation, yet when infused acutely FFAs mediate vasodilatation.[84] FFA mediated vasodilatation may therefore involve $K\text{-}_{ATP}$ channels.

6 AIMS OF THE STUDIES

The present studies were undertaken to answer the following questions:

1) Is interindividual variation in insulin's ability to acutely decrease the AgI specific to insulin or can similar variation be observed in the response of the AgI to GNT? (Study I)

2) Are large arteries of type 2 diabetic patients more resistant to the ability of insulin to acutely decrease the AgI than arteries of non-diabetic subjects? (Study II)

3) Does insulin treatment enhance the ability of insulin to acutely lower the AgI in type 2 diabetic patients? (Study III)

4) Is insulin resistance related to platelet sensitivity to ASA? (Study IV)

7 SUBJECTS AND STUDY DESIGNS

Baseline characteristics of the study subjects are shown in Table 1. Study designs of the individual studies are described below. The nature and potential risks of the study were explained to all subjects prior to obtaining their written informed consent. The study protocols were approved by the ethics committee of Helsinki University Central Hospital. All studies were performed after an overnight fast starting at 8.00-8.30 a.m.

Study I

Dose-response study.

Dose-response characteristics of insulin and GTN on the aortic waveform were first determined using applanation tonometry and pulse wave analysis in seven healthy men (age 26±1 yrs, BMI 25±2 kg/m^2). Three studies were performed in the same subjects on three separate occasions with a one-week interval. Each study consisted of a basal period of 1 hour, during which the haemodynamic measurements (peripheral and central blood pressures, the AgI, heart rate) were performed. The effect of sublingual GTN (500 μg, exposure time 1, 3 or 5 min in random order) on the AgI was then determined using pulse wave analysis with concomitant recording of central and peripheral blood pressures and heart rate. After the AgI returned to the baseline values and stabilized after a minimum of 30 min, vascular (AgI, peripheral blood flow, peripheral and central blood pressures) and metabolic parameters (whole body insulin sensitivity) were determined before and under normoglycemic hyperinsulinemic conditions (insulin infusion rate 0.5, 1 and 2 mU/kg·min for 120 minutes in random order). These conditions were maintained using the euglycemic insulin clamp technique.

Cross-sectional study.

We compared responses to insulin (2 mU/kg·min for 120 min) and sublingual GTN (500 μg for 5 min) in 20 non-diabetic subjects (age 50±2 yrs, BMI 21.0-36.3 kg/m^2). The duration of the basal period was 1 hour, during which the haemodynamic measurements (peripheral and central blood pressures, the AgI, heart rate) were performed. Thereafter effects of sublingual GTN on the AgI was determined using pulse wave analysis with concomitant recording of central and peripheral blood pressures and heart rate. After the AgI had returned to baseline values and stabilized for at least 30 min, vascular (AgI, peripheral blood flow, peripheral and central blood pressures) parameters were determined basally and every 30 min under normoglycemic hyperinsulinemic conditions (insulin infusion rate 2 mU/kg·min) using the euglycemic insulin clamp technique.

Study II

Sixteen type 2 diabetic patients and 19 non-diabetic subjects participated in the study. Except for diabetes, all participants were healthy as judged by history and physical examination, electrocardiogram and routine laboratory tests. Subjects with hypertension or cardiovascular disease (determined by electrocardiogram, history and physical examination) were excluded from the study. Other major systemic diseases were excluded by laboratory tests and by history and physical examination. The diabetic patients were recruited from diabetes outpatient clinics in Helsinki area and had to fulfil, in addition to the above criteria, the following: (1) age 40 to 70 years, (2) treatment with sulphonylurea alone or in combination with metformin, (3) no active retinopathy requiring laser treatment, and (4) no history of ketoacidosis.

Insulin action on glucose uptake, limb blood flow and arterial stiffness were determined under normoglycemic hyperinsulinemic conditions, using the euglycemic insulin (insulin infusion rate 2 mU/kg·min) clamp technique. The subjects were advised not to take any medications the previous evening or in the morning of the study day. Before and during the insulin infusions, metabolic and haemodynamic measurements (recording of the pulse wave, heart rate, blood flow and vascular resistance) were performed at 30 min intervals.

Study III

Thirteen type 2 diabetic patients participated in the study. Except for diabetes, the patients were healthy as judged by history, physical examination, ECG and routine laboratory tests. The diabetic patients were recruited from diabetes outpatient clinics in the Helsinki area on the basis of the following criteria: (1) age 40 through 70 years, (2) treatment with sulphonylurea alone or in combination with metformin, (3) no history of ketoacidosis, (4) and no retinopathy requiring laser treatment.

Each patient was studied before and after 6-month combination therapy with glimepiride and bedtime NPH insulin. Insulin action on glucose uptake, limb blood flow and arterial stiffness were determined under normoglycemic hyperinsulinemic conditions, using the euglycemic insulin (insulin infusion rate 2 mU/kg·min) clamp technique. The patients were advised not to take any medications in the morning of the study day. Before and during the insulin infusions, metabolic and haemodynamic measurements (recording of the pulse wave, heart rate, blood flow and vascular resistance) were performed at 30 min intervals.

Study IV

A total of 21 non-diabetic subjects were recruited for the study. The subjects were divided into an obese and a non-obese group by their median (29.0 kg/m^2) body mass index (BMI). The subjects were healthy as judged by history, physical examination, ECG and routine laboratory tests, and were not taking any regular medications, including use of anti-inflammatory drugs, for 2 weeks prior to the study. Smoking was prohibited on the study day and exercise and alcohol for two days before the study. At the first visit, in vivo insulin action was measured by using the euglycemic insulin clamp technique. At the second visit (2-7 days after the first visit) the blood samples for aggregation tests were withdrawn before and one hour after administration of a tablet containing 50 mg ASA chewed by the patient.

Table 1. Characteristics of the subjects

	Study I	Study II		Study III	Study IV	
Variable	Cross-sectional study	Normal subjects	Type 2 diabetic patients	Type 2 diabetic patients	Non-obese subjects	Obese subjects
Number of the subjects (M/F)	20 (12/8)	19 (13/6)	16 (12/4)	13 (11/2)	10 (5/5)	11 (8/3)
Age (years)	50±2	51±2	54±2	53±2	50±3	52±2
Height (cm)	172±2	174±2	174±2	176±2	171±4	173±3
BMI (kg/m^2)	28.2±1.5	28.9±0.9	29.1±1.1	30.8±1.2	24.9±0.6	31.7±0.8***
Waist/hip ratio	-	0.95±0.02	1.00±0.02	1.02±0.02	0.90±0.02	0.99±0.02**
Body fat (%)	28±2	28±2	28±1	29±2	26±2	30±2
Heart rate (beats/min)	61±2	61±2	70±2	73±2	58±3	63±3
Systolic blood pressure (mmHg)	122±3	125±4	129±4	132±4	124±7	125±4
Diastolic blood pressure (mmHg)	79±2	80±2	79±2	80±2	78±3	82±2
S-cholesterol (mmol/l)	4.9±0.2	4.8±0.2	5.2±0.3	5.3±0.3	4.8±0.3	5.1±0.2
S-HDL cholesterol (mmol/l)	1.3±0.1	1.3±0.1	1.1±0.1	1.1±0.1	1.4±0.1	1.3±0.1
S-LDL cholesterol (mmol/l)	3.0±0.1	3.1±0.1	3.2±0.3	3.4±0.3	2.9±0.2	3.2±0.2
S-triglycerides (mmol/l)	1.2±0.1	1.2±0.1	1.8±0.2**	1.9±0.2	1.1±0.2	1.3±0.1

Data are shown as mean±SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

8 METHODS

8.1 Whole body glucose uptake

Whole body insulin sensitivity was determined by using the euglycemic insulin clamp technique.[94] The studies were performed after an overnight fast starting at 8.00 a.m. An 18-gauge catheter (Venflon; Viggo-spectramed, Helsingborg, Sweden) was inserted into the left antecubital vein for infusion of glucose and insulin. Another 18-cauge catheter was inserted retrogradely in a dorsal hand vein. This hand was kept in a heated chamber (65º C) for sampling of arterialized venous blood as previously described.[365] Insulin (Actrapid Human, Novo Nordisk, Copenhagen, Denmark) was infused in a primed-continuous fashion for 180 min (study II, III and IV) or 120 (study I) min. The rate of the continuous insulin infusion was 0.5, 1 and 2 mU/kg·min (study I dose-response) or 2 mU/kg·min (study I cross-sectional, II and IV). The amount of 20 % glucose infused to maintain normoglycemia during hyperinsulinemia was based on plasma glucose concentrations measured at 5 minute intervals. Whole body insulin sensitivity (the M-value, mg/kg·min) was calculated from the glucose infusion rate after correction for changes in glucose pool size at 30 minute intervals. Blood samples were taken at 30 minute intervals for measurements of serum free insulin concentrations.

8.2 Forearm blood flow and peripheral vascular resistance

Forearm blood flow (study I, II and III) 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 (Model EC-4, Hokanson, Bellevue, WA) as previously described.[365] The gauge was attached around the widest, most muscular segment of the right forearm. Before flow measurements, circulation to the hand was interrupted by inflating a pediatric blood pressure cuff around the wrist to above the systolic blood pressure. Venous return was occluded by a rapid cuff inflation (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 few pulses following cuff inflation. The slope of this line reflects the volume change per unit time. Calibration was performed with the use of the built-in electronic calibration signal for a 1 per cent volume change, the height of which is used for blood

flow calculations. Peripheral vascular resistance was calculated by dividing mean arterial pressure in the brachial artery by forearm blood flow.

8.3 Pulse wave analysis

The technique of pulse wave analysis was used to determine central aortic pressure and the AgI (study I, II and III) as previously described in detail.[265; 393] 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) basally and every 30 minutes during the insulin infusion. Data were collected directly into a desk top computer and processed with 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 artefacts from movement and respiration were minimized. Recordings for pulse wave analysis were made twice basally and every 30 minutes during insulin infusions. The mean of 3 measurements, each consisting of 15 to 20 sequential radial artery waveforms, was used to calculate augmentation and other parameters at the given time point.

In study I the effect of sublingual GTN (500 µg, Cox Pharmaceuticals, Barnstaple, UK; exposure time 1, 3 or 5 min) on the AgI was then determined using pulse wave analysis with concomitant recording of central and peripheral blood pressures and heart rate. Insulin clamp study was started after the AgI had returned to the baseline values and stabilized after a minimum of 30 min, In studies I, II and III, vascular (AgI, peripheral blood flow, peripheral and central blood pressures) parameters were determined before and under normoglycemic hyperinsulinemic conditions. The integral system software was used to calculate an average radial artery waveform, and to generate the corresponding ascending aortic pressure waveform using a transfer function (**Fig. 8**).[61; 170] The transfer function has been validated recently for the present device by comparing the derived the AgI to that measured simultaneously invasively by recording of central pressure in 62 patients going through coronary bypass surgery.[281]

Wave transmission properties in the upper limbs (in contrast to the descending aorta and lower limbs) change little with age, disease, and drug therapy in adults.[258] As suggested by O'Rourke and Gallagher,[265] the radial pressure was calibrated against the sphygmomanometrically determined brachial pressure, ignoring the small degree of amplification between the brachial and radial sites.

The aortic waveform was then subjected to further analysis for calculation of aortic augmentation (the pressure difference between the second and first systolic pressure peaks), the AgI, ejection duration and central systolic and diastolic blood pressure. The AgI was calculated by dividing augmentation with pulse pressure (**Fig. 4**).[178; 265] 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 incision in the aortic pressure wave. In studies I, II, III and IV blood pressure was measured with a calibrated sphygmomanometer and basal value was measured twice after a ten-minute bed rest.

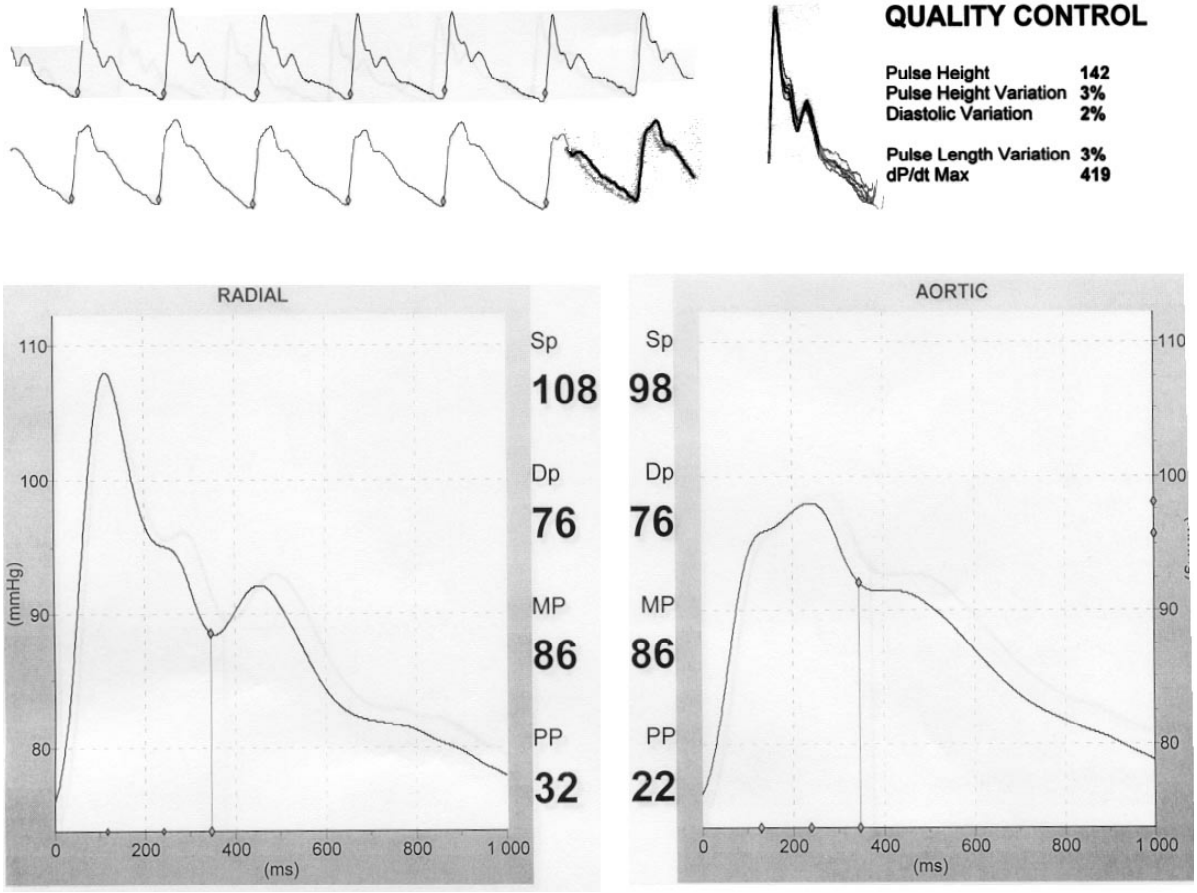

Figure 8. Radial (left panel) and aortic (right panel) waveforms from pulse wave analysis.

8.4 Insulin treatment

Type 2 diabetic patients considered eligible to participate in study III met with the doctor and the diabetes nurse 6 weeks before the start of insulin therapy. At this visit, the patients underwent complete history and physical examination. The patients were instructed to measure their fasting blood glucose concentrations and to record any episodes of hypoglycemia. The patients then visited the laboratory for measurement of fasting blood glucose, HbA_{1C}, serum concentrations of creatinine

and liver enzymes. An electrocardiogram was recorded and the urinary albumin excretion rate measured before start of insulin therapy. The results of the laboratory tests were checked, and if acceptable, all previous antidiabetic drugs were stopped and the patients were treated with 3 mg glimepiride for three weeks before the start of insulin therapy. Thereafter the patients were treated with combination therapy consisting of bedtime human isophane insulin (NPH) and continued glimepiride. The patients were taught self-adjustment of the insulin dose on the basis of plasma glucose measurements. The patients were instructed to increase their bedtime NPH dose by 2 units if the fasting glucose exceeded 6 mmol/l on three consecutive mornings. The patients visited the hospital outpatient clinic monthly. The pulse wave analysis and determination of whole body insulin sensitivity were performed after the outpatient visits at 6 months.

8.5 The effect of ASA on platelet aggregation

The platelet studies were performed after an overnight fast starting at 8.00–8.30 a.m. The subjects (study IV) were not taking any regular medications, including anti-inflammatory drugs, for 2 weeks prior to the study. Smoking was prohibited on the study day and exercise and alcohol for two days before the study. Blood for the initial aggregation test was collected from median antecubital vein via an 18-gauge catheter. The patient thereafter chewed and swallowed a 50 mg tablet of ASA (Disperin 50 mg, Orion, Finland) with some water. After 60 minutes, another catheter was inserted in an intact antecubital vein for sampling of blood for repeated aggregation measurements. Blood for platelet aggregation measurements was collected into tubes containing 0.129 M sodium citrate (9:1) and centrifuged (170 x g, 12 min at 22° C) to obtain platelet rich plasma (PRP). Platelet-poor plasma (PPP) was prepared by centrifugation of PRP at 5000 x g for 5 min. The concentration of platelets in PRP was determined by a cell counter (Sysmex K-1000, TOA Medical Electronics, Japan) and adjusted to 300 x 10^6/ml with autologous PPP. PRP and PPP were stored in capped plastic tubes and kept in room temperature.

In vitro platelet aggregation in PRP was measured using a four-channel turbidometric aggregometer (PACKS-4, Helena Laboratories, Beaumont, TX).[50] The aggregating agents were ADP (final concentrations 1, 1.5, 2 and 3 µmol/l; Sigma Chemicals, St. Louis, MI) and AA (final concentrations 0.5, 0.75, 1 and 1.5 mmol/l; Sigma Chemicals, St. Louis, MI). The samples were stirred at 600 rpm and preincubated for 1 minute at 37°C before the addition of the aggregating agent. The maximal aggregation (%) response at each concentration of the agonist was determined at baseline and after 50 mg oral ASA (**Fig. 9**).

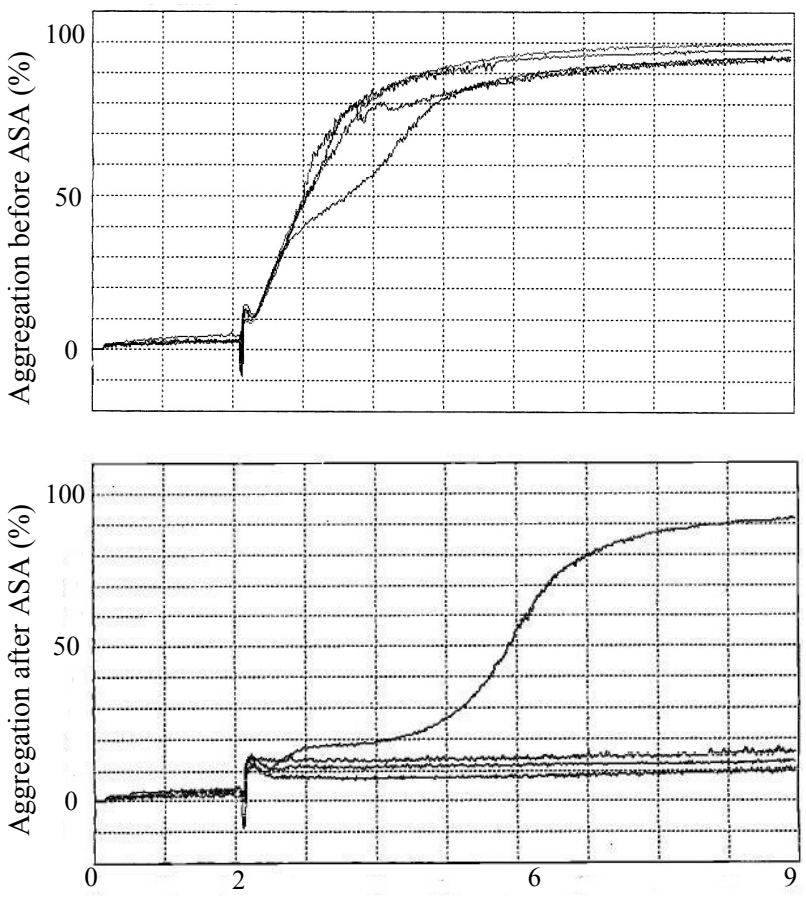

Figure 9. Aggregation with four doses of arachdonic acid before (upper panel) and after (lower panel) 50 mg ASA. 100% denotes fullblown aggregation.

8.6 Body composition

Fat free mass (FFM) and the percent of body fat were determined using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model #BIA-101A; RJL Systems, Detroit, MI).[225] The prediction equation used for estimating fat free mass (FFM; kg) was:

$FFM = 13.74 + 0.34 \cdot (Ht^2/R^2) + 0.33 \cdot Wt - 0.14 \cdot Age + 6.18 \cdot gender$

Where height (Ht) is expressed in cm, resistance (R) in W, weight (Wt) in kg, and age in yr; for gender a value 1 is given for males and 0 for females.[299] To calculate the W/H, waist circumference (W) was measured midway between spina iliaca superior and the lower rib margin, and hip circumference (H) at the level of the greater trochanters.[403]

8.7 Analytical procedures

Plasma glucose concentrations were measured in duplicate with the glucose oxidize method using a Beckman Glucose analyzer II (Beckman Instruments, Fullerton, CA).[163] Serum free insulin concentrations were measured by radioimmunoassay (Phadeseph® Insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) after precipitation with polyethylene glycol[98] and C-peptide concentrations using time-resolved fluoroimmunoassay (AUTOdelfia™ C-peptide, Wallac, Turku, Finland). HbA_{1C} was measured by high pressure liquid chromatography using the fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, CA).[335] Serum total, HDL cholesterol and triglyceride concentrations were measured with respective enzymatic kits from Roche Diagnostics using an autoanalyzer (Roche Diagnostics Hitachi 917, Hitachi Ltd, Tokyo, Japan). Low density lipoprotein (LDL) concentration was calculated using the formula of Friedewald. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) activities were determined as recommended by the European Committee for Clinical Laboratory Standards.

8.8 Statistical methods

The unpaired t-test was used to compare mean values between groups (study I, II and IV) and the paired t-test to compare selected variables before and after intervention (study III), after logarithmic transformation if necessary. Spearman's non-parametric rank correlation coefficient was used to calculate correlation coefficients between selected variables (study I, II, III and IV). Parameters, which were not normally distributed, were log-transformed. Calculations were made by GraphPad Prism version 3.0 (GraphPad Inc, San Diego, CA). The best fit characterizing the relationship between haemodynamic parameters over time and corresponding 50% effective dose/exposure time of the curves were determined by comparing the goodness of fit of linear and multiple non-linear equations using GraphPad Prism. Analyses of ASA effects at various agonist concentrations between lean and obese subjects (study IV) and changes in haemodynamic parameters over the time (study II, III and IV) were made using analysis of variance (ANOVA) for repeated measures using the SYSTAT statistical package (Evanston, IL) followed by Bonferroni's multiple comparisons test. The results are expressed as mean±standard error of mean. All p-values are 2-tailed. P-values less than 0.05 were considered statistically significant.

9 RESULTS

9.1 Comparison of *in vivo* effects of GTN and insulin (Study I)

Dose-response study

Effects of GTN. The AgI averaged 0.5±2.0, -0.1±2.5 and -1.6±2.0 % at baseline before GTN exposure of 1, 3 and 5 min (NS). The AgI decreased significantly after all exposures to maximally -9.6±1.7, -10.6±1.7 and -15.7±2.2 %, respectively. From the best fit for the dose -response curve, the 50 % effective exposure time for GTN to decrease the AgI was 44 s (**Fig. 10**).

Effects of insulin. During the insulin infusions serum free insulin concentrations increased from 6±2, 5±1 and 5±1 mU/l basally to 30±2, 62±2 and 156±4 mU/l between 30-120 min during the 0.5, 1 and 2 mU/kg·min insulin infusions. Plasma glucose concentrations averaged 5.5±0.2, 5.4±0.2 and 5.4±0.2 basally and 5.1±0.1, 5.0±0.1 and 5.0±0.1 mmol/l during insulin infusions, respectively. Whole body glucose uptake averaged 5.4±0.9, 7.6±0.9 and 10.5±1.2 mg/kg·min, respectively (**Fig. 10**). The AgI averaged 0.7±1.7, -0.3±2.3 and -0.9±2.0 % at baseline before insulin infusions of 0.5, 1 and 2 mU/kg·min (NS). The AgI decreased significantly within 60 min during the insulin infusion rates of 0.5 (-6.3±2.8 %, $p<0.05$) and 1 (-6.2±2.2 %, $p<0.01$) mU/kg·min and already at 30 min during the 2 mU/kg·min insulin infusion (-5.0±1.0 %, $p<0.05$). The maximal insulin-induced decrease in the AgI averaged -8.0±2.7, -10.2±1.8 and -13.4±1.8 % ($p=0.001$ for slope relating change in AgI to insulin dose), respectively. From the best fit for dose-response curve, the 50 % effective dose for insulin to decrease the AgI was 0.6 mU/kg·min.

During the insulin infusions, aortic pressure augmentation (i.e. the pressure difference between the first and second systolic pressure peaks at the level of the aorta) decreased significantly during 60 min with insulin infusion rates of 0.5 (0±1 vs. -2±1 mmHg, 0 vs. 60 min, $p<0.05$) and 1 (0±1 vs. -2±1 mmHg, 0 vs. 60 min, $p<0.05$) mU/kg·min and already at 30 min during 2 mU/kg·min insulin infusion (0±1 vs. -2±1 mmHg, 0 vs. 30 min, $p<0.05$). Heart rate remained unchanged during all studies (59±1 vs. 58±1, 58±2 vs. 59±2 and 58±2 vs. 61±2 beats/min 0 vs. 120 min during 0.5, 1 and 2 mU/kg·min insulin infusions). There were no changes in peripheral blood flow during the insulin infusions of 0.5 and 1 mU/kg·min. During the highest insulin dose, the AgI decreased significantly ($p<0.001$) by 30 min. A 31% increase in peripheral blood flow was observed at 2 hours ($p<0.05$), but not at any other time point.

Brachial artery systolic blood pressure did not change during the 0.5 mU/kg·min insulin infusion (118±4 vs. 117±4 mmHg, 0 vs. 120 min, NS), but increased significantly at 120 min during the insulin infusion of 1 mU/kg·min, (113±3 vs. 116±3 mmHg, 0 vs. 120 min, $p<0.01$) and 2 mU/kg·min (114±3 vs. 120±4 mmHg, 0 vs. 120 min, $p<0.01$). Brachial artery diastolic blood pressure did not change during 0.5 mU/kg·min insulin infusion (64±2 vs. 63±2 mmHg, 0 vs. 120 min, NS). Brachial artery diastolic blood pressure decreased significantly at 90 min during the insulin infusion of 1 mU/kg·min, (62±1 vs. 60±2 mmHg, 0 vs. 90 min, $p<0.01$) and at 120 min using 2 mU/kg·min insulin infusion (63±2 vs. 59±1 mmHg, 0 vs. 120 min, $p<0.05$). In contrast to changes in peripheral blood pressures, both aortic systolic and diastolic blood pressure remained unchanged during all three insulin infusions.

Cross-sectional study

The AgI. After 5 min sublingual GTN, the AgI decreased significantly from 25.2±2.5 to 6.4±2.7 % ($p<0.0001$). During the insulin infusion serum free insulin concentrations increased from 10±2 basally to 171±6 mU/l between 30-120 min. Plasma glucose concentrations were maintained at 5.1±0.1 mmol/l during the insulin infusion. Whole body glucose uptake averaged 5.1±0.5 mg/kg·min (range 1.0-8.6 mg/kg·min). During the insulin infusion, the AgI decreased significantly by 30 min (19.7±3.0 vs. 15.7±3.1 %, 0 vs. 30 min, $p<0.0001$). The maximal insulin-induced decrease in the AgI averaged 11.7±3.6 %, $p<0.0001$. The change in the AgI by GTN and insulin were significantly positively correlated (Spearman's $r=0.92$ (95% confidence interval 0.81-0.97), $p<0.0001$, **Fig. 11**). There was no correlation between basal AgI and change in the AgI by either insulin or GTN. Adjustment for age (after adjustment $r=0.93$ between change in AgI by GTN and insulin, $p<0.0001$) or gender (after adjustment $r=0.92$, $p<0.001$) did not change the results. The correlation coefficient between the change in AgI by insulin and GTN was 0.96 ($p<0.0001$) in women and 0.90 ($p<0.0001$) in men.

Other haemodynamic effects. GTN decreased aortic pressure augmentation significantly from 10±2 to a nadir of 3±1 mmHg ($p<0.001$). Heart rate remained unchanged (62±2 vs. 63±2 beats/min, basal vs. nadir). Brachial artery systolic blood pressure did not change by GTN (128±3 vs. 127±3 mmHg) but decreased significantly at the level of the aorta from 119±4 at baseline to a nadir of 112±3 mmHg ($p<0.001$).

Insulin decreased aortic pressure augmentation significantly by 30 min (7±1 vs. 5±1 mmHg, 0 vs. 30 min, $p<0.01$). Heart rate remained unchanged during the insulin infusion (61±2 vs. 60±2 beats/min 0 vs. 120 min). Peripheral blood flow increased significantly by 120 min (1.7±0.1 vs. 2.0±0.1 ml/dl·min, 0 vs. 120 min, $p<0.05$). Brachial artery systolic blood pressure did not change during insulin infusion (122±3 vs. 121±3 mmHg, 0 vs. 120 min), but decreased significantly at the level of the aorta at 30 min (111±3 vs. 107±3 mmHg, 0 vs. 30 min, $p<0.01$).

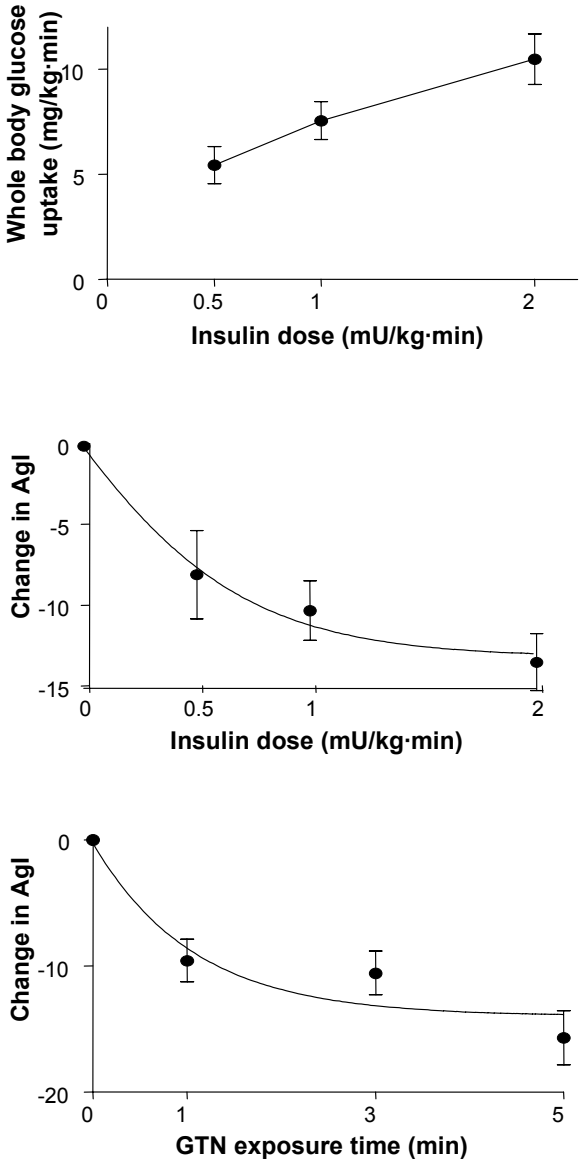

Figure 10. Whole body glucose uptake (upper panel) and change in the AgI (middle panel) during 2-hour insulin infusions of 0.5, 1 and 2 mU/kg·min, and change in the AgI after sublingual GTN (500 µg) for 1, 3 and 5 minutes. Euglycemia was maintained using the euglycemic insulin clamp technique.

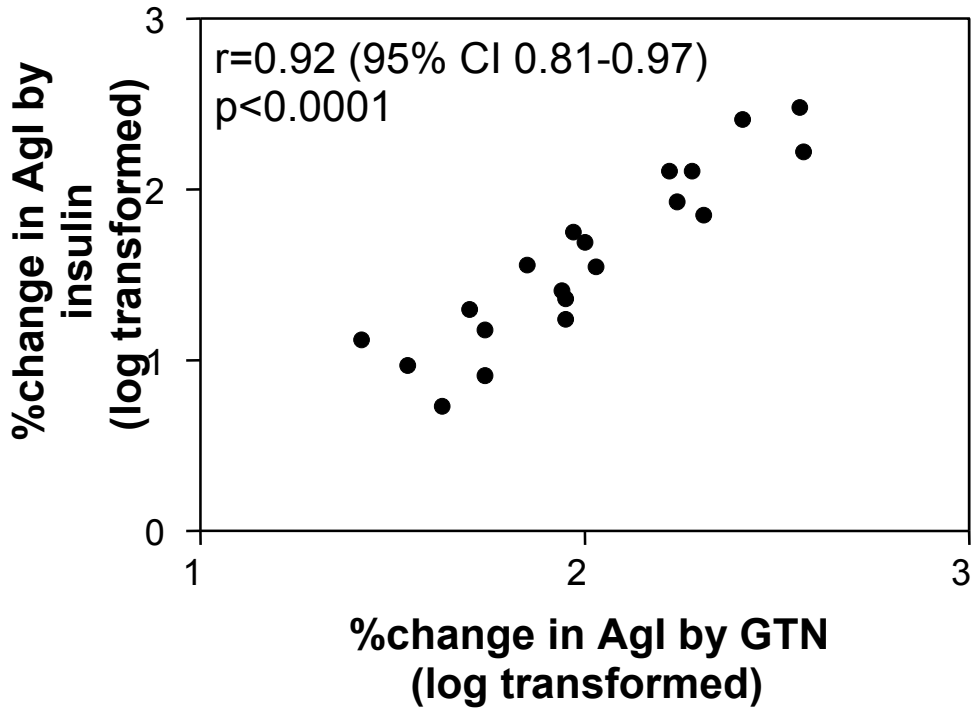

Figure 11. Relationship between percentage change in the AgI by sublingual GTN (500 µg for 5 min) and insulin infusion of 2 mU/kg·min. R denotes Spearman's non-parametric correlation coefficient, CI denotes confidence interval.

9.2 The effect of insulin on the AgI and central blood pressure in type 2 diabetes (Study II)

Glucose and insulin concentrations, insulin sensitivity

Fasting plasma glucose concentrations averaged 5.7±0.1 mmol/l in normal subjects and 11.6±0.7 in type 2 diabetic patients, $p<0.001$. Fasting serum free insulin concentrations were 11±2 mU/l in both groups**.** During the insulin infusions, serum free insulin concentrations averaged 170±7 mU/l in the normal subjects, and 167±7 mU/l in the patients with type 2 diabetes (NS). During the last hour of hyperinsulinemia, plasma glucose averaged 5.1±0.1 mmol/l in the normal subjects, and 5.3±0.1 mmol/l in the patients with type 2 diabetes (NS). Whole body insulin sensitivity of glucose metabolism (M-value 150-180 min) was 31 % lower in patients with type 2 diabetes (4.8±0.6 mg/kg·min) than in the normal subjects (7.0±0.6 mg/kg·min, $p<0.05$).

Augmentation and the augmentation index

Augmentation averaged 8.9±1.3 mmHg at baseline in the normal subjects, and decreased significantly within 30 minutes to 6.1±1.1 mmHg ($p<0.001$ vs. basal), and after 180 minutes to 5.5±1.2 mmHg. ($p<0.001$ vs. basal). The AgI averaged 23.1±2.1% at 0 min and decreased significantly and by 30 % after 30 min to 17.9±2.6 %, ($p<0.001$ vs. basal) and after 180 min to 14.9±3.0 % (**Fig. 13**). The decrease during first hour also could not be attributed to a decrease in peripheral vascular resistance since both forearm blood flow (1.8±0.2 vs. 1.7±0.1 ml/dl·min, 0 vs. 60 min, NS) and peripheral vascular resistance (64±7 vs. 54±4 mmHg/ml/dl·min, respectively, NS) remained unchanged.

Basally, before the insulin infusion, augmentation and the AgI were comparable between type 2 diabetic patients and normal subjects. In contrast to the normal subjects, however, augmentation did not decrease significantly by insulin in the diabetic patients during the first 30 minutes. Mean augmentation averaged 11.1±1.2 mmHg basally and 9.1±1.1 mmHg after 30 minutes (NS vs. basal) (**Fig 13**). The first significant decrease in augmentation was observed at 60 min in the patients with type 2 diabetes, 8.5±1.0 mmHg ($p<0.05$ vs. basal). Minimum augmentation was reached after 180 minutes of insulin infusion, when it averaged 7.2±1.0 mmHg ($p< 0.001$ vs. basal). (**Fig. 13**). The percent change in augmentation was significantly smaller in the patients with type 2 diabetes than in the normal subjects at 30 minutes (-17±6 % vs. -36±7 %, $p<0.05$). Basally, the AgI averaged 27.5± 2.1 % in the diabetic patients. The AgI decreased to 24.4±2.2 % at 30 minutes ($p<0.01$ vs. basal) and reached minimum at 180 minutes 19.1±2.7 % ($p<0.001$ vs. basal). There was a significantly smaller percent change in the AgI in the diabetic patients than in normal subjects at 30 minutes (-13±4% vs. -30±7%, $p<0.05$) and at 60 minutes (-16±5% vs. -39±9%, $p<0.05$). The decrease was significantly lower in the diabetic than non-diabetic group independent of basal AgI. There were no significant correlations between insulin sensitivity and augmentation or the AgI or their changes by insulin and in either group.

*Central and peripheral systolic blood pressur*es

As expected from the change in augmentation and the AgI (**Fig. 13**), systolic aortic blood pressure decreased significantly in the normal subjects by 30 minutes, while there was no significant change in systolic aortic blood pressure at this time point in the type 2 diabetic patients (**Fig. 12**). Aortic systolic blood pressure remained significantly lower than at baseline for the entire period of hyperinsulinemia in the normal subjects (**Fig. 12**). In the type 2 diabetic patients, a significant decrease in aortic systolic

blood pressure was not observed until 120 minutes of hyperinsulinemia (**Fig. 12**). Brachial systolic pressure did not change significantly in either group (**Fig. 12**).

Other haemodynamic parameters

Heart rate remained unchanged in both groups during the 180 min period of insulin infusion (data not shown). A small (21 %) increase in peripheral blood flow was observed at 180 min in the normal subjects (1.8±0.2 vs. 2.2±0.2 ml/dl·min, $p<0.05$) and this was associated with a significant decrease in brachial artery diastolic pressure at 150 (-2.7±1.0 mmHg, $p<0.05$) and 180 min (-3.7±0.9 mmHg, $p<0.01$) compared to basal diastolic pressure (80±2 mmHg). Forearm blood flow and diastolic pressure remained unchanged in the type 2 diabetic patients (data not shown).

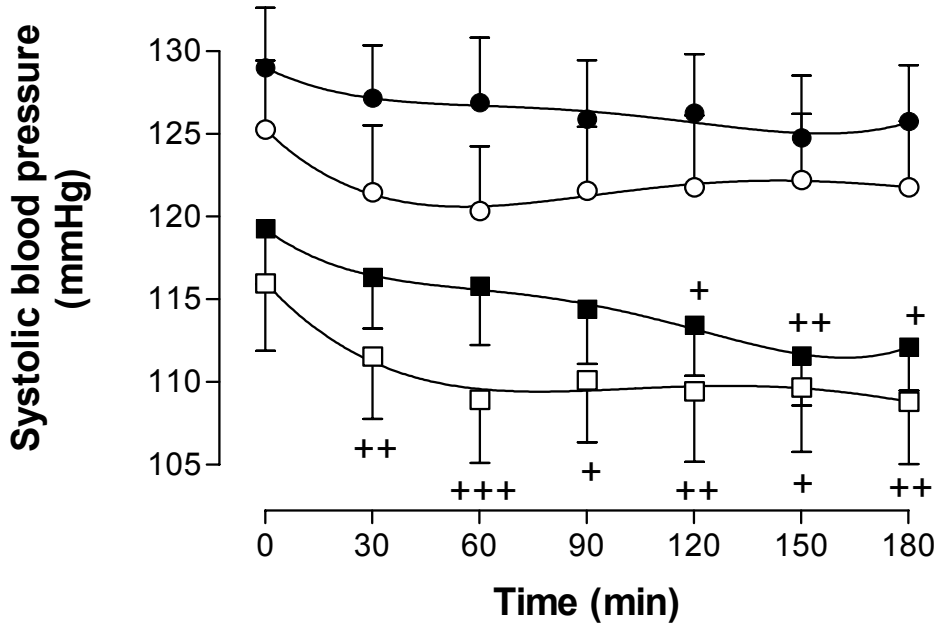

Figure 12. Peripheral (circles) and aortic (squares) systolic blood pressure in the normal subjects (open symbols) and the type 2 diabetic patients (filled symbols). + $p<0.05$, ++ $p<0.01$, +++ $p<0.001$ for change vs. 0 min.

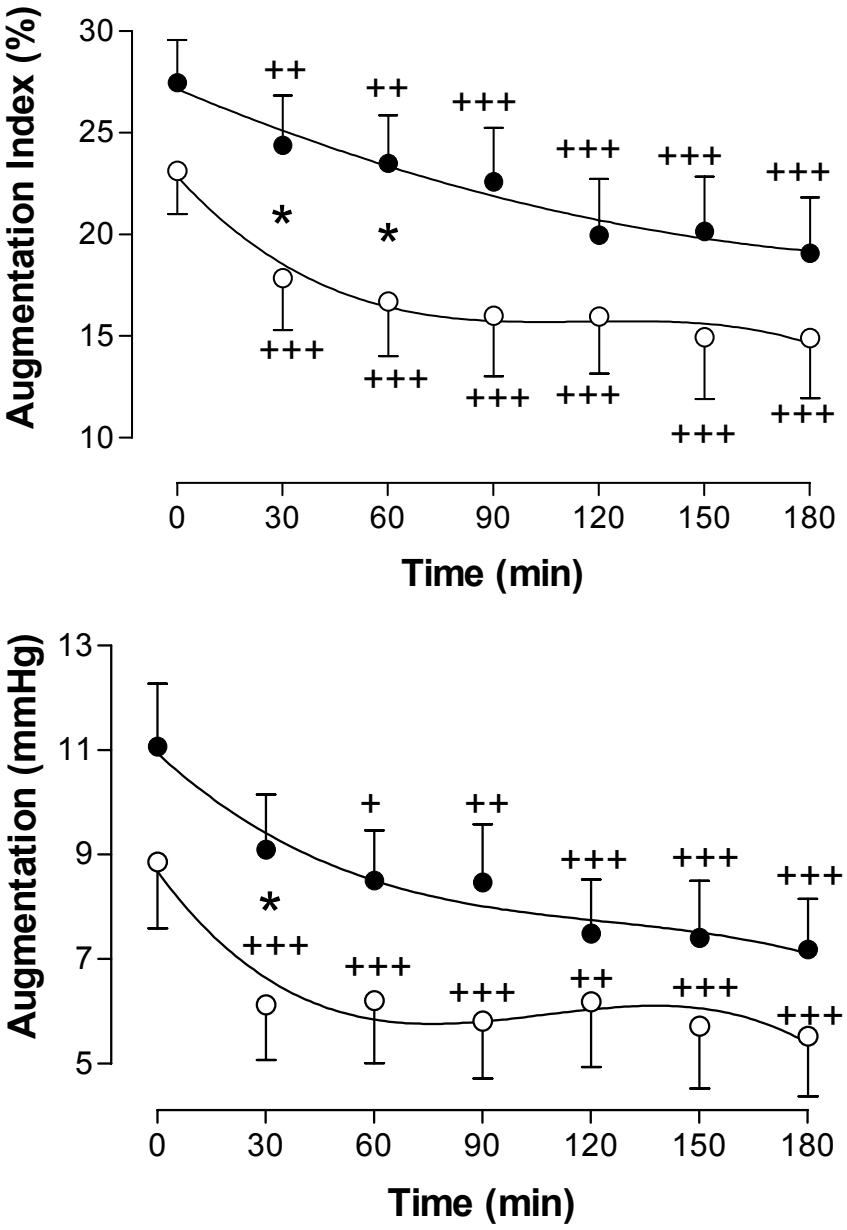

Figure 13. The AgI and augmentation during 180 min of hyperinsulinemia. The open circles (o) denote normal subjects and the filled circles (•) type 2 diabetic patients. * $p<0.05$ for the difference of change in the AgI or augmentation between the groups. + $p<0.05$, ++ $p<0.01$, +++ $p<0.001$ for change vs. 0 min.

9.3 The effect of insulin therapy on glucose metabolism, the AgI and central blood pressure in type 2 diabetes (Study III)

Metabolic effects of insulin therapy

Metabolic effects of insulin therapy have been summarized in Table 2. The mean bedtime insulin dose at 6 months was 76±10 IU/day. HbA_{1C} decreased from 8.8±0.2 to 7.2±0.2 % ($p<0.001$) and body weight increased from 96±4 to 102±5 kg ($p<0.01$) during the six month insulin therapy.

Glucose and insulin concentrations and insulin sensitivity of glucose metabolism

Fasting plasma glucose and serum free insulin concentrations are given in **Table 2**. During the insulin infusion (30-180 min), serum free insulin concentrations were comparable before and after insulin treatment. During the last hour (120-180 min) of hyperinsulinemia plasma glucose averaged 5.4±0.2 mmol/l before insulin treatment and 5.1±0.1 mmol/l after insulin treatment (NS). Insulin treatment increased insulin sensitivity by 35 % from 5.1±0.7 to 6.8±0.6 mg/kg ffm·min ($p<0.001$).

Insulin action on wave reflection, blood pressure and other haemodynamic parameters

Six months of insulin therapy decreased basal AgI from 26.2±1.8 to 22.7±2.3% ($p<0.05$). Insulin acutely decreased AgI progressively until 120 minutes both before and after insulin therapy. The change in the AgI by insulin was similar before and after insulin therapy at all time points (**Fig. 14**). During insulin infusion brachial systolic blood pressure was unchanged both before and after insulin therapy. Mean arterial pressure measured before insulin infusion was similar before and after 6 months of insulin therapy (98 ± 2 vs. 97 ± 2 mmHg). Mean arterial pressure decreased significantly by 120 min of insulin infusion with no significant difference in the change of mean arterial pressure before and after 6 months of insulin therapy. Consistent with the decrease in the AgI, aortic systolic blood pressure decreased similarly acutely by insulin both before and after insulin therapy (**Fig. 15**). Peripheral blood flow, pulse pressure, heart rate and ejection duration were similar before and after insulin treatment and remained unchanged during the insulin infusion (data not shown).

Table 2. Metabolic effects of 6 months of insulin therapy

	Type 2 diabetic patients before insulin therapy	Type 2 diabetic patients after insulin therapy
Men/women	11/2	
Age (years)	53±2	
Height (cm)	176±2	
Weight (kg)	96±4	102±5**
Body mass index (kg/m^2)	30.8±1.2	32.7±1.5**
Body fat (%)	29±2	30±1
Systolic blood pressure (mmHg)	132±4	130±3
Diastolic blood pressure (mmHg)	80±2	80±2
Waist/hip ratio	1.02±0.02	1.02±0.02
Fasting plasma glucose (mmol/l)	12.5±0.8	7.1±0.5***
Fasting serum insulin (mU/l)	11±2	28±4**
SSSI[1] (mU/l)	177±7	197±14
HbA_{1C} (%)	8.8±0.2	7.2±0.2***
Serum cholesterol (mmol/l)	5.3±0.3	5.3±0.4
Serum HDL cholesterol (mmol/l)	1.1±0.1	1.1±0.1
Serum triglycerides (mmol/l)	1.9±0.2	1.9±0.3

Data are shown as mean±SEM. **p<0.01, ***p<0.001 for difference between before and after insulin treatment in diabetic patients, [1]SSSI = steady-state serum free insulin concentration (30-180 min, mU/l).

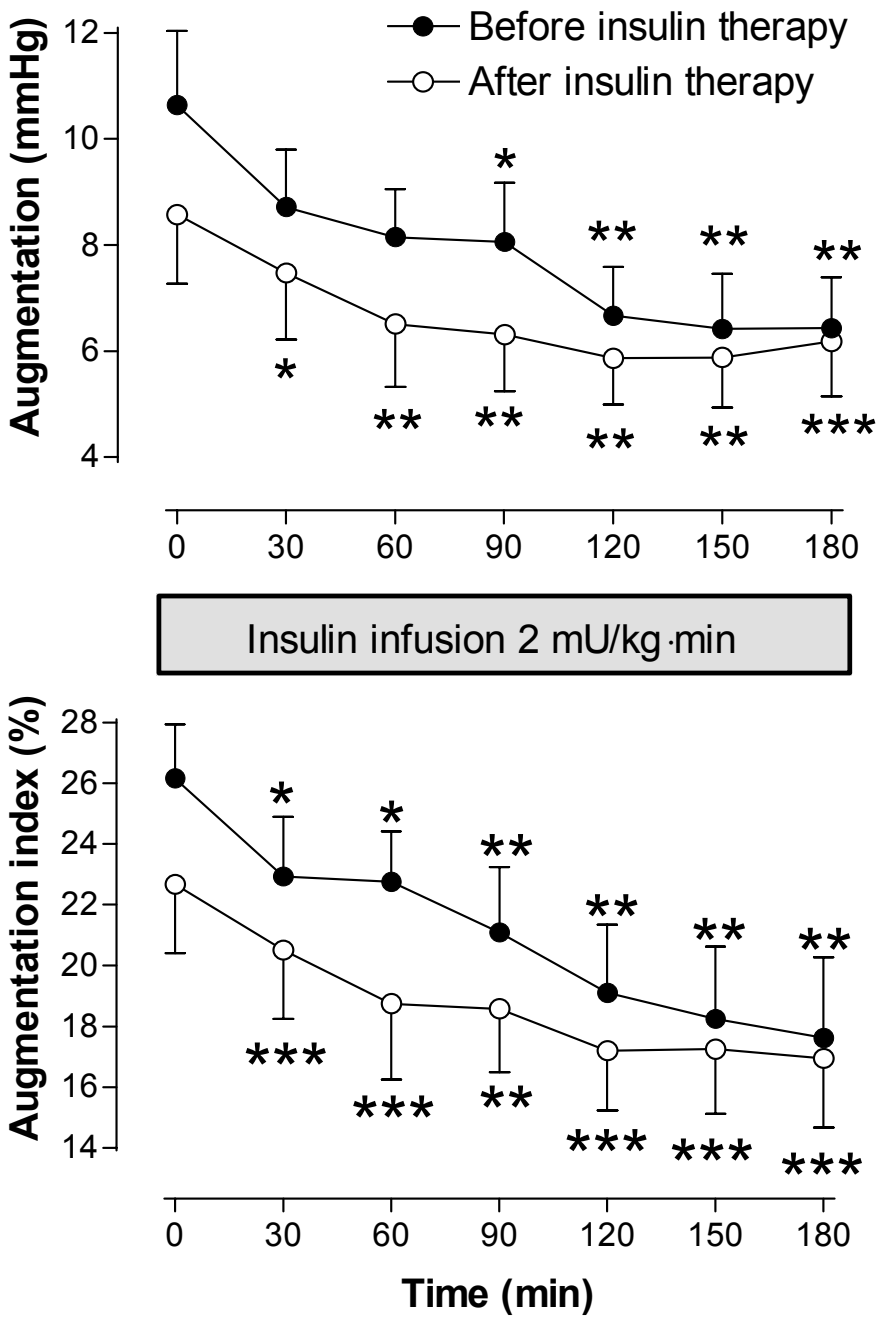

Figure 14. Augmentation and AgI before and after insulin therapy. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ for change vs. 0 min.

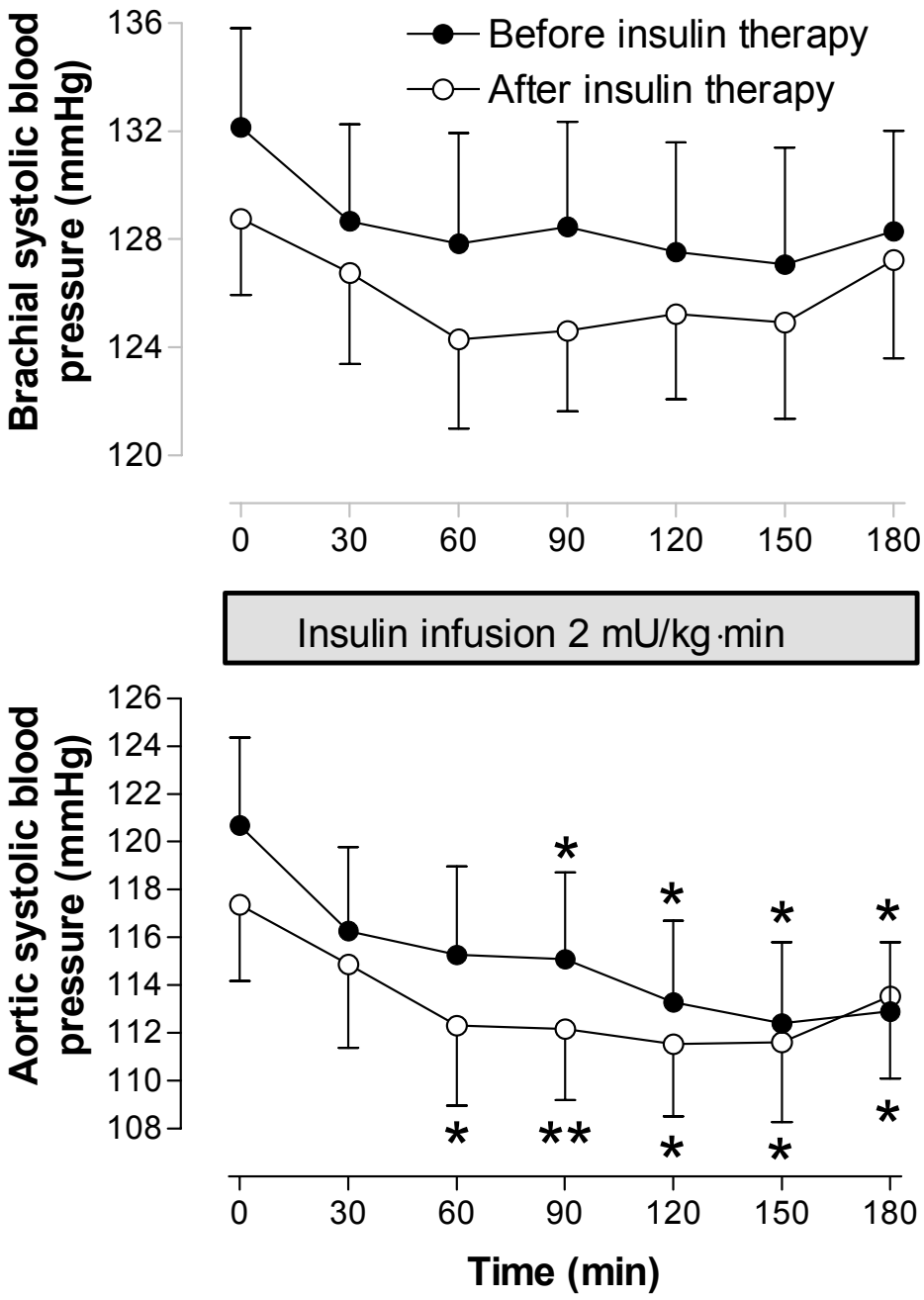

Figure 15. Brachial and aortic systolic blood pressure before and after insulin therapy. *p<0.05, ** p<0.01, *** p<0.001 for change vs. 0 min.

9.4 The effect of ASA on platelet aggregation in obese and non-obese subjects (Study IV)

Whole body insulin sensitivity

During the insulin infusion, serum free insulin concentrations averaged 182±14 in the obese and 164±8 mU/l in the non-obese group (NS). Plasma glucose averaged 5.1±0.1 mmol/l in both groups during hyperinsulinemia. Whole body insulin sensitivity was 36 % lower in the obese (4.5±0.6) than the non-obese (7.1±0.6 mg/kg·min, $p<0.01$) group. Both BMI ($r=-0.73$, $p<0.001$), body weight ($r=-0.60$, $p<0.01$), the waist/hip ratio ($r=-0.43$, $p<0.05$), and concentrations of fasting serum insulin ($r=-0.76$, $p<0.001$), C-peptide ($r=-0.86$, $p<0.001$), serum triglycerides ($r=-0.53$, $p<0.05$) and HDL cholesterol ($r=0.49$, $p<0.05$) were significantly correlated with whole body insulin sensitivity.

Platelet aggregation

Before ASA administration already the lowest concentration of AA (0.5 mmol/l) induced maximal aggregation in each subject without any differences between the groups (95±1 % vs 94±2 %, obese vs non-obese, NS). In the blood sample taken one hour after 50 mg ASA, AA-induced aggregation was inhibited by ASA in the obese group only at the lowest dose of AA, while ASA significantly inhibited aggregation at all doses of AA in the non-obese group (**Fig. 16**). Maximal aggregation after ASA was significantly greater in the obese than the non-obese group ($p=0.016$ for ANOVA, **Fig.16**).

Before ASA, when ADP was used as the agonist, aggregation in response to the two lowest concentrations of ADP was slightly but not significantly greater in platelets of the obese than those of the non-obese group (**Fig. 16**). Aggregation was significantly more inhibited by ASA at ADP concentrations of 2 µmol/l (74±6 vs 91±1 % in non-obese vs obese, $p<0.01$) and 3 µmol/l (79±6 vs 93±1 %, respectively, $p<0.05$) in the non-obese than the obese group (**Fig. 16**).

Before ASA administration, whole body insulin sensitivity in the whole study group was inversely correlated with aggregation induced by the two lowest doses of ADP (1 and 1.5 µmol/l, Table 3). After ASA ingestion, there was a strong negative correlation between insulin sensitivity and maximal aggregation induced by AA at concentrations of 0.75, 1, 1.5 mmol/l, and ADP at concentrations of 1.5 and 3 µmol/l (Table 3). Aggregation induced with 1 mmol/l AA after ASA

also correlated with BMI, body weight, waist/hip ratio, serum triglycerides, C-peptide and fasting serum insulin as well as with ADP induced aggregation both before and after ASA administration**.**

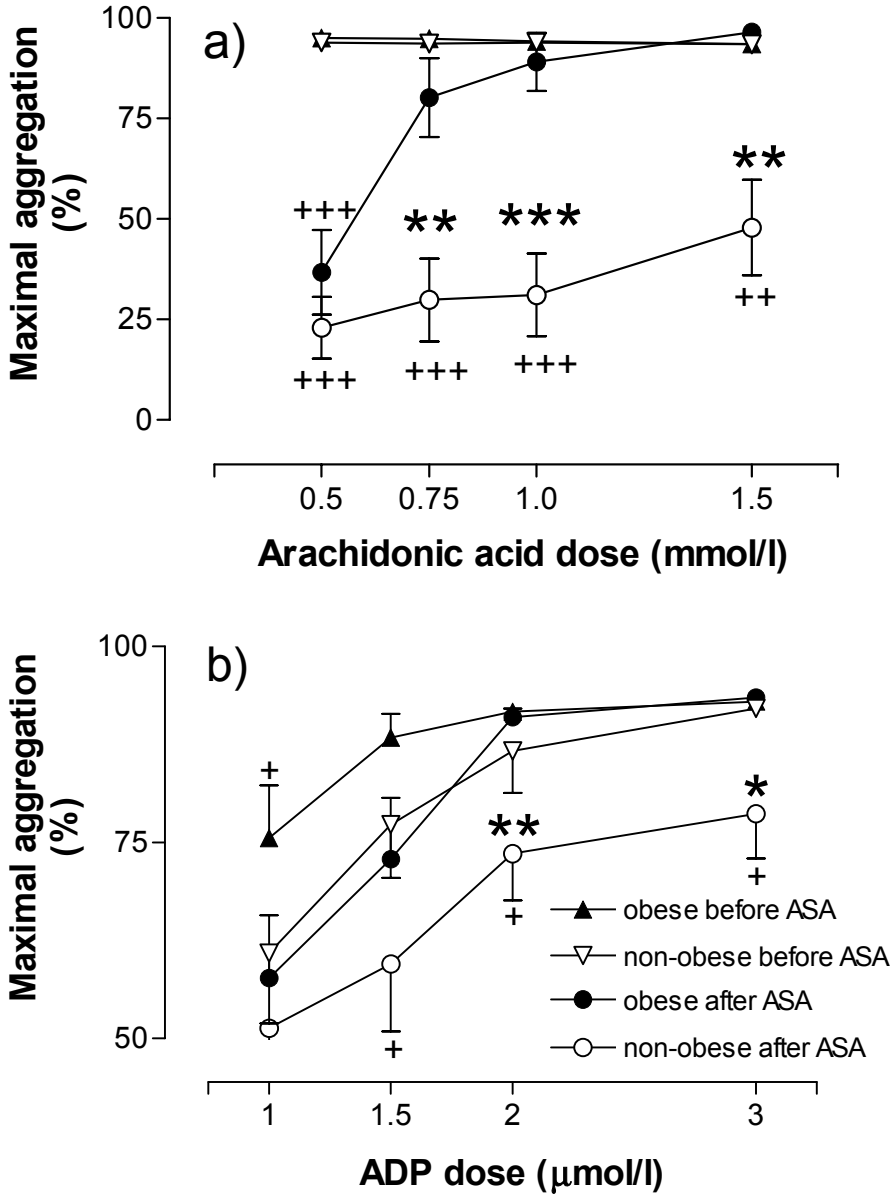

Figure 16. Maximal aggregation at four concentrations of AA (a) and ADP (b) at baseline and after ASA 50 mg. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ for obese vs non-obese after ASA 50 mg. + $p<0.05$, ++ $p<0.01$, +++ $p<0.001$ for difference between aggregation at baseline and after ASA.

Table 3. Correlations between insulin sensitivity (mg/kg·min) determined by the euglycemic clamp technique and maximal aggregation with different concentrations of AA and ADP in the whole study group.

	r	p
AA 0.75 mmol/l after ASA	-0.67	<0.001
AA 1 mmol/l after ASA	-0.68	<0.001
AA 1.5 mmol/l after ASA	-0.63	<0.001
ADP 1 µmol/l at baseline	-0.48	<0.05
ADP 1.5 µmol/l at baseline	-0.48	<0.05
ADP 1.5 µmol/l after ASA	-0.52	<0.05
ADP 3 µmol/l after ASA	-0.45	<0.05

10 DISCUSSION

The present studies were made to investigate the connections between insulin resistance and insulin action on arteries and ASA action on platelets. There are interesting parallels between insulin action on vessels and platelets.[356] Both vascular synthesis of prostacyclin (PGI_2) and platelet sensitivity to PGI_2 may be diminished in diabetes.[10; 136] Insulin promotes endothelium-dependent vasodilatation[373] and inhibits platelet aggregation.[359] Insulin actions on platelet aggregation and the AgI are impaired in insulin resistant subjects.[21; 357; 388; 392] Insulin-induced vasodilatation and insulin-induced inhibition of platelet aggregation can both be blocked by L-NMMA.[314; 333; 358] Both peripheral resistance arteries[229] and platelets[21] have been reported to have diminished sensitivity to NO in patients with type 2 diabetes[229] and in obese non-diabetic as compared to age-matched normal-weight subjects.[21; 366] In study I we also found a correlation between the action of insulin and GTN on large artery function. Impaired bioavailability of NO, due to diminished synthesis, increased destruction or insensitivity of effector pathways, could thus be a common defect predisposing insulin resistant individuals to abnormalities in vascular and platelet functions.

STUDIES I, II and III

In the studies I, II and III, we studied large artery function with pulse wave analysis. In study I we found insulin and GTN to decrease the AgI in dose-dependent manner and that the actions of insulin and GTN on the AgI were closely correlated. In study II we found that the insulin action on the AgI was impaired in type 2 diabetic patients when compared to age and weight matched normal subjects. In study III we found that 6 months of basal insulin therapy decreases the AgI but does not alter the change of AgI induced by insulin infusion. In the studies II and III, insulin sensitivity of glucose metabolism within the group of type 2 diabetic patients or their matched control subjects did not correlate with the insulin-induced change of the AgI, although such relationship has been found in previous studies in both non-diabetic men[390] and a group of obese subjects.[392] In a previous study of non-diabetic group including a total of 50 men with BMIs ranging from 19 to 45 kg/m^2 and age from 18 to 60 years there was a correlation between insulin sensitivity and both basal AgI and the change in AgI by insulin infusion.[390] This group was larger than present study groups and had a larger variation in both basal AgI, its change by insulin and in insulin action on glucose metabolism than in the present studies.

Previous studies have uniformly found endothelium-dependent vasodilatation in the peripheral resistance arteries of type 2 diabetic patients to be impaired. In addition, endothelium-independent vasodilatation has been impaired in some of the studies.[231] Insulin does have a sluggish vasodilatating effect on peripheral resistance vessels.[196; 415] For example, while acetylcholine, a classic endothelium-dependent vasodilatator, or sodium nitroprusside increase blood flow at submaximal doses at least 500 % above basal within a minute, it takes even in young healthy normal subjects 120 min before insulin significantly increases peripheral blood flow and even then, the increase is only 20-80 %. In the present, as well as previous studies,[11; 390; 392; 393] the changes in the AgI occurred within 30-60 minutes at physiological insulin concentrations, unlike in peripheral resistance vessels where the effect of insulin occurs after several hours of supraphysiological concentrations.[415]

In studies I, II and III, peripheral vascular resistance, heart rate and brachial systolic and diastolic blood pressure remained unchanged during the time period when the changes in augmentation occurred. In study II there was a small increase in peripheral blood flow in normal subjects but not in diabetic subjects at 180 minutes. In study I there was an increase in peripheral blood flow in both dose-response and cross-sectional study at 120 minutes at insulin infusion rate 2 mU/kg·min. At the higher insulin doses (2-5 mU/kg·min), peripheral systolic blood pressure increases slightly and diastolic blood pressure decreases concomitant with peripheral vasodilatation.[393] Changes in blood pressure at supraphysiological insulin concentrations[415] are likely to be a consequence of activation of the sympathetic nervous system in response to peripheral vasodilatation.[393] The decrease in wave reflection by insulin could thus have been due to an acute decrease in the tone of muscular arteries greater than those controlling peripheral vascular resistance. Alternatively, it could have been due to a change in elastic properties of the aorta, although given the rapid time course, this possibility seems unlikely.

In the studies I cross-sectional, II and III, the AgI was positive i.e. pressure of the reflected wave was higher than that of the first systolic pressure wave prior to start of the insulin infusion. The higher AgI was most likely explained by age-associated stiffening of arteries in these subjects, who averaged 50 years of age.[178] In studies I cross-sectional, II and III, insulin not only decreased the AgI but also central systolic pressure within 30 min in normal subjects and between 60 and 120 minutes in type 2 diabetic patients. Brachial systolic blood pressure remained unchanged in all groups. In previous studies, where only young healthy volunteers were studied,[11; 391-393] insulin did not decrease central systolic pressure because baseline augmentation was negative.

Pulse pressure, a surrogate measure of arterial stiffness, varies throughout the arterial tree, in part because of differences in vessel compliance and the phenomenon of wave reflection. Normally there is amplification of pulse pressure from the aorta to the periphery, mainly caused by a rise in systolic blood pressure, whereas diastolic pressure and mean arterial pressure are relatively constant.[398] Especially in young subjects high brachial systolic blood pressure may be a sign of systolic hypertension or great pulse wave amplification due to flexible arteries. With increasing age arterial stiffness increases and the difference between central and peripheral systolic blood pressure decreases. Wave transmission properties of the upper limb change little with age or disease in humans, which enables the use of a generalized transfer function to synthesize the aortic waveform from those recorded in the periphery.[258] The transfer function and the device used to record radial artery waveforms used in the present studies were recently validated by invasive measurements in 62 patients undergoing cardiac surgery.[281]

In the present studies, the radial blood pressure was calibrated against the sphygmomanometrically determined brachial blood pressure as suggested by O'Rourke at al [265] thus ignoring the small amplification between these sites. When calibrated against invasively measured aortic blood pressure, there is a strong correlation between central blood pressures and the AgI measured invasively and non-invasively from the radial artery using a generalized transfer function.[281] When calibrated against blood pressure measured with a sphygmomanometer from the brachial artery, the central blood pressures derived from radial measurements are accurate, but the AgI does not correlate well with the AgI measured invasively[147] or from carotid artery without the use of a transfer function.[250] Therefore some authors consider direct carotid artery AgI measurement[250; 269] or even the AgI measured at radial artery without use of transfer function to be a better measure of wave reflection than the AgI measured with the help of a transfer function.[147] However, in the study criticizing use of the transfer function blood pressures were measured oscillometrically rather than using the auscultatory method.[250] Oscillometric measurement provides a more reliable determination of mean arterial pressure, but systolic and diastolic blood pressures are calculated rather than measured directly.[326] Since our main goal was to investigate the change in the AgI caused by insulin in the same individuals at different time points, it is unlikely that use of the generalized transfer function influenced our results. The parallel decreases in aortic systolic blood pressure and the AgI supports this interpretation. It has also been previously shown that the changes in the AgI derived from radial measurements after GTN or norepinephrine administration correlate well with changes in the AgI measured without transfer function.[250]

The AgI measured from carotid artery with pulse wave analysis has been shown to independently predict cardiovascular mortality in patients with end-stage renal failure,[219] providing the first evidence that increased arterial wave reflection is an independent predictor of cardiovascular disease. AgI measured from radial artery correlates with carotid artery intima-media thickness in diabetic and non-diabetic subjects,[295] but it has not been shown to predict mortality. Pulse wave analysis as a method is relatively cheap, rapid and can be performed at an office visit. It is easy to use after a short training period and there is good inter- and intra operator reproducibility.[82; 269] Prospective studies are still needed to show whether the AgI predicts vascular end-points in patients groups other than end-stage renal failure.

The exact mechanisms responsible for the effect of insulin on large arterial function and central haemodynamics remain unclear. Insulin dilates peripheral resistance vessels, in part, via the release of endothelium-derived NO.[314] In resistance arteries, insulin-induced vasodilatation in vivo can be abolished by co-infusion of L-NMMA but not by other vasoconstrictors such as norepinephrine.[415] Insulin has also been shown to potentiate acetylcholine but not sodium nitroprusside induced vasodilatation in human resistance arteries *in vivo*.[341; 389] The fact that peripheral vasodilatation to insulin is blunted in subjects with endothelial dysfunction[334] has lead a number of investigators propose endothelial dysfunction as a cause of insulin resistance, although this has been questioned.[415] Endothelium-derived NO has recently been shown to regulate large arterial stiffness.[185; 400] If the action of insulin on large arteries is mediated in part by NO then endothelial dysfunction rather than insulin resistance may cause the blunted effect of insulin on large artery mechanics. Indeed studies in patients with type 2 diabetes, a condition associated with insulin resistance and endothelial dysfunction, have demonstrated raised central pulse pressure compared to non-diabetic controls.[53]

GTN also exhibits beneficial effects on arterial mechanics by reducing central aortic pressure without changes in heart rate or peripheral blood pressure.[160] As with the haemodynamic effects of insulin, GTN exerts its action on central aortic augmentation at low doses whilst it is only at high doses that GTN produces arteriolar dilation and decreases peripheral vascular resistance.[160] In the study I this action of GTN was confirmed and shown to closely correlate with that of insulin, independent of basal AgI. This implies that the previously described defect in the acute effect of insulin on central aortic augmentation[391; 391; 392] is not specific to insulin and that vascular insulin resistance is associated with resistance to nitrates. This *in vivo* finding is consistent with that of effects of insulin and GTN previously described in platelets.[21; 21; 359] Furthermore, the significant

correlation between the vascular effects of insulin and GTN suggests that at least some of the action of insulin on large arteries is mediated via NO. Although study I was not designed to assess mechanistic backgrounds to heterogeneity of vascular effects of GTN and insulin, one can hypothesize that large arteries may exhibit a generalized defect in vasodilator mechanisms in certain individuals. The close correlation between the vasodilator effects of GTN and insulin may also reflect insulin-mediated nitric oxide release causing vasodilatation specifically in large arteries.

As in previous studies, 6 months of insulin therapy improved insulin-dependent whole body glucose disposal, despite inducing weight gain.[144] Such an improvement could reflect correction of relative insulin deficiency[413] and accompanying metabolic changes such as lowering of glucose and FFA concentrations.[373] Weight gain in the present study was greater than would have been expected based on previous studies, where the average weight gain has been 2 kg for a 1 % unit decrease in HbA_{1C}.[230] In the present study weight gain was 3.75 kg for a 1 % unit decrease in HbA_{1C}. The combination of insulin and glimepiride seemed to cause greater weight gain than expected in this group of type 2 diabetic patients, whose BMI averaged 30.8 kg/m^2 before insulin therapy. Use of metformin instead of glimepiride might have prevented such excessive weight gain.[408]

The novel finding in the present study was that the basal AgI decreased significantly by 6 months of insulin therapy. Given the multiple metabolic changes induced by insulin therapy, it is not possible to identify the factors responsible for this change with certainty. The changes in glucose and lipid metabolism could improve endothelial function and have antiatherogenic effects.[373] Increased bioavailability of NO due to decreases in oxidative stress caused by hyperglycemia could improve vascular function.[350] Small LDL size is also a correlate of endothelial dysfunction.[366] Insulin therapy increases LDL size,[56] which also could improve vascular function. In cultured endothelial cells insulin increases NO production by increasing the expression and activity eNOS.[193; 420] Insulin has been shown to enhance endothelial function measured as ACh induced vasodilatation both acutely and after long-term insulin treatment.[373; 374; 389] Insulin treatment also decreases circulating vascular adhesion molecules such as E-selectin.[304] FFAs cause vasodilatation and increase basal forearm blood flow when infused acutely, but impair ACh induced endothelium-dependent vasodilatation.[83] Acute increases in insulin concentrations have been shown to reverse endothelial dysfunction induced be circulating FFAs[218] and therefore the decrease in FFAs caused by insulin therapy might have contributed to the decrease in the AgI.

Insulin action on vascular tissue could also theoretically be improved by insulin treatment. Insulin therapy increased the basal insulin concentration 2.5-fold to 28 mU/l. We established in study I a dose-response curve for insulin action on the AgI and showed that acutely 30 mU/l of insulin is sufficient to significantly decrease the AgI. Thus, simply the increase in the basal insulin concentration may have decreased basal AgI. The acute decrease induced by insulin before and after insulin therapy remained unchanged. This does not, in view of the sensitivity of decreases in the AgI to small increments in serum insulin concentrations exclude the possibility that sensitivity of the insulin-induced decrease in the AgI was enhanced, because the insulin concentration achieved during the insulin infusion may have been too high to detect a change.

Study I demonstrates that insulin at physiological concentrations decreases the AgI, which is associated with a fall in central systolic pressure in subjects in whom the amplitude of the reflected wave determines systolic pressure. Study II demonstrates that the ability of insulin to decrease aortic systolic pressure is blunted in type 2 diabetic patients. Our findings provide one potential link between insulin resistance and systolic hypertension. The data obviously do not exclude the possibility that insulin resistance affects blood pressure via other mechanisms such as via excessive activation of the sympathetic nervous system.[18; 19] Study III shows that six-month insulin therapy might have beneficial effects on vascular function by decreasing the AgI. The effects of insulin therapy on vascular function is consistent with the results of the DIGAMI study,[235] that showed that insulin therapy can decrease cardiovascular mortality in type 2 diabetes. If these results can be confirmed by other studies it might favour the use of insulin earlier in the course of type 2 diabetes.

STUDY IV

Study IV was undertaken to examine whether insulin resistance is associated with the degree of inhibition of platelet aggregation after *in vivo* ASA administration. We found that the ability of ASA to inhibit platelet aggregation induced by AA and ADP was significantly blunted in a group of obese insulin-resistant subjects compared to age- and gender matched normal-weight subjects. AA-induced aggregation was more strongly inhibited by ASA than ADP-induced aggregation. ADP at higher concentrations (2 and 3 μmol/l) overcame the inhibition of COX1 in the obese subjects, whose platelet aggregation returned to baseline. The difference between obese and non-obese subjects could reflect the impact of ADP-induced release of AA. The obese may continue to activate thromboxane receptors and aggregate platelets in spite of ASA. Also the platelets of the

obese are known to have reduced levels of cAMP and cGMP, which may lower the threshold for activation.[359; 388]

To study the interindividual variation in the platelet ASA responses, we chose to administer the lowest effective dose of ASA targeted to antithrombotic prophylaxis to maximize the likelihood of detecting interindividual differences.[280; 376] The result might have been different if a larger dose or longer period of ASA use had been chosen. At doses smaller than 100 mg the effect of ASA is cumulative over a few days time.[146; 277; 278] In patients with acute myocardial infarction it is recommended that a larger initial dose of ASA should be used to inhibit platelet function. In the Second International Study of Infarct Survival (ISIS-2) study 162.5 mg was proved beneficial.[1; 279] Normally maximal ASA concentration with soluble formulations of 50-1200 mg is reached within 13-27 minutes.[47] Maximal ASA concentrations measured in acute myocardial infarction with 100 mg rapid release tablet were smaller and peak concentration was measured later (0.71 hours vs 0.48 hours) than in control subjects. The difference resulted possibly from reduced flood blow to splachnic bed and delayed gastric emptying.[47] In study IV, we took blood samples 1 hour after chewed ASA tablets. One hour should be enough time to reach maximal absorption in healthy obese or non-obese subjects, but the effect on platelet aggregation may well have been submaximal with a single 50 mg dose. We also only studied aggregation and not thromboxane production or closure times with platelet function analyser (PFA-100). Although optical aggregometry is the traditional and largely used method of studying platelet aggregation, the method is highly unphysiological and the conditions for platelet aggregation bear little resemblance to those in blood flow. Sample handling, preassay conditions and the timing of the preparation have to be standardized as far as possible, but there is still a large day-to-day and between operator variation.[259] There is also no evidence that the effect of ASA on optical aggregation study would correlate with clinical events[142] but there are results, which suggest that closure time with PFA-100 predicts cerebral and myocardial events.[17; 131] Results from optical aggregometry and PFA-100 have however been shown to correlate.[233]

The term 'ASA resistance' has been used in several contexts to describe either individuals who experience vascular events despite using ASA,[297] or who do not respond normally to ASA by some laboratory measure. These include the ability of ASA to prolong bleeding time[174] and to inhibit aggregation responses to various agonists.[134; 141; 142; 367] The causes of interindividual variation in ASA responsiveness are poorly understood and could involve in clinical trials factors such as non-compliance or drug-interactions.[297] There is also some evidence that platelet responses

to ASA vary for other reasons. The antiplatelet effect of a fixed dose of ASA is known not to be constant in all subjects over time.[142] In the study of Kawasaki et al, 8 healthy subjects were classified as non-responders or responders to ASA based on prolongation of their bleeding time. Platelets of 3 subjects classified as non-responders had increased sensitivity to collagen before ASA administration compared to the responders.[174] This finding is reminiscent of that in the study IV, where the subjects who had a blunted response to a single dose of ASA also had increased sensitivity to ADP before ASA administration. In addition, we found that poor response to ASA was confined to obese and insulin resistant subjects suggesting that insulin resistance or obesity may be one cause of impaired antiplatelet effect of ASA. The observed differences cannot be due to differences in ASA drug levels between obese and lean subjects since platelets are inhibited in the portal circulation during drug absorption, thus the antiplatelet effect of aspirin is largely independent of systemic bioavailability. As a consequence, while very little drug is detected systemically, already 95% of platelet COX-1 is inhibited.[283]

The meta-analysis of Antithrombotic Trialists' Collaboration suggests that diabetic (including both type 1 and type 2 diabetic patients) might have smaller relative benefit from ASA use than non-diabetic subjects.[6] Primary Prevention Project also indicates that in diabetic subgroup the effect of ASA may not as good as in non-diabetic group.[308] Unlike ASA, GP IIb/IIIa inhibitors seem to be more effective in diabetic than non-diabetic group.[300] One might speculate that the increase in glycoprotein expression and the inability of ASA to inhibit platelet glycoprotein expression are of significance among the many defects described in diabetic platelets.

Although many abnormalities in platelet function have been ascribed to 'diabetes', the control subjects have in many studies been younger and less obese than the patients with diabetes.[79; 157; 161; 188; 290; 317] These data raise the possibility that while hyperglycemia may be a cause of abnormal platelet function,[405] other factors such as obesity or insulin-resistance associated with obesity could also have contributed to the various abnormalities ascribed to 'diabetes'. Study IV where we found baseline ADP-induced platelet aggregation to correlate with the degree of insulin resistance in non-diabetic healthy subjects, suggests that body weight or insulin sensitivity indeed should be considered whenever diabetic and non-diabetic subjects are compared. Consistent with the importance of obesity or insulin resistance on platelet function, the antiaggregatory *in vitro* effects of both insulin and glyceryl trinitrate have been found to be impaired in obese healthy and type 2 diabetic subjects and preserved in lean type 2 diabetic patients and their weight-matched controls.[21; 359]

It is important to emphasize that abnormalities in platelet function tests *in vitro* and impaired platelet-inhibitory effect of ASA may not translate into an increased risk of cardiovascular events. However, the data suggest that future or ongoing trials examining the impact of ASA or other antiplatelet agents on vascular events or efficacy of ASA in patients with diabetes might want to include relative body weight or some measure of insulin resistance as a potential confounding variable. This would seem important since both obesity and other features of insulin resistance are associated with increased risk of coronary heart disease and stroke.[288] Possibly, insulin-resistant obese subjects might benefit from ASA in combination with clopidogrel or therapies inhibiting platelet aggregation by blocking thromboxane-independent pathways or thromboxane receptors.

11 SUMMARY

Insulin and GTN both decrease the AgI in a dose-dependent manner, although the time course of action for insulin is slower than that of GTN. In a group of apparently healthy middle-aged subjects the haemodynamic effects of insulin and GTN are closely correlated, independently of basal AgI. These data suggest that any variation in the action of insulin on large arteries in insulin-resistant subjects is not specific to insulin and that individuals who respond poorly to insulin also do so to nitrates.

Type 2 diabetic patients are resistant not only to the action of insulin to stimulate glucose uptake but also to its ability to decrease the AgI. The decrease in the AgI in the normal subjects was accompanied by a significant and rapid decrease in aortic systolic blood pressure. The ability of intravenous insulin to decrease the AgI and central systolic blood pressure is delayed in type 2 diabetic patients when compared to age and weight matched normal subjects.

Six months of basal insulin therapy improves insulin sensitivity in type 2 diabetic patient, in poor metabolic control with oral hypoglycemic agents, despite weight gain. Insulin therapy significantly decreases the AgI measured before insulin infusion and the AgI measured during hyperinsulinemia but does not alter the change in the AgI induced by insulin infusion. These data suggest that insulin therapy has beneficial effects on aortic wave reflection.

The ability of ASA to inhibit platelet aggregation induced by AA and ADP is significantly blunted in a group of obese insulin-resistant subjects compared to age- and gender matched normal-weight subjects. *In vivo* insulin sensitivity is closely inversely correlated with aggregation responses induced by ADP before ASA administration, and to aggregation responses to both AA and ADP after oral ASA.

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