Diabetologia (1995) 38: 86–96

Diabetologia

Peripheral arterial disease in relation to glycaemic level in an elderly Caucasian population: the Hoorn Study

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Summary We investigated the cross-sectional association between peripheral arterial disease and glycaemic level in an age, sex, and glucose tolerance stratified random sample from a 50–74-year-old Caucasian population. Subjects treated with oral hypoglycaemic agents or insulin were classified as having known diabetes mellitus (KDM) ($n = 67$). Using two oral glucose tolerance tests, and based on World Health Organisation criteria, all other participants were categorized as having a normal (NGT) ($n = 288$), an impaired (IGT) ($n = 170$), or a diabetic (NDM) ($n = 106$) glucose tolerance. Prevalence rates of ankle-brachial pressure index less than 0.90 were 7.0 %, 9.5 %, 15.1 % and 20.9 % in NGT, IGT, NDM and KDM subjects, respectively (chi-square test for linear trend: $p < 0.01$). Prevalence rates of *any* peripheral arterial disease (ankle-brachial pressure index < 0.90, at least one monophasic or absent Doppler flow curve or vascular surgery) were 18.1 %, 22.4 %, 29.2 % and 41.8 % in these categories (chi-square test for linear trend: $p < 0.0001$). The prevalence of *any* peripheral arterial disease was higher in KDM and NDM than in NGT ($p < 0.03$, $p < 0.0001$, respectively), whereas no statistically significant difference was demonstrated between IGT and NGT. The same applied when using the ankle-brachial pressure index criterion. Logistic regression analyses showed that *any* arterial disease was significantly associated with HbA_{1c}, fasting and 2-h post-load plasma glucose after correction for cardiovascular risk factors (odds ratios and 95 % confidence intervals 1.35; 1.10–1.65 per %, 1.20; 1.06–1.36 and 1.06; 1.01–1.12 per mmol/l, respectively), whereas it was not associated with fasting and 2-h post-load specific insulin. Ankle-brachial pressure indices were not associated with either plasma glucose parameters or insulin in univariate or multivariate analyses. In conclusion, parameters of glucose tolerance are independently associated with *any* peripheral arterial disease, whereas insulin is not. [Diabetologia (1995) 38: 86–96]

Key words Non-insulin-dependent diabetes mellitus, impaired glucose tolerance, specific insulin, peripheral arterial disease, epidemiology, population-based survey, Caucasians.

Received: 11 April 1994 and in revised form: 15 July 1994

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Abbreviations: NGT, Normal glucose tolerance; IGT, impaired glucose tolerance; NDM, newly-diagnosed diabetes mellitus; KDM, known diabetes mellitus (= using hypoglycaemic medication); PAD, peripheral arterial disease; ABPI, (systolic) ankle-brachial pressure index; OR, odds ratio; 95 % CI, 95 % confidence interval; WHR, waist/hip ratio.

Atherosclerotic vascular disease, manifesting itself as coronary artery disease, carotid artery disease and peripheral arterial disease (PAD), is known to be markedly increased in diabetic subjects as compared to non-diabetic subjects [1, 2]. Epidemiological research has focused mainly on diabetes-related coronary and carotid artery disease, because of its high morbidity and mortality rates. Although PAD does not rank among the main primary causes of mortality, it causes serious discomfort and disability, and has been shown to be associated with an increased all-cause and coronary heart disease mortality [3–7].

Furthermore, PAD can have a substantial economic impact because of work absenteeism, medication and utilization of hospital resources for reconstructive arterial surgery or amputation.

Some epidemiological studies have focused either on the prevalence of PAD per se, irrespective of glycaemic level [8–11], or have determined the prevalence of PAD in subjects with non-insulin-dependent diabetes mellitus or impaired glucose tolerance (IGT) in a hospital or out-patient clinical setting [12–16]. Only a few reports have included the total range of glycaemic levels and studied its association with PAD in a population-based sample [17, 18].

The Hoorn Study took place from 1989 to 1992. It was a population-based cross-sectional survey to determine the prevalence of abnormal glucose tolerance and its macrovascular complications in an elderly Dutch Caucasian population [19]. In contrast to the other population-based studies, glucose tolerance was evaluated with two oral glucose tolerance tests (OGTT) to improve the diagnostic precision of this test, and insulin levels were measured with a specific insulin assay.

To study asymptomatic PAD the systolic ankle-brachial-pressure index (ABPI) is used in many epidemiological and clinical studies [3–18]. However, the measured ankle pressures may be elevated if the vascular wall is less compressible, in particular in the case of medial arterial calcification (Mönckeberg's sclerosis). In persons with this disease the presence of PAD is not reflected by a lower ABPI [20]. Since the prevalence of medial arterial calcification is reported to be elevated in elderly men [21], non-insulin-dependent diabetic subjects [21–23] and subjects with neuropathy [23–25], an under-estimation of PAD may be expected in subjects with a disturbed glucose tolerance when the ABPI is used for PAD detection. Therefore, various additional non-invasive tests have been performed [26], resulting in a wide variety of prevalence data for PAD. In the Hoorn Study, qualitative evaluation of continuous wave Doppler flow velocity curves was chosen as an additional non-invasive test, as it is easily and quickly performed without discomfort for the participant [13, 27, 28]. It can be accurately scored on-line by an experienced vascular technician or vascular surgeon.

The aims of this study were to determine: 1) the prevalence of PAD in an elderly Dutch population, 2) the association of PAD with various parameters of glycaemic level and insulin levels, and 3) the possible confounding of this association by other cardiovascular risk factors.

Subjects and methods

Subjects. All study participants were involved in the cohort of the Hoorn Study, a cross-sectional survey on disturbances of glucose tolerance and their complications in a general Caucasian population.

For this study, a random sample of 50–74-year-old subjects was taken from the population register of the town of Hoorn (59,000 inhabitants) in the Netherlands (Fig. 1). Of the 3,553 subjects invited for the study, 2,540 (71.5 %) participated, of whom 56 were excluded from analysis and further study because they were non-Caucasian. Thus, the final Hoorn Study cohort consisted of 2,484 subjects. Information on age, sex, body height, weight, history of diabetes, treatment for hypertension and cardiovascular complaints was obtained by interview from virtually all the non-participants (93 %). The non-participants were only slightly, but significantly older (63.1 vs 61.7 years), taller (171 vs 169 cm), lighter (73.1 vs 75.5 kg) and had a less frequent family history of diabetes (20 vs 27 %). They did not differ significantly from our study population with respect to sex, self-reported diabetes, treatment for hypertension and cardiovascular symptoms.

All cohort members not treated with oral hypoglycaemic agents or insulin, had an initial 75-g OGTT according to the World Health Organisation criteria [29] (phase 1). All participants with a 2-h post-load plasma glucose over 7.5 mmol/l ($n = 436$) and an age and sex-stratified random sample (five strata for both sexes: 50–54, 55–59, 60–64, 65–69, and 70–74) of persons with a 2-h post-load plasma glucose under 7.5 mmol/l ($n = 783$; mean sample fraction 40 %) were invited for a second OGTT within 3–5 weeks (phase 2) to assess the variability of the OGTT (response rate 93 %). In the same sampling procedure, a sub-sample was drawn to select invitees for the diabetes-related disorder study (phase 3). All cohort members with a 2-h post-load glucose over 11.1 mmol/l ($n = 122$), and age and sex-stratified random samples from subjects with a 2-h post-load glucose over 7.5 mmol/l and less than 11.1 mmol/l ($n = 254$; mean sample fraction 81 %), as well as from those with a 2-h post-load glucose less than 7.5 mmol/l ($n = 256$; mean sample fraction 13 %) at the first OGTT were invited for phase 3. The cut-off value of 7.5 mmol/l was deliberately used as a selection criterion to include as many subjects as possible with disturbances in glucose tolerance. Sample fractions were chosen to obtain approximately equal numbers of participants in each age, sex, and glucose tolerance stratum. Cohort members treated with oral hypoglycaemic agents or insulin ($n = 76$) only had their fasting plasma glucose measured once, and were all asked to participate in the diabetes-related disorder study. Thus, a total number of 708 subjects was invited.

The Hoorn Study was approved by the Ethical Review Committee of the Academic Hospital of the Vrije Universiteit and informed consent was obtained from all participants.

Non-invasive assessment of peripheral arterial disease

Ankle-brachial pressure indices (ABPI). Doppler-assisted systolic blood pressure measurements were taken from the brachial and posterior tibial arteries on both sides, using 12-cm cuffs. The cuffs were automatically inflated and deflated (Medasonics Vasculab, Mountainview, Calif., USA). The maximal cuff pressure was 250 mmHg. Recording started after a 15-min resting period in a supine position (room temperature 23 °C). Whenever the ABPI over the posterior tibial artery was less than 0.90, or the Doppler flow signal was not audible, the ankle pressure was also measured over the dorsalis pedis artery or the peroneal artery. ABPIs at rest were calculated using the highest systolic brachial pressure. If the highest ABPI for either leg was less than 0.90, the participant was classified as having PAD [8, 10, 11]. The highest reading on the foot with the lowest ABPIs was used for data-analysis with

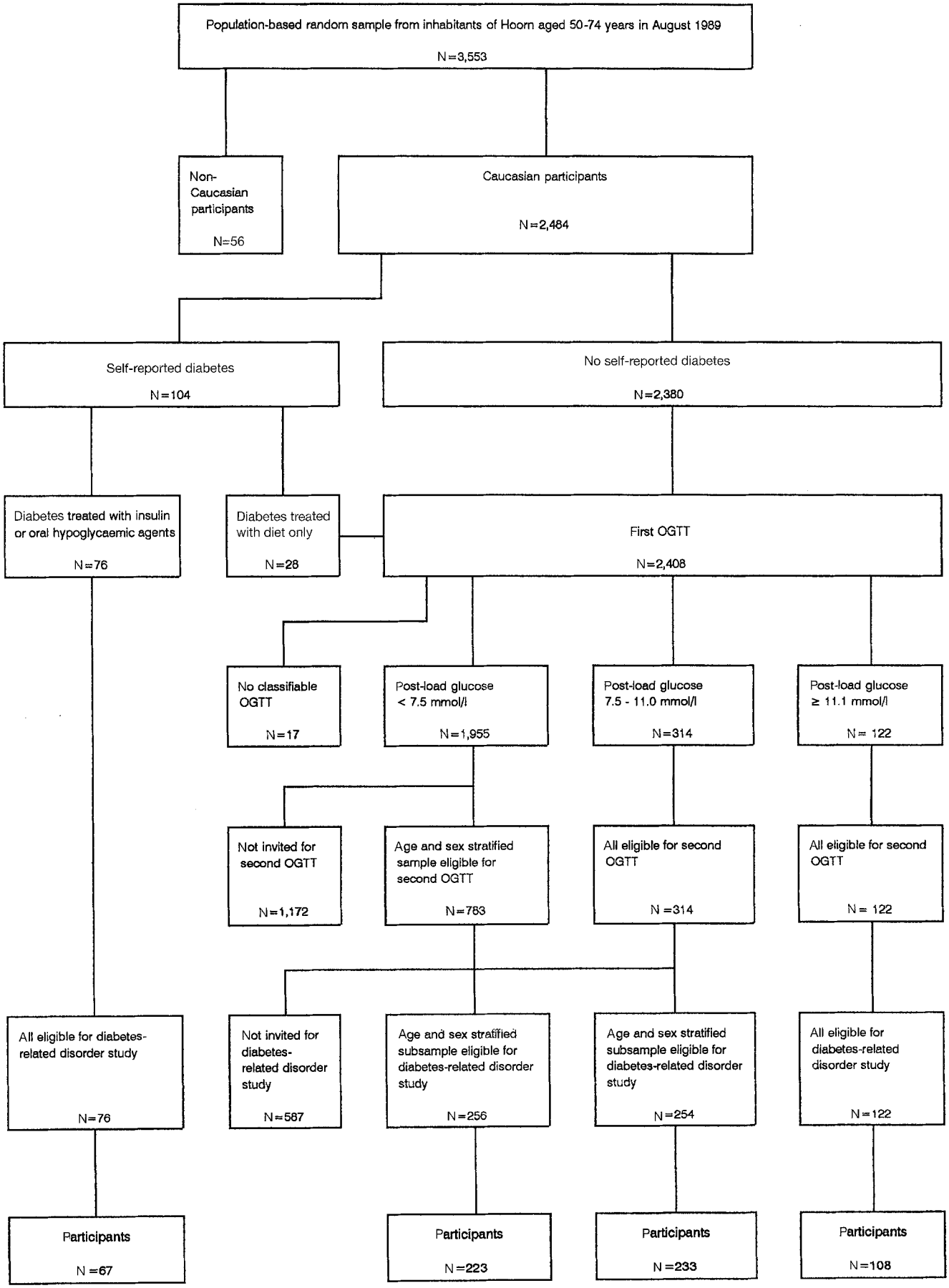

Fig. 1. The Hoorn Study: the subject-sampling procedures for phase 3 concerning the diabetes-related disorder study performed between 1989–1992

the ABPI as a continuous parameter of PAD. Two participants who had undergone surgery on the lower part of the aorta, the iliac or the lower extremity arteries, and had an ABPI greater than 0.90, were excluded from analyses on the basis of the ABPI less than 0.90 criterion.

Doppler flow velocity curves. Using a 5 or 8 mHz bidirectional continuous wave Doppler, connected to a frequency analyser, two experienced technicians recorded flow velocity tracings from the radial, ulnar, femoral, popliteal, posterior tibial, dorsalis pedis and, if possible, peroneal arteries. Tri-or biphasic curves were considered to be normal, whereas a monophasic curve or the absence of a curve was considered to be abnormal [13, 27, 28].

Two criteria for PAD were used in the analyses: a) an ABPI less than 0.90, and b) *any* PAD (vascular surgery on lower part of the aorta, the iliac or the lower extremity arteries, an ABPI less than 0.90, or a monophasic or absent Doppler flow curve for any of the above-mentioned arteries).

A reproducibility study of these non-invasive tests was performed in a random sample ($n = 41$), the second measurement being performed 6 to 9 months after the first registration. The reproducibility of the ABPIs, expressed as the mean difference (SD) between the first and second ABPI registration, were –0.005 (0.095) and 0.000 (0.107) for the right and left ankle, respectively. An ABPI greater than 0.90 ($n = 32$) was confirmed in 29 subjects, whereas an ABPI less than 0.90 ($n = 9$) was confirmed in eight subjects. The agreements between the first and second examination were expressed as kappa values and 95 % confidence intervals (95 % CI) of both criteria for PAD (ABPI < 0.90 and *any* PAD). These kappas were 0.73 (0.49–0.98) and 0.61 (0.38–0.83), respectively, indicating a good agreement [29].

Parameters of glycaemia and insulin levels. Fasting and 2-h post-load venous plasma glucose levels were determined with a glucose dehydrogenase method (Merck, Darmstadt, Germany) in the laboratory of the Academic Hospital of the Vrije Universiteit in Amsterdam. In diabetic subjects treated with oral hypoglycaemic agents or insulin, fasting plasma glucose and fasting serum insulin (in duplicate) were measured on one occasion only.

Fasting and 2-h post-load specific serum insulin levels were quantified (in duplicate) with an insulin-specific double-antibody radioimmunoassay (antibody: Linco SP21, St. Louis, Mo., USA). No cross-reactivity with proinsulin and split proinsulin was found. The intra-assay and inter-assay coefficients of variation for insulin were 5–8 % and 7–11 %, respectively. Glycated haemoglobin (HbA_{1c}) was determined once by ion-exchange high-performance liquid chromatography, using a Modular Diabetes Monitoring System (Bio-Rad, Veenendaal, the Netherlands: normal range 4.3–6.1 %). Serum fructosamine concentrations were measured once, using a second generation serum fructosamine assay (Roche Diagnostica, Basel, Switzerland). The inter-assay coefficient of variation in the normal range was 4.6 %; in the pathological range 3.0 %.

Subjects treated with oral hypoglycaemic agents or insulin were classified as having known diabetes mellitus (KDM). All non-KDM subjects, including previously-diagnosed diabetic patients treated with dietary advice only, were classified according to the World Health Organisation criteria [30] applied to mean fasting and mean 2-h post-load plasma glucose values of two OGTTs. In 25 subjects, at least one glucose value at the second OGTT was missing. They were substituted by values of the first OGTT. Thus, in addition to the KDM category, the following three glucose tolerance categories were recognized: normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and newly-diagnosed diabetes mellitus (NDM).

Potential confounding factors. Concentrations of serum lipids were determined in the fasting blood sample at the first OGTT in all subjects. Total cholesterol, HDL-cholesterol (after precipitation of the low and very low-density proteins) and triglycerides were measured by enzymatic techniques (Boehringer Mannheim, Mannheim, Germany). The Friedewald formula was used to calculate the LDL-cholesterol [31]. This formula was not applied in subjects with serum triglyceride levels greater than 8.0 mmol/l ($n = 3$). As inclusion of subjects with triglyceride levels between 4.5 and 8.0 mmol/l ($n = 20$) hardly influenced the studied associations, they were not excluded from further analyses.

Systolic and diastolic (Korotkov V) blood pressure was determined before the start of both OGTTs on the right arm of seated subjects, after at least 5 min resting, using a random-zero sphygmomanometer (Hawksley-Gelman Ltd., Lancing, Sussex, UK). The average of duplicate measurements on two occasions was used for analysis. Hypertension was defined as present treatment with antihypertensive drugs or having an elevated blood pressure (diastolic > 95 mmHg or systolic > 160 mmHg).

Height, weight and body circumferences were measured with all subjects barefoot and wearing light clothes only, at the end of the first visit. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist/hip-ratio (WHR) was defined as waist circumference divided by hip circumference.

The medical history was obtained by means of a self-administered questionnaire. The subjects were asked whether they had received any specialist medical care during the past 10 years and, if so, for what reason and from which specialty. History of cardiovascular disease – myocardial infarction, angina pectoris, intermittent claudication, stroke, brain haemorrhage or transient palsy – was assessed by using a Dutch translation of the questionnaire from the London School of Hygiene and Tropical Medicine [32], which was kindly made available by Professor D. Kromhout, principal investigator of the Zutphen Study [33]. Information on family history of vascular disease was obtained by asking the participants whether any of their grandparents, parents, brothers or sisters had had any of the above-mentioned diseases before the age of 60 years. Smoking was expressed on a dichotomized scale (ever vs never smoked cigarettes, cigars or pipe) and on a continuous scale (cigarette pack-years). On their first visit to the study centre, the participants brought with them the medication they were using and the completed questionnaire. The answers on the questionnaire were checked for completeness and consistency, and the names of the medications and the dosages prescribed were registered.

Statistical analysis

To asses the population-based prevalence, the frequency of PAD was determined in 24 strata (age: 50–59, 60–69 and 70–74 years old; sex: male and female; glucose tolerance category of the first OGTT: NGT, IGT, NDM and KDM) of the phase 3 cohort ($n = 631$). The prevalence of PAD in the original population-based sample ($n = 2{,}484$) was recalculated from the magnitude of each age, sex, and glucose tolerance category-stratum (based on the result of the first OGTT) combined with the prevalences of PAD for the corresponding strata from the phase 3 cohort.

The chi-square test was used to test differences in PAD prevalence between various glucose tolerance categories (including KDM). The chi-square test for trend was used to investigate linear trends of the prevalence of PAD over glucose toler-

Table 1. Characteristics of the study population stratified for glucose tolerance in phase 3 of the Hoorn Study

Glucose tolerance	NGT (n =288)	IGT (n = 170)	NDM (n = 106)	KDM (n = 67)
Demographic data				
Age (years)	63.1 (7.4)	64.9 (7.0)	65.9 (6.5)	65.5 (7.2)
Sex (% male)	52	47	46	40
Parameters of glucose metabolism				
Fasting plasma glucose (mmol/l)	5.4 (0.5)	6.1 (0.7)	8.4 (3.1)	11.0 (3.7)
2-h plasma glucose (mmol/l)	5.6 (1.4)	9.1 (0.9)	15.6 (5.5)	–
HbA_{1c} (% of total haemoglobin)	5.3 (0.5)	5.6 (0.5)	6.7 (1.9)	8.1 (1.6)
Serum fructosamine (μmol/l)	240 (17)	246 (19)	286 (69)	335 (66)
Fasting specific insulin (pmol/l)	73 (56, 93)	90 (69, 134)	114 (80, 151)	103 (75, 139)
2-h specific insulin (pmol/l)	303 (184, 428)	606 (379, 1050)	507 (268, 845)	–
Confounding risk factors				
Smoking status:				
never smoked (%)	31	35	37	39
ex-smoker (%)	36	39	39	36
current smoker (%)	33	26	25	25
Smoking (pack-years)	17.8 (20.7)	15.9 (20.3)	17.1 (23.5)	20.7 (24.7)
Waist/hip-ratio	0.90 (0.08)	0.93 (0.08)	0.96 (0.09)	0.94 (0.08)
BMI (kg/m^2)	26.0 (3.3)	27.8 (3.7)	28.6 (4.1)	29.0 (5.2)
Total cholesterol (mmol/l)	6.65 (1.14)	6.74 (1.20)	6.54 (1.33)	6.43 (1.15)
LDL-cholesterol (mmol/l)	4.61 (1.04)	4.62 (1.02)	4.26 (1.16)	4.25 (1.00)
HDL-cholesterol (mmol/l)	1.38 (0.39)	1.23 (0.34)	1.13 (0.27)	1.17 (0.27)
Triglycerides (mmol/l)	1.49 (0.74)	1.96 (1.10)	2.50 (1.98)	2.29 (1.45)
Hypertension (%)	24.3	46.1	52.8	58.2
antihypertensive drugs	17.0	33.5	35.8	50.7
Blood pressure[a] (mm Hg)				
diastolic	80 (9)	84 (10)	85 (10)	81 (9)
systolic	132 (18)	141 (19)	145 (18)	138 (18)
Familiy history of:				
myocardial infarction (%)	14.3	13.5	17.9	20.9
stroke (%)	8.0	8.8	2.8	14.9

Values are mean (SD), percentage or median (25th, 75th percentile).
[a] Subjects using antihypertensive drugs are excluded

ance categories and quintiles of glycaemic indices and insulin levels.

KDM subjects were only included in the univariate and multivariate model, with the glucose tolerance category as independent variable. After exclusion of KDM subjects, six univariate logistic regression analyses were performed to assess the contribution of each of the four glycaemic indices (fasting and 2-h post-load plasma glucose, HbA_{1c}, and serum fructosamine) and of the fasting and 2-h post-load specific insulin levels to the risk for PAD. Subsequently, six multivariate logistic regression models, correcting for the influence of age, sex, BMI, WHR, smoking behaviour, hypertension, triglycerides, LDL and HDL-cholesterol, were tested to evaluate the independent contribution of each one of the above-mentioned parameters of glycaemic level to the risk for PAD.

Results

Study population. The response-rate for phase 3 was 89 % (631 of 708). Reasons for non-response were refusal to participate (n = 57), inability to visit the study centre before the end of the study (n = 15), becoming non-resident in Hoorn (n = 2), language problems (n = 2) and hospitalization (n = 1).

In the non-participant group, the percentage of females was significantly higher, whereas age, hypertension, possible stroke, myocardial infarction, angina pectoris and intermittent claudication did not differ significantly between participants and non-participants. The absence of differences in prevalence rates of hypertension and cardiovascular disease suggests that the non-response was not associated with the presence of macrovascular disease.

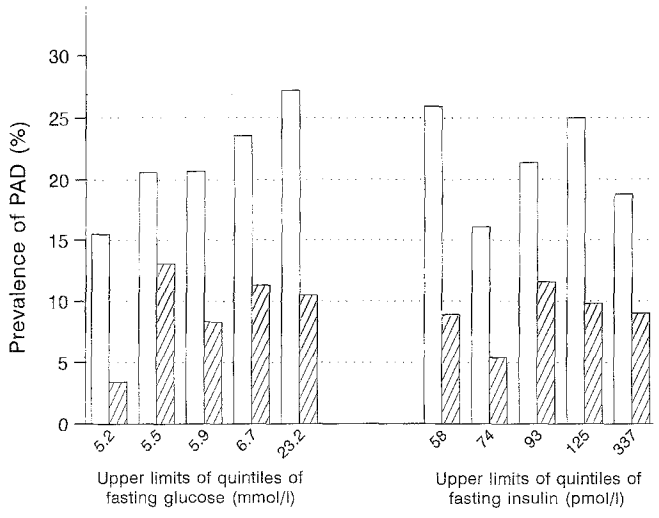

Fig. 2. Prevalence of *any* PAD ☐ and ABPI less than 0.90 ▨ according to quintiles of fasting plasma glucose and fasting specific insulin levels

Characteristics of participants. Based on the mean fasting and 2-h post-load plasma glucose levels of two OGTTs and the World Health Organisation criteria, the study cohort was divided into three glucose tolerance categories: NGT ($n = 288$), IGT ($n = 170$) and NDM ($n = 106$), apart from KDM ($n = 67$ of whom 52 were treated with oral hypoglycaemic agents and 15 with insulin).

Glycaemic indices, insulin levels, and other putative determinants of PAD are listed in Table 1. As expected, glycaemic indices and specific insulin levels increased stepwise from NGT via IGT and NDM to KDM. The only exception was the fasting serum insulin level in the KDM group, which was lower than in the NDM group. Because the distribution of the serum insulin levels is skewed to the left, values are represented as median (25^{th}, 75^{th} percentile).

The percentage of 'never smoked' was highest in KDM subjects, but the KDM smokers had the highest cigarette consumption. WHR, BMI, HDL-cholesterol, triglycerides and systolic blood pressure are less favourable in the IGT, NDM and KDM categories than in the NGT. The percentage of hypertensive subjects increased over the respective glucose tolerance categories.

Prevalence of PAD in a general Dutch Caucasian population

Table 2 shows the recalculated age and sex-specific prevalence rates of PAD in a general Dutch Caucasian population in the town of Hoorn, using the two diagnostic criteria for PAD. Because some contributing strata are small, 95 % CI are wide. The population-based prevalences (95 % CI) in 50–74 year-old subjects of *any* PAD and ABPI less than 0.90 are 19.6 % (15.6–23.7 %) and 7.3 % (4.9–9.7 %), respectively. No statistically significant difference between male and female subjects in any age-class could be demonstrated. The prevalence of PAD increased with age, irrespective of the diagnostic criterion. Abnormal Doppler flow curves were consistently more prevalent than an ABPI less than 0.90.

Prevalence of PAD in relation to glucose tolerance category

For the whole range of glucose tolerance the prevalence of *any* PAD – including the Doppler flow curve criterion – is higher than when using the ABPI criterion only. The crude prevalence of *any* PAD increased stepwise from 18.1 % in NGT, via 22.4 % in IGT and 29.2 % in NDM to 41.8 % in KDM (Table 4). The prevalence of *any* PAD was significantly higher in KDM and NDM than in NGT ($p < 0.03$, $p < 0.0001$, respectively), whereas no statistically significant differences were found between IGT and NGT ($p = 0.319$), IGT and NDM ($p = 0.198$) and NDM and KDM ($p = 0.090$). The same results were found using the ABPI less than 0.90 criterion (data not shown). The chi-square test for linear trend is highly significant for both PAD criteria (ABPI < 0.90: $p < 0.003$; *any* PAD: $p < 0.0001$). The prevalence of intermittent claudication was 3.1 %, 3.5 %, 3.9 % and 7.5 %, in the respective glucose tolerance categories. The presence of symptoms was accompanied by *any* PAD and ABPI less than 0.90 in 17 (71 %) and 14 (58 %) out of 24 persons with intermittent claudication, respectively.

Association between prevalence of PAD and quintiles of glycaemic indices and insulin

The glucose cut-off points chosen for the commonly used World Health Organisation glucose tolerance categories are originally based on the development of microvascular diabetic complications [34, 35]. We used quintiles of glycaemic indices and specific insulin to describe the relation between PAD and glucose tolerance more precisely. Figure 2 shows the prevalence rates of PAD in quintiles of fasting plasma glucose and fasting specific insulin. All previously-diagnosed diabetic subjects treated with oral hypoglycaemic agents or insulin were excluded. The chi-square test for linear trend between the prevalence of *any* PAD in respective quintiles of fasting plasma glucose was significant ($p < 0.03$), whereas the linear trend tests for quintiles of HbA_{1c} ($p < 0.08$) and 2-h post-load plasma glucose ($p = 0.20$) were not. The same test showed a linear association between ABPI less than 0.90 and quintiles of HbA_{1c} ($p = 0.03$) and 2-h post-load plasma glucose ($p < 0.05$), but not be-

Table 2. Calculated population-based age and sex-specific prevalences of PAD in Hoorn, the Netherlands, 1989–1992

Age-group (years)	Male		Female		Both sexes	
	ABPI < 0.90	Any PAD	ABPI < 0.90	Any PAD	ABPI < 0.90	Any PAD
50–59	3.5 %	19.4 %	2.5 %	16.2 %	3.0 %	17.7 %
60–69	12.2 %	14.4 %	4.3 %	17.3 %	7.8 %	16.0 %
70–74	20.8 %	32.8 %	15.9 %	33.7 %	18.0 %	33.3 %
All ages 50–74	9.4 %	19.6 %	5.6 %	19.7 %	7.3 %	19.6 %

Diagnostic criteria for PAD; ABPI < 0.90: ankle-brachial pressure index less than 0.90, any PAD: ABPI less than 0.90, at least one monophasic or absent flow curve, or vascular surgery in lower extremities

Table 3. Prevalence of PAD according to glucose tolerance in various age groups

Age-group (years)		Normal		Impaired		New diabetes		Known diabetes	
	PAD[a]	%	(n1/n2)	%	(n1/n2)	%	(n1/n2)	%	(n1/n2)
50–59	ABPI	2.7	(3/112)	4.3	(2/47)	4.8	(1/21)	6.3	(1/16)
(n = 197)	Any	16.8	(19/113)	17.0	(8/47)	14.3	(3/21)	37.5	(6/16)
60–69	ABPI	7.3	(8/110)	6.8	(5/74)	14.0	(7/50)	13.3	(4/30)
(n = 265)	Any[b]	14.5	(16/110)	14.7	(11/75)	24.0	(12/50)	30.0	(9/30)
70–74	ABPI[c]	13.8	(9/65)	18.8	(9/48)	22.9	(8/35)	42.9	(9/21)
(n = 169)	Any[c]	26.2	(17/65)	39.6	(19/48)	45.7	(16/35)	61.9	(13/21)
All ages 50–74	ABPI[d]	7.0	(20/287)	9.5	(16/169)	15.1	(16/106)	20.9	(14/67)
(n = 631)	Any[d]	18.1	(52/288)	22.4	(38/170)	29.2	(31/106)	41.8	(28/67)

[a] Diagnostic criteria for PAD; ABPI: ABPI < 0.90, any: ABPI < 0.90, at least one monophasic or absent curve or vascular surgery. % = percentage PAD per stratum., n1: number of persons with PAD per stratum; n2: total number of persons per stratum. Chi-square test for linear trend over glucose categories, [b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$, respectively

tween ABPI less than 0.90 and quintiles of fasting plasma glucose ($p = 0.138$). No significant association was found between *any* PAD or ABPI less than 0.90 on the one hand, and quintiles of serum fructosamine, fasting insulin or 2-h post-load insulin on the other (data not shown).

Determinants of any PAD

Firstly, the odds ratios (OR) for IGT, NDM and KDM vs NGT were calculated to estimate the relative risk for *any* PAD. In this univariate analysis, KDM and NDM subjects had significantly higher risks for PAD than NGT subjects (OR: 3.26; $p = 0.0001$, OR: 1.88; $p < 0.02$, respectively). In the multivariate analysis KDM remained a significant risk factor (OR: 3.43; $p < 0.001$), whereas NDM lost its significance and became a trend (OR: 1.76; $p = 0.065$). No increased risk could be demonstrated for IGT subjects in univariate or multivariate analyses.

After exclusion of diabetic subjects treated with oral hypoglycaemic agents or insulin, logistic regression analyses showed that fasting and 2-h post-load plasma glucose (OR: 1.20 and 1.06 per mmol/l increase, respectively) and HbA_{1c} (OR: 1.35 per % increase) are significantly associated with PAD. This applies to univariate as well as to multivariate analyses. No association was found between fasting or 2-h post-load specific insulin and PAD. The best fitting multivariate logistic regression model included HbA_{1c} as the parameter of glycaemic level, and was used to assess the contribution of other confounding factors to the risk for PAD. Age (OR: 1.05; 95 % CI: 1.02–1.09 per year), smoking behaviour (OR: 1.91; 95 % CI: 1.09–3.35 ever vs never smoked) and hypertension (OR: 2.08; 95 % CI: 1.33–3.25 present vs absent) are other factors which significantly contribute to the risk for *any* PAD. BMI was consistently and negatively associated with any PAD (OR: 0.93; 95 % CI: 0.86–0.99 per kg/m^2). Neither triglycerides, nor LDL- or HDL-cholesterol were associated with PAD. In KDM subjects (n = 67), only age was significantly associated with *any* PAD (OR: 1.08; 95 % CI: 0.99–1.18), whereas all other cardiovascular risk factors were not, in either univariate or multivariate logistic regression analyses.

Determinants of ABPI

Only one NDM subject had a unilateral ankle-brachial pressure difference of more than 75 mmHg indicating the presence of medial arterial calcification. Since it is reported that a difference of more than

Table 4. Odds ratios for any PAD[a] of various parameters of glucose tolerance

Parameter of glucose metabolism	Univariate		Multivariate	
	Odds ratio	95 % CI	Odds ratio[b]	95 % CI
Degree of glucose tolerance[c]				
IGT vs NGT	1.31	0.82–2.09	1.14	0.68–1.89
NDM vs NGT	1.88	1.21–3.14	1.76	0.97–3.21
KDM vs NGT	3.26	1.84–5.77	3.43	1.78–6.62
Fasting plasma glucose[d] (per mmol/l)	1.16	1.05–1.28	1.20	1.06–1.36
2-h plasma glucose[d] (per mmol/l)	1.06	1.02–1.10	1.06	1.01–1.12
HbA_{1c}[d] (per %)	1.32	1.11–1.58	1.35	1.10–1.65
Serum fructosamine[d] (per μmol/l)	1.00	1.00–1.01	1.00	1.00–1.01
Fasting specific insulin[d] (per pmol/l)	1.00	0.99–1.00	1.00	0.99–1.00
2-h specific insulin[d] (per pmol/l)	1.00	1.00–1.00	1.00	0.99–1.00

[a] Any PAD: ABPI < 0.90, at least one monophasic or absent flow curve or vascular surgery. [b] Multivariate logistic regression analyses; each model consisted of the parameter of glucose metabolism at issue and the following confounding cardiovascular risk factors: age, sex, smoking behaviour, WHR BMI, LDL-cholesterol, HDL-cholesterol, triglycerides and hypertension. [c] Diabetic subjects treated with oral hypoglycaemic agents or insulin included. NGT: Normal glucose tolerance; IGT: impaired glucose tolerance; NDM: newly-diagnosed diabetes mellitus; KDM: previously-diagnosed diabetes mellitus. [d] Diabetic subjects treated with oral hypoglycaemic agents or insulin excluded

75 mmHg is highly specific, but not very sensitive, we did not consider it worthwhile to exclude this one subject from analysis.

Neither univariate nor multivariate logistic regression analyses showed any significant association between glycaemic indices and serum insulin levels on the one side, and ABPI less than 0.90 on the other (data not shown). Age (OR: 1.09; 95 % CI: 1.04–1.15 per year), smoking behaviour (OR: 3.53; 95 % CI: 1.35–9.19 ever vs never smoked) and hypertension (OR: 3.03; 95 % CI: 1.59–5.77 present vs absent) were associated risk factors for ABPI less than 0.90 in the multivariate model including HbA_{1c} as the parameter of glycaemic level. No association between glycaemic indices and insulin levels on the one hand, and ABPI on a continuous scale on the other, could be demonstrated by using multiple linear regression analysis (data not shown).

Discussion

In the general Caucasian population of Hoorn the recalculated prevalence rate of ABPI less than 0.90 was 7.3 % (4.9–9.7 %) in 50–74 year-old participants. In the Edinburgh Artery Study [11], the crude population-based prevalence rate of PAD (ABPI < 0.90), was reported to be 18 % in a 55- 74-year-old Scottish group, irrespective of glycaemic level. The difference may be attributed to an over-representation of older age groups in the Scottish study, because of the age-stratified sampling procedure. In the Hoorn Study a random sample was drawn from the population register.

Prevalence data concerning non-invasively diagnosed PAD in various glucose tolerance categories, obtained in one study sample, are sparse [17, 18]. All former studies used only one OGTT to assess the glucose tolerance. Because of the poor reproducibility of the OGTT, we performed two OGTTs to measure the glucose tolerance more precisely. Our data show that the prevalence of non-invasively diagnosed PAD is significantly higher in newly-diagnosed (NDM) as well as previously-diagnosed diabetic (KDM) subjects than in normoglycaemic (NGT) subjects, irrespective of the diagnostic criterion for PAD. No significant difference in prevalence was found between IGT and NGT subjects. The multiple logistic regression analyses with *any* PAD as the dependent variable also demonstrated that KDM and NDM subjects had an increased risk for PAD (OR: 3.43 and 1.76, respectively), whereas IGT subjects did not. The risk for KDM was about twice as high as the risk for NDM subjects.

In the Islington Diabetes Survey [18], the crude prevalences of ABPI less than 0.90 in NGT, IGT and NDM subjects were about twice as high as in the Hoorn Study. An explanation for our lower prevalence rates may be the fact that we used the ABPI over the least diseased crural artery, whereas in Islington the ABPI was only determined over the posterior tibial artery. The Hoorn Study prevalence data were similar to those of a study among second generation Japanese-American men [17], and of an

Italian study [16]. The absence of statistically significant differences in PAD prevalences between IGT and NGT subjects conforms with the results of three other studies [16, 18, 36], but seems to contradict the results of a further two studies [17, 37]. However, in the first study the difference was not statistically significant [17], and in the second one the definition of 'IGT' did not conform with the World Health Organisation criteria [36]. It may be assumed that IGT subjects have been exposed to the milder hyperglycaemia for much shorter periods of time than diabetic subjects. This may lead to relatively low HbA_{1c} and fasting glucose levels in IGT subjects. Accordingly, our IGT subjects had only slightly higher HbA_{1c} and fasting plasma glucose levels than the NGT subjects. This small contrast may result in the inability to show an increased risk for PAD in IGT subjects.

In the KDM subjects we found prevalences of *any* PAD and ABPI less than 0.90 of 41 % and 21 %, respectively. The prevalences, reported elswhere, are also highly influenced by the applied diagnostic criterion for PAD, and vary from 38 % using a combination of three PAD criteria to about 20 % using the ABPI less than 0.90 [12–15, 37]. The relatively high prevalence of *any* PAD in Hoorn may, at least in part, be explained by the fact that we assessed the flow in all three crural branches, whereas the flow in the peroneal artery was not evaluated in other studies.

After excluding the participants treated with oral hypoglycaemic agents or insulin, a positive and statistically significant linear association between quintiles of fasting plasma glucose and prevalence rate of *any* PAD was found (Fig. 2). Quintiles of HbA_{1c} were linearly associated with ABPI less than 0.90 and showed a linear trend for *any* PAD. This suggests a gradual increase in the risk for PAD with increasing fasting plasma glucose and HbA_{1c} levels. A cut-off point above which the risk for PAD rapidly increases, as described for diabetic microangiopathy [34, 35], could not be demonstrated.

In logistic regression analyses, HbA_{1c}, fasting and 2-h post-load glucose were found to be risk factors for PAD, even after correction for other cardiovascular risk factors. This is in agreement with various other studies [37, 38]. Age, smoking and hypertension are the most important risk factors for PAD, which is in agreement with the general findings in epidemiological studies [3]. Serum lipids (triglycerides, LDL or HDL-cholesterol) were not statistically associated with PAD, which might, at least in part, be explained by the cross-sectional study design. Conflicting results have been reported concerning the role of serum lipids as cardiovascular risk factors [3]. The negative association between BMI and *any* PAD has also been reported in two other studies [15, 39]. However, an explanation is still lacking. One explanation might be the selective mortality of obese persons with PAD resulting in a disappearance or, even, a reversal of the association between BMI and PAD. The low number of KDM subjects ($n = 67$) did not allow further analysis of risk factors for PAD in this sub-group.

In contrast to *any* PAD, no association was found between ABPI and glycaemic indices or insulin levels. After exclusion of KDM subjects, univariate and multivariate analyses correcting for various risk factors (age, sex, smoking, BMI, WHR, LDL and HDL-cholesterol, triglycerides and hypertension), did not show any association between ABPI (continuous parameter of PAD) or ABPI less than 0.90 and the continuous parameters of glucose tolerance (HbA_{1c}, fasting and 2-h post-load plasma glucose, serum fructosamine, fasting and 2-h post-load insulin). This may be due to the measurement of ABPI over the least diseased crural artery, which was clinical practice in our vascular laboratory to obtain a general impression of the presence of serious arterial disease in the lower extremity [26]. This resulted in a low sensitivity for PAD and may explain why no association was found. Our findings seem to support the advice to measure the ankle pressure over at least two, or, preferably, three crural arteries in both legs [40].

Serum insulin has been reported to be an independent risk factor for the development of coronary heart disease [41–43]. However, population-based studies evaluating the role of specific insulin as a risk factor for non-invasively diagnosed PAD are rare. One study reported an association between aspecific insulin and non-invasively diagnosed PAD, and another an association between aspecific insulin and intermittent claudication [44, 45]. In the present study, neither fasting nor 2-h post-load specific insulin levels are associated with any criterion for PAD in univariate and multivariate analyses. A reason may be the inter-individual variability of insulin, which may be explained by two concurring phenomena in diabetic subjects: peripheral insulin resistance and deficient insulin production in the pancreas. Assuming that in NGT and IGT subjects pancreatic beta-cell insuffiency is a minor issue, we also studied the association between insulin and PAD in this sub-group. However, even in this sub-set of participants with normal and impaired glucose tolerance no association was found (data not shown).

In an epidemiological setting, one has to rely on non-invasive techniques for the assessment of PAD. At present the most widely-used and advocated non-invasive technique to determine PAD in population-based prevalence studies is the ankle pressure measurement [8–11, 15, 17, 18, 46]. An ABPI less than 0.90 has been reported to correlate very well with angiographically-diagnosed PAD [47, 48]. Since the prevalence of medial arterial calcification has been reported to be elevated in elderly men [21], non-insulin-de-

pendent diabetic subjects [21–23] and subjects with neuropathy [23–25], an under-estimation of PAD may be expected in subjects with a disturbed glucose tolerance when ABPI is used for PAD detection. Choosing qualitative Doppler flow velocity curve analysis, as an additional non-invasive test, resulted in a two-fold increase in diagnosed PAD. This, in turn, increased the statistical power to demonstrate associations between glycaemic indices or insulin levels and PAD. Therefore, our data support the need to perform an additional non-invasive vascular test in addition to the ankle pressure measurements [40]. If possible, all three crural arteries should be evaluated to provide better information about the peripheral vascular status, especially if one bears in mind that in diabetic subjects distal PAD is reported to be more prevalent than proximal PAD [49, 50]. This may result in an earlier detection of subjects who are at risk of developing macrovascular complications.

Our data support the fact that the most important interventions in clinical practice are to motivate the patients who smoke to give up smoking and to treat existing hypertension.

The final conclusion is that, after correction for cardiovascular risk factors, glycated haemoglobin, fasting and 2-h post-load plasma glucose are independent determinants of the presence of *any* PAD, whereas specific insulin is not found to be a determinant of PAD. As these data result from a cross-sectional survey, longitudinal studies are needed to further explore these associations, the underlying mechanisms and the clinical consequences.

Acknowledgements. We gratefully acknowledge the technical assistance of Mrs. M. Bischoff and Mrs. J. Scholma who were responsible for all vascular laboratory tests.

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