Insulin resistance in the elderly
The Rotterdam Study

Ronald Stolk

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Chapter 1

Insulin resistance: assessment

Insulin resistance is a diminished ability to keep the serum glucose low with insulin levels in the normal range. Subjects with raised insulin resistance therefore usually have increased serum insulin levels. When the ß-cells of the pancreas are no longer able to produce these increased amounts of insulin, serum glucose increases and diabetes mellitus develops. Raised insulin resistance and the ensuing hyperinsulinemia increase with age. Because hyperinsulinemia is a risk factor for several (chronic) diseases which are common in the elderly, insulin resistance was assessed as part of a large population-based study to chronic diseases in the elderly, the Rotterdam Study. In this first chapter a general description of the Rotterdam Study is given, with an overview of the measurements of the glucose metabolism. This is followed by a review on the oral glucose tolerance test. Finally, the results of a validation study are reported on the non-fasting oral glucose tolerance test, as used in the Rotterdam Study.

Glucose metabolism in the Rotterdam Study

The proportion of elderly people in the Netherlands and other affluent societies has been rapidly increased during the last decades.[1] This is mainly caused by an increased life expectancy at birth due to diminished perinatal and infant mortality. In addition, life expectancy at middle and old age has also slightly increased.[2] However, many people suffer from chronic diseases during these added years of life. The compression of mortality has not been paralleled by a compression of morbidity.[3] To postpone the onset of age-related chronic diseases will improve the quality of the last part of life. Before intervention can be initiated, potentially modifiable risk factors for the occurrence and progression of these diseases have to be identified.

The Rotterdam Study is a population-based cohort study of determinants of chronic disabling diseases in the elderly. The study focuses on four areas of chronic disease: cardiovascular diseases, neurogeriatric diseases, osteoporosis, and ophthalmologic diseases. Insulin resistance is commonly studied as cardiovascular risk factor, which is also the emphasis in the Rotterdam Study. However, the design of the Rotterdam Study provides an unique opportunity to also examine insulin in relation to several other chronic diseases.

General description of the Rotterdam Study

The Rotterdam Study is a single-center, prospective study of a large cohort of subjects, aged 55 years and over.[4] All inhabitants of Ommoord, a suburb of Rotterdam, in this age range were invited to participate (3726 men and 5434 women). The names and addresses were provided by the municipality of Rotterdam. From all subjects written informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School. The baseline examinations were carried out between 1990 and 1993; after one reminder 7983 subjects participated, which gives a response rate of 77.7%. The demographic characteristics of the study population are given in Table 1.

After the invitation letter all subjects were contacted by telephone to make an appointment for the first part of the study: an interview at their home. The home interview consisted of a structured computerized questionnaire by specially trained technicians using a portable personal computer. The items covered in the interview are given in Table 2. Family history was confined to first degree relatives. The

Table 1 Demographic characteristics of the study population.

Age (years)	Men		Women	
	number	response	number	response
55 - 59	513	79.8%	718	85.7%
60 - 64	649	83.2%	838	85.1%
65 - 69	654	83.3%	765	79.2%
70 - 74	521	79.2%	766	78.4%
75 - 79	388	72.3%	644	73.7%
80 - 84	229	71.1%	518	68.2%
85 - 89	112	66.7%	403	66.0%
90 - 94	34	70.8%	187	72.8%
≥ 95	5	55.6%	39	67.2%
Total	3105	78.6%	4878	77.1%

interview comprised questions on smoking habits, the cardiovascular Rose-questionnaire,[5] the Stanford Health Questionnaire on activities of daily living,[6] and medical consumption. The generic and trade names of the medications the participant used was entered into the database using the ATC (Anatomical Therapeutical Chemical) classification index codes.[7] The medication data were verified by a physician at the research center, who also recorded the indication for the used medication.

Subsequently the participants came to the research center twice for several clinical examinations (Table 2). In total 7129 (69.4%) subjects participated in this part of the study. Anthropometric measures were obtained with light indoor clothes and no shoes, and included height, weight, waist and hip circumference. Blood pressure was measured with a randomzero-sphygmomanometer. In addition to the blood pressure in the arm, blood pressure was also measured in the ankle. Bone density was assessed by dual-energy X-ray absorptiometry (DXA), using a Lunar DPX-L densitometer. Cognitive function was assessed by the Mini Mental State Examination;[8] screen-positives received an extensive cognitive screening during the second center visit.[9] The eye examination included ocular pressure, visual acuity, perimetry, slit-lamp examination and fundus examination. In addition two 35° colour slides centred on the macular area were taken of each eye (Diabetic Retinopathy Study (DRS) Standard Field 2). Ultrasound images were made of the

Table 2 Measurements in the Rotterdam Study.

Home interview	*Clinical examinations*	*Laboratory measurements*
Activities of daily living	Anthropometry	Clinical chemistry
Diet	Blood pressure	Coagulation factors
Family history	Bone densitometry	Glucose tolerance test
Medical history	Cognitive function	Hematology
Medication use	Electrocardiogram	Urine examination
Smoking habits	Eye examination	
Social economic status	Physical examination	
	Ultrasound measurements	
	X-rays	

heart, the abdominal aorta and the carotid arteries. X-rays were taken of the lumbar spine, hands, hips, and knees.

After the home interview participants were asked to collect a timed overnight urine sample in the night before the first center visit. As part of the first center visit blood was obtained by venepuncture with minimal stasis and using a 12 gauge Butterfly needle. This was followed by an oral glucose tolerance test. In Table 2 an overview of the laboratory measurements is given. In addition, biologic material was stored for future analyses in selected subgroups. This included serum, plasma, urine and lymphocytes (DNA).

Subjects living in residential homes of Ommoord were also invited for the Rotterdam Study. To improve the response-rate among these, usually very old, subjects, the clinical measurements were performed in the home itself. As part of the equipment could not be moved to these homes, the measurements were somewhat limited compared to the research center.

The follow-up started once the participant had completed the examinations. All general practitioners of Ommoord collaborate with the Rotterdam Study and provide morbidity and mortality data of the participants at a regular basis. Moreover, data on prescribed medication are provided by the pharmacist of Ommoord.

Assessment of the glucose metabolism

Both at the home interview and the research center, data on the glucose metabolism were collected (Table 3). A blood sample was obtained in 7050 participants. Blood was drawn by venepuncture and allowed to coagulate for 30 minutes. This period was strictly adhered to because the glucose concentration

in whole blood decreases approximately 7% in the first hour, due to glycolytic activity of the blood cells.[10] Subsequently serum was separated by centrifugation and quickly frozen in liquid nitrogen. Fructosamine was measured in the first 5248 participants only. All participants who did not use antidiabetes medication received a glucose drink of 75 grams in 200 ml water after the first venepuncture (2240 men and 3303 women). Two hours later a second venous blood sample was obtained, in which glucose and insulin were measured. Glucose levels were measured in both samples by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix, Brussels, Belgium). This assay has a cross-reaction with proinsulin of 40%. The intra- and inter-assay coefficients of variation of these measurements are less then 2.5% and less then 6.0%, respectively. The ratio of post-load insulin over glucose was used as a measure of insulin resistance, which provides a good estimate in subjects without diabetes mellitus.[11] Because subjects using antidiabetes medication did not undergo the glucose tolerance test, insulin was not measured in this group.

The distribution of serum glucose, fructosamine, insulin and insulin resistance are given in Figures 1 till 4 in strata of age and gender. The figures show that all indicators of the glucose metabolism increase with age. Moreover, the post-load insulin levels as well as the ratio of insulin over glucose were higher in women than in men at all ages.

Diabetes mellitus was defined as the use of antidiabetes medication or a random or post-load serum glucose level greater than 11 mmol/l. Of all participants of the Rotterdam Study 796 (10.0%) met this definition. The age and gender specific prevalence is given in Figure 5. Subjects with glucose level in the diabetic range and not using antidiabetes medication were defined as newly diagnosed. This were 174 men (5.9% of all men) and 252 women (5.2%). Figure 5 shows that the proportion newly diagnosed of the subjects with diabetes mellitus decreases with age.

Table 3 Data on glucose metabolism collected in the Rotterdam Study.

Home interview	*Examination at the research center*
Presence of diabetes mellitus	Random serum glucose
Age of onset of diabetes mellitus	Time since last meal
Use of antidiabetes medication	Serum fructosamine
Duration of use of antidiabetes medication	Post-load serum glucose
Family history of diabetes mellitus	Post-load serum insulin

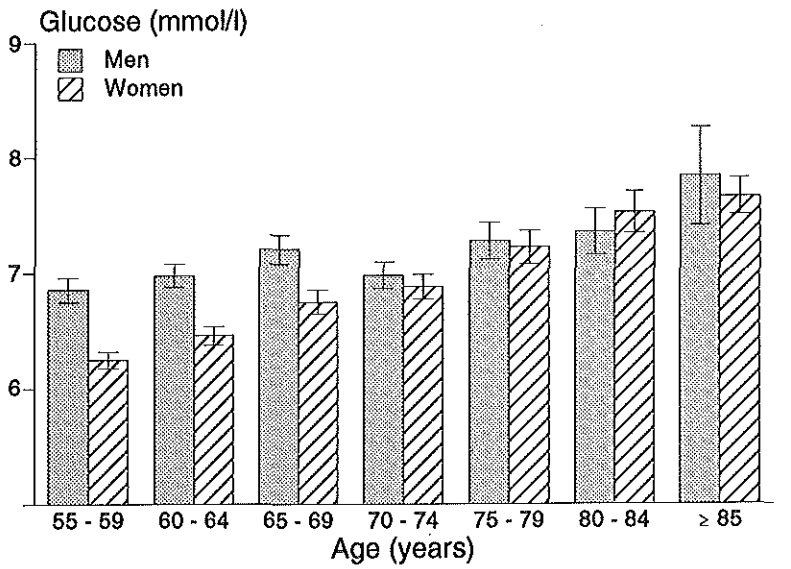

Figure 1 Non-fasting serum glucose in 5-year age-categories.
Values are means with standard error.

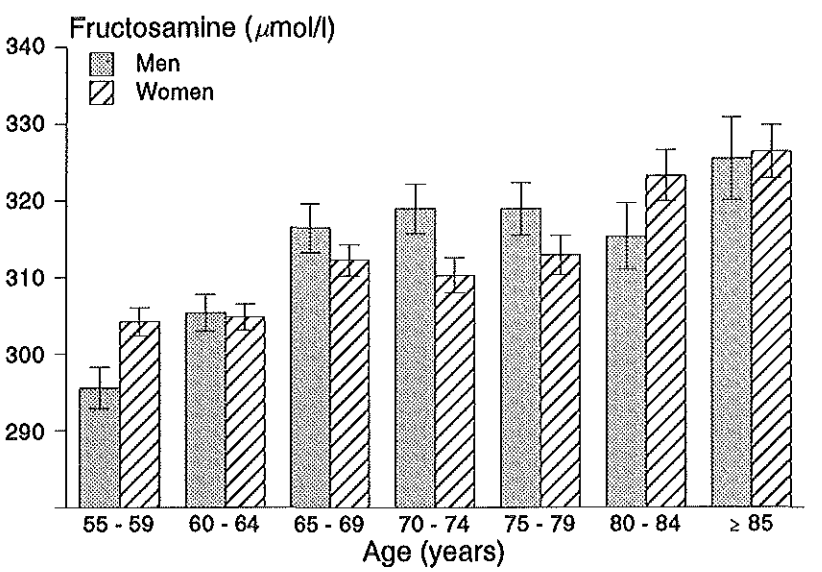

Figure 2 Fructosamine in 5-year age-categories.
Values are means with standard error.

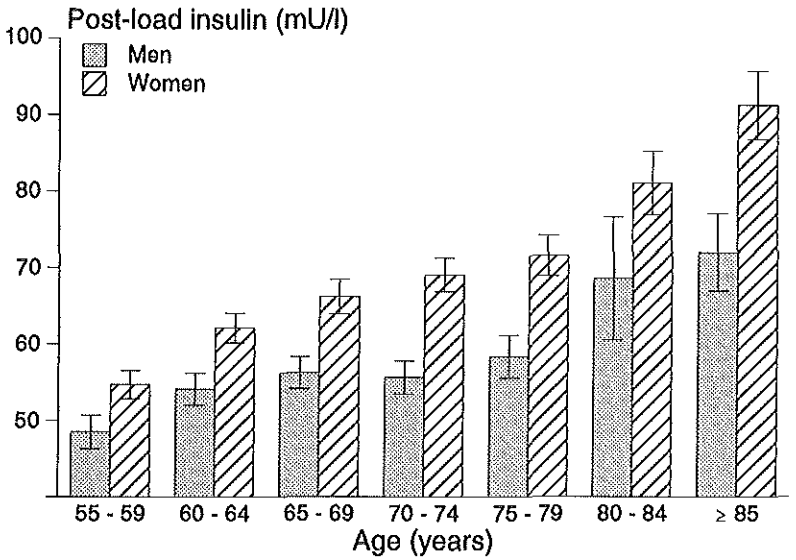

Figure 3 Post-load insulin level in 5-year age-categories.
Values are means with standard error.

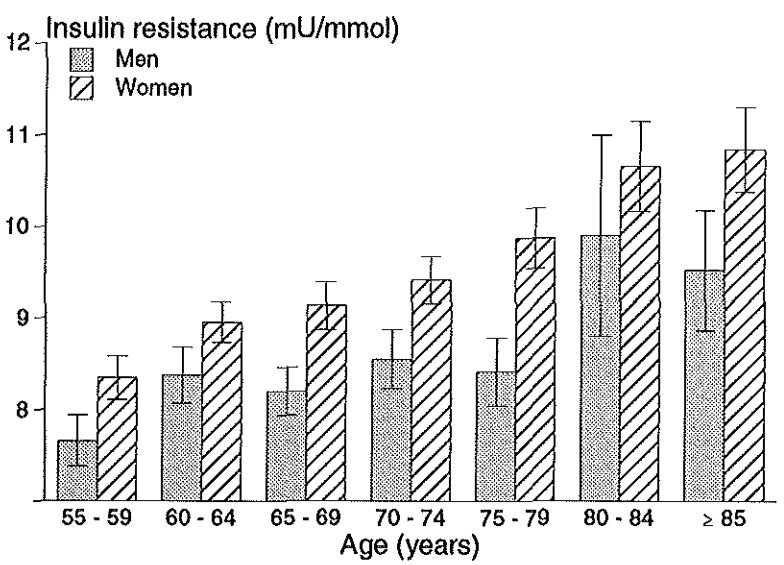

Figure 4 Insulin resistance, assessed by the ratio of post-load insulin over glucose in 5-year age-categories.
Values are means with standard error.

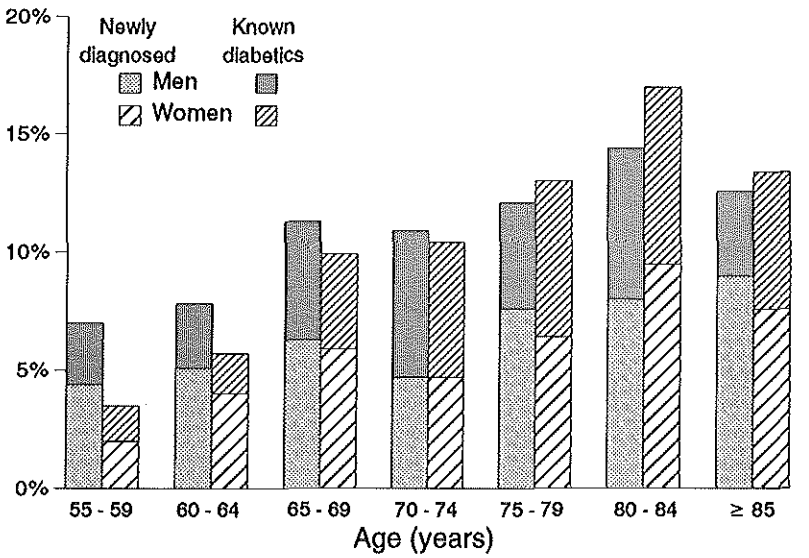

Figure 5 Prevalence of diabetes mellitus in 5-year age-categories.

Additional metabolic and endocrine examination

A sample of participants from the Rotterdam Study was invited for an additional examination. The study population included 219 persons aged 55 to 80 years, who had completed the baseline visit of the Rotterdam Study not more than six months earlier. Subjects with psychiatric or endocrine disease, including diabetes mellitus treated with medication, were not invited. Compared to the other participants of the Rotterdam Study of the same age without known diabetes mellitus, there were no differences in age and gender distribution, blood pressure, use of antihypertensive medication, evidence of atherosclerotic plaques in the carotid arteries by ultrasound, and electrocardiographic abnormalities.

Participants were seen at the research center after an overnight fast. Blood was drawn between 8.00 and 9.00 am. They were asked for any change in their health status since the examinations of the Rotterdam Study. A dexamethasone suppression test (DST) was carried out.[12] Participants were given a tablet of 1 mg dexamethasone and were instructed to take this at 11.00 PM. Next morning a fasting blood sample was obtained at the same time as the previous day. Blood was drawn by venepuncture and allowed to coagulate for 30 minutes. Subsequently serum was separated by centrifugation and quickly frozen in liquid nitrogen. In Table 4 measurements performed in the obtained blood samples are given. Figure 6 gives the gender-specific distributions of the fasting cortisol levels, whereas in

Table 4 Measurements of the additional examination.

Fasting blood sample		Fasting blood sample after 1 mg dexamethasone
Clinical chemistry	*Steroids*	*Clinical chemistry*
Apo A1	Androstenedione	Insulin
Cholesterol	Cortisol	
Free fatty acids	Dehydroepiandrosterone	*Steroids*
Fructosamine	sulphate	Androstenedione
Glucose	Oestradiol	Cortisol
HDL-cholesterol	Testosterone*	Dexamethasone
Insulin		
Lipoprotein(a)	*Binding proteins*	*Growth factors*
Triglycerides	Cortisol binding globulin	Insulin like growth factor 1
	Sex-hormone binding protein	
Growth factors	IGF binding protein 1	*Binding proteins*
Insulin like growth factor 1	IGF binding protein 3	Cortisol binding globulin
		IGF binding protein 1
		IGF binding protein 3

* Measured in men only.

Figure 7 distributions of the cortisol level after the DST are given. Four subjects did not fulfil the clinical criteria of a normal DST (suppression of cortisol below 50 nmol/l). However, also in the group of 'suppressors' a wide range of cortisol levels was found. This was not related to the serum level of dexamethasone.

As part of this study the activity of the cortisol receptor was investigated. The number and affinity of glucocorticoid receptors on mononuclear leucocytes was analyzed as well as the biological responses of these cells to glucocorticoids in a mitogen-stimulated lymfocyte proliferation assay. Using polymerase chain reaction based analyses of single strand conformation, the glucocorticoid receptor gene was screened for the presence of mutations.

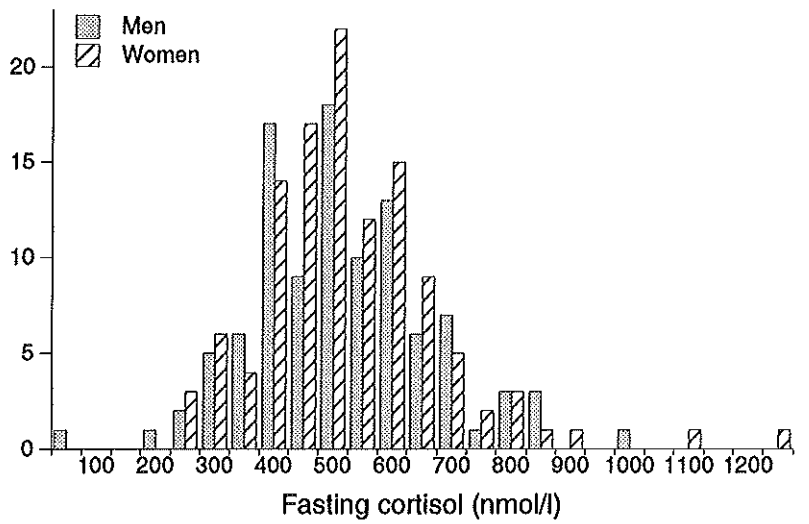

Figure 6 Distribution of fasting cortisol levels.

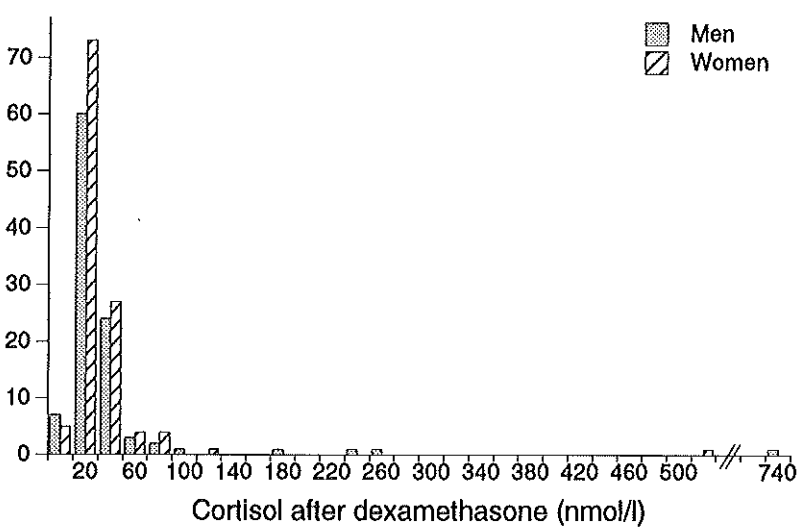

Figure 7 Distribution of fasting cortisol levels after 1 mg dexamethasone.

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The oral glucose tolerance test

The hallmark of diabetes mellitus is a chronic elevation of the blood glucose level. Thus a single blood glucose measurement is not always sufficient to make the diagnosis. In particular recent food intake strongly affects the levels of glucose and glucose regulating hormones. To overcome these problems, fasting blood samples are taken to assure a glucose measurement that can be compared with previous values and with those in other patients. For a more detailed and sensitive assessment of the glucose metabolism the oral glucose tolerance test (OGTT) was introduced, particularly for those without overt symptoms. However, in clinical practice the OGTT is often regarded as a cumbersome, time-consuming, and patient-unfriendly procedure. When diabetes mellitus is suspected in symptomatic subjects, presenting with typical diabetes symptoms ranging from thirst and frequent urination to ketoacidotic coma, or subjects with complications, the (random) serum glucose level is usually unequivocally raised. This suggests that for a clinician the use of the OGTT to diagnose symptomatic diabetes mellitus may be very limited in those cases. Nonetheless in screening programmes, clinical research, and population-based epidemiologic studies, where participants often lack diabetes symptoms or complications, an OGTT is commonly used to detect diabetes mellitus thus adding to the diabetic population an equal sized group of subjects with unrecognized diabetes. As a result, a potential divergence between clinical practice and (clinical) epidemiologic research, which should provide knowledge relevant to clinical practice, may exist.

In this chapter the diagnosis of diabetes mellitus, impaired glucose tolerance (IGT) and insulin resistance by the OGTT is discussed, in the context of clinical practice and epidemiologic research.

Criteria for the OGTT

Over a decade ago the National Diabetes Data Group of the National Institutes of Health (NDDG)[1] and an Expert Committee of the World Health Organization (WHO)[2] published guidelines for the OGTT based on a glucose load of 75 grams, which have been internationally accepted. The criteria for the diagnosis of diabetes mellitus and impaired glucose tolerance are given in Table 1. In addition to the WHO criteria, the NDDG requires an intermediate glucose measurement between

Table 1 Diagnostic criteria for the oral glucose tolerance test according to WHO guidelines.[2]

	Diabetes mellitus	Impaired glucose tolerance	Normoglycemia
Fasting	≥ 7.8 mmol/l	< 7.8 mmol/l	< 7.8 mmol/l
2 hours post-load	≥11.1 mmol/l	7.8 - 11.1 mmol/l	< 7.8 mmol/l

Values are venous plasma glucose concentrations.

the fasting and 2-hour sample to be higher than 11.1 mmol/l (200 mg/dl) for the diagnosis diabetes mellitus or impaired glucose tolerance (IGT). In epidemiologic studies the intermediate blood sample may be omitted, making the two criteria identical.[1] In children 1.75 grams of glucose per kg body weight is used up to a total of 75 grams. In pregnant women the OGTT is performed with a glucose load of 50 grams.[3] With regard to the diagnosis of insulin resistance no uniform criteria exists.

Diagnosing diabetes mellitus in clinical practice

Physicians diagnose and treat diabetes mellitus primarily to reduce the risk of diabetes complications, rather than to treat the disease itself. Recently McCance *et al* have reported on the association between different glycemic measures and the incidence of diabetic retinopathy and nephropathy in a group of Pima Indians who did not use antidiabetes medication at baseline.[4] In their study fasting plasma glucose (FPG), two-hour post-load plasma glucose and glycated hemoglobin were all good predictors of the incidence of diabetes complications. The onset of diabetes mellitus was equally well predicted by the three glycemic variables.[4]

Clinically, it seems reasonable not to routinely use the OGTT to diagnose diabetes mellitus. Both the NDDG and the WHO guidelines indicate that in the presence of symptoms the diagnosis can be made using a single (fasting or non-fasting) blood glucose measurement. This is also recommended in guidelines for clinical practice, such as those from the American Diabetes Association: diabetes mellitus is diagnosed when the plasma glucose exceeds 11.1 mmol/l and 'classic symptoms of diabetes' (polydipsia, polyuria, polyphagia, weight loss) are present, or when the fasting plasma glucose twice exceeds 7.8 mmol/l.[5] If diabetes is not confirmed by one of these tests, but still suspected, an OGTT is performed.

A survey among 174 physicians in Pittsburgh showed that this corresponds well with current practice.[6] The physicians were randomly selected from the Internal Medicine and Family Practice listings in the telephone directory and the attending faculty in the Department of Internal Medicine. They were asked to indicate which tests they use if diabetes mellitus is suspected. 76 physicians returned the questionnaire, mainly internists (84%). The majority (68%) worked in a hospital. The median time since their graduation in medicine was 10 years. Only one of the physicians used the OGTT as a first test, and over two-thirds (70%) never used it. FPG (64%), random blood glucose (28%), and urinanalysis (13%) were most frequently used. Of the hospital based physicians 25% occasionally used the OGTT, mostly as a second test, whereas 42% of the non-hospital physicians used it, mainly as third test. Reported diagnostic cut-off values differed remarkably. The range for FPG was 5.6 to 11.1 mmol/l, whereas upper limits for the random blood glucose ranged between 6.1 and 16.7 mmol/l. The guidelines of the American Diabetes Association (7.8 and 11.1 mmol/l, respectively) were used by 50% and 33% of the physicians, more so by hospital physicians.

Diagnosing diabetes mellitus in epidemiologic research

The number of epidemiologic studies on diabetes mellitus and its complications is constantly increasing. Commonly, in these studies the OGTT is used. According to the WHO guidelines only the two hour post-load sample is sufficient to diagnose diabetes in these studies. When a number of single glycemic measures is compared, the two hour post-load glucose provides the best indicator of the presence of diabetes mellitus.[7,8]

The OGTT is a non-physiological procedure and the inter-person variability is rather high. This may be due to a number of factors, including diet and exercise during the days before the test, caffeine use, smoking, medications, and stress. In a population of elderly people examined annually for five years, the reliability coefficient of the diagnosis according to the WHO-criteria was 0.62.[9] Other researchers have found that only about 50 percent of the oral glucose tolerance tests are reproducible,[10] which may be partly explained by changes in ambient temperature.[11] Of subjects with IGT on the first OGTT only 40 to 60% is diagnosed as IGT or diabetes on a second test.[12,13,14] The biological variation (20% to 35% for the post-load glucose) is difficult to control, but can be minimized by more careful attention to the protocol.[15] Because of its high variability and low specificity, epidemiologic studies based on a single OGTT may overestimate the prevalence of diabetes mellitus by as much as 16%.[16] In spite of poor precision, the post-load

glucose and the post-load insulin level have been identified as important risk factors for diabetes complications and cardiovascular disease.[17]

Using the 2-hour post-load glucose measurement of the OGTT, more subjects are diagnosed with diabetes compared to a single FPG. In population studies fasting blood levels of subjects with newly diagnosed diabetes show a wide distribution, ranging in one study from lower than 5.0 mmol/l to higher than 30.0 mmol/l.[18] As a consequence, the sensitivity of the OGTT is naturally higher, given the current criteria. Using the OGTT more diabetes subjects will be normal on repeated testing than using a FPG ('false positives'), whereas after a FPG more normal subjects will have diabetes on repeated testing than after an OGTT ('false negatives'). When the most frequent clinical approach to diabetes diagnosis (FPG) is compared with the standard epidemiological diagnosis (OGTT), this means that virtually all clinical cases are diagnosed in epidemiological studies. However, nearly as many unrecognized cases with non-diagnostic fasting levels are identified. Consequently, epidemiologic data may not always reflect the natural history to be expected in clinical practice. Indeed, complication rates and risk factors associations may be very different. In contrast, clinical criteria may classify subjects as normal in spite of deviations in glucose metabolism that have prognostic relevance.

Diagnosing impaired glucose tolerance and insulin resistance

Impaired glucose tolerance (IGT) is determined by the post-load glucose value of the OGTT (Table 1). The concept of IGT has been introduced to identify subjects with a moderately disturbed glucose metabolism. Although there is a considerable difference between the NDDG and WHO criteria for IGT,[19] subjects with IGT are generally hyperinsulinemic and have an increased risk of developing diabetes mellitus, as reflected in the now obsolete term 'pre-diabetes'. In follow-up studies deterioration to diabetes mellitus has been reported from 1.5 to 4% per year.[20,21] Another reason to identify IGT is the increased risk of cardiovascular diseases.[22]

Intermediate glucose measurements during the OGTT may give a better estimate of the presence of IGT. This is reflected in the NDDG guidelines which require at least one intermediate glucose level above 11.1 mmol/l for the diagnosis IGT.[1] However, one disadvantage of more measurements (beyond the necessity of an extra sample) is a substantial number of non-classified subjects, as shown in a large population study.[23] This may be avoided using a single continuous measure rather than diagnostic categories. A good measure of the overall glucose response is the area under the glucose tolerance curve (AUC),[24] but this method is too complex for clinical use.

Insulin resistance, probably the key factor of a cluster of cardiovascular risk factors (Syndrome X or insulin resistance syndrome),[25] has been shown to precede the onset of NIDDM.[26,27] The gold standard for the assessment of insulin resistance is the glucose clamp technique.[28] Basically, insulin resistance is a diminished ability to keep the glucose levels low with insulin levels in the normal range. In these subjects glucose levels are slightly increased but remain below the diabetes range at the expense of raised insulin levels. This becomes more apparent if the insulin need increases, for example after a glucose load. Therefore, insulin levels are often used as proxy for insulin resistance. The association between insulin levels and whole-body glucose uptake during the euglycemic hyperinsulinemic clamp was studied in 132 subjects with varying degrees of glucose intolerance.[29] This study shows that the fasting, but especially the post-load insulin level are good measures of insulin resistance in subjects without NIDDM. In subjects with a normal and impaired glucose tolerance, the correlation coefficients with the log fasting insulin level were -0.68 ($p < 0.01$) and -0.47 ($p < 0.05$), respectively. For the log two-hour post-load insulin level -0.74 ($p < 0.01$) and -0.54 ($p < 0.01$) were found.[29] Recently, in non-diabetic subjects a strong correlation was found between the mean of two fasting insulin levels and insulin sensitivity assessed by Bergman minimal model technique ($r = 0.57$, $p < 0.025$) and with the hyperglycemic clamp ($r = 0.71$, $p < 0.005$) (M. Korytkowski, personal communication).

A further improvement may be made by using the ratio of the post-load insulin over glucose. The inclusion of glucose in the measure of insulin resistance is based on the notion that in non-diabetic subjects insulin resistance results in a higher insulin level to preserve normoglycemia.[30] In spite of this hyperinsulinemia there is a slight increase of the post-load glucose level, until the criteria of IGT are met.[26] To compare subjects in population-based studies, this ratio has been shown to be an adequate approximate measure.[31] In addition, there is a large variation in insulin-stimulated glucose uptake in subjects with a normal glucose tolerance.[32]

Data from the Rotterdam Study, a population-based study to chronic diseases in the elderly,[33] may give additional support for the use of the insulin/glucose ratio. Figure 1 gives the post-load serum insulin and glucose levels from a sample of 500 subjects without antidiabetes medication, aged 55 to 75. Firstly, the figure demonstrates the variation in insulin response, which indicates that adjustment for the glucose level may be useful. Moreover, the insulin response is higher in the upper part of the non-diabetic glucose distribution (< 11.1 mmol/l), but the variation is also greater. Some subjects in the diabetic glucose range have high insulin levels suggesting recent onset diabetes, while others have a low insulin response suggesting a longer lasting disease.

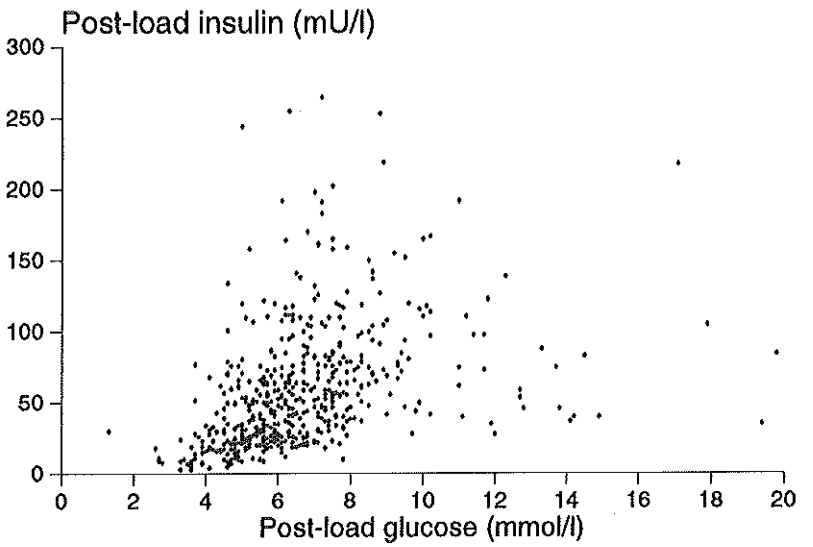

Figure 1 Post-load glucose and insulin values of a random sample of 500 participants of the Rotterdam Study without antidiabetes medication, aged 55 to 75.

The use of both fasting and post-load glucose levels may help to distinguish ß-cell dysfunction from insulin resistance. Low insulin levels in the fasting state have only limited effect on peripheral muscle cells. An oral glucose load is largely cleared from the plasma by insulin-sensitive tissues (particulary skeletal muscle), the response of which is diminished in insulin resistance. As a consequence, subjects with only a ß-cell defect may have a modestly increased post-load glucose despite fasting hyperglycemia, whereas insulin resistant subjects show markedly increased post-load glucose levels.[34] In established NIDDM both glucose levels are often increased, reflecting both defects being present.[35]

The requirement of the fasting state may sometimes give logistic problems for the use of the OGTT in large (screening) studies, which are now restricted to the early morning. There is some evidence suggesting that the glucose and insulin levels after a glucose load given in a non-fasting state are as good as those given in a fasting state.[36] Moreover, the first population-based epidemiologic studies on risk factors of cardiovascular disease used a non-fasting oral glucose tolerance test, which revealed similar associations with the prevalence and incidence of cardiovascular diseases.[37,38] Because the post-load glucose value is the most

valuable to diagnose diabetes (see above) this would permit the use of a non-fasting test in certain circumstances.

To investigate the influence of (non)fasting an OGTT was performed twice in 69 subjects without diabetes mellitus, once non-fasting and once fasting. The glucose levels, but in particular the insulin levels two hours after the oral glucose load were quite comparable after the fasting and the non-fasting OGTT (correlation coefficient 0.61 and 0.74 respectively, $p < 0.001$). More details of that study are given in Chapter 1.3. At least, the results suggest that it is not necessary to give the glucose load in the fasting state to estimate insulin resistance, raising the possibility that the OGTT, though not ideal for the clinical diagnosis of diabetes, may have a role as (clinical) marker of insulin resistance.

A small number of subjects have a normal fasting but diabetic post-load glucose level, who are neither covered by the NDDG nor by the WHO criteria. In a population-based study in the elderly this was found in 56% of the men and 74% of the women,[8] indicating that insulin resistance increases with age. In a follow-up study among family members of the Pittsburgh IDDM cohort, subjects with 'non-fasting diabetics' have a slightly lower risk on subsequent complications than 'normal (fasting) diabetics'.[6] Because these subjects are probably insulin resistant,[34] their risk may be comparable to IGT subjects.

Future of the OGTT

While in clinical practice glucose intolerance is classified as diabetes or not, it is important to realise that both hyperglycemia and hyperinsulinemia represent a continuum. Any cut-off value remains arbitrary. Furthermore, within the diabetic range increased serum glucose levels are associated with increased severity of diabetes complications, both microvascular, like retinopathy[39] and macrovascular.[40] Results from trials show that increased metabolic control decreases the incidence and progression of retinopathy.[41,42] In non-diabetic populations the risk of diabetes complications, both microvascular and macrovascular, increases gradually with an increase in serum glucose,[43] HbA1c[44] and insulin.[45] This suggest that also in non-diabetic subjects an increased levels of glycemic measures is associated with an increased risk of 'diabetes' complications.

In conclusion, it would seem desirable that clinical and epidemiologic definitions (and methodology) are comparable, and that the role of the OGTT in the clinical diagnosis of diabetes mellitus is limited. As McCance *et al* demonstrated in terms of prediction of diabetic complications, it can easily be replaced by FPG or HbA1c. Both of which are, it seems, preferentially used by clinicians anyway.

The other argument for the diagnostic use of the OGTT, to detect the unknown diabetic patient rests upon the assumption that subclinical deviations in glucose metabolism have prognostic importance. Knowledge as whether and how to treat discovered asymptomatic diabetic subjects is clearly needed.[46] If the data of McCance *et al* from the Pima Indians[4] are confirmed in other studies, it would seem that the high risk of unknown diabetic subjects can be identified by simpler tests.

On the other hand, the OGTT may have a role in the evaluation of IGT and insulin resistance. In this regard it may be used to identify subjects with an increased risk of diabetes mellitus and cardiovascular disease more precisely than with a random measurement of the serum glucose or insulin. Currently, however, there is no definitive treatment for IGT or insulin resistance. Further research, using the OGTT, may help elucidate the role of IGT and insulin resistance and indicate ways for early treatment in order to prevent or postpone subsequent morbidity and complications.[46] Eventually, the place of IGT and insulin resistance in the risk profile of cardiovascular and diabetic diseases should be more clearly defined so that preventive approaches, similar to those currently in operation for hypertension and hypercholesterolemia, can be developed.

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The use of a non-fasting oral glucose tolerance test in epidemiologic studies

Blood samples for the measurement of serum glucose and insulin are usually obtained in the fasting state. This logistically cumbersome and patient-unfriendly method is needed to assure a glucose measurement that can be compared with previous values and with those in other patients, because the levels of glucose and glucose regulating hormones are strongly influenced by the intake of food, notably carbohydrates. To obtain a more detailed assessment of the glucose metabolism the oral glucose tolerance test (OGTT) is used: serum glucose level is measured in the fasting state and two hours after an oral glucose load.[1,2] This approach has been internationally accepted as standard for the evaluation of glucose metabolism in comparative studies. In subjects without diabetes mellitus the post-load insulin levels showed a consistent correlation with insulin resistance measured by the whole-body glucose uptake during clamp studies.[3]

As part of the Rotterdam Study the glucose metabolism of the participants is studied. In this study, for logistic reasons it is not possible to obtain a fasting blood sample from all participants. Therefore we considered the possibility of assessing glucose metabolism in subjects without known diabetes mellitus by measuring random serum glucose and insulin levels and two hours after a standard oral glucose load. For the diagnosis of diabetes mellitus it might be used as first screening instrument, reserving further diagnostic work-up for only a small proportion of eligible subjects. To study the potential of the non-fasting oral glucose tolerance test we performed both a fasting and non-fasting OGTT in 69 elderly subjects.

Materials and methods

Study population

The Rotterdam Study is a population-based cohort study of determinants of chronic disabling diseases in the elderly. All inhabitants of the suburb Ommoord of Rotterdam, The Netherlands, aged 55 years and over are invited to participate. An outline of the study and its objectives has been published previously.[4] As part of the study an assessment is made of the glucose metabolism.

For the present study, a random selection of 69 subjects from the first 1000 participants with a complete non-fasting test was made. To obtain better estimates of the test characteristics, those who had glucose levels higher than 10 mmol/l during the non-fasting test were over-sampled in the study group. None of the participants was ever diagnosed with diabetes mellitus.

Measurements

The participants attended the Rotterdam Study research center for several measurements. The participants were asked for the time of their last meal (either breakfast or lunch). After a first venepuncture all subjects without antidiabetes medication received a glucose drink of 75 grams in 200 ml water. Two hours later a second venous blood sample was obtained. Serum was separated by centrifugation and quickly frozen in liquid nitrogen. Glucose levels were measured by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium). The coefficients of variation of these measurements are less then 2.5% and less then 6.0%, respectively.

For the present study an oral glucose tolerance test according to the WHO-criteria was performed.[2] The participants came to the research center in the fasting state, and after a venepuncture they received a standard glucose load (75 grams of glucose in 200 ml water). Two hours later a second blood sample was obtained.

Data analysis

Linear regression analysis was used to estimate the association between the glucose and insulin levels during the fasting and non-fasting oral glucose tolerance test, as well as the relation between serum levels and the time since last meal. The analyses were performed in the whole group and in strata of possible confounders, notably age, gender and body mass index. The data were also analyzed using the fasting OGTT as diagnostic standard, using the WHO-criteria. Subjects with fasting serum glucose $\geq$ 7.8 mmol/l or post-load glucose $\geq$ 11.1 mmol/l were classified as having diabetes mellitus (DM). If the fasting serum glucose was $<$ 7.8 mmol/l and the post-load value was between 7.8 and 11.1 mmol/l the diagnosis impaired glucose tolerance (IGT) was made. In the analysis those with IGT and DM were considered together as a group with disturbances of the glucose metabolism. The non-fasting values were used as categorical variables to calculate the optimal cut-off level, defined as maximal discrimination between normal and abnormal glucose tolerance. 95% confidence intervals for the sensitivity and specificity were calculated using the method described by Simel *et al.*[5]

Table 1 Baseline characteristics of the participants.

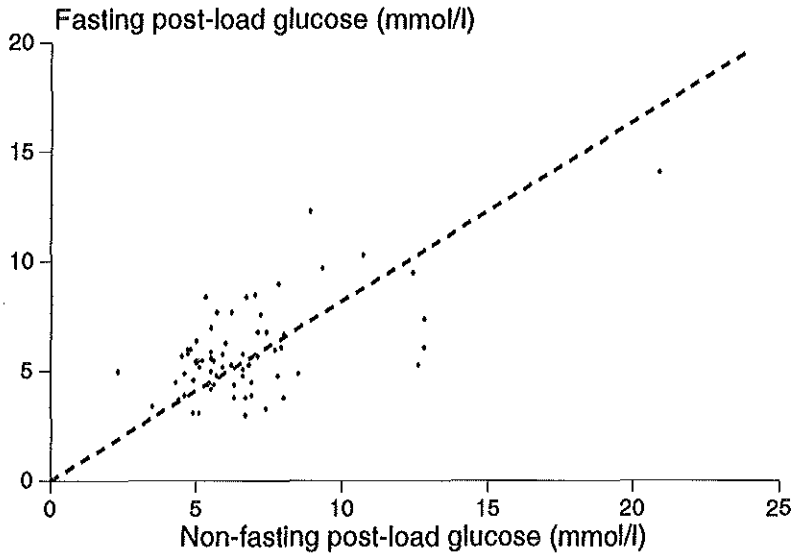
Number		69	
Men		42%	
Age (years)		69.9	(8.4)
Body mass index (kg/m^2)		27.0	(3.9)
Glucose (mmol/l)	Non-fasting baseline	6.5	(2.1)
	Non-fasting two hours post-load	6.7	(2.7)
	Fasting	5.7	(1.0)
	Fasting two hours post-load	5.8	(2.1)
Insulin (mU/l)	Non-fasting baseline	43.8	(33.4)
	Non-fasting two hours post-load	64.1	(41.2)
	Fasting	18.3	(9.8)
	Fasting two hours post-load	81.9	(71.8)

Values are means with standard deviation in parentheses.

Results

The study was performed in 69 persons without known diabetes mellitus, 29 men and 40 women. The time between the non-fasting and the fasting test was at maximum 6 months (median 4.6 months). In Table 1 the baseline characteristics of the study population are given. Women had a higher body mass index (27.9 vs. 25.6 kg/m^2, $p = 0.01$) and a lower random glucose level (6.0 vs. 7.3 mmol/l, $p = 0.02$). The other variables did not differ significantly between men and women. Table 2 shows the regression coefficients of the relations between the different glucose and insulin measurements. All associations were highly statistically significant. In Figure 1 the relations between the post-load glucose and insulin levels are given. This suggested that the serum levels obtained after an oral glucose load are relatively little influenced by the fasting state at baseline. The results were essentially the same if the analyses were repeated in separate strata for age, gender, and body mass index.

The random glucose values were not associated with the time since last meal ($p = 0.60$), whereas the random insulin levels significantly decreased with the time since the subject had eaten (regression coefficient -0.17 mU/min, $p = 0.03$) (Figure 2). The post-load glucose levels showed a slight increase with the time since last meal (regression coefficient 0.014 mmol/min, $p = 0.03$). The post-load



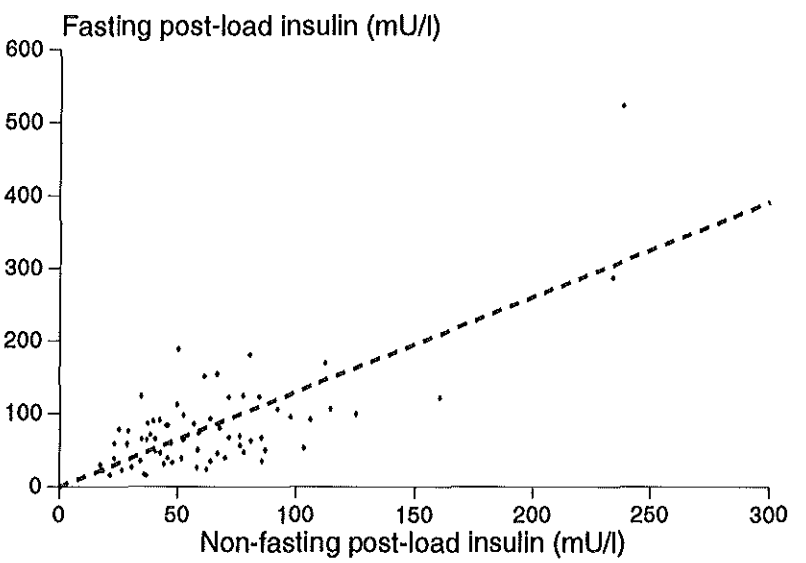


Figure 1 Serum glucose and insulin two hours after the non-fasting and fasting oral glucose tolerance test.
Dotted lines are regression lines.

Table 2 Linear regression analyses of glucose and insulin levels during fasting and non-fasting oral glucose tolerance test.

Serum glucose before glucose load (mmol/l)	0.25	(0.16 - 0.34)
Serum glucose after glucose load (mmol/l)	0.47	(0.33 - 0.62)
Serum insulin before glucose load (mU/l)	0.11	(0.04 - 0.18)
Serum insulin after glucose load (mU/l)	1.30	(1.01 - 1.59)

Dependent variable: fasting serum level, independent variable: non-fasting serum level.
Values are regression coefficients with 95% confidence interval in parentheses.

insulin levels were not associated with the time of the last food intake ($p = 0.80$). All associations were essentially the same after adjustment for age, gender and body mass index. In Figure 2 the random glucose and insulin levels are presented by the time since last meal. The figure shows that in this non-diabetic population random insulin levels were strongly influenced by the time since last meal, resulting in a stable random glucose level.

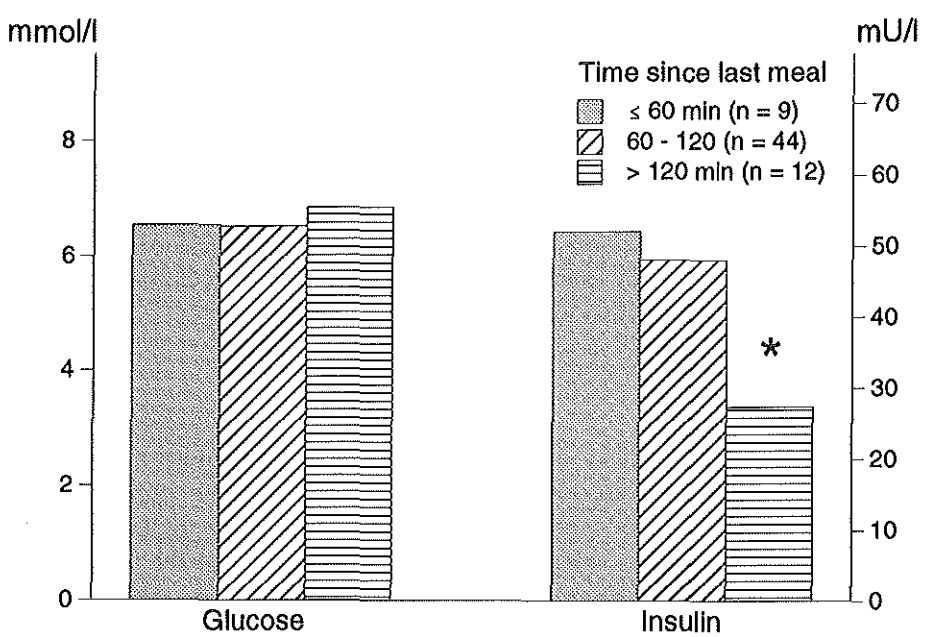


Figure 2 Non-fasting glucose and insulin levels by time since last meal.
* Test for trend: $p < 0.05$

Table 3 Classification of subjects with a normal and disturbed glucose tolerance according to the results of the fasting and non-fasting oral glucose tolerance test.

	Fasting		
	normal	disturbed*	
Non-fasting			
normal	51	2	53
disturbed†	9	7	16
	60	9	69

Values are the numbers of persons in each category.

* Impaired glucose tolerance and diabetes mellitus according to the WHO-criteria for the oral glucose tolerance test.[2]

† Non-fasting baseline glucose level higher then 8.0 mmol/l and/or post-load glucose level higher then 7.8 mmol/l.

The prevalence of disturbances of the glucose metabolism according to the fasting glucose tolerance test in the selected study group was 13% (8 persons with impaired glucose tolerance, 1 with diabetes mellitus). The optimal discriminatory cut-off levels of the baseline and post-load non-fasting glucose values were calculated as 8.0 and 7.8 mmol/l respectively. Using these cut-off levels a 2x2 table was constructed (Table 3). The sensitivity of the non-fasting test was 78% (95% confidence interval 68 - 88%), with a specificity of 85% (95% CI 77 - 93%). The positive predictive value was 44% (95% CI 32 - 56%), whereas the negative predictive value was 96% (95% CI 91 - 100%). If only the baseline non-fasting glucose level was used, 5 (in stead of 7) of the 9 persons with a disturbed glucose metabolism would be classified as abnormal.

Discussion

In this paper the non-fasting and fasting oral glucose tolerance tests were compared. The study showed that the glucose and insulin levels during the non-fasting OGTT were significantly associated with those during the fasting OGTT (for all associations $p < 0.001$). The glucose levels, but in particular the insulin levels two hours after the oral glucose load were comparable after the fasting and the non-fasting OGTT (regression coefficients 0.47 and 1.30 respectively). The post-load insulin levels of the non-fasting OGTT were hardly influenced by the time of the last meal. The non-fasting OGTT appeared to be a good predictor of a normal

glucose metabolism, using the fasting glucose tolerance test with the WHO-criteria as the gold standard. The sensitivity and specificity of the non-fasting OGTT were 78% and 85% respectively, with a positive predictive value of 44% and a negative predictive value of 96%.

The use of fasting blood samples to assess glucose metabolism is firmly based in the tradition of clinical practise. In epidemiologic studies, however, in particular in the framework of large scale population-based studies, a disadvantage of a fasting test is the restriction to early morning examinations. In these situations non-fasting measures of glucose and insulin could be useful.

The baseline value of a fasting OGTT is a fasting blood sample, whereas in a non-fasting test this is a random blood sample. Because the serum glucose and insulin levels are influenced by the intake of food, it can be expected that these values are not similar. Although the associations found were statistically significant, the regression coefficients were small (0.25 and 0.11 respectively). In non-diabetic subjects the serum glucose levels are maintained at a fairly constant level, which may partly explain the small regression coefficients. However, the association between insulin levels two hours after the fasting and non-fasting glucose load was good: regression coefficient of 1.30 with an intercept of -0.15. It can be assumed that the 75 grams glucose orally 'overrules' the differences between fasting and non-fasting.

The WHO guidelines from 1985 suggest that the fasting value of the glucose tolerance test is of limited significance: 97% of the persons who have a raised fasting glucose level ($\geq$ 7.8 mmol/l) also show a raised 2-hour glucose ($\geq$ 11.1 mmol/l).[2] The same conclusion emerges from a population survey in Rancho Bernardo, California: of the persons with diabetes according to the WHO-criteria 94% had raised 2-hour glucose levels.[6] However, in these studies the glucose load was given in the fasting state, in contrast with our study in which the OGTT was performed randomly over the whole day. Recently in Finland a study of an oral glucose tolerance test without fasting has been performed (J. Tuomilehto, personal communication). From this study a sensitivity of 80% and a specificity of 96% was reported for exclusion of a disturbed glucose metabolism. Moreover, the diagnosis of gestational diabetes is based on the one hour glucose levels after an oral glucose load of 50 grams given without respect to the time since last meal.[7]

A limitation of our study is that a relatively small number of subjects was studied. As a consequence the confidence intervals of sensitivity and specificity are wide. However, even in this limited group the approach did not result in a considerable misclassification of persons with a disturbed glucose tolerance: only 4% of them was classified as normal.

We used the fasting oral glucose tolerance test as the gold standard, but it is known that the inter-person variability of this 'gold standard' is rather high. In a population of elderly people examined annually for five years, the reliability coefficient of the diagnosis according to the WHO-criteria was 0.62.[8] Other researchers found that only about 50 percent of the oral glucose tolerance tests were reproducible.[9] Diabetes mellitus diagnosed by a single OGTT has a poor reproducibility.[10] As we have compared our findings in the non-fasting state to the fasting OGTT, it is important that the performance of the non-fasting test observed in our study is considered with the results of the above mentioned studies in mind. If the diagnosis of a disturbed glucose metabolism is confirmed by a second OGTT in 50% of the cases, we can expect the same predictive value of the non-fasting OGTT, which was found indeed (Table 3). Moreover, it remains to be shown what the clinical and pathophysiological significance is of elevated post-load levels in subjects that are tested in a non-fasting state, irrespective of the results of a fasting OGTT. The first population-based epidemiologic studies on risk factors of cardiovascular disease were performed before the guidelines of the WHO were established. Most of these studies used a non-fasting oral glucose tolerance test to evaluate the glucose metabolism. The glucose levels obtained in this way showed significant associations with the prevalence and incidence of cardiovascular disease.[11,12]

In any event, given the high negative predictive value it seems possible to fruitfully use the results of the non-fasting test as a first screening filter, leading to further diagnostic evaluation in only a subset of the population. Some of the patient unfriendly aspects of a glucose tolerance test are not removed with a non-fasting test, like the two hours waiting time and the unpalatable glucose drink. In studies performed in asymptomatic populations with a low prevalence of these disorders, however, the non-fasting test may have significantly logistic benefits at the expense of relatively little misclassification. The use of a fasting test and further diagnostic work-up could then be reserved for only a small proportion of eligible subjects.

In conclusion, the non-fasting oral glucose tolerance test may give appropriate estimates of the serum glucose and insulin levels two hours after the glucose load, comparable to those after the glucose load in a fasting situation. The test may be used to adequately select the majority of subjects with normal glucose tolerance, which suggest that it may be used as first screening instrument, especially in large scale population-based studies.

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Chapter 2

Insulin resistance: characteristics

It is well known that increased age, obesity and physical inactivity are characteristics of subjects with increased insulin resistance. In this chapter additional characteristics of insulin resistance are discussed. First some associations with the steroid metabolism are explored, based on the results of an additional endocrine examination in a sample of participants of the Rotterdam Study. This chapter starts with the association between cortisol and insulin resistance. Next the relation of insulin with another adrenal steroid, dehydroepiandrosterone sulfate (DHEAS), is presented. The effects of DHEAS differ markedly between men and women, therefore the presented analyses are restricted to men. The liver has a central role in the carbohydrate and lipid metabolism. The third part of the chapter describes the associations between liver function, assessed by serum liver enzymes, and insulin resistance in the total population of the Rotterdam Study.

Insulin resistance and hypertension: relationship with cortisol levels

Aging in man is associated with hyperinsulinaemia and increased insulin resistance,[1] which have been related to many different conditions frequently seen in the elderly, such as abdominal obesity,[2] impaired glucose tolerance,[3] and a gradual increase in blood pressure and the development of signs and symptoms of atherosclerosis.[4,5] The origin of insulin resistance and its relation with hypertension are poorly understood. Both conditions are present during glucocorticoid excess. Age-related changes in the activity of the hypothalamo-pituitary adrenal axis in animals strongly suggest that the decline in organ functions and body composition as well as the development of hypertension and atherosclerosis are more or less directly related to increased corticosteroid production, as well as to a diminished sensitivity to glucocorticoid feedback suppression.[6,7] Cortisol is an important glucose counter-regulatory hormone, which results in raised insulin resistance.[8]

To further examine the role of cortisol in the etiology of insulin resistance and hypertension in elderly subjects, we measured these variables in a group of 218 healthy non-hospitalized elderly men and women.

Materials and methods

Study population

For the present study, a sample of participants from the Rotterdam Study was invited for an additional examination. The Rotterdam Study is a population-based cohort study of the determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over are invited to participate as described elsewhere.[9]

The population for the present study included 219 persons aged 55 to 80 years, who had completed the baseline visit of the Rotterdam Study not more than six months earlier. Subjects with psychiatric or endocrine disease, including diabetes mellitus treated with medication, were not invited. The one person using hydrocortisone was excluded from all analyses. Compared to the other participants of the Rotterdam Study of the same age without known diabetes mellitus, there were no differences in age and gender distribution, mean blood pressure, use of antihypertensive medication, echocardiographic evidence of atherosclerotic plaques

in the carotid arteries, and electrocardiographic abnormalities. The participants were seen at the research centre after an overnight fast and blood was drawn between 8.00 and 9.00 AM. They were asked for any changes in their health status since the examination of the Rotterdam Study. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School.

Measurements

Blood was drawn by venepuncture and allowed to coagulate for 30 minutes. Subsequently serum was separated by centrifugation and quickly frozen in liquid nitrogen. Measurements included insulin, glucose, cortisol, and corticosteroid binding globulin (CBG). Glucose levels were determined by the glucose hexokinase method. Radioimmunoassays were used for determination of insulin and CBG (Medgenix diagnostics, Brussels, Belgium) and of cortisol (Diagnostic Product Corporative, Los Angeles, CA). Insulin resistance was assessed by the ratio of serum insulin over glucose. To estimate the levels of the free circulating cortisol the ratio of cortisol over CBG was used.

Blood pressure was measured with a random-zero sphygmomanometer, and the mean of two measurements was used in the analyses. Hypertension was defined as systolic blood pressure of 160 mmHg or over, or diastolic blood pressure of 95 mmHg or over, or use of anti-hypertensive medication. Body mass index was defined as weight divided by the square of height (kg/m^2), body fat distribution was assessed by the ratio of waist and hip circumferences.

Data analysis

Analysis of covariance was applied to estimate the age-adjusted differences in baseline characteristics between men and women, and between subjects with and without hypertension. Pearson correlation coefficients were calculated to assess the association between variables. Multiple linear regression analysis was used to estimate the associations between cortisol and insulin resistance. The analyses were performed both univariate and with adjustment for confounding variables, notably age, body mass index, and waist/hip ratio. Results are presented as regression coefficients with corresponding two-sided p-values.

Table 1 Baseline characteristics.

	Total		Men		Women	
	mean	(SD)	mean	(SE)	mean	(SE)
Number	218		102		116	
Age (years)	67.0	(5.9)	67.7	(0.56)	65.8	(0.57)
Body mass index (kg/m^2)	26.4	(3.7)	26.4	(0.29)	26.4	(0.39)
Waist/hip ratio	0.92	(0.09)	0.97	(0.005)	0.87	(0.009)
Hypertension*	30.3%		31.4%		29.3%	
Creatinine (μmol/l)	83.5	(16.4)	91.0	(1.73)	77.0	(1.12)
Glucose (mmol/l)	5.9	(1.0)	6.0	(0.10)	5.8	(0.08)
Insulin (mU/l)	13.5	(7.9)	14.3	(0.80)	12.9	(0.72)
Insulin/glucose ratio (mU/mmol)†	2.26	(1.19)	2.35	(0.12)	2.19	(0.11)
Cortisol (nmol/l)	523.6	(150.5)	520.3	(14.5)	526.6	(14.4)
CBG (nmol/l)	776.6	(206.2)	696.8	(12.9)	846.0	(21.9)
Cortisol/CBG ratio‡	0.70	(0.22)	0.76	(0.02)	0.64	(0.02)

SD = standard deviation, SE = standard error
CBG = corticosteroid binding globulin

* Systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or using antihypertensive medication.

† The insulin/glucose ratio is used as an estimate of insulin resistance.

‡ The cortisol/CBG ratio is used as an estimate of the free cortisol level.

Results

Baseline characteristics of the study population are given in Table 1. Men were slightly older then women. After adjustment for age, the waist/hip ratio and mean creatinine level were higher in men than in women ($p < 0.01$). Corticosteroid binding globulin (CBG) was higher in women than in men; this difference remained statistically significant after adjusting for age ($p < 0.01$). In Figure 1 CBG levels are given by 5-year age categories. The level in women was higher at all ages, but the difference decreased at higher ages. The age-adjusted ratio of cortisol over CBG was higher in men than in women ($p < 0.01$). These results were essentially the same after excluding the four women using estrogens.

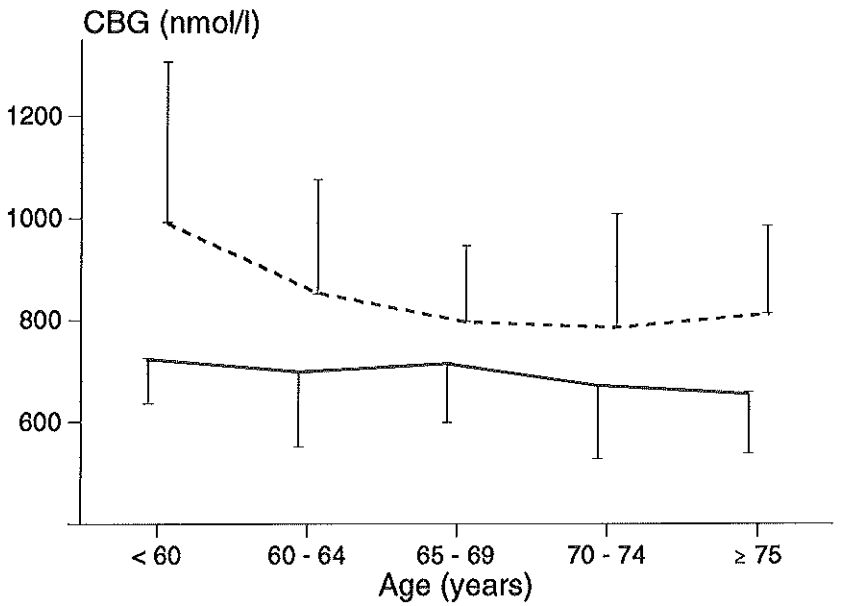

Figure 1 Corticosteroid binding globulin (CBG) by age and gender.
Values are means with standard error.

In women higher age was significantly associated with an increase in fasting serum insulin, serum glucose and insulin/glucose ratio (Table 2). In men only the association between age and serum glucose was statistically significant. An increased body mass index as well as an increased waist/hip ratio was associated with elevated insulin levels, glucose levels and an increased insulin/glucose ratio. In men, however, the association between serum glucose and body mass index did not reach statistical significance (Table 2).

Free cortisol, assessed by the ratio of fasting cortisol over corticosteroid binding globulin (CBG), was not associated with age in both sexes. The relation to body mass index was different in men and women. A significant inverse association was present in men which was not observed in women. Adjustment for serum creatinine levels did not change these findings. Body fat distribution, assessed by the waist/hip ratio, was not related to serum cortisol or the cortisol/CBG ratio.

In women an increased cortisol/CBG ratio was associated with increased serum insulin levels (age-adjusted regression coefficient 10.11 per mU/l insulin, 95% confidence interval 2.3 - 17.9). In men no association between cortisol/CBG ratio and insulin was found (1.50 per mU/l, 95% CI -5.0 - 8.0). After further adjustment for body mass index the regression coefficient was 7.84 in women

Table 2 Pearson correlation coefficients between selected clinical characteristics.

Men

	age	BMI	WHR
Glucose	0.22*	0.044	0.25¶
Insulin	0.092	0.26¶	0.22*
Insulin/glucose ratio†	0.028	0.29¶	0.18§
Cortisol	0.071	-0.35¶	0.082
Cortisol/CBG ratio‡	0.11	-0.26¶	0.13

Women

	age	BMI	WHR
Glucose	0.20*	0.25¶	0.25¶
Insulin	0.24¶	0.53¶	0.43¶
Insulin/glucose ratio†	0.22*	0.51¶	0.40¶
Cortisol	-0.21*	-0.086	-0.048
Cortisol/CBG ratio‡	0.16	0.10	0.045

BMI = body mass index
WHR = waist/hip ratio
CBG = corticosteroid binding globulin

§ $p < 0.10$, * $p < 0.05$, ¶ $p < 0.01$

† The insulin/glucose ratio is used as an estimate of insulin resistance.
‡ The cortisol/CBG ratio is used as an estimate of the free cortisol level.

(95% CI 1.0 - 14.6), and 4.14 in men (95% CI -2.3 - 10.5). Additional adjustment for the serum creatinine level did not change the relations. The same associations were found when the insulin/glucose ratio was used as an estimate of insulin resistance, and after further adjustment for waist/hip ratio. Figure 2 gives the serum insulin levels by quintiles of the cortisol/CBG ratio, adjusted for age.

Subjects with hypertension showed an increased insulin resistance (Table 3). In women this difference was no longer statistically significant after adjustment for body mass index. Cortisol levels were increased in men with hypertension with or without adjustment for body mass index. Adjusting for waist/hip ratio left the results essentially the same.

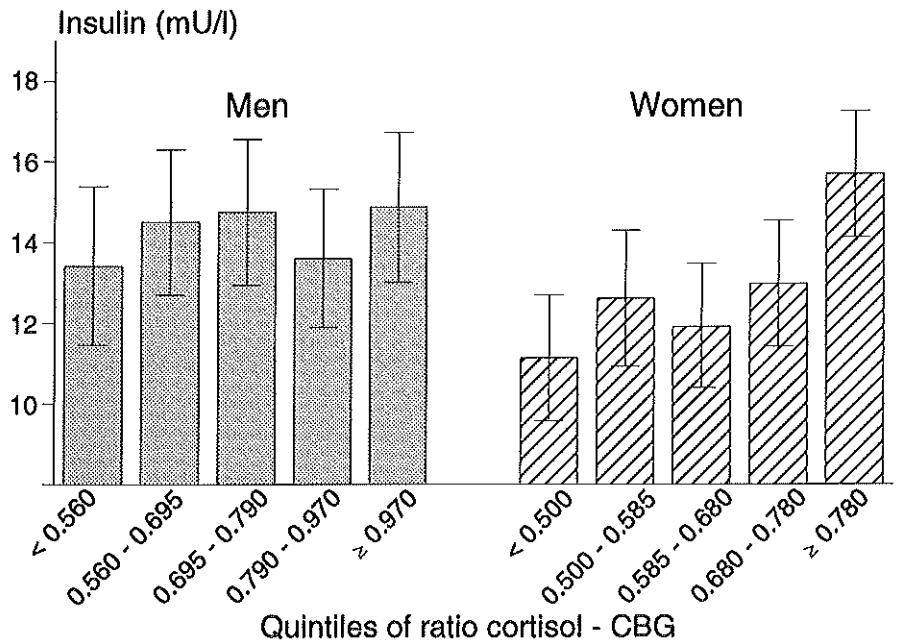

Figure 2 Serum insulin level by (gender-specific) quintiles of the ratio cortisol over corticosteroid binding globulin (CBG).
Values are means with standard errors, adjusted for age.
The cortisol/CBG ratio is used as an estimate of the free cortisol level.

Discussion

In this study of 218 healthy non-hospitalized elderly men and women a higher CBG level was found in women compared to men. The early morning free cortisol level did not change with age. In men an inverse association was present between cortisol and body mass index. In women higher cortisol levels were associated with increased insulin resistance. Men with hypertension had increased insulin and cortisol levels, whereas hypertensive women only showed increased insulin levels.

The approach used in a non-hospitalized population study has certain limitations which have to be considered before these findings can be discussed in more detail. There was a single measurement of the serum cortisol in each participant. The blood samples were obtained between 8.00 and 9.00 AM, and all participants were seen within one month, leaving little room for circadian or seasonal effects. The fasting serum insulin level is commonly used in epidemiologic

Table 3 Insulin and cortisol in subjects with and without hypertension.

Men

	Hypertension				p-value, adjusted for:	
	present		absent		age	age, BMI
Insulin (mU/l)	16.5	(1.27)	12.0	(0.84)	<0.01	<0.01
Insulin/glucose ratio (mU/mmol)†	2.63	(0.18)	2.05	(0.14)	0.01	0.01
Cortisol (nmol/l)	546.4	(21.0)	492.0	(19.2)	0.03	0.02
Cortisol/CBG ratio‡	0.82	(0.04)	0.72	(0.03)	0.03	0.03

Women

	Hypertension				p-value, adjusted for:	
	present		absent		age	age, BMI
Insulin (mU/l)	15.6	(1.34)	11.0	(0.72)	0.01	0.67
Insulin/glucose ratio (mU/mmol)†	2.57	(0.21)	1.93	(0.12)	0.03	0.86
Cortisol (nmol/l)	505.7	(19.6)	540.9	(20.1)	0.46	0.44
Cortisol/CBG ratio‡	0.65	(0.03)	0.64	(0.02)	0.94	0.67

Values are means with standard errors in parentheses.

BMI = body mass index
WHR = waist/hip ratio
CBG = corticosteroid binding globulin

† The insulin/glucose ratio is used as an estimate of insulin resistance.
‡ The cortisol/CBG ratio is used as an estimate of the free cortisol level.

studies as a measure of insulin resistance. It has been shown to have a consistent correlation with insulin resistance measured by the whole-body glucose uptake during clamp studies.[10] The use of the ratio of fasting insulin level over fasting glucose level to assess insulin resistance is based on the notion that in non-diabetic subjects insulin resistance results in a higher insulin level to preserve normoglycaemia.[11] To compare subjects in population-based studies, this ratio provides an adequate approximate measure.[12] Additional reason to include glucose levels in the measure of insulin resistance is the large variation in insulin-stimulated glucose uptake in individuals with a normal glucose tolerance.[13]

In women the total cortisol level decreased with age, but the CBG levels decreased as well. This was most evident in the women aged 55 to 65, which is likely to result from increased estrogen levels due to the recent menopause in these women. However, in this older population CBG levels remained significantly higher in women than in men at all ages (Figure 1). Because total cortisol level did not differ between men and women, men had an increased cortisol/CBG ratio. After adjustment for age, body mass index, waist/hip ratio or serum creatinine the difference between men and women remained statistically significant. This indicates that elderly men have higher free serum cortisol levels than elderly women. Most studies of cortisol in the elderly have been restricted to men. In a study of 15 healthy elderly men and women it was found that women had higher evening cortisol levels.[14] Further studies in elderly men and women are needed to clarify these gender differences.

Body mass index and body fat distribution are associated with insulin resistance,[15] which was confirmed in this study. The effect of obesity on cortisol is less clear. Abdominal obesity, indicated by a high waist/hip ratio, is a well known feature of Cushing's syndrome.[16] An increase in fat mass in healthy adults is accompanied by an increased adrenal production of cortisol, as well as by an accelerated metabolic clearance rate of cortisol. As a consequence, in obese subjects the urinary excretion of 17-hydroxycorticosteroids is increased, in the presence of normal plasma cortisol levels. However, also in obese individuals the urinary excretion of 17-hydroxycorticosteroids is normal when it is corrected for urinary creatinine excretion.[17,18] This suggests that the increased production of cortisol is a compensation for the increased metabolic clearance, which is probably a consequence of increased muscle mass in obesity, rather than of the increased fat mass.

In the present study there was an inverse association between cortisol and body mass index in men, but not in women. The reason of this discrepancy between the sexes is not immediately clear. On average, women have a larger increase in body fat with age than men.[19,20] Therefore, the negative correlation between cortisol and body mass index in men appears to be a consequence of the increased muscle mass. However, adjustment for the level of serum creatinine, an indicator of muscle mass, did not change the associations. This negative relation has also been found in previous studies in healthy middle-aged subjects.[21,22] Conversely, it has been proposed by Björntorp that stress, including smoking and alcohol consumption, may lead to hyperactivity of the hypothalamo-adrenal axis, inducing abdominal obesity and subsequently hyperinsulinemia and hypertension.[23] However, this is mainly based on animal and clinical studies. Our data obtained in

a population of healthy elderly subjects do not support the hypothesis of a metabolic syndrome caused by increased cortisol levels (Table 2).

Hypertension is a well known feature of increased insulin resistance and the resulting hyperinsulinaemia,[24] which was also found in our study (Table 3). Disorders with an increased secretion of corticosteroids are associated with a raised blood pressure.[25] We found higher cortisol levels in elderly hypertensive men, but not in women. One hypothesis is that cortisol-induced hypertension is a consequence of cortisol-induced insulin resistance. In a study of 6 men during 5 days this effect was not found.[26] However, it usually takes some time before the effects of corticosteroids are achieved.

The association between cortisol and raised insulin resistance has been demonstrated in other populations. Patients with Cushing's disease are insulin resistant.[27] Increased insulin resistance and diabetes mellitus are recognized side effects of the treatment with synthetic corticosteroids.[28] Animal studies and studies under laboratory conditions like hypoglycaemia and clamp-studies have shown an insulin-antagonistic effect of cortisol and other corticosteroids.[29,30,31] Moreover, in a population-based follow-up study fasting cortisol was associated with a deterioration of glucose tolerance.[32] The results of these and the presented studies indicate that serum cortisol may have an antagonizing effect on the action of insulin. It is well known that insulin resistance increases with age.[1,33] From the results of this study in elderly subjects it can be hypothesized that an increased cortisol level contributes to insulin resistance and associated hypertension. However, insulin and cortisol were assessed at the same time. As a result it is not possible to confirm a causal relationship between these two hormonal systems on the basis of the present study only.

In summary, the data presented suggest that in elderly persons serum cortisol may play a role in the pathogenesis of increased insulin resistance and hypertension. Further investigations are needed to elucidate the pathophysiologic and aging mechanisms involved in the relation between cortisol and the insulin resistance syndrome.

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Insulin and dehydroepiandrosterone sulfate in healthy elderly men: influence of smoking and alcohol consumption

The quantitatively most important C19 steroids produced by the adrenal gland are dehydroepiandrosterone (DHEA) and its sulfated conjugate DHEAS. It has been suggested that in men DHEA(S) has predominantly oestrogenic effects.[1] Androgens and corticosteroids raise insulin resistance, leading to higher insulin levels.[2] Oestrogens have the opposite effect. As a consequence, circulating insulin and DHEAS levels would be expected to be inversely associated in men, which indeed has been found.[3] Smoking increases serum androgens[4] and worsens insulin resistance;[5] alcohol consumption increases insulin resistance.[6] The effect of alcohol consumption on serum androgens is not well known, while also a potential modifying effect of smoking and alcohol on the association between insulin and DHEAS has not been studied. Moreover, because circulating insulin levels increase with age,[7] the relation between insulin and DHEAS may be different in elderly men.

Therefore we assessed the association between serum insulin and DHEAS concentrations in a group of 102 healthy non-hospitalized elderly men. In addition, the effect of smoking and alcohol consumption was examined.

Materials and methods

Study population

For the present study, 103 men from the Rotterdam Study were invited for an additional examination. The Rotterdam Study is a population-based cohort study of the determinants of chronic disabling diseases in the elderly as described elsewhere.[8] All inhabitants of a suburb of Rotterdam, aged 55 years and over were invited to participate. As part of the baseline examination the participants were interviewed in their homes by trained research assistants, using a computerized questionnaire. This included questions on smoking habits.

The men participating in the present study were 55 to 80 years old, and had completed the baseline visit of the Rotterdam Study not more than six months earlier. Subjects with psychiatric or endocrine disease, including diabetes mellitus treated with medication, were not invited. One subject with a DHEAS level of

15.0 μmol/l was considered to have subclinical disease and excluded from the present analysis. Compared to the other men of the Rotterdam Study of the same age without known diabetes mellitus, there were no differences in blood pressure, use of antihypertensive medication, evidence of atherosclerotic plaques in the carotid arteries by ultrasound, and electrocardiographic abnormalities. Participants were seen at the research center after an overnight fast and blood was drawn between 8.00 and 9.00 AM. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School.

Measurements

Fasting blood was drawn by venepuncture and allowed to coagulate for 30 minutes. Subsequently serum was separated by centrifugation and quickly frozen in liquid nitrogen. Glucose levels were determined by the glucose hexokinase method. Insulin and DHEAS were determined by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium and Diagnostic Products Corporation, Los Angeles, CA, respectively). Body mass index was defined as weight divided by the square of height (kg/m^2), body fat distribution was assessed by the ratio of waist and hip circumferences. The participants were asked to complete a food frequency questionnaire, which covered the previous year. Alcohol consumption was asked separately for weekdays and the weekend in three categories (beer, wine, liquor). Based on these answers, the mean pure alcohol consumption was calculated in grams per day. One drink is roughly equivalent to 10 grams of alcohol.

Data analysis

Analysis of covariance was applied to estimate the age-adjusted mean values by smoking habits and alcohol consumption. The associations between glucose, insulin and DHEAS were assessed by multiple linear regression analysis to adjust for potential confounding variables, notable age, body mass index, and waist/hip ratio. The relation between insulin and DHEAS was also analyzed in strata of smoking habits and alcohol consumption. Results are presented as regression coefficients with standard errors and corresponding two-sided p-values.

Table 1 Baseline characteristics of the study population.

Number	102	
Age (years)	67.7	(5.6)
Body mass index (kg/m^2)	26.4	(2.9)
Waist/hip ratio	0.97	(0.05)
Current smokers	29%	
Former smokers	67%	
Never smokers	4%	
Alcohol consumption		
never	12%	
< 10 gr/day	30%	
10 - 20 gr/day	20%	
≥ 20 gr/day	38%	
Glucose (mmol/l)	6.0	(1.1)
Insulin (mU/l)	14.2	(8.1)
DHEAS (μmol/l)	3.96	(2.17)

Values are means with standard deviation.

DHEAS = dehydroepiandrosterone sulfate

Results

Baseline characteristics of the study population are given in Table 1. Serum DHEAS slightly decreased with age (0.06 μmol/l per year, SE 0.04, $p = 0.14$). DHEAS was not associated with body mass index or waist/hip ratio ($p > 0.5$). Serum DHEAS was higher in smokers than in non-smokers, both former and never smokers (Figure 1). In men who smoked, DHEAS increased with the number of cigarettes smoked, without, however, reaching statistical significance. Fasting insulin levels showed an inverse association with smoking: the highest levels were found in subjects who never smoked, and the lowest level in current smokers (Figure 1). Alcohol consumption was also significantly associated with serum DHEAS levels (Figure 2). Men with the highest alcohol consumption had the highest DHEAS levels. For the association between serum insulin and alcohol consumption again an opposite trend as with DHEAS was found (Figure 2) without, however, reaching statistical significance. In Table 2 DHEAS levels are given for combinations of smoking and drinking habits. The results in this table show that the observed

Table 2 DHEAS levels by smoking status and alcohol consumption.

	Alcohol consumption				p-value
	Yes		No		
Smoking					
Yes	5.27	(0.42)	4.68	(0.49)	< 0.10
No	3.59	(0.26)	2.09	(0.34)	< 0.05
p-value	< 0.01		< 0.01		

Values are age-adjusted means with standard error.

differences were independently of each other. Fasting serum glucose was not associated with smoking or alcohol consumption. Further adjustment for body mass index or body fat distribution did not change these results.

Fasting insulin was inversely associated with DHEAS levels (coefficient of linear regression -0.062 μmol/mU, SE 0.026, p = 0.02, adjusted for age). When analyses were performed according to smoking status serum DHEAS appeared to be negatively associated with insulin concentrations only in men who smoked (Table 3). The associations did not differ in strata of alcohol consumption. Also further adjustment for body mass index or waist-hip ratio did not change the associations.

Table 3 Associations between fasting serum insulin and DHEAS by smoking status.

	coeff	(SE)	p-value
Current smoking			
Yes	-0.16	(0.063)	0.02
No	-0.02	(0.027)	0.44

Values are age-adjusted regression coefficients in μmol/l DHEAS per mU/l insulin with standard errors in parentheses and the appropriate p-values.

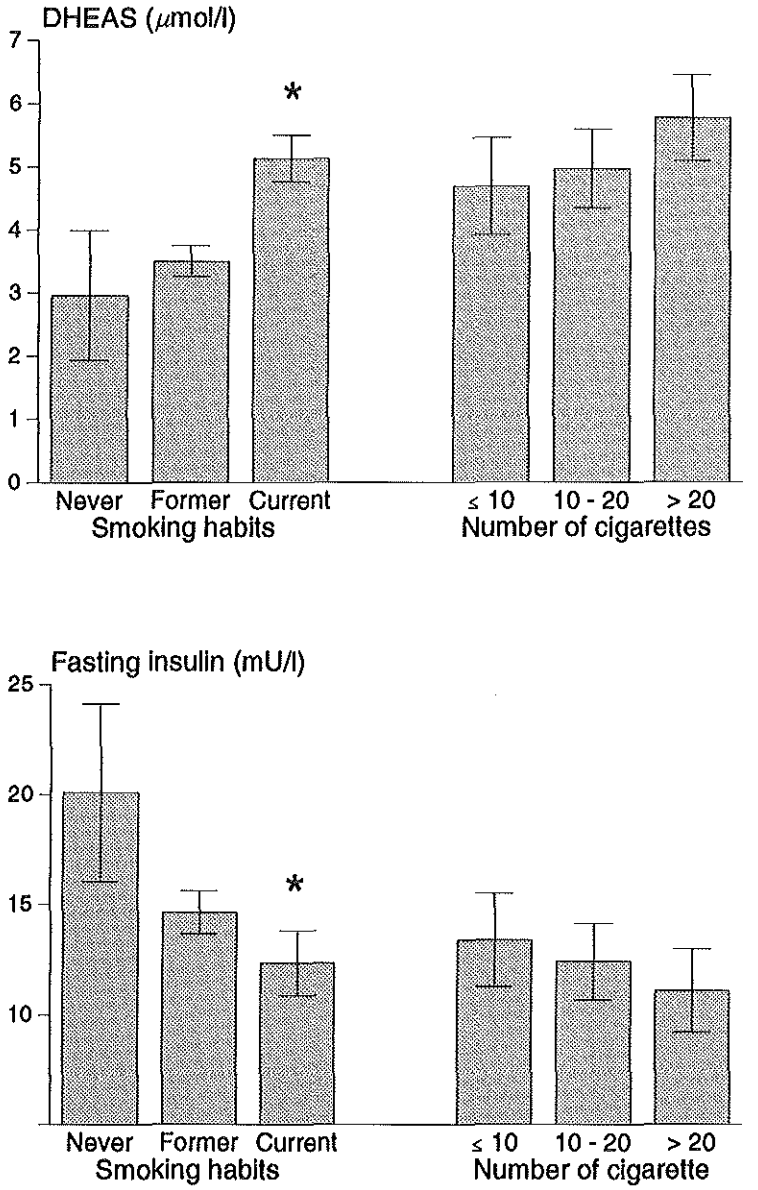
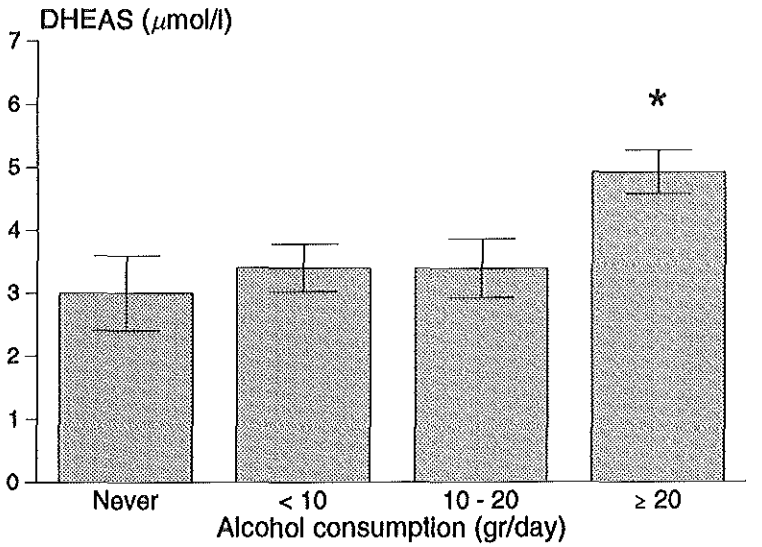


Figure 1 Dehydroepiandrosterone sulfate (DHEAS) and fasting serum insulin by smoking habits and number of cigarettes currently smoked.

Values are age-adjusted means with standard errors.

* Test for trend $p < 0.05$



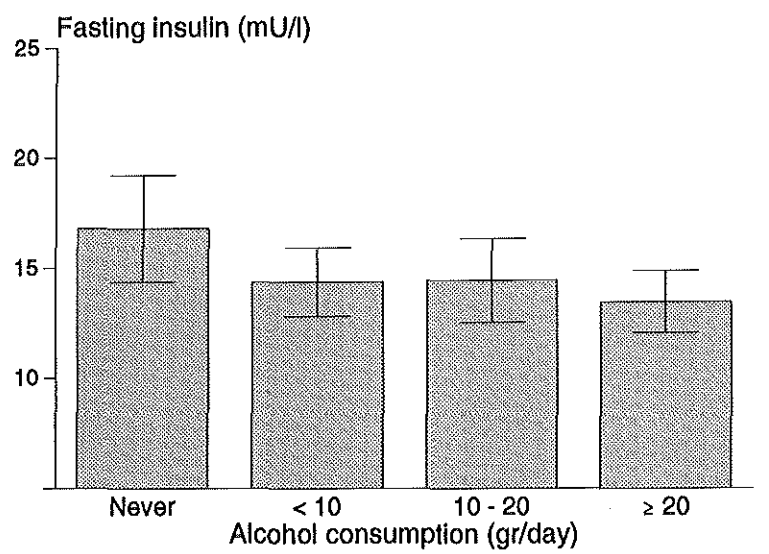


Figure 2 Dehydroepiandrosterone sulfate (DHEAS) and fasting serum insulin by alcohol consumption.
Values are age-adjusted means with standard errors.
* Test for trend $p < 0.05$

Discussion

In this study of 102 healthy non-hospitalized elderly men we found that smokers and men using alcohol have raised DHEAS and lower insulin levels. Insulin was inversely associated with DHEAS. Further analyses suggested that this association is restricted to smokers, whereas alcohol consumption has no influence.

All measurements were performed in single fasting blood samples obtained between 8.00 and 9.00 AM, and all participants were seen within one month, leaving little room for circadian or seasonal effects. The fasting serum insulin level is commonly used in epidemiologic studies of non-diabetic subjects as a measure of insulin resistance, as it has been shown to have a consistent correlation with insulin resistance measured by the whole-body glucose uptake during clamp studies.[9,10]

Within the age range reflected in the study (55 - 80 years) the decrease in DHEAS with age was small, which is in accordance with results from studies across a wider age range. From those studies a sharp decrease in DHEAS between 20 and 50 years was reported whereafter the decline diminishes.[11,12] This may reflect a decreased 17,20-desmolase activity.[13] Apart from age and gender there appears to be a strong genetic component which influences the levels of DHEAS, as well as other sex-hormones.[14]

The increased serum DHEAS in male smokers (Figure 1) has also been found in other populations.[15] Smoking acutely increases the secretion of some adrenal steroids, including DHEAS.[16] Increasing glucose counterregulatory hormones may account for the decreased insulin resistance in smokers (Figure 1).[5,17,18] To our knowledge there is no report in the literature concerning the association between alcohol consumption and DHEAS. Table 2 shows that this effect is independent of smoking habits. The lower insulin levels in subjects with moderate alcohol consumption have been described before.[6,19]

Several investigators have reported an inverse association between insulin and DHEAS in men.[3,20] Men with non-insulin dependent diabetes mellitus, an insulin resistant state, also have lower serum DHEAS.[21] Moreover, reduction of serum insulin in non-diabetic men with metformin increased the DHEAS levels.[22] It has been found that insulin increases the metabolic clearance rate of DHEA, which might be the result of the vasodilatory action of insulin.[20] In addition, the association may be explained by the inhibitory effect of insulin on the male adrenal 17,20-lyase activity.[23] Smoking and hyperinsulinemia therefore might decrease the synthesis of DHEAS by different mechanisms, which may work synergistic. This could explain the differences in the relation between DHEAS and insulin resistance between smokers and non-smokers.

The analyses presented here are based on cross-sectional data, and no direct causal relationship can be inferred. However, in prospective studies increased serum DHEAS has been associated with reduced occurrence cardiovascular disease and mortality in men.[16,24] Insulin resistance and cardiovascular diseases are promoted by androgens, but prevented by oestrogens, as evidenced by the finding that oestrogens in post-menopausal women may protect against the development of cardiovascular diseases.[25] The inverse association with insulin and the potential favourable cardiovascular effects of DHEAS support the hypothesis that in elderly men the effects of DHEAS are oestrogenic.[1] In spite of the apparent positive effect of smoking on DHEAS, there is abundant evidence that the net effect of smoking on the cardiovascular is disastrous.[26]

In conclusion, the data presented here support the hypothesis that in men insulin decreases serum DHEAS, which is in accordance with the idea that DHEAS has predominantly oestrogenic effects in men. In addition we found that smoking affects both serum DHEAS and insulin, as well as their interrelationship. Further investigations are needed to elucidate the pathophysiologic mechanisms involved in the relation between insulin resistance, steroids and aging.

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Liver enzymes and insulin resistance

An increased frequency of diabetes mellitus in patients with liver disease has been known for a long time.[1] The majority of patients with liver cirrhosis develop glucose intolerance: about 50-80% have impaired glucose tolerance and up to 30% overt diabetes mellitus.[2] Hyperinsulinemia is also a common finding in cirrhosis. Studies using the euglycemic clamp technique have shown increased insulin resistance in cirrhotic patients,[3] but its etiology remains unknown. Glucose intolerance has also been found in other forms of liver disease, like acute liver failure[4] and alcohol abuse.[5] The liver has a central role in glucose homeostasis; it is the only site of glucose production. Liver dysfunction has been proposed as a causal factor of non-insulin dependent diabetes mellitus.[6] However, little is known of the relation between liver enzymes and glucose metabolism in subjects without liver disease.

Insulin levels and the prevalence of diabetes mellitus increase with age.[7] Therefore, we studied the association between liver enzymes and insulin resistance in a large population-based study in the elderly.

Subjects and methods

Study population

The Rotterdam Study is a population-based cohort study of determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over were invited to participate. An outline of the study and its objectives has been published previously.[8] The baseline examination of the Rotterdam Study was conducted from 1990 to 1993. The participants were interviewed in their homes by trained research assistants, using a computerized questionnaire. This included an assessment of current medication use. Subsequently the participants came to the research center for several measurements. In addition, an oral glucose tolerance test was performed. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School. Overall 7983 participants were examined in the Rotterdam Study (response rate 77.7%). Standard clinical chemistry measurements were performed during the first two years of the study only. The analyses in this article are restricted to the 4178 subjects in whom serum insulin levels and liver enzymes were measured.

Measurements

The participants came to the research center throughout the day. Blood was drawn by venepuncture and subjects not using antidiabetes medication (tablets or insulin) received a glucose drink of 75 grams of glucose. Two hours later a second blood sample was obtained. Glucose levels were measured in both samples by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium) in the post-load sample only. Diabetes mellitus was defined as the use of antidiabetes medication or a random or post-load glucose level greater than 11 mmol/l. Insulin resistance was assessed by post-load insulin level and the ratio of post-load insulin over glucose. Blood chemistry measurements were performed using standard methods. The assessed liver enzymes included alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALKPH), gamma glutamyltransferase (γGT), and lactate dehydrogenase (LDH). The mean pure alcohol consumption was calculated from a food frequency questionnaire, which covered the previous year. Body mass index was defined as weight divided by the square of height (kg/m^2), and the ratio of waist and hip circumferences was used as measure of body fat distribution.

Data analysis

The baseline characteristics of the study population were compared between men and women after adjustment for the different age-distribution, using analysis of covariance. The associations of liver enzymes and insulin resistance were assessed by multiple linear regression analyses, to adjust for potential confounding variables, notable age, body mass index, and body fat distribution. The analyses were also performed in subjects with liver enzymes below the upper limit of clinical reference values. Age-adjusted mean values (with standard errors of the mean) of post-load insulin by categories of liver enzymes were calculated by analysis of covariance. In addition, the attributable risk was calculated of liver dysfunction for increased insulin resistance, defined as the upper quintile of the post-load insulin distribution. The age-adjusted prevalence of increased insulin resistance in subjects with normal serum liver enzymes was subtracted from the prevalence in those with raised serum liver enzymes and subsequently divided by this prevalence.

Table 1 Baseline characteristics.

	Total		Men		Women	
	mean	SD	mean	SE	mean	SE
Number	4178		1611		2567	
Age (years)	69.5	(8.9)	68.5	(0.2)	70.1	(0.2)
Body mass index (kg/m^2)	26.4	(4.1)	25.8	(0.1)	26.7	(0.1)
Serum glucose (mmol/l)	6.7	(2.2)	6.9	(0.1)	6.6	(0.1)
Post-load insulin (mU/l)	63.2	(54.4)	56.4	(1.3)	67.5	(1.1)
Insulin resistance (mU/mmol)*	9.0	(6.7)	8.5	(0.2)	9.4	(0.1)
Diabetes mellitus†	7.5%		7.2%		7.7%	
Albumin (g/l)	42.9	(2.7)	43.1	(0.1)	42.7	(0.1)
Total protein (g/l)	71.7	(5.1)	71.8	(0.1)	71.6	(0.1)
ALAT (U/l)	18.3	(12.8)	19.4	(0.3)	17.7	(0.3)
ASAT (U/l)	21.0	(14.0)	21.4	(0.4)	20.6	(0.2)
ALKPH (U/l)	79.6	(26.3)	78.0	(0.7)	80.6	(0.5)
γGT (U/l)	30.0	(30.2)	34.9	(0.8)	20.6	(0.2)
LDH (U/l)	327.0	(63.5)	316.8	(1.6)	333.3	(1.2)

SD = standard deviation, SE = standard error

* Ratio of post-load insulin over glucose.

† Random or post-load glucose ≥ 11.1 mmol/l or using antidiabetes medication.

ALAT = alanine aminotransferase
ASAT = aspartate aminotransferase
ALKPH = alkaline phosphatase
γGT = gamma glutamyltransferase
LDH = lactate dehydrogenase

Results

The baseline characteristics of the study population are given in Table 1. After adjustment for age, the variables were significantly different between men and women ($p < 0.01$), except for the prevalence of diabetes mellitus and the level of total protein ($p > 0.5$). No subjects with severe liver disease were included in the study population, which is illustrated by the normal serum albumin (range: 27.9 - 60.0 g/l) and total protein (49.0 - 102.0 g/l) levels (Table 1).

Table 2 Associations between post-load insulin (mU/l) and liver parameters.

2a Total study population

	Men		Women	
ALAT (U/l)	0.58	(0.34 - 0.82)	0.81	(0.63 - 0.99)
ASAT (U/l)	0.19	(0.03 - 0.35)	0.44	(0.24 - 0.64)
ALKPH (U/l)	-0.12	(-0.22 - -0.02)	0.09	(0.01 - 0.17)
γGT (U/l)	0.19	(0.11 - 0.27)	0.21	(0.13 - 0.29)
LDH (U/l)	-0.01	(-0.05 - 0.03)	-0.004	(-0.04 - 0.04)

2b Subjects with elevated liver enzymes excluded

	Cutoff value	Total excluded	Men		Women	
ALAT (U/l)	35	183	0.89	(0.48 - 1.30)	1.44	(1.09 - 1.79)
ASAT (U/l)	35	117	0.86	(0.31 - 1.41)	1.30	(0.83 - 1.77)
ALKPH (U/l)	300	8	-0.13	(-0.26 - 0.01)	0.12	(0.02 - 0.22)
γGT (U/l)	50	371	0.87	(0.60 - 1.14)	1.38	(1.13 - 1.63)
LDH (U/l)	525	31	-0.02	(-0.06 - 0.02)	-0.005	(-0.04 - 0.03)

Values are age-adjusted regression coefficients with the 95% confidence interval in parentheses.

In Table 2 the age-adjusted associations between liver enzymes and post-load insulin concentrations are given. In both men and women liver enzymes were significantly associated with insulin, except for LDH. After excluding subjects with liver enzymes above the clinical reference values, the associations were even stronger (Table 2b). Higher body mass index was positively associated with most liver enzymes (data not shown). However, the results were essentially the same after further adjustment for body mass index, body fat distribution, or after excluding subjects with diabetes mellitus. If the ratio of post-load insulin over glucose was used as measure of insulin resistance the results did not change either. Figure 1 gives the age-adjusted insulin levels by categories of liver enzymes. The figure shows that the association is constant over the whole range of values.

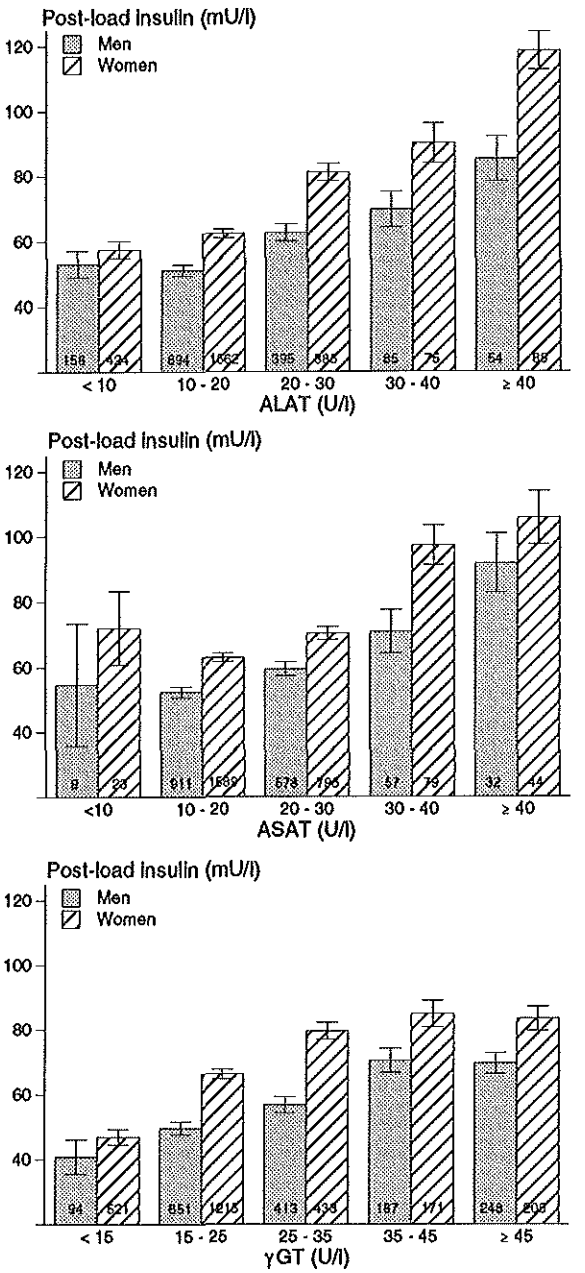

Figure 1 Post-load insulin levels in categories of liver enzymes.
Values are age-adjusted means with standard errors. The numbers at the bottom of each bar indicate the number of subjects in that category.

In subjects who used alcohol no association between insulin and the amount of alcohol was found ($p > 0.5$). Liver enzymes did not show a strong association with the amount of alcohol consumed, but all enzymes were increased in those subjects using more than 30 grams of alcohol per day (roughly equivalent to three drinks). The associations between liver enzymes and insulin resistance did not change after adjustment for alcohol use.

Additional analyses were performed using dichotomous variables. Liver dysfunction was defined as one or more liver enzymes above the clinical reference values, whereas increased insulin resistance was defined as the upper quintile of the gender-specific, age-adjusted distribution of post-load insulin levels. The attributable risk of liver dysfunction for the presence of raised insulin resistance was 0.39 in men and 0.45 in women.

Discussion

In this study of healthy elderly men and women we found that raised serum levels of liver enzymes were associated with an increased insulin resistance, assessed by the post-load insulin levels and the ratio of post-load insulin over glucose. These association were independent of gender, age, body mass index, body fat distribution and alcohol use. About 40% of the elevated insulin resistance could be attributed to liver dysfunction.

In the Rotterdam Study no fasting blood sample was obtained, the insulin levels were measured after a non-fasting oral glucose load. We reported previously that these insulin levels are similar to the fasting post-load levels.[9] Insulin resistance was assessed by the ratio of post-load insulin over glucose. In subjects without diabetes mellitus this ratio is a good measure of insulin resistance.[10]

Serum transaminases are considered to provide specific measures of liver function, whereas ALAT is more liver specific than ASAT.[11] This liver-specific enzyme showed the strongest association with insulin resistance in this study. In clinical practice ALKPH, γGT and LDH are used as markers of liver function, but these enzymes are present in a number of other tissues, in particular in muscle and bone, and their elevation does not always indicate liver dysfunction.[11] To assess liver function in healthy asymptomatic subjects this may be more important than in patients with liver disease.

Subjects with abdominal obesity, which is associated with raised insulin resistance, have increased hepatic steatosis.[12] Hyperinsulinemia is a common finding in patients with liver disease.[2] Insulin resistance, as measured by the euglycemic clamp technique, has been reported in patients with liver cirrhosis[13] and in alcoholics.[5] Several studies have indicated that in liver disease a decreased

hepatic insulin uptake is responsible for the increased peripheral insulin level.[14] In addition, patients with liver cirrhosis have an increased portocaval and intrahepatic shunting, which may contribute to an increase in peripheral insulin levels.[15] Another possible explanation is substrate competition between glucose and free fatty acids (FFA), known as the Randle cycle.[16] Indeed FFA levels are increased in subjects with liver disease. Also an increased insulin secretion has been suggested.[4,17] It is important to realise that one can not distinguish between these different forms of liver dysfunction by assessment of elevated serum liver enzymes only. Hyperinsulinemia itself can induce insulin resistance,[18,19] which explains the similar results when the ratio of insulin over glucose is used.

An association between liver enzymes and insulin resistance has been reported in subjects with impaired glucose tolerance[20] and with untreated hypertension.[21] In a follow-up study of middle-aged men disturbed liver function was associated with the development of non-insulin dependent diabetes mellitus (NIDDM).[22] In elderly subjects without apparent liver disease liver function abnormalities may be one of the explanations for the development of NIDDM. Both peripheral (muscle, fat) and hepatic insulin resistance are present in subjects with impaired glucose tolerance.[23] It has been suggested that the liver might be the most important site of insulin resistance in NIDDM.[24] The suppression of hepatic glucose production by insulin is impaired, leading to higher glucose levels, especially in the fasting state. Moreover, the diminished hepatic insulin clearance results in peripheral insulin resistance, which precedes NIDDM.[25] Both hyperglycemia and hyperinsulinemia may impair ß-cell function, leading to diabetes mellitus.[2]

In summary, this study indicates an association between serum liver enzymes and insulin resistance in healthy elderly men and women. These results suggest that the liver may play a role in the development of NIDDM.

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Chapter 3

Insulin resistance syndrome

In subjects without diabetes mellitus insulin resistance is not only associated with raised insulin concentrations, but also with a number of cardiovascular risk factors, notably obesity, dyslipidemia and elevated blood pressure. This cluster of risk factors is called 'insulin resistance syndrome'. In the first part of this chapter the elements of this syndrome in elderly subjects are discussed, as well as the question which risk factor is the best marker of this cluster. The relation between insulin and hypertension is further elaborated in the remaining parts of the chapter. Several antihypertensive drugs are notorious for their effect on insulin resistance. Therefore the influence of these drugs on the associations of insulin resistance with blood pressure and age is evaluated. Finally some results from the follow-up of a population survey conducted in the Dutch town of Zoetermeer are presented: the risk of high blood pressure (an insulin resistant state) on the incidence of diabetes mellitus (a severe insulin resistant state).

Insulin as marker for the clustering of metabolic risk factors

The clustering of several metabolic cardiovascular risk factors in a single subject has in recent years been studied by several investigators.[1] The risk factors involved in this clustering are raised insulin, lowered HDL-cholesterol, raised triglycerides, obesity, increased abdominal fat and hypertension. The clustering has been referred to as Syndrome X,[2] the deadly quartet[3] and insulin resistance syndrome.[4,5] Most studies have so far been conducted in middle-aged subjects, often with some form of symptomatic cardiovascular disease present. Until now only a few investigators have studied the associations between risk factors without relating these to cardiovascular disease or NIDDM.[6] Furthermore it is not clear whether the same clustering is present at old age. For the etiology and possible prevention of this clustering the question of the nature of the primary abnormality is important. A common view is that insulin resistance is the underlying defect.

To examine whether and which cardiovascular risk factors cluster in elderly subjects, and which component best identifies this clustering, we measured risk factors in healthy non-hospitalized older men and women.

Material and methods

Study population

A sample of participants from the Rotterdam Study was invited for an additional study on metabolic and endocrine abnormalities and disease risk. The Rotterdam Study is a population-based cohort study of the determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over are invited to participate as described elsewhere.[7]

The population for the present study included 219 persons aged 55 to 80 years, who had completed the baseline visit of the Rotterdam Study not more than six months earlier. Subjects with psychiatric or endocrine disease, including diabetes mellitus treated with medication, were not invited. The participants were seen at the research center after an overnight fast. They were asked for any changes in their health status since the examination of the Rotterdam Study. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School.

Measurements

Blood was drawn by venepuncture and allowed to coagulate for 30 minutes. Subsequently serum was separated by centrifugation and quickly frozen in liquid nitrogen. All samples were measured in one assay to avoid inter-assay variability. Measurements included insulin, glucose, total and HDL-cholesterol, triglycerides, and free fatty acids. Insulin was determined by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium). LDL-cholesterol was calculated from the total cholesterol, triglycerides and HDL-cholesterol using the Friedewald formula.[8] Height and weight were measured with the participant wearing indoor clothing and no shoes. Waist circumference was measured midway between the lower rib and the iliac crest, while hip circumference was measured at the greater trochanter. Body mass index was defined as weight divided by the square of height (kg/m^2), body fat distribution was assessed by the ratio of waist and hip circumferences. Blood pressure was measured with a random-zero sphygmomanometer, and the mean of two measurements was used in the analyses. Hypertension was defined as systolic blood pressure of 160 mmHg or over, or diastolic blood pressure of 95 mmHg or over, or use of antihypertensive medication.

Data analysis

Analysis of covariance was used to estimate age-adjusted baseline characteristics in men and women, as well as age-adjusted prevalences in metabolic disorders. Pearson correlation coefficients were calculated to assess the association between the variables. The multiple R^2 of the linear regression model of each variable with the other variables was used to estimate the relative importance of a variable as a marker of the cluster. Analyses were performed with the BMDP statistical package.[9] Those subjects using antihypertensive medication (15 men and 19 women) were excluded from the analyses using blood pressure as a continuous variable.

Results

The baseline characteristics of the study population are given in Table 1. After adjustment for age, the waist/hip ratio was higher in men than in women ($p < 0.01$), but the body mass index was similar. Women had higher age-adjusted levels of LDL-cholesterol, HDL-cholesterol, and free fatty acids, whereas diastolic blood pressure was higher in men ($p < 0.01$).

Table 2 shows Pearson correlation coefficients between the variables. The strong correlations between insulin, glucose, HDL-cholesterol, triglycerides, body mass index, waist/hip ratio and systolic blood pressure indicate clustering of these variables in this elderly population. Analyses in men and women separately revealed

Table 1 Baseline characteristics.

	Total		Men		Women	
	mean	(SD)	mean	(SE)	mean	(SE)
Number	219		103		116	
Age (years)	66.7	(5.9)	67.6	(0.55)	65.8	(0.57)
Body mass index (kg/m^2)	26.4	(3.7)	26.4	(0.29)	26.4	(0.39)
Waist/hip ratio	0.92	(0.09)	0.97	(0.05)	0.87	(0.10)
Systolic blood pressure (mmHg)	139.3	(19.4)	141.1	(1.90)	137.6	(1.81)
Diastolic blood pressure (mmHg)	74.8	(9.9)	76.8	(0.98)	73.1	(0.90)
Hypertension†	47%		51%		41%	
Insulin (mU/l)	13.5	(7.9)	14.2	(0.80)	12.9	(0.72)
Glucose (mmol/l)	5.9	(1.0)	6.0	(0.10)	5.8	(0.08)
LDL-cholesterol (mmol/l)*	5.27	(1.19)	5.04	(0.10)	5.47	(0.12)
HDL-cholesterol (mmol/l)	1.35	(0.38)	1.17	(0.03)	1.51	(0.04)
Triglycerides (mmol/l)	1.96	(1.04)	2.01	(0.11)	1.91	(0.09)
Free fatty acids (mmol/l)	0.58	(0.25)	0.52	(0.02)	0.64	(0.03)

* Calculated with the Friedewald formula.

† Hypertension was defined as systolic blood pressure ≥ 160 mmHg, or diastolic blood pressure ≥ 95 mmHg, or use of antihypertensive medication.

the same pattern of correlations. If subjects using antihypertensive medication were included in the analyses, the coefficients with insulin, triglycerides, and body mass index also reached statistical significance.

Table 3 gives the R^2 of the regression model of each variable of the cluster with the remaining variables, adjusted for age. The R^2 estimates the approximate proportion of the variation in the variable explained by the variation in the cluster variables. This can be used as an estimate of the variation in the cluster explained by this variable. Overall insulin had the highest R^2, explaining 42% of the cluster variables. In women this was higher than in men, but the ranking order of variables was the same for both sexes (Table 3).

Table 2 Pearson correlation coefficients.

	Insulin	Glucose	LDL-chol	HDL-chol	Trigly-cerides	FFA	BMI	W/H ratio	Systolic bp
Insulin	1.00								
Glucose	0.46¶	1.00							
LDL-cholesterol	0.02	0.08	1.00						
HDL-cholesterol	-0.38¶	-0.19†	-0.16*	1.00					
Triglycerides	0.43¶	0.28¶	0.34¶	-0.49¶	1.00				
Free fatty acids	0.03	0.12§	-0.08	0.27‡	-0.02	1.00			
Body mass index	0.42¶	0.16*	0.07	-0.21†	0.19†	0.09	1.00		
Waist/hip ratio	0.34¶	0.25‡	-0.02	-0.42¶	0.24‡	-0.13§	0.31¶	1.00	
Systolic blood pressure**	0.11	0.23‡	0.03	0.05	0.08	0.13§	0.11	0.27‡	1.00
Diastolic blood pressure**	0.06	0.15*	-0.10	0.02	-0.07	0.02	0.13§	0.18*	0.55¶

§ $p < 0.10$, * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$, ¶ $p < 0.0001$.

** Subjects using antihypertensive medication (n=34) excluded.

Table 3 Multiple R^2 for the regression model with the listed variable as dependent variable and the other variables and age as independent variables.

	Total	Men	Women
Insulin	0.42	0.36	0.50
Glucose	0.27	0.32	0.25
HDL-cholesterol	0.37	0.33	0.36
Triglycerides	0.34	0.36	0.49
Body mass index	0.22	0.29	0.28
Waist-hip ratio	0.35	0.26	0.33
Systolic blood pressure	0.17	0.16	0.23

The subsequent analyses were performed according to the presence or absence of 'metabolic disorders', dichotomous variables defined by sex-specific upper quintiles of each variable included in the cluster. For blood pressure, the WHO-definition of hypertension was used. The cutpoints used to define the disorders resemble clinical cut-points. The frequency distribution of the disorders is given in Figure 1. The figure shows that the proportion of subjects with no disorder present, as well as the proportion of subjects with four or more metabolic disorders are higher than expected if the presence of metabolic disorders would be independent of each other. The number of other metabolic disorders present was highest in subjects with hyperinsulinemia compared to those with another disorder (Figure 2).

Discussion

In this study among 219 healthy non-hospitalized elderly men and women without diabetes mellitus, we observed that fasting insulin, glucose, HDL-cholesterol, triglycerides, body mass index, waist/hip ratio and systolic blood pressure were significantly interrelated. Moreover, insulin appeared to have the strongest relations with the other risk factors in both men and women.

The data presented were obtained in elderly subjects without known diabetes mellitus. The fasting serum insulin level is commonly used in epidemiologic studies as a measure of insulin resistance. In non-diabetic subjects it shows a good correlation with insulin resistance as measured by the whole-body glucose uptake.[10] The use of antihypertensive medication may have adverse effects on the glucose and lipid metabolism.[11] However, in the analyses with blood pressure as a

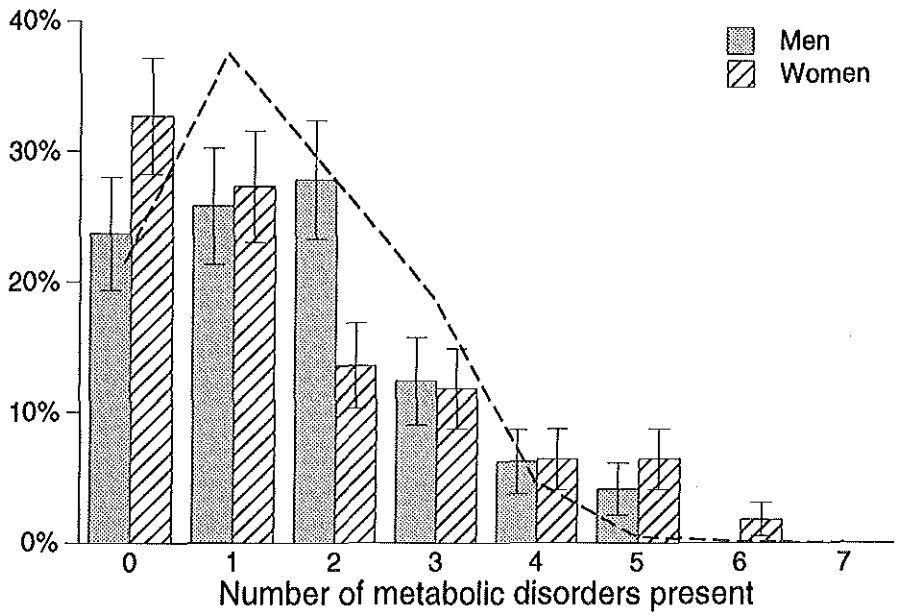

Figure 1 Proportion of the population with increasing numbers of metabolic disorders present.

For definition of metabolic disorders see text. Error bars indicate standard errors of the mean.

The dotted line represent the hypothetical proportions, assuming independency of the seven disorders. These proportions were calculated by the formula $0.20^{n} \times 0.80^{7-n} \times 100\% \times m$, where n is the number of metabolic disorders and m is the number of possible combinations (1 when no disorder present, 7 when one disorder present, 21 when two disorders are present, etc).

continuous variable, subjects using antihypertensive medication were excluded. As these analyses revealed the same pattern of associations as the analyses where hypertension was used (raised blood pressure or using antihypertensive medication), it is unlikely that the associations with serum insulin are confounded by the use of antihypertensive drugs.

In several previous studies the combined presence of multiple metabolic abnormalities in subjects with cardiovascular disease, notably coronary artery disease, and myocardial infarction was documented.[12,13] To some extent the results of these studies may have been influenced by the presence of cardiovascular disease and the prescribed medication. This is even more true for the presence of subjects with non-insulin dependent diabetes mellitus in the study population. Moreover, the levels of metabolic risk factors tend to rise with age, and as a

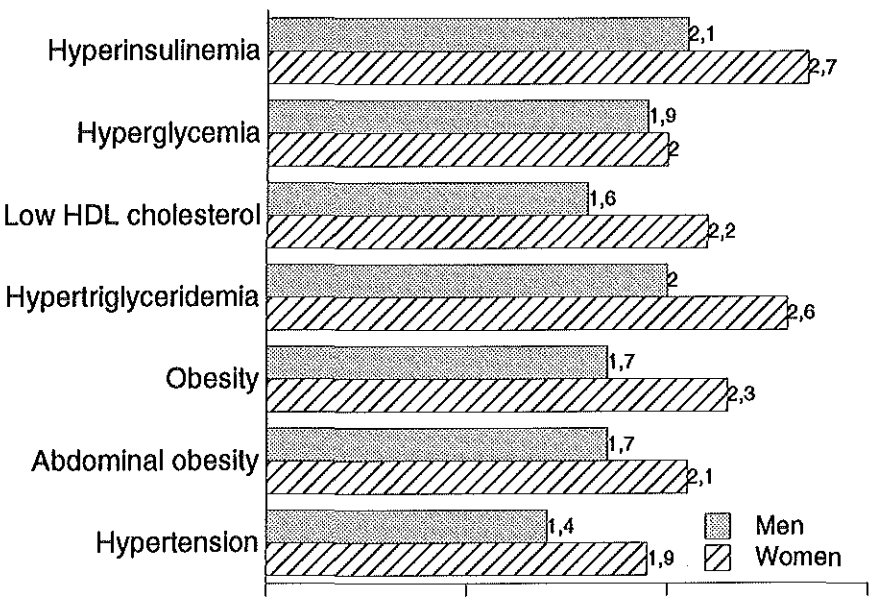

Figure 2 Graphic expression of the number of metabolic disorders present in subjects with the listed disorder.

consequence the prevalence of metabolic disorders is higher in the elderly.[14] The present study was performed in a population of elderly subjects without evident diseases. The results show that the pattern of clustering is the same as found in middle aged subjects. The strongest associations were found between insulin, HDL-cholesterol, triglycerides, and waist/hip ratio. These findings are in accordance with those obtained in a population-study of non-diabetic elderly in Finland,[15] as well as in a study in elderly women.[16]

Although a clustering of metabolic risk factors has been found in many studies, its etiology remains controversial. Several authors have proposed that insulin resistance is the underlying defect.[1,2,4] The ensuing increased insulin levels may inhibit lipoprotein lipase, which then results in increases in serum triglycerides and free fatty acids. A study in middle aged women showed that HDL-cholesterol and triglycerides are strongly associated with hepatic lipase and the ratio of hepatic lipase to lipoprotein lipase,[17] which has been shown to lead to an altered, more atherogenic, lipoprotein composition.[18] Moreover, there could be a direct relation of insulin with the development of atherosclerosis and hypertension by its effect on smooth muscle cells, the water and salt regulation by the kidneys, and its modification of cation fluxes across cell membranes.[19,20]

An alternative explanation is that increased abdominal fat is the driving force behind the cluster of metabolic disorders. In abdominal obesity, fat cells are hypertrophied and basal lipolysis increased which may stimulate VLDL production in the liver. Moreover, raised fatty acids in the portal circulation lead to an increase in lipoproteins with associated dyslipidemia, and may compete with the insulin mediated glucose metabolism of the liver with associated increase of insulin resistance.[21,5] Serum triglycerides also showed a strong association with the other metabolic risk factors (Table 3, Figure 2). However, it is difficult to conceive a mechanism that explains the clustering by increased triglycerides.[22]

Although a high correlation can both be explained by a real association and by the degree of variation in the variables used, the cross-sectional data of our study suggest hyperinsulinemia as the primary defect of the clustering. This has not yet been extensively examined in follow-up studies. One prospective follow-up study among Mexican-Americans and non-Hispanic whites showed that high serum insulin levels were associated with increased triglycerides, increased blood pressure and decreased HDL-cholesterol.[23] As discussed above, a raised fasting insulin level is regarded as an estimate of insulin resistance. If hyperinsulinemia is the primary defect, this would imply that resistance to the effects of insulin-mediated glucose uptake (primary in skeletal muscle) does not imply similar resistance to other metabolic effects of insulin.

If insulin resistance is a marker of the cluster of increased risk factors, one would expect an association between insulin levels and the development of cardiovascular disease. In several population based studies this association has been found indeed.[24,25,26] Still, this does not imply a direct effect of insulin on atherosclerosis. Further research is needed to elucidate to what extent the clustering variables have clinical implications, or are just statistically related to hyperinsulinemia, which makes insulin resistance a common denominator for different phenomena.

In summary, the results of this study demonstrate clustering of cardiovascular risk factors in healthy elderly persons, and suggest that fasting insulin levels may be a better marker for this cluster than waist/hip ratio.

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Insulin, hypertension and antihypertensive drugs

Raised insulin resistance and ensuing hyperinsulinemia in subjects with hypertension has repeatedly been demonstrated.[1,2] Most of these studies, however, have been performed in middle-aged subjects. Non-insulin dependent diabetes mellitus (NIDDM) is common in the elderly, as is the prevalence of hyperinsulinemia,[3] which precedes the onset of NIDDM.[4] Moreover, the prevalence of hypertension and use of antihypertensive drugs steeply increase with increasing age.[5] Because many antihypertensives influence glucose metabolism,[6] this may further worsen the metabolic condition in the elderly. On the other hand one may wonder whether the association between insulin and blood pressure is still present in those subjects who survive till older ages, and whether antihypertensive drugs still have an adverse effect on glucose metabolism in subjects with age-associated insulin resistance.

To answer these questions, we examined blood pressure, use of antihypertensive drugs and glucose metabolism in older subjects as part of the population-based Rotterdam Study. This paper describes the associations of insulin resistance with hypertension and the use of antihypertensive drugs.

Material and methods

Study population

The Rotterdam Study is a population-based cohort study of determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over were invited to participate. An outline of the study and its objectives has been published previously.[7] The baseline examination of the Rotterdam Study was conducted from 1990 to 1993. The participants were interviewed in their homes by trained research assistants, using a computerized questionnaire, which included an assessment of current medication use. Subsequently the participants came to the research center for several measurements, including blood pressure. In addition, an oral glucose tolerance test was performed, which was introduced a few months after the start of the study. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School. Overall 7983 participants were examined in the Rotterdam Study (response rate 78%). The present analyses are restricted to the

5453 subjects in whom serum insulin levels were measured and the presence of hypertension was known.

Measurements

During the home interview the participant was asked to show all medications she or he was using at that time. Information about generic and trade names was entered into the database using the ATC (Anatomical Therapeutical Chemical) classification index codes.[8] The data were verified by a physician at the research center, who also recorded the indication for the used medication. For the present analyses antihypertensive drugs were classified into diuretics (ATC code C03), ß-blockers (C07), ACE inhibitors (C02EA) and calcium antagonists (C02DE). Other antihypertensive drugs (eg α-blockers) were seldomly prescribed.

The participants came to the research center throughout the day. Blood was drawn by venepuncture and subjects not using antidiabetes medication (tablets or insulin) received a glucose drink of 75 grams of glucose. Two hours later a second blood sample was obtained. Glucose levels were measured in both samples by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium) in the post-load sample only. Diabetes mellitus was defined as use of antidiabetes medication or a random or post-load serum glucose level greater than 11 mmol/l. Insulin resistance was assessed by post-load insulin level and the ratio of post-load insulin over glucose. Blood pressure was measured with a random-zero sphygmomanometer, and the mean of two measurements was used in the analyses. Hypertension was defined as a systolic blood pressure of 160 mmHg or over, or a diastolic blood pressure of 95 mmHg or over, or current use of medication for hypertension. For body mass index weight divided by the square of height (kg/m^2) was used.

Data analysis

Analysis of covariance was used to estimate the age-adjusted baseline characteristics of subjects with and without hypertension, as well as adjusted mean values by groups of hypertension and antihypertensive drugs. Multiple linear regression analysis was used to assess the associations between age, blood pressure and insulin, as well as to adjust blood pressure level for use of antihypertensives. The results are presented as regression coefficients with 95% confidence interval. The difference in the use of antihypertensive drugs with increasing age was estimated by logistic regression analysis with odds ratios as an approximation of relative risk. Insulin resistance was compared between subjects with and without hypertension in strata of age by t-tests. The association between age and insulin was further examined by stratified linear regression analysis.

Table 1 Baseline characteristics of the total study population, and by presence or absence of hypertension.

	Total*		Hypertension†			
			Present		Absent	
Number	5453		1554		3899	
Women	59.7%		64.0%		58.0%	
Age (years)	68.8	(8.9)	71.0	(0.22)	68.0	(0.14)
Body mass index (kg/m^2)	26.3	(4.0)	27.2	(0.12)	26.0	(0.06)
Random serum glucose (mmol/l)	6.7	(2.2)	6.9	(0.06)	6.6	(0.03)
Post-load serum insulin (mU/l)	62.7	(52.8)	71.3	(1.45)	59.3	(0.81)
Insulin resistance (mU/mmol)‡	9.0	(6.5)	9.7	(0.17)	8.7	(0.10)
Diabetes mellitus§	7.1%		11.0%		5.5%	
Systolic blood pressure (mmHg)¶	138.8	(22.3)	160.0	(0.55)	130.4	(0.26)
Diastolic blood pressure (mmHg)¶	73.8	(11.5)	81.4	(0.30)	70.7	(0.16)
Use of antihypertensive medication	18.0%		58.9%			
Diuretics	16.9%		29.7%		9.7%	
ß-blockers	14.7%		29.9%		8.0%	
ACE inhibitors	6.0%		13.3%		2.7%	
Calcium antagonists	6.4%		9.2%		4.8%	

* Values are means with standard deviation in parentheses.

† Systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or use of antihypertensive medication. Values are means with standard error in parentheses.

‡ Assessed by the ratio of post-load insulin over glucose levels.

§ Random or post-load glucose level ≥ 11.1 mmol/l, only in subjects who did not use antidiabetes medication.

¶ Subjects using antihypertensive medication included.

Results

Hypertension was present in 28.5% of the participants, 560 men (25.5%) and 994 women (30.5%). In Table 1 the baseline characteristics of the study population are given by the presence of hypertension. All measures of glucose metabolism were

significantly higher in subjects with hypertension than in those without, which remained after adjustment for age, gender and body mass index ($p < 0.0001$).

Post-load serum insulin increased 0.87 mU/l by each year of age (95% confidence interval 0.71 - 1.03, adjusted for gender). In men and women the increase was 0.57 mU/l (95% CI 0.32 - 0.83) and 1.02 mU/l (95% CI 0.82 - 1.22), respectively. The results were essentially the same if the ratio of insulin over glucose was used as measure of insulin resistance. The proportion of subjects using antihypertensive drugs increased with age (increase 23% per 10 years of age, 95% CI 13 - 34). In men the corresponding relative risk was 1.14 (95% CI 0.98 - 1.34), whereas in women 1.26 (95% CI 1.14 - 1.40) was found.

Post-load insulin levels were associated with systolic blood pressure level; coefficient of linear regression 0.025 mmHg per mU/l (95% CI 0.015 - 0.035, adjusted for age, gender and antihypertensive drug use). For diastolic blood pressure the change was 0.007 mmHg per mU/l (95% CI 0.001 - 0.013). In men coefficients for systolic and diastolic blood pressure were 0.016 mmHg per mU/l (95% CI -0.004 - 0.036, adjusted for age and antihypertensive drug use) and 0.003 (95% CI -0.007 - 0.013), respectively. In women the corresponding regression coefficients were 0.029 mmHg per mU/l (95% CI 0.015 - 0.043) and 0.009 (95% CI 0.001 - 0.017). If subjects with diabetes mellitus were excluded from the analyses, the results remained essentially the same.

Subjects using antihypertensive drugs for hypertension had higher post-load insulin levels than those with hypertension but not currently using antihypertensives (74.9 vs 67.2 mU/l, $p < 0.01$). Mean insulin levels in subjects without hypertension were considerably lower than in both hypertensive subgroups (59.3 mU/l, for both comparisons: $p < 0.0001$). The same pattern was found in men and women separately, after adjustment for age and systolic blood pressure, or when the ratio of insulin over glucose was used as measure of insulin resistance. Insulin levels in subjects using antihypertensive drugs for angina pectoris (20% of the users) were the same as in subjects using these drugs for hypertension (60%). Subjects with antihypertensive drugs for heart failure (15%) had still higher insulin levels then subjects using these drugs for hypertension: 91.0 mU/l ($p < 0.01$, adjusted for age, gender and systolic blood pressure).

Table 2 gives some characteristics of the users of four major groups of antihypertensive drugs. As expected, in spite of the medication, mean blood pressure was higher in users compared to non-users. Users of antihypertensive drugs also on average had higher insulin levels. In Figure 1 the insulin levels, adjusted for systolic blood pressure, are given by four groups of antihypertensive drugs. In both men and women insulin levels adjusted for age and systolic blood pressure were significantly higher in those who used diuretics and ß-blockers than

Table 2 Selected clinical characteristics, by category of antihypertensive drug use.

	None		Diuretics		ß-blockers		ACE inhibitors		Calcium antagonists	
Number	3701		377		458		138		144	
Women	58.3%		76.7%†		58.1%		50.1%		54.2%	
Age (years)	67.7	(0.1)	74.8†	(0.5)	68.6*	(0.4)	69.3†	(8.2)	72.3†	(0.7)
Systolic blood pressure (mmHg)	136.9	(0.4)	142.5	(1.1)	143.5†	(1.1)	151.7†	(1.9)	142.3	(1.9)
Diastolic blood pressure (mmHg)	73.5	(0.2)	72.8	(0.6)	75.9†	(0.5)	79.0†	(1.0)	73.6	(1.0)
Hypertension‡	16.5%		51.5%†		58.5%†		73.2%†		40.3%†	
Post-load insulin (mU/l)	57.1	(0.8)	83.1†	(3.2)	68.8†	(3.0)	75.9†	(6.7)	69.4	(5.1)

Values are means with standard error in parentheses.
Subjects using combinations of antihypertensive drugs were excluded from the analysis.

* $p < 0.05$, † $p < 0.01$, compared to subjects without antihypertensive drugs, adjusted for age and gender.
‡ Systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or use of antihypertensive medication.

in subjects without antihypertensive drugs. This was also found for thiazides and loop-diuretics separately (data not shown). Women using ACE inhibitors also had higher insulin levels. The same results were found after adjustment of insulin levels for diastolic blood pressure.

In Figure 2 mean insulin levels are given by 5-year age-categories for subjects with and without hypertension. The number of subjects in each category ranged from 66 to 680. In women the difference in insulin levels decreased with age, and disappeared in the very old (75 years and older). When examined more closely, it appeared that the increase in insulin level with age was much more apparent in those without hypertension: in normotensive men insulin increased 0.61 mU/l per year (95% CI 0.32 - 0.90), whereas an increase of 0.40 (95% CI -0.15 - 0.95) was found in hypertensive men. This difference was more pronounced in women: 1.16 mU/l per year (95% CI 0.93 - 1.40) in normotensive women, versus 0.26 mU/l per year (95% CI -0.15 - 0.67) in women with hypertension. Figure 3 gives the

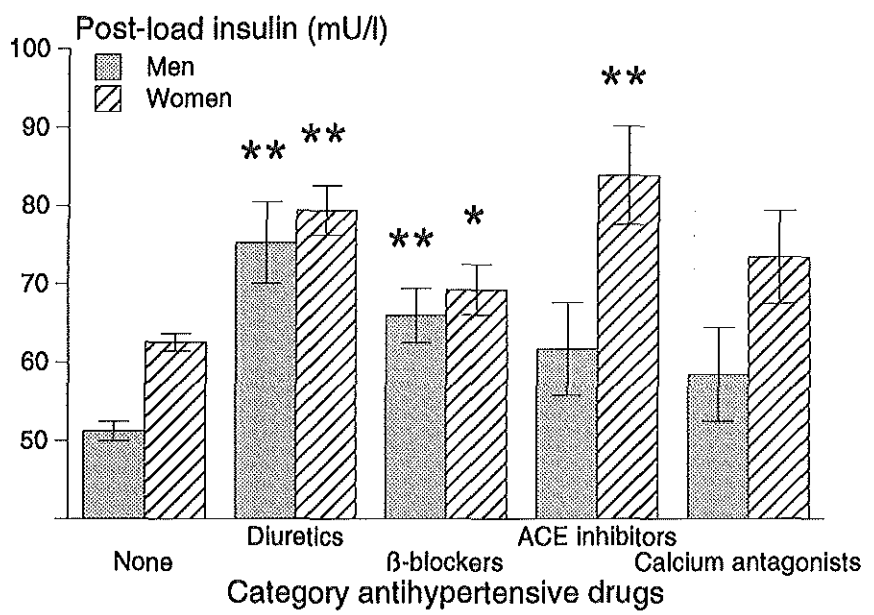

Figure 1 Mean post-load insulin levels, adjusted for systolic blood pressure, by group of antihypertensive drug use.

Values are means with standard error. Subjects using combinations of antihypertensive drugs were excluded from the analysis.

** $p < 0.01$ compared to subjects not using antihypertensive drugs, adjusted for age and systolic blood pressure.

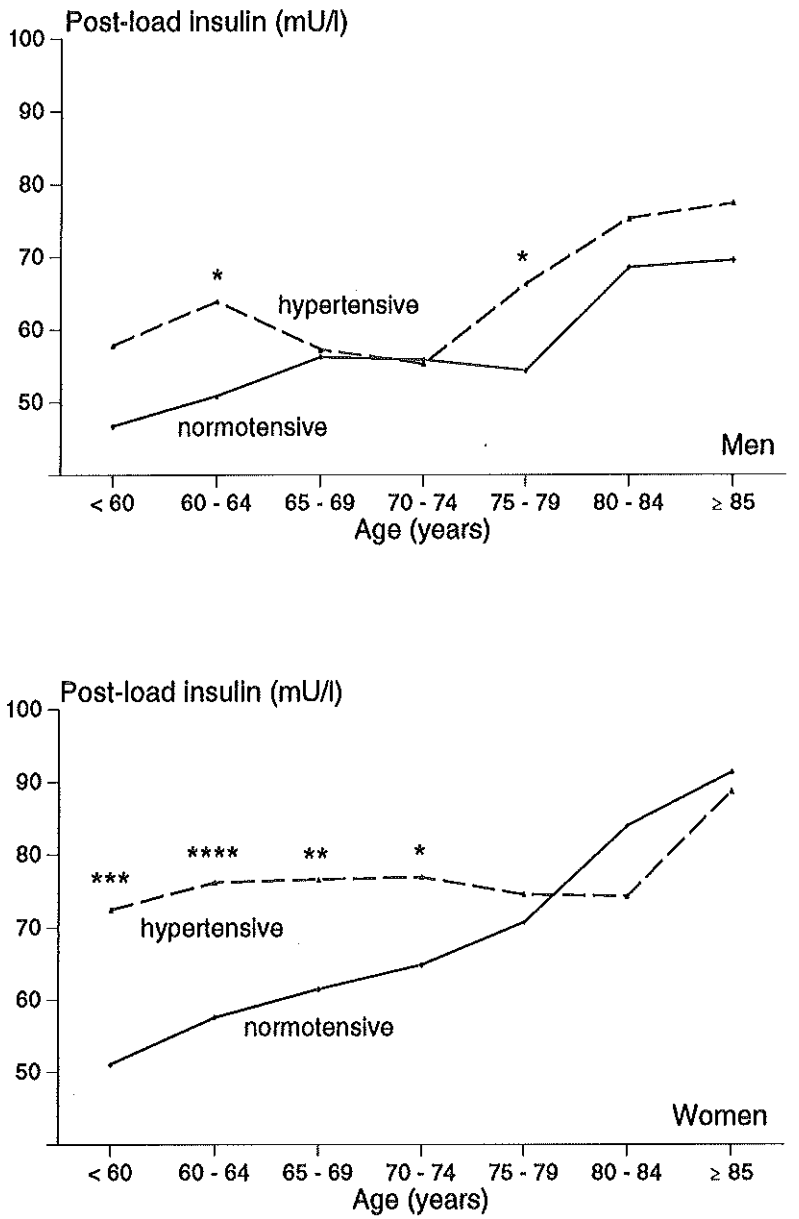

Figure 2 Mean insulin levels by 5-year age categories, for subjects with and without hypertension.

Difference between subjects with and without hypertension:
**** $p < 0.001$, *** $p < 0.005$, ** $p < 0.01$, * $p < 0.05$.

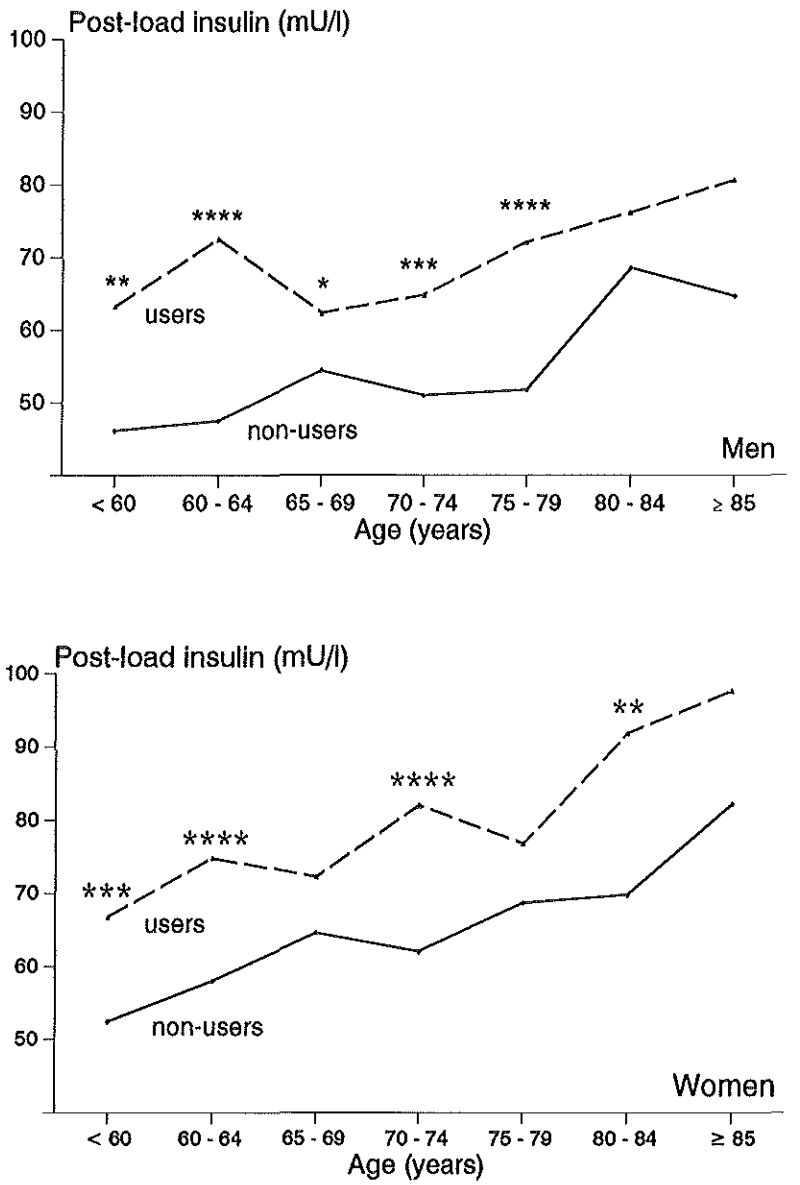

Figure 3 Mean insulin levels, adjusted for systolic blood pressure, by 5-year age categories, for subjects who did and did not use antihypertensive drugs.

Difference between subjects who did and did not use antihypertensive drugs: **** $p < 0.001$, *** $p < 0.005$, ** $p < 0.01$, * $p < 0.05$.

insulin levels, adjusted for systolic blood pressure, for subjects who did and did not use antihypertensive drugs. The figure shows that antihypertensive drug users had higher insulin levels at all ages, both in men and women. The increase in insulin resistance with age was present in both users and non-users.

Discussion

In this population based study of 5453 elderly men and women who did not use antidiabetes medication, insulin resistance was assessed using an oral glucose tolerance test. Insulin resistance, estimated by post-load insulin levels and the ratio of post-load insulin over glucose, and the use of antihypertensive drugs increased with age. Higher insulin levels were associated with increased systolic and diastolic blood pressure and the presence of hypertension. However, the age-associated increase of insulin resistance was much more apparent in subjects without than in those with hypertension. This results in similar insulin levels in hypertensives and normotensives at high ages. Subjects using antihypertensive drugs have higher insulin levels at all ages, also after adjustment for blood pressure.

A limitation of the Rotterdam Study is the use of a non-fasting blood sample. To overcome this disadvantage insulin was measured two hours after an oral glucose load. We reported previously that these insulin levels are similar to the fasting post-load levels.[9] Insulin resistance was assessed by the ratio of post-load insulin over glucose. In subjects without diabetes mellitus this ratio provides a good measure of insulin resistance.[10] The indication for the medication used was obtained from the participants which may have introduced some misclassification.

The increase of insulin resistance with age has been described in other population-based studies and is thought to be a general aging phenomenon.[11] The association between serum insulin and blood pressure is well known,[12] and has been discussed in several recent reviews.[2,6,13] Most studies, however, are restricted to middle-aged subjects. In the elderly study population of Rancho Bernardo a similar association between hypertension and post-load insulin levels was found.[14] However, the association was no longer statistically significant after adjustment for age, gender, obesity, and glucose tolerance. In obese elderly subjects, hypertension was associated with a decrease of both insulin sensitivity and maximal responsiveness to insulin.[15] The results of these and our study suggest that the association between insulin and blood pressure remains at higher ages.

Findings from several other studies strengthen the hypothesis that insulin resistance is related to blood pressure. The prevalence of hypertension is increased in subjects with impaired glucose tolerance and NIDDM.[16] Moreover, insulin sensitivity is improved by metformin in subjects without diabetes mellitus,[17] and

reducing the amount of administered insulin in diabetic hypertensive patients lowers blood pressure.[18] Insulin may increase blood pressure by changes in the vascular muscle cells, renal retention of sodium and water, activation of the sympathic nervous system, and modification of cation fluxes across cell membranes.[19,20] The association between insulin and blood pressure, however, forms part of a cluster of cardiovascular risk factors, which finally results in hypertension, diabetes mellitus, obesity and dyslipidemia.[1,21] The sequence of occurrence of the components of this syndrome is still poorly understood.

The use of several antihypertensive drugs is associated with adverse effects on glucose metabolism[6,22] and the development of NIDDM.[23,24] Figure 3 shows that also at advanced age insulin resistance is increased in subjects using antihypertensive drugs. The pathophysiologic mechanisms to explain the increase in insulin resistance by the different drugs are not well known. Among others, blood flow to skeletal muscles, serum electrolytes, and bradykinin might be involved.[6,25] It has been suggested that drug-induced worsening of glucose metabolism may be an explanation for the lesser than expected reduction of morbidity and mortality from coronary heart disease in trials of mild to moderate hypertension.[26] The hypothesis that the diabetogenic effect is restricted to diuretics and ß-blockers is not supported by our findings (Figure 1). However, these results have to be interpreted cautiously, because the current study was not designed to study the association of antihypertensives with insulin resistance. In particular, confounding by indicating may pose a serious problem in this analysis. Those subjects with more severe hypertension (eg. long lasting, difficult to control) or with an increased risk for diabetes mellitus (eg. obese, positive family history) are more likely to receive the newer classes of antihypertensive drugs. In addition, in the present analyses dose and duration of the antihypertensive medication were not taken into account. It has been shown that low dose diuretics have the same effect on blood pressure as conventional doses, without influencing insulin sensitivity.[27]

Differences in insulin level between users of antihypertensives for hypertension and other indications are difficult to interpret, because the other indications may have been accompanied by an increased blood pressure. However, it has been reported that heart failure is associated with increased insulin resistance.[28,29]

The finding that insulin resistance did apparently not further increase with age in those subjects with hypertension suggests that there may be an upper limit to insulin resistance, which could be reached by either antihypertensive drug use or aging. This upper limit may reflect the development of non-insulin dependent diabetes mellitus, which is considered as a state of severe insulin resistance.[30] In these subjects insulin resistance can not be estimated by an oral glucose tolerance test, and therefore they were not included in the present study.

In conclusion, the results of this study suggest that the age-associated increase in insulin resistance is diminished in subjects with raised blood pressure in the presence raised insulin resistance. In addition, the use of antihypertensive drugs gives an independent additional increase of insulin resistance at all ages. Follow-up studies are needed to evaluate the effects of age and the different classes of antihypertensive drugs on insulin resistance in the elderly.

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High blood pressure and the incidence of non-insulin dependent diabetes mellitus: findings in a 11.5 year follow-up study

In several epidemiologic studies risk factors for the development of non-insulin dependent diabetes mellitus have been identified. Among these, the most important are age, obesity and glucose intolerance.[1,2,3,4] Studies on the association between other cardiovascular risk factors, e.g. hypertension, and the incidence of diabetes mellitus, however show conflicting results.[5,6,7] This is partly due to differences in definition of diabetes. Abnormal glucose tolerance tests, fasting blood glucose levels above certain levels, health-questionnaires, hospital records, and the use of hypoglycemic medication are the most commonly used definitions. In addition, most studies have been cross-sectional, which precludes to investigate the time-sequence of the potential risk factor and the incidence of diabetes mellitus.

In the present study we used data obtained in a 11.5 year follow-up study of a large population in the Netherlands to prospectively assess the relation between cardiovascular risk factors and the incidence of diabetes mellitus.

Subjects and methods

Study population

Between 1975 and 1978 a population survey was conducted in the Dutch town of Zoetermeer, among 5681 men and women aged 20 to 65. Details of the study have been published previously.[8] The study was conducted among all the inhabitants of two suburbs (respons rate 78%). The main objective of this population survey was to study determinants and prevalence of rheumatic diseases, chronic pulmonary diseases and cardiovascular diseases. In 1988 a questionnaire on the presence of chronic diseases was sent to those between 20 and 65 years of age at the initial survey. The questionnaire included two questions on diabetes mellitus: "Did you develop diabetes mellitus since the examination in 1975 - 1978?" and "Do you presently have diabetes mellitus?".

The questionnaire was send to the 4968 subjects who were alive and whose current addresses were available in 1988. After one reminder, 3973 questionnaires were returned (response rate 80%) and 133 participants answered positively to one

of the diabetes questions. The general practitioners of the persons who indicated on the questionnaire that they had diabetes mellitus were contacted to confirm the diagnosis. Diabetes mellitus was defined as the current use of oral hypoglycemic drugs and/or insulin. Of each confirmed case, the general practitioner also provided the date of diagnosis. From all 133 persons, information was obtained from the general practitioners in 1989. At the initial survey in 1975 - 1978 the diagnosis diabetes mellitus was made by a physician, using the same criteria. These prevalent cases were excluded from the analysis. The age of onset of all incident diabetic cases in this study was 36 years and over. The mean age of onset was 50.4 years for the 13 subjects using insulin; the insulin therapy started on average 3 years after the time of diagnosis. So we assumed that they all have non-insulin dependent diabetes. All others responding to the questionnaire who were not classified as having diabetes, and had complete data of the initial survey, served as controls (n = 3744).

Measurements

Blood pressure was measured on the left arm using a random zero sphygmomanometer. The mean of two readings in a sitting position was used in the analysis. Height and weight were measured without shoes and with indoor clothing. Body mass index was calculated as the ratio of weight to the square of height. Triceps skinfold was measured at the right and left arm in 73% of the participants. The mean of these two measurements was used in the analysis.

For an additional analysis of the association between hypertension and the incidence of diabetes mellitus, the population was categorized according to the WHO-guidelines in normotensives (diastolic blood pressure lower then 90 mmHg), borderline hypertensives (diastolic 90 - 94 mmHg) and hypertensives (diastolic 95 mmHg or over).[9]

Data analysis

To investigate the association between cardiovascular risk factors and the incidence of diabetes mellitus, and to adjust for possible confounders, proportional hazard analysis was used. Analyses were performed both by entering determinants as continuous variables in the model and by using categorical variables based on quintiles of the distribution in the total population. For each cardiovascular risk factor the relative risk for diabetes mellitus and the 95% confidence interval was computed.

Table 1 Baseline characteristics of participants in the initial survey, according to subsequent development of diabetes mellitus.

	Men				Women			
	DM+*		DM-		DM+		DM-	
Number	33		1747		32		1997	
Age (years)	47.6	(10.5)	41.7	(11.0)	51.9	(8.1)	41.5	(11.0)
Body mass index (kg/m^2)	26.6	(3.0)	24.2	(2.9)	28.6	(4.5)	23.8	(3.3)
Skinfold thickness (mm)	11.2	(4.4)	10.1	(4.4)	25.0	(6.7)	19.0	(6.6.)
Heart rate (beats/min)	81.6	(14.0)	75.5	(12.8)	79.3	(12.8)	78.4	(12.9)
Systolic blood pressure (mmHg)	144.6	(18.7)	132.3	(17.0)	144.3	(25.1)	127.4	(18.4)
Diastolic blood pressure (mmHg)	87.1	(11.5)	79.3	(11.8)	88.1	(15.5)	78.1	(12.0)
Cholesterol (mmol/l)	6.0	(1.2)	5.8	(1.1)	6.5	(1.2)	5.7	(1.1)
Hypertension†	36.4%		15.3%		56.2%		17.6%	
Use of diuretics	9.1%		3.2%		37.5%		8.4%	
Coffee use (cups/day)	4.7	(2.1)	5.3	(2.4)	4.2	(1.9)	4.5	(2.1)
Current smoking	53.3%		58.9%		54.5%		61.9%	

Values are means with standard deviation in parentheses.

* DM+ are persons who developed diabetes mellitus during the follow-up period, DM- persons who did not develop diabetes mellitus during the follow-up period.

† Systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or using anti-hypertensive medication.

Results

After exclusion of the known diabetes patients in 1975 - 1978 (n = 28) and the persons who did not had diabetes according to their general practitioner (n = 40), 65 incident cases of diabetes mellitus remained, 33 men and 32 women (incidence rate 1.5/1000 person-years). The baseline characteristics at the survey in 1975 - 1978 of the persons who later developed diabetes mellitus and who did not develop diabetes mellitus, are given in Table 1.

Age was significantly associated with the incidence of diabetes mellitus, with a relative risk of 1.05 per year for men (95% confidence interval 1.02 - 1.09) and 1.10 for women (95% CI 1.06 - 1.14). The results of the proportional hazard

Table 2 Relative risks of diabetes mellitus for several cardiovascular risk factors.

	Adjusted for age				*Adjusted for age and BMI*			
	Men		**Women**		**Men**		**Women**	
Body mass index (5 kg/m^2)	2.78	(1.78 - 4.35)	3.44	(2.35 - 5.02)	-		-	
Skinfold thickness (mm)*	1.05	(0.97 - 1.13)	1.09	(1.04 - 1.16)	0.94	(0.86 - 1.03)	1.03	(0.96 - 1.10)
Heart rate (10 beats/min)	1.37	(1.08 - 1.73)	1.08	(0.84 - 1.40)	1.34	(1.05 - 1.71)	1.07	(0.82 - 1.40)
Systolic blood pressure (10 mmHg)	1.34	(1.12 - 1.60)	1.24	(1.06 - 1.46)	1.28	(1.06 - 1.54)	1.08	(0.91 - 1.29)
Diastolic blood pressure (10 mmHg)	1.51	(1.17 - 1.95)	1.44	(1.13 - 1.85)	1.40	(1.06 - 1.85)	1.13	(0.87 - 1.47)
Cholesterol (mmol/l)	1.00	(0.73 - 1.38)	1.28	(0.94 - 1.75)	0.93	(0.68 - 1.28)	1.23	(0.90 - 1.67)
Hypertension*	2.35	(1.12 - 4.96)	3.17	(1.49 - 6.72)	1.84	(0.86 - 3.92)	1.98	(0.92 - 4.26)
Use of diuretics	2.02	(0.60 - 6.78)	3.46	(1.64 - 7.31)	1.82	(0.54 - 6.11)	2.26	(1.04 - 4.90)
Coffee use (cups/day)	0.90	(0.76 - 1.06)	0.94	(0.78 - 1.13)	0.88	(0.74 - 1.04)	0.87	(0.72 - 1.05)
Current smoking	1.05	(0.73 - 1.50)	1.10	(0.72 - 1.67)	1.05	(0.73 - 1.50)	1.11	(0.73 - 1.68)

Values are relative risks, with 95% confidence interval in parentheses.

* Systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or using anti-hypertensive medication.

analysis of the cardiovascular risk factors assessed at baseline are given in Table 2. Body mass index, systolic and diastolic blood pressure were significantly associated with the incidence of diabetes mellitus in men and women, after adjustment for age. Heart rate in men, and skinfold thickness and use of diuretics in women also showed significant associations with the incidence of diabetes.

After adjustment for age and body mass index, heart rate, systolic and diastolic blood pressure remained significantly associated with the incidence of diabetes mellitus in men. In women, only the relative risk associated with the use of diuretics remained statistically significant. By using categorical variables the risk of diabetes mellitus appeared to rise gradually with increasing blood pressure level and body mass index. By contrast, for heart rate in men the relative risk of diabetes was raised in particular in the highest quintile. After adjustment for age, body mass index and systolic blood pressure, the associations of heart rate and diuretics with the incidence of diabetes were no longer statistically significant. In men the relative risk was 1.25 (95% CI 0.97 - 1.62) for heart rate and 1.62 (95% CI 0.48 - 5.44) for diuretics. In women these relative risks were 1.05 (0.80 - 1.38) and 2.18 (1.00 - 4.78) respectively. Similar results were obtained after adjustment for diastolic blood pressure.

To exclude the effect of anti-hypertensive medication on the association of blood pressure and the development of diabetes mellitus, those subjects using anti-hypertensive medication were excluded from the analysis. The results were essentially the same as in the group as a whole: adjusted for age, the relative risks for systolic blood pressure were 1.38 per 10 mmHg (95% confidence interval 1.15 - 1.66) in men, and 1.22 (95% CI 1.01 - 1.48) in women respectively. When the analysis was restricted to the subjects with normal blood pressure (systolic blood pressure $\leq$ 160 mmHg) the relative risks for systolic blood pressure were 1.41 per 10 mmHg (95% CI 1.06 - 1.89) in men and 1.16 (95% CI 0.87 - 1.55) in women, adjusted for age.

In an additional analysis of the risk related to elevated blood pressure, the population was categorized into three groups: normotensives, borderline hypertensives, and hypertensives. The findings in this analysis are shown in Figure 1, adjusted for age. In men and women the relative risk of diabetes mellitus increased with each blood pressure category. In both men and women this trend was statistically significant.

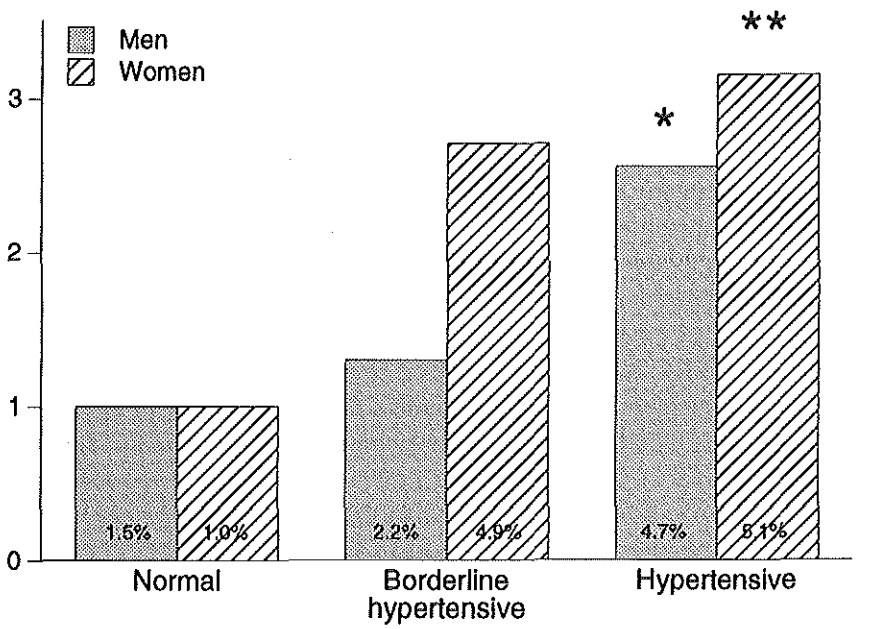

Figure 1 Relative risk of diabetes mellitus in normotensive subjects and categories of hypertension, adjusted for age.
For the definition of the different hypertensive categories, see text. Numbers indicate the proportion of cases in each category.
Test for trend: * $p < 0.05$, ** $p < 0.01$

Discussion

The results of our study show that age, body mass index, systolic and diastolic blood pressure are associated with the incidence of diabetes mellitus during a follow-up period of 11.5 years. In men there is also an association with heart rate, whereas in women skinfold thickness and the use of diuretics are significantly associated with the incidence of diabetes mellitus. After adjustment for age and body mass index, in men heart rate, systolic and diastolic blood pressure and in women the use of diuretics remain significantly associated with the incidence of diabetes mellitus. When the effect of anti-hypertensive medication (especially thiazide diuretics) is excluded, the association of blood pressure and diabetes mellitus remains statistically significant.

Diagnosis of diabetes mellitus in our study was based on participant response to the questionnaire with subsequent confirmation by general practitioners. By using current hypoglycemic medication as criterion for the diagnosis of diabetes mellitus,

false-positive misclassification is not likely to occur. It is known that the rate of undiagnosed diabetes mellitus is about the same as the rate of diagnosed diabetes.[10] So the use of these strict criteria may have led to an underestimation of the incidence of diabetes. However, when the number of incident cases is small compared to the whole study population, false-positive misclassification is more important then false-negative misclassification.[11] This will, if anything, reduce the magnitude of the risk estimates.

Several epidemiologic studies have shown that age is an important risk factor for the development of non-insulin dependent diabetes mellitus. Also, overweight and body fat distribution are well documented risk factors.[2] In our study we were able to confirm that age and overweight are significantly associated with the development of diabetes mellitus. Triceps skinfold thickness, the only measure of body fat obtained in the initial survey, is not appropriate to assess body fat distribution, and has found in other studies to have only a weak association with the incidence of non-insulin dependent diabetes mellitus.[12] Unfortunately, no blood glucose levels were measured at baseline, so no information could be obtained about the third important risk factor for the development of non-insulin dependent diabetes mellitus, glucose intolerance.[6] Because the mean time between the initial survey and the diagnosis of diabetes mellitus was 7.3 year, it is not likely that a substantial proportion of the 'incident' diabetic cases actually had diabetes mellitus during the initial survey.

Of the cardiovascular risk factors studied, serum cholesterol and smoking showed no association with the development of non-insulin dependent diabetes mellitus. However, the mean cholesterol level was slightly higher in persons who later developed diabetes mellitus ($p = 0.28$), so the possibility of a small risk associated with serum cholesterol can not completely ruled out. Coffee consumption, although not a cardiovascular risk factor,[13] has been suggested to be a risk factor for the development of insulin dependent diabetes mellitus.[14] We found no association between coffee consumption and the incidence of non-insulin dependent diabetes mellitus.

The risk associated with the use of diuretics was remarkably high in women, but the risk associated with raised blood pressure, both systolic and diastolic, was lower than in men. After controlling in the analysis for blood pressure the risk associated with diuretic use moved close to one. Although the diabetogenic effects of diuretics, in particular thiazides, have been reported by several authors,[15,16] our findings may indicate that the risk associated with the use of diuretics mainly reflects the risk of (treated) hypertension. In accordance with this, when blood pressure was included in the analysis, diuretic use lost its relation with the development of diabetes mellitus. When the subjects using antihypertensive

medication were excluded from the analysis, the association between blood pressure and the incidence of diabetes mellitus remained, also in the normopressure range.

It should be stressed that, because prevalent cases of diabetes were excluded from this prospective study, blood pressure was recorded before diabetes mellitus developed. In addition, the results given in Figure 1 suggest a dose-response relation between blood pressure and the incidence of non-insulin dependent diabetes mellitus. Together with the results from the analysis of the anti-hypertensive medication, this supports the view that NIDDM and hypertension may have a similar origin.[17,18] According to several authors this common cause is a raised insulin resistance.[19,20,21,22] This would imply that elevated blood pressure, whether treated or not, at the survey in 1975 - 1978 was a first sign of hyperinsulinemia in those subjects who later developed diabetes mellitus.

In summary, from our study it appears that, in addition to age and obesity, high blood pressure is a risk factor for non-insulin dependent diabetes mellitus.

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Chapter 4

Insulin resistance: consequences

In this chapter the relation of insulin resistance with morbidity is discussed. Each part deals with one of the diseases studied in the Rotterdam Study: cardiovascular diseases, eye diseases, dementia and osteoporosis. The discussion on cardiovascular morbidity covers both the associations of insulin resistance with the degree of atherosclerosis, as well as the relations with clinical diseases, like myocardial infarction and stroke. Diabetic retinopathy might be regarded as vascular disorder, and is discussed in the next part. The final parts deal with dementia and osteoporosis, two chronic diseases in which the association with insulin have not extensively been studied. The associations of insulin with cognitive function and bone mineral density are presented. In contrast to the vascular diseases, which is regarded as a consequence of the metabolic effects of insulin resistance, these associations are probably the consequence of the anabolic effects of insulin.

Insulin, atherosclerosis and cardiovascular disease

There is ample evidence from autopsy, clinical and epidemiological studies that cardiovascular disease (CVD) is more common in subjects with diabetes mellitus than in those without.[1] Subjects with minor disturbances of the glucose metabolism, impaired glucose tolerance and hyperinsulinemia, also have an increased risk of developing CVD.[2] Symptomatic atherosclerotic events are the eventual result of a thrombotic occlusion of an atherosclerotic artery.[3] It has been suggested that hyperinsulinemia promotes atherosclerosis, probably due to a direct effect of insulin on the vascular wall, or to an insulin-mediated increase in cardiovascular risk factors, notably dyslipidemia, obesity and hypertension.[4]

To study these associations in the elderly, we assessed glucose metabolism, the degree of atherosclerosis and the presence of CVD in a population-based study in the elderly. Two non-invasive measurements were used to assess the degree of atherosclerosis: ankle/arm index (ratio of systolic blood pressure in the ankle over systolic blood pressure in the arm),[5] and the number of atherosclerotic plaques on ultrasonographic images of the carotid arterial wall.[6,7]

Subjects and methods

Study population

The Rotterdam Study is a population-based cohort study of determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over were invited to participate. An outline of the study and its objectives has been published previously.[8] The baseline examination of the Rotterdam Study was conducted from 1990 to 1993. The participants were interviewed in their homes by trained research assistants, using a computerized questionnaire. Subsequently the participants came to the research center for several measurements. In addition, an oral glucose tolerance test was performed. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School. Overall 7983 participants were examined in the Rotterdam Study (response rate 78%). The analyses in this article are restricted to the 6618 subjects in whom serum glucose levels were measured.

Measurements

The participants came to the research center throughout the day. Blood was drawn by venepuncture and subjects not using antidiabetes medication (tablets or insulin) received a glucose drink of 75 grams of glucose. Two hours later a second blood sample was obtained. Glucose levels were measured in both samples by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium) in the post-load serum only. Diabetes mellitus was defined as the use of antidiabetes medication or a random or post-load glucose level greater than 11 mmol/l. Insulin resistance was assessed by post-load insulin level and the ratio of post-load insulin over glucose. Blood pressure was measured with a random-zero sphygmomanometer, and the mean of two measurements was used in the analyses. For body mass index weight divided by the square of height (kg/m^2) was used, whereas body fat distribution was assessed by the ratio of hip over waist circumference.

Two measures of degree of atherosclerosis were used: ankle/arm index, and number of atherosclerotic plaques in the carotid arteries. Ankle/arm index was calculated as the ratio of systolic blood pressure in the ankle over the systolic blood pressure in the arm.[9] Ankle systolic blood pressure was measured at the posterior tibial artery with a Doppler ultrasound transducer while the subject was in supine position. The lowest ankle/arm index in either leg as used in the analyses. Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear array transducer (ATL UltraMark IV, Advanced Technology Laboratories, Bothell, Washington, USA). The common carotid artery, the carotid bifurcation, and the internal carotid artery were evaluated on line for the presence (yes/no) of atherosclerotic lesions. Plaques were defined as a focal widening relative to adjacent segments, with protrusion into the lumen either composed of only calcified deposits or a combination of calcification and non-calcified material.[10] Because of logistic limitations the presence of plaques was determined in 5051 subjects.

The medical history of the participants was obtained by a structured interview, which included the history of myocardial infarction and stroke. For the present analyses only myocardial infarctions requiring hospitalisation were considered. The presence of angina pectoris and intermittent claudication was assessed by the Rose questionnaire.[11] Cardiovascular disease was defined as the presence of at least one of these four conditions.

Data analysis

Age-adjusted linear regression analysis was used to assess the associations of glucose and insulin resistance with the degree of atherosclerosis. Mean levels of serum glucose and insulin resistance, as well as the prevalence of diabetes mellitus,

Table 1 Baseline characteristics.

	Total		Men		Women	
	mean	(SD)	mean	(SE)	mean	(SE)
Number	6618		2668		3950	
Age (years)	69.4	(9.2)	68.2	(0.2)	70.2	(0.2)
Body mass index (kg/m^2)	26.3	(4.0)	25.7	(0.1)	26.7	(0.1)
Waist/hip ratio	0.91	(0.09)	0.96	(0.001)	0.87	(0.001)
Serum glucose (mmol/l)	6.9	(2.7)	7.1	(0.05)	6.8	(0.04)
Post-load insulin (mU/l)*	62.7	(52.8)	55.6	(1.0)	67.5	(1.0)
Insulin resistance (mU/mmol)†	8.9	(6.5)	8.4	(0.1)	9.3	(0.1)
Diabetes mellitus‡	10.7%		10.4%		10.9%	
Systolic blood pressure (mmHg)	139.5	(22.5)	138.7	(0.4)	140.0	(0.4)
Diastolic blood pressure (mmHg)	73.7	(11.7)	74.6	(0.2)	73.1	(0.2)
Ankle/arm index	1.05	(0.23)	1.09	(0.005)	1.03	(0.004)
Number of plaques in carotid arteries	1.52	(1.67)	1.75	(0.04)	1.36	(0.03)
Cardiovascular disease¶	16.7%		20.7%		14.0%	

SD = standard deviation, SE = standard error

* Only measured in subjects not using antidiabetes medication (n = 5532).

† Ratio of post-load insulin over post-load glucose.

‡ Use of antidiabetes medication or random glucose value ≥ 11.1 mmol/l or post-load glucose value ≥ 11.1 mmol/l.

¶ History of myocardial infarction or stroke, or presence of angina pectoris or intermittent claudication.

were compared between subjects with and without CVD. The association of diabetes mellitus and presence of CVD was estimated by logistic regression analysis with odds ratios as an approximation of relative risk. To control for possible confounders, the analyses were adjusted for age, body mass index, waist/hip ratio and systolic blood pressure, if appropriate. Furthermore, age-adjusted prevalence of CVD was given by categories of serum glucose and insulin.

Table 2 Associations between some metabolic parameters and measures of atherosclerosis.

	Men		Women	
Ankle/arm index				
Serum glucose (mmol/l)	-0.007	(-0.010 - -0.003)	-0.009	(-0.012 - -0.007)
Post-load insulin (10 mU/l)	-0.001	(-0.002 - 0.001)	-0.003	(-0.004 - -0.002)
Insulin resistance (mU/mmol)	0.001	(-0.001 - 0.002)	-0.002	(-0.003 - -0.001)
Number of plaques in carotid arteries				
Serum glucose (mmol/l)	0.050	(0.023 - 0.077)	0.050	(0.030 - 0.070)
Post-load insulin (10 mU/l)	-0	(-0.016 - 0.016)	0.017	(0.007 - 0.027)
Insulin resistance (mU/mmol)	-0.002	(-0.014 - 0.010)	0.011	(0.002 - 0.020)

Values are age-adjusted coefficients of linear regression with 95% confidence interval between parentheses.

Results

The baseline characteristics of the study population are given in Table 1. Increased serum glucose was associated with a decreased ankle/arm index, and an increased number of plaques in the carotid arteries (Table 2). After excluding subjects with diabetes mellitus the associations remained essentially the same. In women insulin resistance was associated the three measures of atherosclerosis. In men, however, no significant association was observed (Table 2). Adjustment for body mass index or waist/hip ratio did not change the results essentially, while adjustment for systolic blood pressure weakened the associations with maintenance of statistical significance. Excluding subjects with prevalent symptomatic CVD did not markedly change these results.

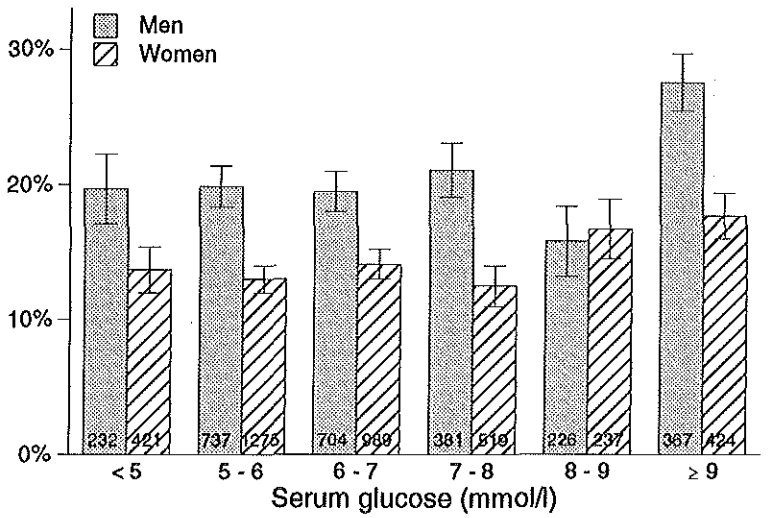
In Table 3, age-adjusted mean values of parameters of the glucose metabolism are given for subjects with and without CVD. Both in men and women serum glucose and insulin resistance were increased in subjects with CVD. This was most clear for coronary heart disease (angina and myocardial infarction). After further adjustment for body mass index, waist/hip ratio or systolic blood pressure the

Table 3 Metabolic parameters in subjects with and without cardiovascular disease.

	No CVD		Angina pectoris		Intermittent claudication		Myocardial infarction		Stroke		Any CVD	
Men												
Number	2116		171		56		323		128		552	
Serum glucose (mmol/l)	7.0	(0.1)	7.6*	(0.2)	7.8	(0.4)	7.6¶	(0.2)	7.5	(0.2)	7.5¶	(0.1)
Post-load insulin (mU/l)	53.0	(1.1)	65.0*	(4.2)	65.8	(7.3)	71.1¶	(3.1)	63.3	(5.1)	66.8¶	(2.4)
Insulin resistance (mU/mmol)	8.0	(0.2)	9.8*	(0.6)	11.0†	(1.0)	10.4¶	(0.4)	9.8*	(0.7)	10.0¶	(0.3)
Women												
Number	3397		277		51		170		142		553	
Serum glucose (mmol/l)	6.8	(0.1)	7.3†	(0.2)	7.9†	(0.4)	7.1	(0.2)	7.1	(0.3)	7.1*	(0.1)
Post-load insulin (mU/l)	65.6	(1.0)	79.3‡	(3.7)	72.6	(9.0)	79.0*	(5.0)	81.6†	(5.3)	79.1¶	(2.7)
Insulin resistance (mU/mmol)	9.1	(0.1)	11.2¶	(0.4)	10.0	(1.0)	10.6*	(0.6)	10.9†	(0.6)	10.8¶	(0.3)

Values are age-adjusted mean values with standard error in parentheses.
CVD = cardiovascular disease

Test for difference between subjects with no cardiovascular disease: * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.005$, ¶ $p < 0.001$.



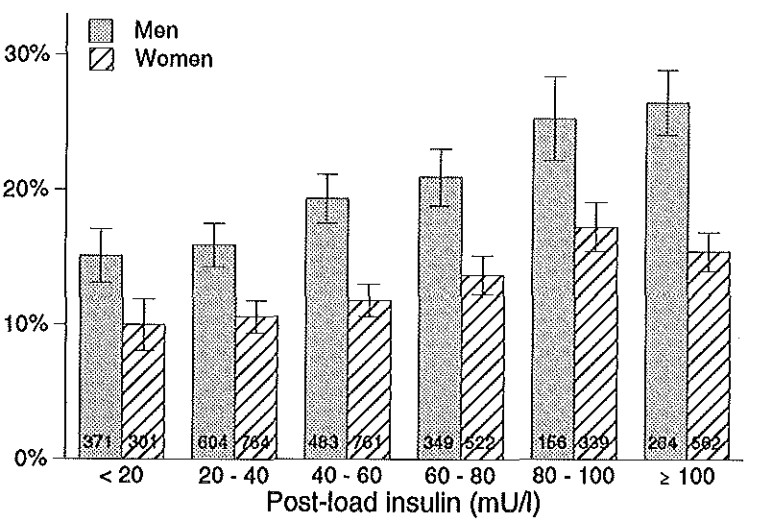


Figure 1 Age-adjusted prevalence of cardiovascular disease by categories of serum glucose and post-load insulin.

Values are means with standard deviation. Figures at the bottom of each bar indicate the number of subjects in that category.

differences were essentially the same and remained statistically significant. Figure 1 gives the prevalence of CVD by categories of serum glucose and post-load insulin. The figure shows that the higher glucose levels in subjects with CVD mainly reflect subjects in the upper glucose category, which represents diabetic patients. This was confirmed by the relative risk of diabetes mellitus for CVD: 1.54 for men (95% confidence interval 1.16 - 2.05) and 1.33 for women (95% CI 1.02 - 1.74). Moreover, if subjects with diabetes mellitus were excluded from the analyses, the associations with serum glucose were no longer statistically significant ($p > 0.3$). Insulin on the other hand showed a linear association with the presence of CVD in both men and women (Figure 1), which remained essentially the same after excluding subjects with diabetes mellitus.

Discussion

In this population-based study serum level of glucose and the prevalence of diabetes mellitus were associated with an increased degree of atherosclerosis, whereas insulin resistance was directly related to atherosclerosis in women only. A history of myocardial infarction or stroke, or the presence of angina pectoris or intermittent claudication was defined as cardiovascular disease (CVD). Insulin resistance and the prevalence of diabetes mellitus were increased in both men and women with CVD. The associations did not change after adjustment for body mass index, waist/hip ratio or systolic blood pressure.

In this cross-sectional study only data from survivors of myocardial infarction and stroke are available. It has been shown that hyperglycemia is associated with a worse prognosis after myocardial infarction,[12] which suggests that the associations between glucose metabolism and cardiovascular disease might be different in subjects who survive till old age. In addition, the medical history was obtained from the participants only, which may misclassify the prevalence of CVD.[13] As a consequence, the reported associations are likely to have been underestimated. Another limitation of the Rotterdam Study is the use of a non-fasting blood sample. To overcome this, insulin was measured two hours after an oral glucose load. We reported previously that these insulin levels are similar to the fasting post-load levels.[14] Insulin resistance was assessed by the ratio of post-load insulin over glucose. In subjects without diabetes mellitus this ratio provides a good measure of insulin resistance.[15]

Non-invasive measures of the degree of atherosclerosis have only recently been introduced in epidemiologic research. Blood pressure drops after a stenosis in an artery, which is the rationale for using the ratio of the ankle over arm systolic blood pressure. In subjects with normal vessels this ratio is close to one. When the

arteries in the leg become narrow due to atherosclerosis, the ratio decreases. The association of the ankle/arm index with the presence of atherosclerosis at various arteries is high, as is the prediction of subsequent cardiovascular morbidity.[5] High-resolution ultrasonography enables direct visualisation of the arterial wall. This technique has a high validity for the detection and evaluation of atherosclerotic plaques.[6]

In non-diabetic subjects an association has been found between increased insulin levels and degree of atherosclerosis, measured by carotid artery wall thickness,[16,17] and distensibility of the aorta.[18] The reported associations with insulin were rather weak, whereas other investigators found no association at all.[19,20] In our study the association was limited to women, which may in part be explained by a different insulin-androgen interaction in men and women.[21] Therefore, it can be hypothesised that those men who survive till older age have been less sensitive for insulin than women of the same age. In animal studies and cell cultures a direct effect of insulin on the smooth muscle cells in the artery wall has been demonstrated.[2] However, our cross-sectional findings and the results presented by others are compatible with the view that insulin itself has a modest, if any, effect on the development of atherosclerosis. Several authors have reported raised insulin levels in subjects with CVD,[22,23,24] including subjects with non-insulin dependent diabetes mellitus,[25] but others did not find an association.[26,27] Only few studies have reported on this association in the elderly.[28,29,30] The incidence of cardiovascular diseases in subjects with hyperinsulinemia, however, has been reported to be higher, unchanged and lower as discussed in a recent review.[31]

Hyperinsulinemia is the consequence of an increased insulin resistance, which is associated with a number of other cardiovascular risk factors, notably obesity, dyslipidemia, impaired glucose tolerance and raised blood pressure. This cluster of risk factors is known as 'syndrome X' or 'insulin resistance syndrome'.[32,33] It has been suggested that this clustering of risk factors is more important than a direct effect of insulin.[34,35] Moreover, insulinomas do not increase the risk of cardiovascular disease.[36] In the present study adjustment for the available components of the cluster (body mass index, waist/hip ratio, glucose, HDL-cholesterol, blood pressure) only slightly diminished the associations between insulin and degree of atherosclerosis (data not shown).

Symptomatic cardiovascular disease is not the result of the thickened arterial wall, but of a thrombotic occlusion of the artery. Reduced fibrinolysis may be a stronger determinant of an atherosclerotic event then increased hemostasis.[37] Insulin is positively associated with plasminogen activator inhibitor 1 (PAI1),[38] which may explain in part the higher insulin levels in subjects with symptomatic

cardiovascular disease. Measures of fibrinolytic function were not available in the current study.

In conclusion, the results of this study are in agreement with the hypothesis that hyperinsulinemia in subjects with symptomatic cardiovascular disease is an expression of increased cardiovascular risk factors ('insulin resistance syndrome'). Our findings suggest that insulin itself may only play a limited role in the progression of atherosclerosis in the elderly.

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Glucose, insulin and retinopathy

A description of diabetes mellitus is to be found in Hindu manuscripts of the 5th century BC,[1] but retinal hemorrhages and cotton-wool spots were first described in diabetes patients in 1856.[2] Studies in animals[3] and diabetic patients[4] have shown that a longer duration of the disease and a lower level of glycemic control are associated with a higher prevalence of diabetic retinopathy. Trials in patients with insulin dependent diabetes mellitus (IDDM) have shown that improved glycemic control lowers the incidence of diabetic retinopathy.[5] Consequently, it has been proposed that retinopathy is caused by hyperglycemia, and not by other metabolic disturbances.[6] As serum glucose level and the prevalence of non-insulin dependent diabetes mellitus (NIDDM) increase with age,[7] a high prevalence of diabetic retinopathy may be expected in elderly people. Until now, few population based studies of retinopathy have included subjects without diabetes mellitus.

We evaluated the presence of retinopathy by fundus photographs in all participants of the population-based Rotterdam Study, while the glucose metabolism was assessed by serum fructosamine and an oral glucose tolerance test. This paper describes the associations of retinopathy with serum glucose, fructosamine and insulin resistance in 6191 elderly non-hospitalized men and women.

Subjects and methods

Study population

The Rotterdam Study is a population-based cohort study of determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited to participate. The design of the study and its objectives have been published previously.[8] The participants attended the research center for several measurements. These included anthropometry, blood pressure measurements, and an extensive ophthalmological examination. In addition, an oral glucose tolerance test was performed. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School.

The ophthalmological examination was part of the measurements in the Rotterdam Study from March 1990 to the end of the baseline examination in July 1993. Totally, 7983 subjects participated in the Rotterdam Study (response

rate 78%), of whom 7129 completed the examinations at the research center. In 6251 persons it was possible to evaluate at least one fundus photograph. Significantly more women than men had absent or ungradable fundus photographs, and they were older than the remaining study population (75.3 vs 68.9 years, $p < 0.001$). However, after adjustment for age and gender, the serum glucose level and the prevalence of diabetes mellitus and hypertension did not differ between those with and without gradable fundus photographs. Data on eyes with retinal vein occlusion or age-related macular degeneration were excluded. The analyses presented here were restricted to the remaining 6191 persons.

Measurements

Retinopathy was assessed on fundus photographs. Of each participant both eyes were dilated with tropicamide 0.5% and phenylephrine 5%. After an average period of 45 minutes two 35° colour slides (Kodak Ektachrome 64 ASA, Topcon TRV-50VT fundus camera) centred on the macular area were taken of each eye (Diabetic Retinopathy Study standard field 2). The slides were examined on a portable stereo viewer with fluorescent back light (Philips PL-S 9W/84; 4,000° K) with 5x magnification. Combined with a 2.5x magnification of the fundus camera, the total magnification was approximately 12.5x. The graders were blinded for the status of the glucose metabolism of the participants. The presence of cotton wool exudates and the presence and number of dot/blot hemorrhages were graded, without differentiation between microaneurysms and hemorrhages. In an additional procedure photographs with laser photocoagulation scars were categorized into either diabetic retinopathy or other diseases (most often retinal vein occlusion), using the photograph of the other eye and available clinical data. Retinopathy was defined as the presence of one or more hemorrhages/microaneurysms and/or cotton wool spots (which corresponds to level 15 to 51 of the modified Airlie House classification[9]) or laser coagulation scars due to diabetic retinopathy. The eye with the most severe retinopathy was used in the analyses. The slides were graded by one of three graders. All questionable lesions were discussed and adjudicated by two ophthalmologists. A reproducibility study in 29 subjects revealed a kappa of 0.71 between the observers and 0.86 within observers.

The participants came to the research center throughout the day. They were asked for the time of last food intake. Blood was drawn by venepuncture and subjects not using antidiabetes medication (tablets or insulin) received a glucose drink of 75 grams of glucose. Two hours later a second blood sample was obtained. Glucose levels were measured in both samples by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium) in the post-load serum only. Diabetes mellitus was defined as the use of

antidiabetes medication or a random or post-load glucose level greater than 11 mmol/l. Insulin resistance was assessed by post-load insulin level and the ratio of post-load insulin over glucose. Fructosamine was measured by the test-combination 1054686 of Boehringer Mannheim during the first two years of the study (n = 4458). Blood pressure was measured with a random-zero sphygmomanometer, and the mean of two measurements was used in the analyses. For body mass index weight divided by the square of height (kg/m^2) was used, whereas body fat distribution was assessed by the ratio of hip over waist circumference.

Data analysis

Mean levels of serum glucose, fructosamine, insulin and insulin resistance were compared between subjects with and without retinopathy, adjusted for age by analysis of covariance. The analyses were performed in the whole population, and in men and women separately and reported with corresponding 95% confidence intervals. Also, separate analyses were performed in those subjects who had not eaten for at least three hours, and in subjects with and without diabetes mellitus and hypertension. Associations between variables were evaluated using linear and logistic regression analysis. Odds ratio were calculated as an approximation of the relative risk. Adjustments were made for age, body mass index and systolic blood pressure, if appropriate.

Results

The baseline characteristics of the study population are given in Table 1. Retinopathy was found in 296 subjects (120 men and 176 women). Women were slightly older than men. After adjustment for age, random serum glucose and post-load insulin levels were significantly higher in women compared to men. This was also found for diastolic blood pressure level and hypertension, but not for the presence of diabetes mellitus and retinopathy.

In Table 2, age-adjusted mean values of some metabolic parameters are given for subjects with and without retinopathy. Serum glucose and fructosamine levels were increased in subjects with retinopathy. The same differences were found in subjects with and those without diabetes mellitus. In the 128 men who had not eaten for at least three hours before the venepuncture (fasting group), the age-adjusted glucose levels in those with and without retinopathy were 10.3 and 6.2 mmol/l, respectively ($p < 0.001$). In the 181 fasting women these values were 9.5 and 6.2 mmol/l ($p < 0.001$).

Table 1 Baseline characteristics of the study population.

	Total		Men		Women	
Number	6191		2522		3669	
Age (year)	68.9	(8.8)	67.9	(8.0)	69.4	(9.1)
Random glucose (mmol/l)	6.9	(2.7)	7.1	(2.6)	6.8	(2.7)
Fructosamine (μmol/l)	310	(52)	309	(55)	310	(50)
Post-load insulin (mU/l)*	62.4	(52.9)	55.7	(49.1)	67.0	(54.8)
Insulin resistance (mU/l)†	8.9	(6.5)	8.4	(6.6)	9.3	(6.4)
Diabetes mellitus‡	10.2%		10.2%		10.2%	
Systolic blood pressure (mmHg)	139.3	(22.4)	138.6	(21.9)	139.9	(22.8)
Diastolic blood pressure (mmHg)	73.7	(11.6)	74.5	(11.6)	73.2	(11.5)
Hypertension§	29.5%		25.7%		32.1%	
Hemorrhages¶	4.6%		4.6%		4.5%	
Diabetes lasercoagulation scars	0.2%		0.1%		0.3%	
Retinopathy‖	4.8%		4.8%		4.8%	

Numbers are means with standard deviation between parentheses.

* Only measured in subjects not on antidiabetes medication (n = 5049).
† Ratio of post-load insulin over post-load glucose.
‡ Use of antidiabetes medication or random glucose value ≥ 11.1 mmol/l or post-load glucose value ≥ 11.1 mmol/l.
§ Use of antihypertensive medication or systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg.
¶ One or more dot/blot hemorrhages/micro aneurysms, or cotton wool spots without retinal vein occlusion or macular degeneration.
‖ Hemorrhages or diabetes lasercoagulation scars.

Hypertension was more prevalent and blood pressure was higher in subjects with retinopathy compared with those without (age- and gender-adjusted levels 146/75 mmHg and 138/74 mmHg, respectively). The differences in the serum glucose and fructosamine levels remained if the analyses were stratified to the presence of hypertension (Table 3). The differences of the post-load insulin levels were only present in women with hypertension (Table 3).

The risk of retinopathy significantly increased with an increase of the serum glucose level. The age-adjusted relative risk in the whole study population was 1.13 per mmol/l (95% confidence interval 1.10 - 1.16). When subjects with diabetes mellitus were excluded, the relative risk was 1.18 (95% CI 1.08 - 1.30). After further adjustment for systolic blood pressure the corresponding relative risks were 1.13 and 1.17, respectively. The post-load insulin level was not associated with retinopathy: the relative risk was 1.00 per mU/l. The same pesult was found when the ratio of post-load insulin over glucose was used. After adjustment for age the relative risk of diabetes mellitus for the presence of retinopathy was 3.13 (95% confidence interval 2.37 - 4.14). For men and women the relative risks were 2.94 (95% CI 1.87 - 4.60) and 3.21 (95% CI 2.24 - 4.59), respectively. Further adjustments for body mass index or systolic blood pressure did not substantially alter these risk estimates. In spite of the high risk of diabetes, most cases of retinopathy in the general population occur in subjects without diabetes mellitus (73.9%, Table 2). The reason is that subjects with diabetes mellitus are a minority of the study population (10.2%, Table 1).

In Figure 1 the prevalence of retinopathy, adjusted for age, is given by different levels of the serum glucose, fructosamine. The figure suggests that the risk of retinopathy increased linearly when the serum glucose was above 6 mmol/l. Within the fructosamine distribution above 350 µmol/l a linear increase of retinopathy was also found (data not shown). When the analyses were restricted to subjects without antidiabetes medication, the same associations were found.

Table 2 Age-adjusted mean values of some metabolic parameters in subjects with and without retinopathy.

	Retinopathy			
	absent	present	difference (95% CI)	
Random glucose (mmol/l)	6.8	8.4	1.6	(1.3 - 1.9)
Fructosamine (µmol/l)	309	330	21	(14 - 28)
Post-load insulin (mU/l)*	61.7	70.4	8.7	(1.4 - 16.0)
Insulin resistance (mU/l)†	8.9	9.7	0.8	(-0.1 - 1.7)
Diabetes mellitus (%)	9.4	26.1	16.8	(11.6 - 21.9)
Hypertension (%)	28.7	44.1	15.4	(9.5 - 21.3)

* Two hours after an oral glucose load of 75 grams; only measured in subjects not on antidiabetes medication.

† Ratio of post-load insulin over post-load glucose.

Table 3 Age-adjusted mean values of the glucose metabolism in subjects with and without retinopathy, by the presence of diabetes mellitus and hypertension.

	No hypertension				Hypertension			
	Retinopathy				*Retinopathy*			
	absent	present	difference (95% CI)		absent	present	difference (95% CI)	
No diabetes mellitus								
Random glucose (mmol/l)	6.3	6.6	0.3	(0.1 - 0.5)	6.5	6.8	0.3	(0.1 - 0.7)
Fructosamine (μmol/l)	302	306	4	(-4 - 13)	305	299	-6	(-16 - 3)
Post-load insulin (mU/l)*	56.3	59.8	3.5	(-5.6 - 12.6)	66.2	77.0	10.8	(-0.7 - 22.4)
Diabetes mellitus								
Random glucose (mmol/l)	12.4	13.9	1.5	(-0.4 - 3.4)	10.9	12.8	1.9	(0.5 - 3.4)
Fructosamine (μmol/l)	372	431	59	(23 - 95)	350	396	46	(17 - 76)
Post-load insulin (mU/l)*	97.2	79.0	-18.2	(-84.8 - 48.5)	106.0	124.2	18.2	(-31.9 - 68.4)

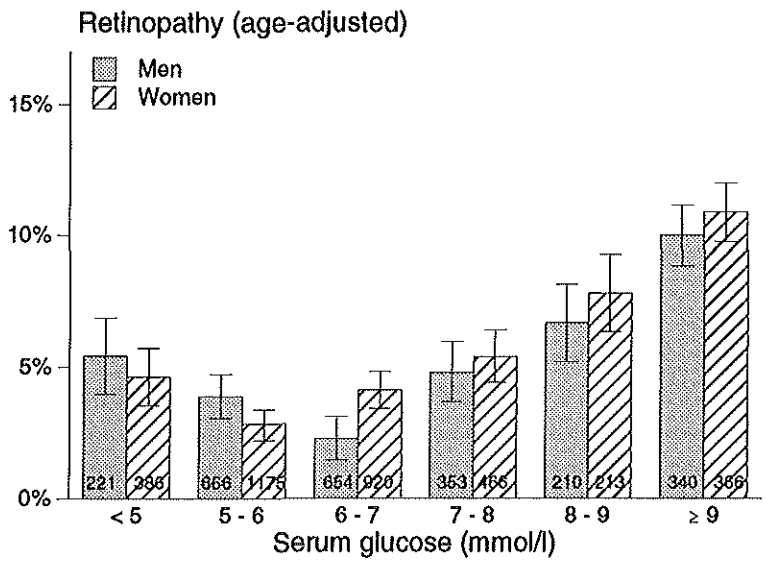
* Two hours after an oral glucose load of 75 grams; only measured in subjects not on antidiabetes medication.

All subjects with laser coagulation scars used antidiabetes medication and had higher glucose and fructosamine levels than those with hemorrhages. Within subjects who had at least one hemorrhage/microaneurysm the number of hemorrhages was significantly associated with the serum glucose level. In men the regression coefficient was 0.41 per mmol/l (SE 0.13, $p < 0.01$) after adjustment for age, whereas in women a coefficient of 0.18 per mmol/l (SE 0.06, $p < 0.01$) was found. The coefficients did not change after adjustment for body mass index or systolic blood pressure.

Discussion

Retinopathy was found in 4.8% of the participants of the Rotterdam Study and was associated with higher serum glucose and fructosamine levels, but not with insulin resistance. These associations were similar in men and women with and without diabetes mellitus, and did not change after adjustment for age, body mass index or systolic blood pressure. The prevalence increased linearly in subjects with a random serum glucose level above 6 mmol/l. Among subjects who had retinopathy, a significant association was present between serum glucose level and the number of hemorrhages.

The association between serum glucose and retinopathy is well known from clinical studies among diabetic patients, but there are only few data from population-based studies that also included non-diabetic subjects. The results presented here are based on a population of non-hospitalized subjects aged 55 years and over both with and without diabetes. The approach in our population-based study has certain limitations when compared with a clinical study. For the assessment of retinopathy, fundus photographs of 35° from the macular area were used. There is ample evidence that fundus photography is superior over an examination by an ophthalmologist to detect diabetic retinopathy.[10] The graders of the fundus photographs were blinded to all characteristics of the participants. Given the high reproducibility, it is unlikely that the results can be explained by misclassification in the assessment of retinopathy. Usually, more than one photograph is taken to cover an extended area of the retina, whereas stereo pictures are more liable to detect macular edema. Findings in a study which compared 30° fundus photographs of Diabetic Retinopathy Study fields 1 and 2 with all seven standard fields to detect any retinopathy showed a sensitivity of 0.87.[11] This limitation of the procedure used in the Rotterdam Study may have introduced only false-negative misclassification. As a consequence, the reported associations are likely to represent an underestimation.



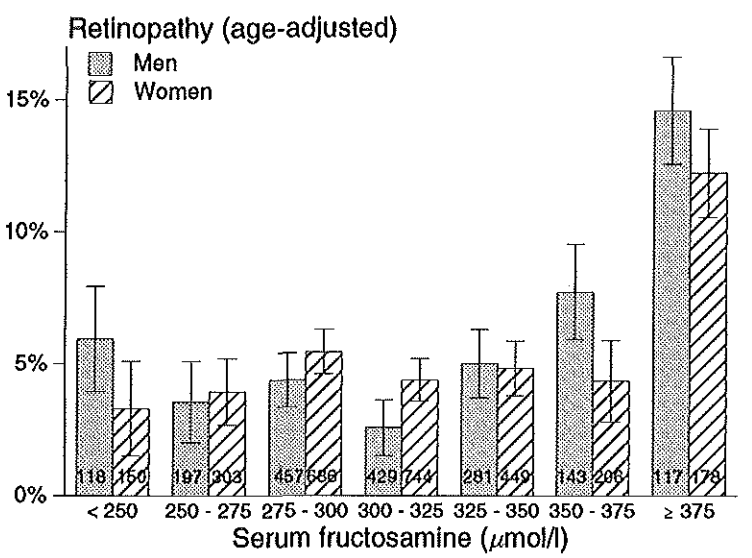


Figure 1 The prevalence of retinopathy, adjusted for age, by categories of serum glucose and fructosamine.

Values are means with standard error. Figures at the bottom of each bar indicate the number of subjects in that category.

In the Rotterdam Study blood samples were obtained in a non-fasting state. However, the associations with the random glucose levels were essentially the same, both after adjustment for the time since the last meal and after restriction of the analyses to those subjects who had their last meal more than three hours before the venepuncture. This indicates that recent food intake does not obscure the relation between serum glucose and retinopathy. Moreover, the same associations were found using serum fructosamine, which is not influenced by the time of last food intake. It is not possible, however, to compare the glucose levels measured in this study with those reported in studies based on a fasting blood sample. Insulin resistance was assessed by the insulin and glucose levels two hours after an oral glucose load. The ratio of insulin over glucose after an oral glucose load given in the fasting state is a good measure of insulin resistance.[12] We reported previously that non-fasting post-load insulin levels are similar to the fasting post-load levels.[13]

The duration of diabetes mellitus is associated with retinopathy. Furthermore, the prevalence of retinopathy has been shown to increase with age, even after adjustment for duration of disease.[14] The importance of age was confirmed in the Beaver Dam Study (Wisconsin), in which the prevalence of retinopathy in elderly IDDM patients was about the same as in NIDDM patients of the same age.[15] Because serum glucose also increases with age,[16] all relations in this study were adjusted for age.

The prevalence of retinopathy increased linearly with an increase of serum glucose and fructosamine levels. Fructosamine reflects the average serum glucose of the last three days.[17] Importantly, when subjects with diabetes mellitus were excluded from the analyses, the same associations were found. The risk associated with increasing serum glucose level for retinopathy found in this study was of the same magnitude as observed in the elderly population of the Beaver Dam Study.[15] However, the frequency of retinopathy found in the latter study was higher than found in the Rotterdam Study. This difference may be explained by the fact that in the Beaver Dam Study a larger area of the retina was photographed then in the Rotterdam Study and stereographic pictures were used. In the elderly non-diabetic study population in Rancho Bernardo (California) the prevalence of retinopathy was lower than in the Rotterdam Study population, and there was no association found with the serum glucose level.[18] In that study only a single photograph, taken with a 45° non-mydriatic camera of the foveal area was used to detect retinopathy, which might account for these differences. Apart from the difficulties associated with the assessment of non-mydriatic photographs, a number of early small lesions may be missed on 45° fundus photographs compared with photographs taken by cameras using a smaller angle.

In several population-based studies in diabetic patients an association has been found between serum glucose level and the severity of retinopathy.[19] Findings in trials conducted in IDDM patients aiming at increased metabolic control have suggested that improved glucose control leads to a decrease of the incidence and progression of retinopathy.[20,21] The central role of serum glucose in the etiology of diabetic retinopathy is supported by findings in animal and biochemical studies using cultures of retinal pericytes.[3,22] Diabetic retinopathy is a vascular disorder, a thickening of the basement membrane. The uptake of glucose by the pericytes of the retinal capillaries is not insulin mediated.[23] As a consequence, with an increasing serum glucose level, the intra cellular glucose level increases. This causes a stimulation of the polyol pathway, leading to the accumulation of sorbitol in the cells of the capillary wall.[24] The increased glucose levels also cause an acceleration of the non-enzymatic glycation,[25] the second pathophysiologic mechanism involved in the pathogenesis of diabetic retinopathy. Sorbitol dehydrogenase is able to keep the cellular glucose low only up to a certain serum glucose level. This may explain the presence of a glucose threshold for the development of retinopathy, as well as the linear increase of the prevalence and the number of hemorrhages with increasing glucose levels. It has to be stressed that the present analyses are based on cross sectional data, which means that no direct causal relationship can be inferred. Retinopathy is the result of longstanding changes in the retinal capillaries, whereas the serum levels of glucose and insulin represent glucose metabolism at one point in time.

Because insulin is an important anabolic hormone and stimulates the proliferation of the vascular endothelium,[26] and diabetic retinopathy is a vascular disorder, it has been suggested that serum insulin is associated with retinopathy.[27] The results of our study, however, do not confirm this view (Table 2). The lack of an association between insulin resistance and retinopathy may be explained by the absence of insulin mediated glucose metabolism in the retinal capillary vessels. The borderline significant association between insulin and retinopathy disappeared after adjustment for blood pressure. The relation between insulin and blood pressure is well known,[28] and blood pressure is a risk factor for (diabetic) retinopathy.[29] It was not possible in our study to classify the retinal hemorrhages into diabetic retinopathy and hypertensive retinopathy. However, adjustment for blood pressure had only a limited effect on the association between serum glucose and retinopathy. When the analyses were performed separately in subjects with and without hypertension, the differences were present in both groups (Table 3). Others have shown that blood pressure is more associated with the severity than with the presence of retinopathy.[30]

Proliferative retinopathy was not seen in any of the 6191 subjects. It might be that subjects with severe retinopathy were less likely to participate in the Rotterdam Study than subjects without retinopathy of the same age. In addition, the limited area visualized on the fundus photographs may have contributed to the absence of proliferative retinopathy, although new vessels most frequently develop within 45° of the optic disk.[4] Also the progression from background retinopathy to proliferative retinopathy is less common in the elderly, especially neovascularization of the disk.[31,32] Moreover, current clinical practice in the Netherlands is to treat macular edema and proliferative retinopathy with laser photocoagulation.[33] As a consequence, some of the subjects with lasercoagulation scars will have had proliferative retinopathy in the past. Subjects with diabetes laser photocoagulation scars had higher serum glucose and fructosamine levels, which suggest a more severe form of diabetic retinopathy.

In conclusion, in this non-hospitalized elderly population we found that the presence and severity of retinopathy were associated with increased serum glucose and fructosamine levels. These linear associations were present in diabetic and non-diabetic subjects. There was no association with serum insulin levels obtained after an oral glucose load.

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Insulin and cognitive function

Cognitive function decreases with age, but this decline is not uniformly distributed in the population. Several risk factors have been identified for an accelerated cognitive decline and dementia.[1] It has been suggested that increased insulin levels play a role in the impairment of cognitive function.[2] Because the presence of cardiovascular disease has been shown to be associated with impaired cognitive function,[3] the association between insulin and cognitive function may be the reflection of the 'insulin resistance syndrome', a clustering of cardiovascular risk factors.[4] However, the results from animal studies indicate that insulin may have a direct effect on the brain and cognition. This would imply that elevated insulin levels, probably in response to raised insulin resistance, can also directly affect cognitive function.

As part of the population-based Rotterdam Study in elderly men and women insulin and cognitive function were assessed. This paper describes the associations of insulin level, as well as insulin resistance, with cognitive function. In addition we analyzed differences in insulin between subjects with and without dementia.

Subjects and methods

Study population

The Rotterdam Study is a population-based cohort study of determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited to participate. The design of the study and its objectives have been published previously,[5] as well as the procedure of the cognitive screening.[6] The participants attended the research center for several measurements, including anthropometry, blood pressure measurements, and an assessment of cognitive function. In addition, an oral glucose tolerance test was performed. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School.

Overall 7983 subjects participated in the Rotterdam Study (response rate 78%). The analyses presented here are restricted to the 5510 subjects of whom the serum insulin level and a complete assessment of cognitive function were available.

Measurements

Global cognitive function was assessed with the Mini Mental State Examination (MMSE). This is a brief cognitive test that covers several cognitive functions, and yields a maximum best score of 30.[7] The test was administered by a trained research assistant. Subjects who had a MMSE-score of 25 or lower or a score greater than zero on the Geriatric Mental Schedule examination (organic level) were further evaluated by a physician, using the CAMDEX diagnostic interview, which included a structured psychiatric interview, neuropsychological testing (CAMCOG) and an interview with an informant.[8] All subjects suspected of dementia were subsequently evaluated by a neurologist, and underwent extensive neuropsychological testing and neuroimaging. A diagnostic panel assessed, based on all available information, wether a dementia syndrome was present.

The participants came to the research center throughout the day. Blood was drawn by venepuncture and subjects not using antidiabetes medication (tablets or insulin) received a glucose drink of 75 grams of glucose. Two hours later a second blood sample was obtained. Glucose levels were measured in both samples by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium) in the post-load serum only. Diabetes mellitus was defined as the use of antidiabetes medication or a random or post-load glucose level greater than 11 mmol/l. Insulin resistance was assessed by the ratio of post-load insulin over glucose. Blood pressure was measured with a random-zero sphygmomanometer, and the mean of two measurements was used in the analyses. For body mass index weight divided by the square of height (kg/m^2) was used.

The medical history of the participants was obtained by a structured interview, which included the history of myocardial infarction and stroke. For the present analyses only myocardial infarctions requiring hospitalisation were considered. The presence of angina pectoris and intermittent claudication was assessed by the Rose questionnaire.[9] Cardiovascular disease was defined as the presence of at least one of these four conditions.

Data analysis

The changes of insulin and cognitive function with age were assessed by linear regression analyses. For the associations of insulin level and insulin resistance, and MMSE-score multiple linear regression analyses was used with adjustments for potential confounding variables. The analyses were also performed in subgroups by the presence of diabetes mellitus, dementia, and age. Analysis of covariance was used to calculate age-adjusted mean values of MMSE-score for categories of insulin, as well as age-adjusted mean values of insulin for subjects with and without dementia. The analyses were performed in men and women separately.

Table 1 Baseline characteristics.

	Men		Women		p-value*
Number	2232		3278		
Age (years)	67.9	(0.2)	69.5	(0.2)	-
Body mass index (kg/m^2)	25.8	(0.1)	26.7	(0.1)	<0.001
Serum glucose (mmol/l)	6.9	(0.05)	6.5	(0.04)	<0.001
Post-load serum insulin† (mU/l)	55.6	(1.0)	67.2	(1.0)	<0.001
Insulin resistance (mU/mmol)‡	8.4	(0.1)	9.3	(0.1)	<0.001
MMSE	27.6	(0.05)	27.3	(0.05)	0.09
Dementia	2.7%		4.0%		0.79

Values are means with standard error in parentheses.

* Test for the difference between men and women, adjusted for age.

† Two hours after an oral glucose load of 75 grams, only measured in subjects not on antidiabetes medication (n = 5510).

‡ Ratio of post-load insulin over post-load glucose.

§ Use of antidiabetes medication or random serum glucose level ≥ 11.1 mmol/l or post-load serum glucose level ≥ 11.1 mmol/l.

Results

The baseline characteristics of the study population are given in Table 1. In both men and women insulin level and insulin resistance increased with age, whereas cognitive function significantly decreased with age (for all associations: $p < 0.01$).

In women insulin level, but not insulin resistance, was inversely associated with cognitive function (Table 2). In men insulin was not associated with cognitive function. Further adjustment for random serum glucose, body mass index, systolic blood pressure, smoking or the presence of cardiovascular disease did not change these results. Excluding subjects with diabetes mellitus did not change the results either. After adjustment for HDL-cholesterol, however, the association with insulin level was no longer statistically significant in women. Since in demented subjects low MMSE-scores primarily reflect the underlying cause of dementia, the analyses were restricted to subjects without dementia. However, the associations were essentially the same when performed in the whole study population. Figure 1 gives the age-adjusted MMSE score by categories of post-load insulin, showing that in women the MMSE-score steadily decreased over the whole range of insulin values.

Table 2 Associations between insulin and MMSE-score in subjects without dementia (n = 5310).

	Men		Women	
Post-load insulin (per 50 mU/l)	0.03	(-0.04 - 0.10)	-0.10	(-0.16 - -0.04)
Insulin resistance† (per 5 mU/mmol)	0.03	(-0.02 - 0.08)	-0.03	(-0.09 - 0.02)

Values are age-adjusted coefficients of linear regression, with the 95% confidence interval in parenthesis.

† Ratio of post-load insulin over post-load glucose.

In subjects younger than 75 years the associations were weaker than in the total population and not statistically significant. In women older than 75 years the regression coefficients of MMSE-score were -0.21 per 50 mU/l insulin (95% confidence interval -0.40 - -0.03) and -0.13 per 5 mU/mmol insulin resistance (95% CI -0.29 - 0.04).

The age-adjusted mean post-load insulin level in women with and without dementia was 76.3 mU/l (SE 4.9) and 66.8 mU/l (1.0) (test for difference: $p = 0.06$), whereas insulin resistance was 10.4 (0.6) and 9.3 mU/mmol (0.1) ($p = 0.04$), respectively. In men with dementia the mean insulin level was 62.3 mU/l (SE 6.5), which was 55.4 mU/l (1.0) in non-demented men. For insulin resistance 9.4 mU/mmol (0.9) and 8.3 mU/mmol (0.1) was found (for both comparisons: $p > 0.20$).

Discussion

The results of this cross-sectional population-based study among 5510 elderly men and women indicate that increased post-load insulin levels are associated with decreased cognitive function in women only. Adjustment for age, body mass index, systolic blood pressure, smoking, or the presence of cardiovascular disease, and excluding subjects with diabetes mellitus, did not change the results.

In the Rotterdam Study no fasting blood sample was obtained, insulin levels were measured after a non-fasting oral glucose load. We reported previously that these insulin levels are comparable with the fasting post-load levels.[10] Insulin resistance was assessed by the ratio of post-load insulin over glucose. In subjects without diabetes mellitus this ratio is a good measure of insulin resistance.[11] The presented results were based on those subjects who had completed all

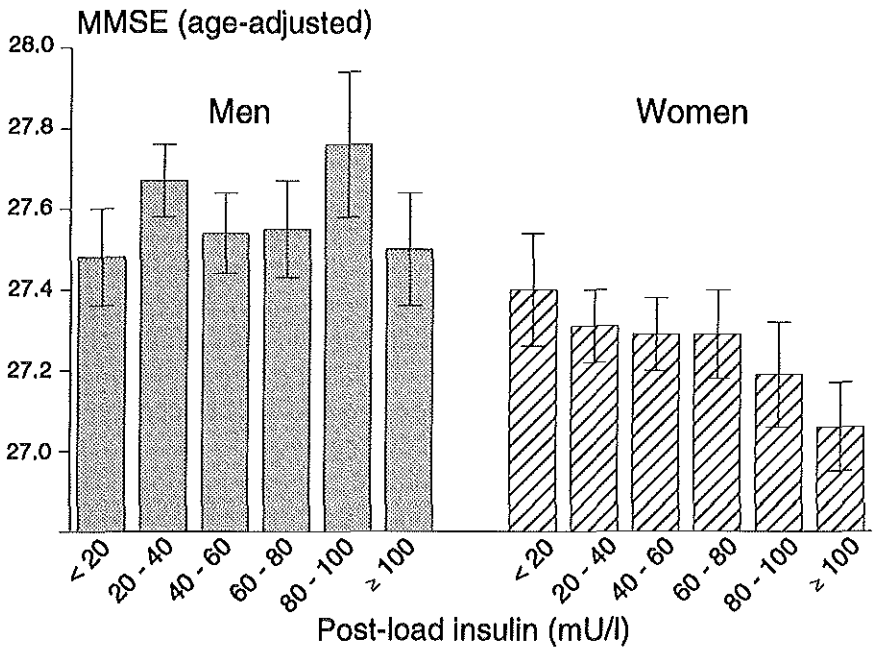

Figure 1 MMSE-score by level of post-load serum insulin.
Values are means with standard error.

examinations at the research center, which may have introduced selective non-response towards less impaired cognitive function. Although the prevalence of dementia in this study population is lower than in the total population of the Rotterdam Study, we do not see how this could influence the associations between cognitive function and insulin.

Men have higher glucose and insulin levels than women (Table 1). The associations between insulin and cognitive function were limited to women, which might in part be explained by a different insulin-androgen interaction in men and women.[12] Moreover, it has been shown that hyperglycemia is associated with a worse prognosis after myocardial infarction.[13] Therefore, it can be hypothesised that those men who survive till older age are less sensitive for insulin than women of the same age.

Little data are available on the association between insulin and cognitive function in subjects without dementia. In a preliminary report from a population-based study of elderly men in the Netherlands lower MMSE-scores were found in subjects with insulin levels in the upper quartile.[14] Moreover, in a Finnish population-based study hyperinsulinemia was associated with impaired cognitive function in subjects with hypertension.[15]

Raised insulin resistance is associated with a number of other cardiovascular risk factors, notably obesity, dyslipidemia, impaired glucose tolerance and raised blood pressure.[4] The sequence of occurrence of the components of this cluster is still poorly understood, but it has been suggested that insulin resistance is the underlying defect.[16] With this in mind, the increased insulin levels in cognitive dysfunction could reflect the adverse cardiovascular risk profile, as reported by several,[3,17] but not all,[18] authors. On the other hand, in animal studies evidence has been found for a direct effect of insulin on the brain and cognitive function.[2,19,20,21] Serum insulin is significantly associated with the insulin level in the cerebrospinal fluid.[22] Therefore, serum insulin may be associated with glucose utilization in those areas of the brains with a dense distribution of insulin receptors, the hypothalamus, olfactory bulb and hippocampus.[23] As a result the functions supported by these areas, such as memory, may be related to plasma insulin levels.

Because insulin levels in subjects without diabetes mellitus reflect insulin resistance, it is difficult to distinguish the effect of insulin level and insulin resistance. However, the association between insulin and cognitive function was independent of several cardiovascular risk factors and the presence of cardiovascular disease, and the association of cognitive function with insulin level was stronger than with insulin resistance. Therefore, our results might support a direct effect of insulin on the brain in women.

It has been reported that subjects with Alzheimer's Disease have higher,[22,24] normal[18,25] and lower[26] insulin levels than control subjects. Although these are all small studies, an explanation for these different findings might be that insulin levels are elevated in the early stages of Alzheimer's Disease and decline as dementia progresses. Findings from a recent follow-up study of patients with Alzheimer's Disease support this hypothesis.[2] An alternative explanation for the increased insulin level is a reduced physical activity of subjects with dementia, which is associated with an increased insulin level.[27]

In conclusion, the results of this study suggest that increased serum insulin level may be associated with decreased cognitive function and dementia in women. These findings are more comparable with a direct effect of insulin on the brain than with an effect through an increase in cardiovascular risk factors. Follow-up studies are needed to study the associations between insulin, other cardiovascular risk factors and cognitive function in the elderly.

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Insulin and bone mineral density

With increasing age bone mineral density (BMD) decreases, leading to an elevated risk of osteoporotic fractures.[1,2,3] The etiology of this age related disorder is not fully understood. Obesity is associated with increased BMD,[4] as well as with raised insulin resistance and hyperinsulinemia.[5] Animal studies and studies in patients with diffuse idiopathic skeletal hyperostosis have shown that insulin stimulates bone formation.[6,7] Some investigators have found an elevated BMD in patients with NIDDM,[8,9] which is, at least in the early phase, characterized by raised insulin levels.[10] Based on these findings one could hypothesize that insulin is related to bone metabolism. The first studies carried out to examine the relation between insulin and BMD have shown conflicting results,[11,12] but recent results indeed suggest a positive association.[13,14] However, most studies are conducted among small and mostly selected groups, and tend to be restricted to women. Moreover, only few population based studies have been conducted in this area which included both men and women of older age.[13]

As part of a large population based cohort study in the elderly we assessed BMD and glucose metabolism. This paper describes the associations between BMD and serum insulin and glucose levels in non-hospitalized elderly men and women. In addition we analyzed the difference in glucose and insulin concentration between subjects with and without a history of non-vertebral fractures.

Materials and methods

Study population

The Rotterdam Study is a population-based cohort study of the determinants of chronic disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited to participate. An outline of the study and its objectives has been published previously.[15] The baseline examination of the Rotterdam Study was conducted from 1990 to 1993. The participants were interviewed in their homes by trained research assistants, using a computerized questionnaire. This included an assessment of smoking habits, current medication use, and a history of different types of fractures in the last five years. For the present analyses only non-vertebral fractures were considered. Subsequently the participants came to the research center for several measurements, including

standard clinical chemistry, anthropometry, and BMD measurements. In addition, an oral glucose tolerance test was performed. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School. Inhabitants of nursing homes (1114) were not examined at the research center, so no bone densitometry results were obtained. The overall response rate of the remaining study population was 77.3%. In 563 subjects no data on BMD were obtained, mainly due to the fact that BMD measurements were not included in the pilot phase of the Rotterdam Study. The analyses in this article are restricted to the 5931 subjects with at least one BMD measurement available.

Measurements

BMD measurements were performed by dual-energy X-ray absorptiometry (DXA), using a Lunar DPX-L densitometer (Lunar Radiation Corporation, Madison, WI, USA). Standard positioning was used with anterior-posterior scans of the lumbar spine and the right proximal femur. If there was a history of hip fracture or prothesis implantation, the left femur was scanned. Using standard software the vertebrae L2 to L4, and at the proximal femur the femoral neck, Ward's triangle and the greater trochanter were analyzed. Quality assurance included calibration with the standard of the machine, and was performed routinely every morning. The in vivo coefficient of variation for the BMD measurements was 0.9% in the lumbar spine, 3.2% in the femoral neck, 3.1% in Ward's triangle, and 2.5% in the greater trochanter.

The participants came to the research center throughout the day. Blood was drawn by venepuncture and subjects not using antidiabetes medication (tablets or insulin) received a glucose drink of 75 grams of glucose. Two hours later a second blood sample was obtained. Glucose levels were measured in both samples by the glucose hexokinase method, while insulin was measured by radioimmunoassay (Medgenix diagnostics, Brussels, Belgium) in the post-load serum only. Diabetes mellitus was defined as the use of antidiabetes medication or a random or post-load glucose level greater than 11 mmol/l. Insulin resistance was assessed by post-load insulin level and the ratio of post-load insulin over glucose. For body mass index weight divided by the square of height (kg/m^2) was used, whereas body fat distribution was assessed by the ratio of hip over waist circumference.

Data analysis

The baseline characteristics of the study population were compared between men and women after adjustment for the different age-distribution, using analysis of covariance. The associations between BMD and parameters of the glucose

metabolism with anthropometric measures (body mass index and waist/hip ratio) were assessed by linear regression analyses. To estimate the associations between BMD and glucose and insulin levels multiple linear regression analysis was used. The analyses were performed univariate and with adjustment for potential confounding variables. In addition age-adjusted mean values (with standard errors of the mean) of BMD for categories of serum glucose and insulin were calculated by analysis of covariance. Finally, serum glucose and insulin were compared between subjects with and those without anamnestic fractures. The difference of the age-adjusted values are presented with the 95% confidence interval.

Results

The baseline characteristics of the study population are given in Table 1. Women were slightly older than men, but also after adjustment for age all studied variables remained significantly different between men and women. Therefore, subsequent analyses were performed in men and women separately. Initially, the analyses were performed with all BMD measurements. However, the results obtained using the individual hip measures (femoral neck, Ward's triangle and the greater trochanter) were similar. The results presented here are restricted to the lumbar spine and the femoral neck.

In both men and women age-adjusted random serum glucose and post-load serum insulin were significantly associated with body mass index and waist/hip ratio (Table 2). The BMD measurements at the different sites also showed a positive association with body mass index and waist/hip ratio (Table 2).

Higher serum glucose and post-load insulin levels were associated with increased BMD at all sites. The age-adjusted associations were statistically significant in men and women (Table 3). After further adjustment for waist/hip ratio the associations remained essentially the same, and statistically significant. Adjusting for body mass index, however, reduced the regression coefficients, and in women the associations with insulin lost statistical significance (Table 3). Figures 1 and 2 give the mean BMD by categories of serum glucose and post-load serum insulin of the lumbar spine and the femoral neck, respectively. When subjects with diabetes mellitus ($n = 578$) were excluded from the analysis, the associations were the same after adjusting for age. After further adjustment for body mass index, however, the regression coefficients were reduced and no longer statistically significant.

Age-adjusted BMD of the femoral neck was 0.03 g/cm^2 lower in men and women who reported at least one non-vertebral fracture when compared to men without fractures (95% confidence interval 0.01 - 0.05 in men; 0.02 - 0.04 in

Table 1 Baseline characteristics.

	Whole group		Men		Women	
Number	5931		2481		3450	
Age (years)	68.0	(8.0)	67.5	(7.6)	68.4	(8.3)
Body mass index (kg/m^2)	26.3	(3.7)	25.7	(3.0)	26.7	(4.0)
Waist/hip ratio	0.91	(0.09)	0.96	(0.07)	0.87	(0.09)
Anamnestic non-vertebral fractures in the last 5 years*	13.3%		10.0%		15.7%	
Bone mineral density (g/cm^2)						
Lumbar spine	1.09	(0.20)	1.17	(0.20)	1.04	(0.18)
Femoral neck	0.84	(0.14)	0.88	(0.13)	0.81	(0.13)
Ward's triangle	0.70	(0.15)	0.73	(0.15)	0.67	(0.15)
Greater trochanter	0.76	(0.15)	0.85	(0.14)	0.72	(0.13)
Glucose metabolism						
Serum glucose (mmol/l)	6.87	(2.56)	7.06	(2.56)	6.73	(2.55)
Post-load serum insulin (mU/l)†	60.2	(50.2)	54.8	(48.3)	64.0	(51.1)
Diabetes mellitus‡	9.7%		9.8%		9.7%	

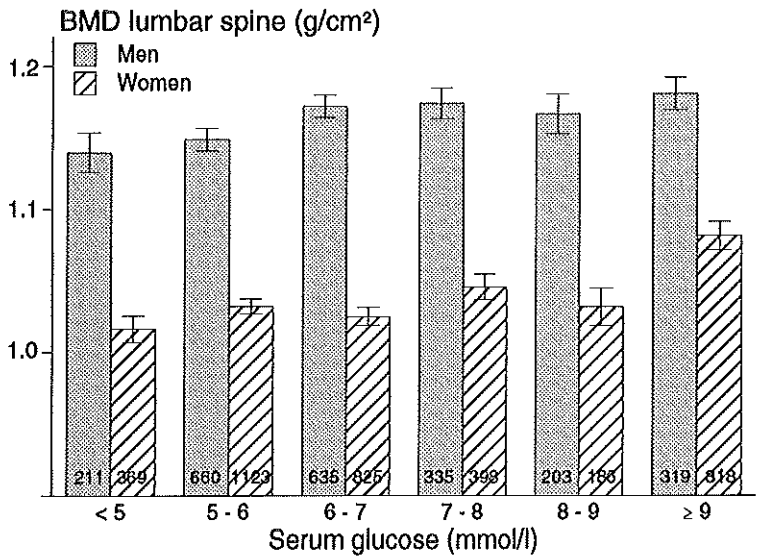
Numbers are means with standard deviation in parentheses.

* Fractures of the ankle, foot, leg, knee, hip, shoulder, arm, elbow, wrist, or hand.

† Two hours after an oral glucose load of 75 grams, only measured in subjects not on antidiabetes medication (n=4917).

‡ Use of antidiabetes medication or random serum glucose level ≥ 11.1 mmol/l or post-load serum glucose level ≥ 11.1 mmol/l.

women). The same differences were present in the lumbar spine and remained essentially unchanged after further adjustment for body mass index. The age-adjusted post-load serum insulin level was 12.5 mU/l lower in men with a previous non-vertebral fracture compared to those without (95% CI 5.4 - 19.7). When men with diabetes mellitus were excluded the difference was 13.0 mU/l (95% CI 6.1 - 20.0). In women serum insulin levels were also lower among those with a fracture in the last five years, but this did not reach statistical significance (61.9 vs 64.3 mU/l). Further adjustment for all potential confounders did not change these differences substantially.



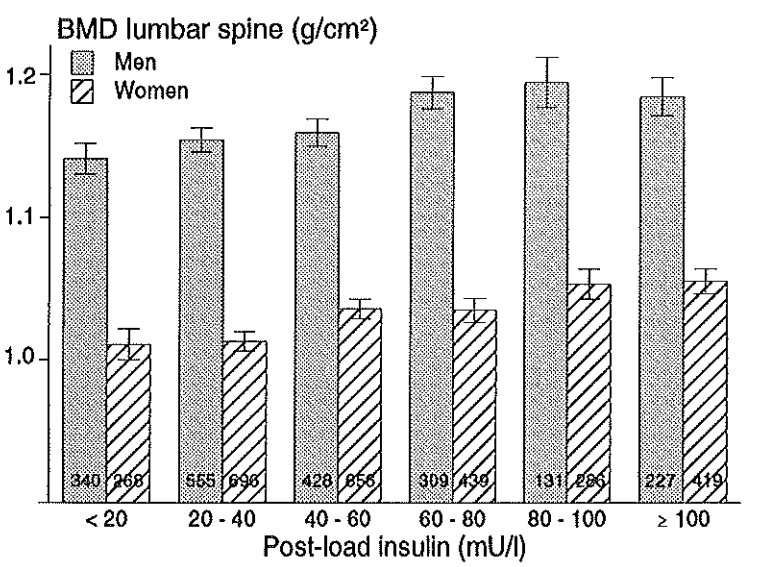
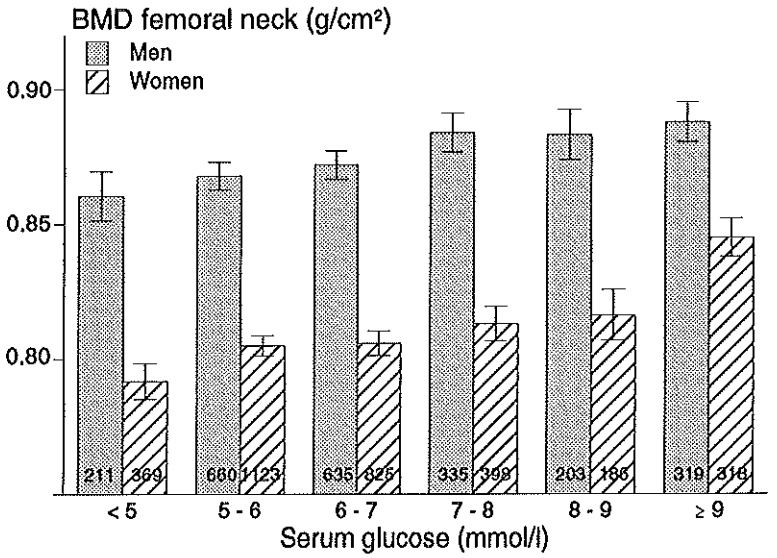


Figure 1 Mean bone mineral density (BMD) of the lumbar spine by categories of serum glucose and post-load serum insulin.
Values are age-adjusted means with standard error. Figures at the bottom of each bar indicate the number of subjects in that category.



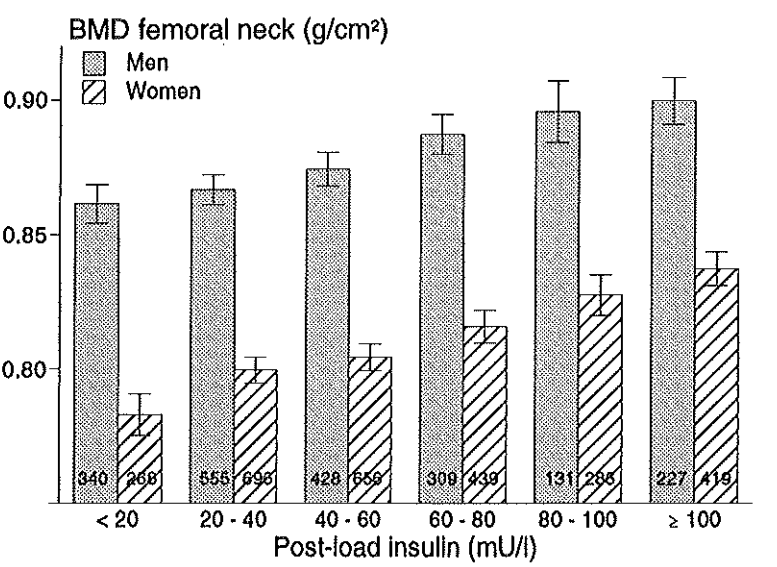


Figure 1 Mean bone mineral density (BMD) of the lumbar spine by categories of serum glucose and post-load serum insulin.
Values are age-adjusted means with standard error. Figures at the bottom of each bar indicate the number of subjects in that category.

Table 2 Associations between bone mineral density, glucose and insulin, and anthropometric measures.

	Men				Women			
	BMI (kg/m^2)		waist/hip ratio		BMI (kg/m^2)		waist/hip ratio	
Bone mineral density								
Lumbar spine (per mg/cm^2)	16.36	(13.80 - 18.92)	214.8	(97.2 - 332.4)	12.71	(11.2 - 14.2)	204.7	(131.8 - 277.6)
Femoral neck (per mg/cm^2)	12.20	(10.51 - 13.89)	133.7	(55.5 - 211.9)	11.20	(10.16 - 12.24)	173.9	(121.8 - 226.0)
Glucose metabolism								
Serum glucose (per mmol/l)	0.048	(0.013 - 0.083)	4.22	(2.71 - 5.73)	0.072	(0.050 - 0.094)	3.60	(2.97 - 4.23)
Post-load serum insulin (per mU/l)†	3.51	(2.82 - 4.20)	120.2	(89.4 - 151.0)	3.10	(2.65 - 3.55)	119.5	(97.7 - 141.3)

Numbers are age-adjusted regression coefficients with 95% confidence interval in parentheses.

† Two hours after an oral glucose load of 75 grams; only measured in subjects not on antidiabetes medication.

Table 3 Associations between bone mineral density (BMD, per mg/cm^2), and glucose and insulin levels.

	Men				Women			
	Serum glucose (mmol/l)		Post-load insulin (mU/l)†		Serum glucose (mmol/l)		Post-load insulin (mU/l)†	
Lumbar spine BMD								
Adjusted for age	4.64	(1.46 - 7.82)	0.35	(0.17 - 0.53)	6.88	(4.37 - 9.39)	0.25	(0.11 - 0.39)
Adjusted for age and WHR	4.02	(0.83 - 7.21)	0.30	(0.10 - 0.50)	6.10	(3.57 - 8.63)	0.18	(0.04 - 0.32)
Adjusted for age and BMI	3.79	(0.75 - 6.83)	0.16	(-0.02 - 0.33)	4.31	(1.90 - 6.72)	0.01	(-0.13 - 0.15)
Femoral neck BMD								
Adjusted for age	3.60	(1.50 - 5.70)	0.26	(0.14 - 0.38)	4.65	(2.85 - 6.45)	0.29	(0.19 - 0.39)
Adjusted for age and WHR	3.25	(1.15 - 5.35)	0.24	(0.12 - 0.36)	3.99	(2.19 - 5.79)	0.24	(0.14 - 0.34)
Adjusted for age and BMI	2.66	(0.68 - 4.64)	0.12	(0.01 - 0.24)	2.43	(0.74 - 4.12)	0.08	(-0.02 - 0.18)

Numbers are regression coefficients with 95% confidence interval parentheses.

† Two hours after an oral glucose load of 75 grams; only measured in subjects not on antidiabetes medication.

Discussion

The results of this population based study among 5931 elderly men and women indicate that bone mass is associated with serum glucose and insulin levels. The associations did not change after adjustment for age or waist/hip ratio, but adjustment for body mass index diminished the relations. In subjects without diabetes mellitus the same associations were found. Subjects who reported a non-vertebral fracture in the last five years had lower BMD and lower insulin levels than those without. The difference in insulin levels reached statistical significance in men.

The data presented were obtained in a population of ambulatory non-institutionalised elderly, who were able to come to the Rotterdam Study research center. Dual-energy X-ray absorptiometry was used for the measurement of BMD. This method has a good reproducibility and accuracy, and a low radiation exposure. A limitation of the Rotterdam Study is the use of a non-fasting blood sample. To overcome this disadvantage insulin was measured two hours after an oral glucose load. We reported previously that the non-fasting post-load levels are comparable with fasting post-load levels.[16]

The association between BMD and body mass index is well known,[4] which was confirmed in our study (Table 2). Obesity may raise BMD of the lower extremities by an increased load to the skeleton. Moreover, in women an increased body mass index (BMI) is associated with lower levels of sex hormone-binding globulin (SHBG) and elevated free oestrogen levels,[17] which may increase BMD.[18] On the other hand obesity, notably abdominal fat, is associated with increased insulin resistance and hyperinsulinemia (Table 2).[5] The increase in serum insulin in obese subjects is likely to result from a number of processes in fat cells (especially abdominal fat) and an increase of fatty acids in the portal blood stream.[19] These findings implies that obesity is part of the causal chain (it causes the hyperinsulinemia) and therefore by definition cannot be a confounding factor in the relation insulin-BMD. As a consequence, adjustment for BMI may lead to over-adjustment which will diminish the associations, especially in women (Table 3). Body fat distribution, another marker of obesity, is more strongly associated with insulin resistance than with BMD. Adjusting for the waist/hip ratio did hardly change the association between insulin and BMD. This is supported by the study of Reid *et al.* in which fat mass was used as marker of obesity.[14] They reported that the association between insulin and BMD was independent of fat mass, suggesting a direct effect of insulin on BMD.

To our knowledge the San Antonio Heart Study is the only other population based study where the association between serum insulin and BMD has been studied.[13] The participants in this study were younger than in the Rotterdam Study.

They found an association between insulin and glucose levels and BMD measured in the femur, but not with the lumbar BMD measurement. Like the present study, they also found that the associations were no longer statistical significant after further adjustment for BMI. In our study there was also a difference between the associations with BMD of the proximal femur and of the lumbar spine (Table 3). Due to the large numbers in the Rotterdam Study the weaker associations with the lumbar measurements did reach statistical significance. The difference of the associations between the lumbar and femoral measurements can be explained by the fact that especially in elderly subjects osteoarthritis of the lumbar spine disturbs the assessment of BMD,[20,21] which was also found in this cohort.[22]

The pathophysiological mechanisms of the relation between insulin and BMD are not fully understood. Insulin is a potent anabolic hormone. Studies in vitro and animal studies have shown that insulin promotes cell proliferation in bone cells.[6,23] This may be brought about via the receptors of insulin-like growth factor 1 (IGF-1), which have been identified on osteoblasts. Consequently the positive effect on bone formation could result in a higher BMD.[24,25] Another possible explanation for the increased BMD in subjects with raised insulin levels is the negative association of insulin with SHBG.[26,27] Lower SHBG levels are associated with higher free oestrogen and testosterone levels, causing an increased BMD. Another explanation for the association between insulin and BMD might be confounding by other variables. However, adjusting for factors known to influence BMD (serum creatinine, smoking, use of diuretics and oestrogens) did not change the results (data not shown).

Glucose levels in subjects without diabetes mellitus are closely related to insulin levels: subjects with raised insulin levels have a higher glucose level as well.[10] This may be the most important explanation for the association between glucose and BMD found in this study. Whether non-enzymatic glycation of bone proteins (eg. collagen, osteocalcin) has an additional role in this relation is currently unclear.[28] Moreover, raised serum glucose levels and non-insulin dependent diabetes mellitus arise when the pancreas fails to secrete sufficient insulin to overcome the increased insulin resistance. This means that these subjects have had a period of hyperinsulinemia.[29] It is possible that the raised BMD resulted from that period, probably by a delayed age-related bone loss. The analyses presented here are based on cross-sectional data, which means that no direct causal relationship can be established.

The different insulin levels in subjects with non-insulin dependent diabetes mellitus (high at the onset of the disease, lower in long-standing cases) might be one of the explanations for the different findings in studies of BMD in subjects with diabetes mellitus.[8,9,30,31,32] In our study the analyses were performed irrespective of

the diagnosis of diabetes mellitus. We found a linear association across the whole range of glucose and insulin values. Post-load insulin levels were not obtained in subjects with medical treated diabetes mellitus (in general corresponding to a longer duration of the disease) leading to higher insulin levels in the remaining subjects with diabetes mellitus, for the greater part diagnosed in this study. After excluding all subjects with diabetes mellitus the associations remained the same. However, because of the restriction of the range of exposure the associations were weaker in the group without diabetes mellitus.

Most fractures in the elderly are related to osteoporosis,[3,33] which was reflected by a lower BMD in subjects with a previous fracture. The lower insulin level in subjects with a history of non-vertebral fractures is in accordance with the relation between insulin and BMD. In women the differences were much smaller and did not reached statistical significance. Elderly women have a lower BMD than men, causing a higher fracture rate (Table 1), which may overwhelm any effect of insulin. Moreover, women with low insulin levels benefit from the positive effect of oestrogen before the menopause, which is probably superior to the effect of insulin on bone mass.[34,35] In particular with respect to fracture rates, prospective studies are needed to provide conclusive data.

In conclusion, the results of this population based study in elderly men and women show that higher serum glucose and insulin levels are associated with an increased BMD and lower fracture rate, which may be the key of the raised BMD in obesity. The association may be explained by a direct effect of insulin on bone formation, probably by the IGF-1 receptor, and the inverse effect of insulin on SHBG. Further research will be necessary to elucidate the physiologic mechanisms involved in this relation.

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Chapter 5

Insulin resistance: marker of aging?

In this dissertation the relation of insulin resistance with several diseases has been described. This co-morbidity is the theme of the general discussion in this final chapter. First the clustering of diseases with insulin resistance is examined, and possible explanations are reviewed. Is insulin resistance a common risk factor for several chronic diseases, or do elderly subjects just have more diseases because of their age? The discussion on 'elderly' is extended by addressing the issue of biologic aging. The 'amount' of disease, degree of disability and perceived health are used to approximate biologic age of the participants of the Rotterdam Study. Based on the associations between insulin levels and these measures the potential of insulin resistance as marker of biologic age is discussed.

Insulin resistance: associations with clustering of disease and biologic aging

Insulin resistance is defined as a subnormal biologic response to a given concentration of insulin.[1] Usually it refers to a diminished insulin-mediated glucose uptake, which can be located in the liver and in peripheral tissues (predominantly muscle cells). When glucose disposal decreases, the ß-cells of the pancreas increase the production of insulin to keep the serum glucose levels low. This may occur in the presence of normoglycemia or hyperglycemia.[2] Several causes of insulin resistance have been identified in animal, laboratory and clinical studies. Besides defects intrinsic to the action of insulin (e.g. mutations of the insulin-receptor gene and autoantibodies to insulin), a number of conditions have been described that inhibit the action of insulin. These include hormonal and metabolic diseases (e.g. Cushing's syndrome, acromegaly, insulinoma, diabetes mellitus), abnormal physiologic states (e.g. fever, cirrhosis, starvation, obesity) and normal physiologic states (eg pregnancy, advanced age).[1,3]

As discussed in chapter 1, in epidemiologic studies the serum insulin level (or the ratio of insulin over glucose) can be used to estimate insulin resistance. The results of the studies presented in this dissertation indicate that in elderly subjects without diabetes mellitus, serum insulin is associated with adrenal steroid metabolism (chapter 2), liver function (chapter 2), lipid metabolism (chapter 3), blood pressure (chapter 3), and the presence of chronic diseases (chapter 4).

Because insulin resistance is associated with this wide range of different diseases it may be an universal 'disease marker'. The clustering of diseases is regarded as measure of increased biologic age, which may suggest that insulin resistance is an indicator of biologic age.

Part I: Insulin and clustering of diseases

If insulin resistance and/or hyperinsulinemia is associated with several chronic diseases, one would expect more of these diseases present in the same subject. From clinical practice it is well known that patients with diabetes mellitus, an insulin resistant state, have an increased risk of other diseases. These comprise not only micro- and macrovascular complications, but also diseases such as dementia and arthritis (see also chapter 4). In the Rotterdam Study several chronic diseases are

studied, therefore it was possible to examine the association of insulin resistance with clustering of diseases. These analyses are based on cross sectional studies, and no direct causal inferences can be made. Based on the literature the potential role of insulin resistance in the development of chronic diseases is discussed.

Evidence from the Rotterdam Study

To study the clustering of diseases the presence of the following diseases was assessed: cardiovascular disease (history of myocardial infarction or stroke with hospitalisation), dementia (diagnosed by a neurologist according to the Rotterdam Study protocol[4]), chronic obstructive lung diseases (COPD, use of specific anti-asthmatic medication (ATC code R03)), and cataract (aphakia or presence of cortical and nuclear cataract assessed by slit-lamp examination). These are the chronic diseases with the highest morbidity and mortality in the Netherlands.[5] The overall prevalence of the different diseases in those subjects in whom serum insulin levels were available was 10%, 3%, 5% and 18%, respectively.

Men had on average 0.41 of the diseases mentioned, whereas the mean number of diseases in women was 0.33. The increase per 10 years of age was 0.31 (95% confidence interval 0.29 - 0.33), in both men and women. After adjustment for age, the number of diseases increased significantly with an increase of the post-load serum insulin concentration: in men the regression coefficient was 0.023 per 50 mU/l (95% CI 0.009 - 0.036), whereas in women an increase of 0.039 per 50 mU/l was found (95% CI 0.015 - 0.064). If the ratio of post-load insulin over glucose was used as estimate of insulin resistance, the results were essentially the same. Systolic blood pressure is also common in the elderly and an important risk factor for cardiovascular disease. However, for systolic blood pressure no increase with the average number of chronic diseases was found.

In Figure 1 the mean number of chronic diseases is given by categories of insulin resistance and hypertension. In both men and women hyperinsulinemic subjects suffer from more diseases, whereas diabetic patients have even more co-morbidity (test for trend $p < 0.01$). No significant trend was observed over the categories of hypertension ($p > 0.4$).

A more general estimation of the burden of disease for an individual may be the number of different medications used. In the Rotterdam Study the average number of medications prescribed per person was 2.30 (vitamins and homeopathic preparations excluded), which increased 0.68 per 10 years of age (95% CI 0.63 - 0.74) in both men and women. The post-load insulin level was positively associated with the number of drugs used. In men the number of medications increased 0.19 per 50 mU/l insulin (95% CI 0.11 - 0.27, antidiabetes drugs excluded, adjusted for age), whereas 0.16 per 50 mU/l was found in women (95% CI 0.09 - 0.22).

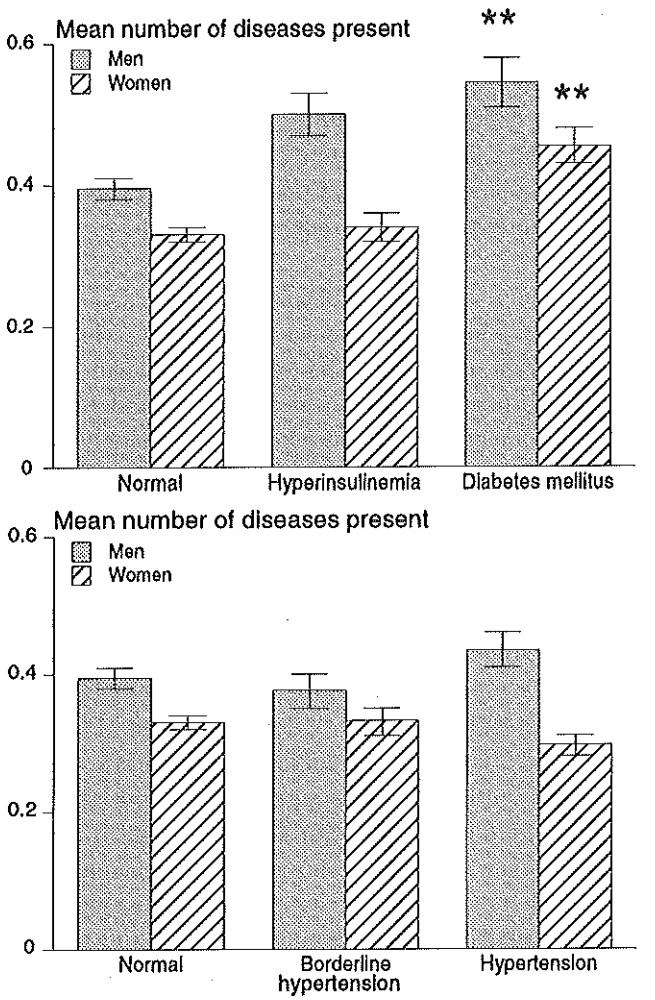

Figure 1 The number of chronic diseases present (CVD, dementia, COPD, cataract) by categories of insulin resistance and hypertension.

Values are age-adjusted means with standard error.

** test for trend: $p < 0.01$

Hyperinsulinemia was defined as the upper quintile of the gender-specific distribution of serum post-load insulin.

Diabetes mellitus was defined as random or post-load glucose ≥ 11.1 mmol/l, or use of antidiabetes medication.

Borderline hypertension was defined as blood pressure ≥ 140/90 mmHg.

Hypertension was defined as blood pressure ≥ 160/95 mmHg, or use of anti-hypertensive medication.

It is important to realize that the participants of the Rotterdam Study have survived to their current old age, which implies that subjects with early fatal diseases are not included in the analyses. Moreover, subjects with (a history of) severe disease are less likely to participate in population surveys and even to complete the examinations at the research center (including the assessment of glucose metabolism), resulting in selective non-response. Therefore, the reported associations probably represent an underestimation.

Insulin resistance risk factor for chronic diseases?

Insulin has different effects in several tissues of the body, which may explain the associations with different diseases. The effect of insulin resistance on the cardiovascular system has been most extensively investigated. Several investigators have shown that it is associated with a number of established cardiovascular risk factors, notably obesity, dyslipidemia, impaired glucose tolerance and raised blood pressure.[6] Insulin may raise blood pressure by a direct effect on the smooth muscle cells in the arterial wall, the water and salt regulation by the kidneys, and the hemostatic system.[7] Besides the effects on the carbohydrate and lipid metabolism, insulin is also involved in the steroid metabolism. Among others, insulin decreases the production of sex-hormone binding globulin (SHBG)[8] and dehydroepiandrosterone (DHEA).[9] The resulting increased androgenicity is associated with an increased risk of cardiovascular diseases.[10] However, several follow-up studies to the incidence of cardiovascular disease could not demonstrate an increased risk of hyperinsulinemia per se.[11] It has been suggested that the clustering of cardiovascular risk factors is more important than a direct effect of insulin.[12,13]

Cardiovascular disease has been shown to be associated with impaired cognitive function,[14] which may be the reason for the decreased cognitive function associated with hyperinsulinemia. Alternatively, in animal studies it has been shown that insulin has a direct effect on the brain.[15] Moreover, insulin stimulates the autonomic nervous system,[16] which may be the explanation for the increased levels in subjects with COPD.

Insulin resistance in subjects without diabetes mellitus is commonly accompanied by moderate hyperglycemia, which is associated with an increase of advanced glycation end products (AGE). These are formed by non-enzymatic glycation of proteins, at a rate directly proportional to the glucose concentration.[17] The best known AGE is glycated hemoglobin (HbA_{1c}), but virtually all proteins in the human body, both extra and intracellular, can be irreversible changed by glycation. This includes, among others, lipoproteins, neurofilaments, and collagen. Glycated collagen and changes in the extracellular matrix are associated with diabetes complications,[18] notably decreased vascular elasticity[19] and opacification of the eye

lens.[20] Moreover, AGE-specific receptors have been identified on monocytes and macrophages.[17] Binding of AGE proteins to this receptor induce the production of interleukin-1 and tumor necrosis factor α. These cytokines may contribute to injuries of the arterial wall (atherosclerosis), glomerular membrane (nephropathy) and bronchial wall (COPD).[21]

Another explanation for the association between insulin resistance and the clustering of diseases might be a less active way of life because of existing illness. Both diminished physical activity and obesity are strongly associated with insulin resistance.[22] However, the presented associations were independent of obesity. Although sick people are definitely less active, this does not imply that they had normal insulin resistance before the onset of the disease(s).

Part II: Insulin and biologic aging

Aging occurs only in humans and domestic and zoo animals.[23] It may be thought of as an aberration of civilization; from the viewpoint of evolution there is no advantage to live beyond the age of sexual maturity and the rearing of progeny. In humans this is about the age of 30, which is generally believed to be the time when age changes become detectable.

In spite of acquired worldly wisdom, age has its infirmities. However, the human aging process (decline of vision and memory, greying of the hairs, wrinkling of the skin, etc.) is not completely determined by the time since birth, some subjects seem unaffected by their increasing age. In other words, chronologic age does not always equal biologic age.[24] Until now, their are no specific measures of biologic age, the best approximation is chronologic age. However, generally people agree in their judgement of 'young elderly'; these subjects are relatively free of disease and active, both physically and socially.

From these observations one could infer that the presence of (chronic) diseases, as well as diminished disability, are markers of advanced biologic age. Both aspects are reflected in perceived health: 'young eldery' are likely to feel themselves younger and healthier than other persons of the same age.

Evidence from the Rotterdam Study

To investigate if insulin resistance is related to biologic age, the associations between insulin levels and the above mentioned markers were analyzed. First of all, insulin resistance increased with chronologic age. The increase of post-load serum insulin level was 0.6 mU/l per year in men (95% confidence interval 0.3 - 0.8), whereas 1.0 mU/l per year (95% CI 0.8 - 1.2) was found in women. Figures 4 and

5 of chapter 1.1 show that insulin gradually increased over the whole age range of the participants of the Rotterdam Study. The age-associated increase has been found in other studies as well.[25,26]

As discussed in the first part of this chapter, insulin resistance is associated with chronic diseases, independently of chronologic age. In addition, insulin resistance represents the early phase of non-insulin dependent diabetes mellitus,[27] and therefore is an important risk factor for diabetes, another chronic disease common in the elderly.[5]

In the Rotterdam Study activities of daily living (ADL), as measure of disability, was assessed by the Stanford Health Questionnaire.[28] Post-load insulin level was positively associated with both activity score and disability index, after adjustment for age (Table 1). If the ratio of post-load insulin over glucose was used, the same associations were found. As part of the home interview, the participants were asked how they perceive their health, compared to subjects of their own age. Of the men, 57.3% answered that their health was better, 34.4% the same, and 8.3% felt less healthy than their peers. In women these figures were 51.0%, 39.6%, and 9.4%, respectively. These proportions were independent of age. The post-load insulin level was associated with the mean score ($p < 0.01$, adjusted for age). Figure 2 shows that with increasing insulin levels the proportion of subjects who felt better than their peers decreased, whereas the number of subjects who felt worse decreased.

Further evidence for the association of insulin resistance with biologic age comes from observations in other type of studies. Insulin resistance is a well known feature of Werner's Syndrome, a genetic disorder of premature aging.[29] A decreased insulin sensitivity has also been described in patients with progeria (Hutchinson-Gilford Syndrome).[30] In studies using cell cultures changes observed in diabetes mellitus are similar to those observed in natural aging.[31]

Table 1 Associations between post-load insulin level (per 50 mU/l) and activities of daily living.

	Men		Women	
Activity score*	0.021	(-0.025 - 0.067)	0.075	(0.046 - 0.105)
Disability index†	0.013	(-0.006 - 0.031)	0.050	(0.033 - 0.068)

Values are age-adjusted coefficients of linear regression, with the 95% confidence interval between parentheses.

* 0: no difficulties with ADL, 1: some difficulties, 2: much difficulties, 3: unable to do.

† ranging from 0: no impediments to 3: unable to live independently.

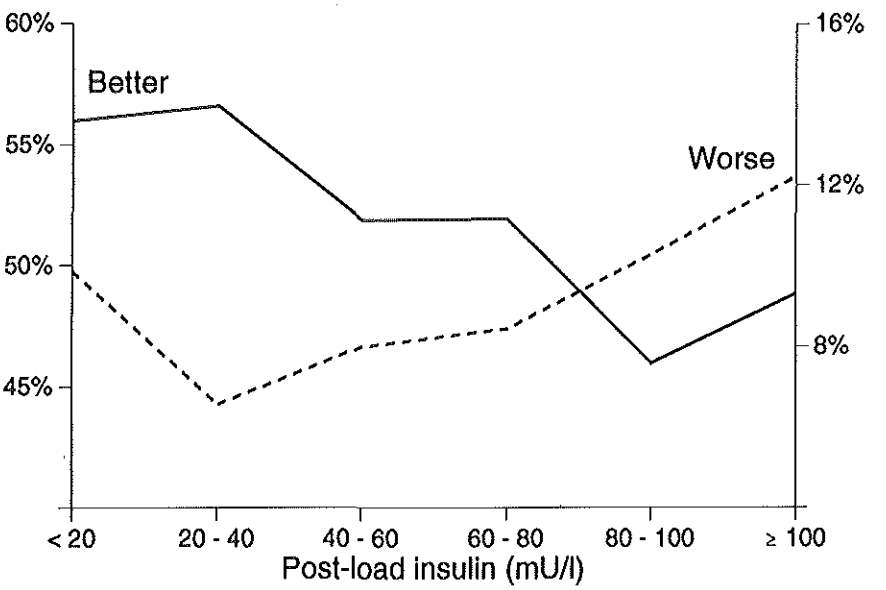

Figure 2 Proportion of subjects who perceived their health better and worse compared to subjects of their own age, by categories of post-load insulin.

A perspective for intervention?

It is conceivable that young biologic age decreases the risk of dying, which means that it increases the lifespan of that individual.[32] Besides to longevity, slow biologic aging is also related to postponement of chronic diseases. However, the concept of biologic aging is only of interest for medicine if intervention can dissociate it from chronologic aging, if 'usual' aging can be converted into 'successful' aging.[33] If, in non-pathologic circumstances, these two invariably proceed with the same speed it may be of interest for gerontologists, but not for physicians. A clear definition of biologic age does not exist and therefore neither appropriate pathophysiologic models for a possible etiologic role of insulin resistance. However, if improvement of insulin resistance induces changes in the approximate measures of biologic age this would be of interest for physicians and medical researchers.

Weight loss and physical activity are two non-pharmacological interventions known to decrease insulin resistance. Several investigators have shown that insulin resistance and the prevalance of NIDDM is associated with physical activity.[34,35,36] Studies in elderly subjects have shown that training programs and diet intervention decrease insulin resistance and are associated with less disease and increased vitality,[22,37] which was also found in patients with NIDDM.[38] In addition, young

subjects with paralysis due to spinal cord injury have the same degree of insulin resistant as elderly subjects, which is most likely caused by their extreme and prolonged physical inactivity.[39] Pharmacological treatment of insulin resistance has been largely restricted to patients with NIDDM.[40] Biguanides and sulfonylureas decrease insulin resistance in these patients, although their mechanism of action is not well known. In a small study in subjects without diabetes mellitus metformin improved insulin sensitivity and lowered blood pressure.[41] In addition, preliminary findings on troglitazone, a new antidiabetic drug, show a decrease of insulin resistance and blood pressure in obese subjects without diabetes mellitus.[42] So it seems possible to intervene in the course of insulin resistance and biologic age.

In spite of possible intervention, it is clear that biologic age increases continuously when time since birth passes. Theoretically, when an intervention is finished there are two alternatives. First, the increase in aging may proceed with the same speed as before, but starting with the younger biologic age achieved. Alternatively, after some time the biologic age returns to the level which would also be reached without intervention. In the latter situation intervention should be continued during the entire lifespan. It has been shown that the effects of exercise training on insulin sensitivity are rapidly reversible.[22,38] Also participants in dietary intervention programs for obesity often relapse to their former weight within a few years.[43,44] These findings suggest that intervention has a transient effect on insulin resistance, which has to be confirmed in studies using a prolonged intervention period.

Conclusion

There is ample evidence that hyperinsulinemia is associated with the presence of several chronic diseases. In addition to the clustering of diseases, insulin resistance is related to vitality, subjective health and physical activity, which are measures of biologic age. Despite the incomplete knowledge, insulin resistance may be used as marker of biologic age and guidance for interventions (both in research projects and in patient care) aimed at improving longevity and postponing chronic diseases in the elderly.

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Summary

Insulin resistance is a diminished ability to keep the serum glucose low with insulin levels in the normal range. Subjects with insulin resistance therefore usually have increased serum insulin levels. Insulin resistance increases with age. The aim of the studies presented in this dissertation was to investigate several aspects of insulin resistance and the ensuing hyperinsulinemia in an elderly population. The results are based on the baseline data of the Rotterdam Study, a population-based cohort study of determinants of chronic disabling diseases in the elderly. Overall 7983 subjects, aged 55 years and over, participated in this study. Besides the assessment of the glucose metabolism in all participants, an additional metabolic and endocrine examination was performed in a sample of 219 participants.

Chapter 1 gives an overview of the Rotterdam Study. In this study the glucose metabolism was assessed by serum fructosamine and a non-fasting oral glucose tolerance test (OGTT). Insulin was assessed in the post-load samples only. All these measures increased with age, as well as the prevalence of diabetes mellitus. About half of the subjects with diabetes were diagnosed during the Rotterdam Study *(1.1)*. This raises the question if the diagnosis based on a (single) OGTT is of equally importance for clinicians and epidemiologists. Based on the literature it is shown that for clinicians the use of the OGTT is very limited. However, to evaluate impaired glucose tolerance and insulin resistance in (large) population-based studies the OGTT remains the preferred approach. Insulin levels, fasting or after a glucose load, and the ratio of post-load insulin over glucose are adequate estimates of insulin resistance *(1.2)*. In addition, the results of a small study are presented on the effect of non-fasting on the results of the OGTT. It is shown that the post-load levels of both glucose and insulin are similar when the glucose load is given in the fasting or non-fasting state *(1.3)*.

Subjects with insulin resistance are generally characterized by increased age, obesity, and decreased physical activity. In *Chapter 2* several additional characteristics are investigated. Cortisol has an antagonistic effect on insulin (it increases the serum glucose level), as a consequence pathologically increased cortisol levels are associated with insulin resistance. This was also found in healthy elderly women, whereas insulin and cortisol levels were increased in men with hypertension. These findings suggest that cortisol may play a role in the pathogenesis of insulin resistance and hypertension *(2.1)*. Dehydroepiandrosterone sulfate (DHEAS) is another steroid hormone produced by the adrenal gland. Insulin and DHEAS levels were inversely associated in healthy elderly men. Because

androgens raise insulin resistance, this supports the hypothesis that DHEAS has predominantly oestrogenic effects in men. In addition, it was found that smoking and alcohol consumption increase the DHEAS level, and modify the association between insulin and DHEAS *(2.2)*. Furthermore, the association between liver function, assessed by serum liver enzymes, and insulin resistance was studied. Raised levels of liver enzymes were associated with increased insulin resistance, independent of gender, age, body mass index, body fat distribution, and alcohol use. These results suggest that impaired liver function is another characteristic of insulin resistance *(2.3)*.

Several investigators have reported that insulin resistance is associated with a number of cardiovascular risk factors, including obesity, dyslipidemia, and high blood pressure. This cluster of risk factors is called 'insulin resistance syndrome'. *Chapter 3* describes some studies on this clustering. Fasting insulin, glucose, HDL-cholesterol, triglycerides, body mass index, waist/hip ratio, and systolic blood pressure were significantly interrelated. This indicates that the components of the cluster in the elderly is the same as in middle-aged subjects. In addition, it was shown that fasting insulin level may be the best marker for this syndrome *(3.1)*. The diabetogenic effect of antihypertensive drugs is well known, and this may influence the associations between glucose metabolism and hypertension. Indeed, it was found that subjects using antihypertensive drugs have higher insulin levels, which remained after adjustment for blood pressure. Raised blood pressure was associated with increased insulin levels, and diminished the age-associated increase in insulin resistance, whereas the effect of antihypertensive drugs was independent of age *(3.2)*. Data from a 11.5 year follow-up study of a population in Zoetermeer show that subjects with hypertension have an increased risk of developing diabetes mellitus. This gives indirect evidence for the increased insulin resistance in subjects with hypertension *(3.3)*.

The Rotterdam Study covers four areas of chronic diseases: cardiovascular diseases, ophthalmologic diseases, neurogeriatric diseases, and osteoporosis. The associations of insulin resistance with these diseases are presented in *Chapter 4*. The degree of atherosclerosis was assessed by the ankle/arm index, and the number of plaques in the carotid arteries. Symptomatic cardiovascular disease was defined as a history of myocardial infarction or stroke, or the presence of angina pectoris or intermittent claudication. Insulin resistance was slightly increased in women with atherosclerosis. In both men and women insulin resistance and the prevalence of diabetes mellitus were increased in subjects with symptomatic cardiovascular disease. This supports the hypothesis that hyperinsulinemia in subjects with cardiovascular disease is predominantly the expression of increased cardiovascular risk factors ('insulin resistance syndrome'), whereas serum insulin

has no consistent effect on the development of atherosclerosis *(4.1)*. Raised serum glucose and fructosamine levels, but not insulin resistance, were associated with the presence and severity of retinopathy, assessed on fundus photographs. These associations were similar in subjects with and without diabetes mellitus, which indicate that the increased risk of hyperglycemia is already apparent in the normoglycemic range *(4.2)*. The Mini Mental State Examination (MMSE) was used to evaluate cognitive function. Increased post-load insulin level was associated with decreased cognitive function in women, but not in men. This association was independent of body mass index and systolic blood pressure. These findings are more in favour of a direct effect of insulin on the brain than of an effect by an increase in cardiovascular risk factors *(4.3)*. Finally, increased insulin and glucose levels were associated with raised bone mineral density. This suggest that, in contrast to other chronic diseases, insulin resistance is associated with a decreased risk of osteoporosis, which is explained by a direct effect of insulin on bone formation. Indeed, insulin levels are lower in subjects who reported a history of non-vertebral fractures in the previous five years *(4.4)*.

The role of insulin resistance as marker of biologic aging is examined in *Chapter 5*. First, insulin as risk factor of several chronic diseases is investigated, based on data from the Rotterdam Study and the literature. Insulin resistance is associated with the clustering of selected chronic diseases. Moreover, subjects who used a larger number of medications had an increased insulin resistance. The co-morbidity may be a measure of biologic age. In addition, degree of disability and perceived health were used to assess biologic age. Insulin resistance is associated with these measures. Therefore, insulin resistance may be used as marker of biologic age and guidance for interventions (both in research projects and in patient care) aimed at improving longevity and postponing chronic diseases in the elderly.

Samenvatting

Insulineresistentie is een verminderd vermogen om de bloedglucosespiegel laag te houden met een normale insulineconcentratie. Personen met insulineresistentie hebben hierdoor meestal een verhoogd insulinegehalte in het bloed. Insulineresistentie neemt toe met de leeftijd. Het doel van de onderzoeken die in dit proefschrift beschreven worden is het bestuderen van verschillende aspecten van insulineresistentie en de bijbehorende hyperinsulinemie bij ouderen. De resultaten zijn gebaseerd op de uitgangsgegevens verzameld in het ERGO onderzoek (Erasmus Rotterdam, Gezondheid en Ouderen), een longitudinaal bevolkingsonderzoek naar oorzaken van chronische ziekten en beperkingen bij ouderen. Aan dit onderzoek hebben 7983 personen van 55 jaar en ouder deelgenomen. Naast een meting van het glucosemetabolisme bij alle deelnemers, werd een aanvullend metabool en endocrinologisch onderzoek uitgevoerd bij een steekproef van 219 deelnemers.

Hoofdstuk 1 bevat een overzicht van het ERGO onderzoek. In dit onderzoek werd het glucosemetabolisme bestudeerd door middel van een niet-nuchtere orale glucose tolerantie test (OGTT). Serum insuline werd bepaald twee uur na de glucosebelasting. Bovendien is de serum fructosaminespiegel gemeten. Al deze maten namen toe met de leeftijd alsmede het vóórkomen van suikerziekte. Ongeveer de helft van de personen met suikerziekte werd gediagnostiseerd tijdens het ERGO onderzoek *(1.1)*. De vraag rijst of deze diagnose gebaseerd op een enkele OGTT terecht is. Op basis van de literatuur blijkt dat de waarde van de OGTT voor de diagnose van de individuele patiënt beperkt is. Echter, in bevolkingsonderzoek heeft de OGTT de voorkeur voor het aantonen van gestoorde glucosemetabolisme en insulineresistentie. Insulinespiegels, nuchter of na glucosebelasting, en de verhouding van de twee-uurs insuline- over glucosespiegel zijn een goede benadering van de insulineresistentie *(1.2)*. Verder worden de resultaten van een klein onderzoek beschreven naar de invloed van niet-nuchter zijn op de OGTT. Het blijkt dat de belaste waarden van zowel glucose als insuline gelijk zijn als de glucosebelasting gegeven wordt in de nuchtere of niet-nuchtere staat *(1.3)*.

Personen met insulineresistentie worden in het algemeen gekarakteriseerd door hogere leeftijd, overgewicht en verminderde lichamelijke activiteit. In *Hoofdstuk 2* wordt een aantal andere karakteristieken beschreven. Cortisol heeft een remmend effect op de werking van insuline (het verhoogt de bloedglucosespiegel). Hierdoor veroorzaakt een pathologisch verhoogde cortisolspiegel insulineresistentie. Dit werd ook gevonden bij gezonde oudere vrouwen. Bij mannen met hypertensie waren de insuline- en cortisolconcentraties verhoogd. Deze bevindingen geven aan dat cortisol

mogelijk betrokken is bij het ontstaan van insulineresistentie en hypertensie *(2.1)*. Dehydroepiandrosterone sulfaat (DHEAS) is een ander steroïdhormoon dat geproduceerd wordt in de bijnier. Insuline- en DHEAS-spiegels vertonen een negatief verband bij gezonde oudere mannen. Omdat androgenen insulineresistentie verhogen, ondersteunt dit de hypothese dat DHEAS bij mannen voornamelijk een oestrogene werking heeft. Daarnaast werd gevonden dat roken en alcoholconsumptie de DHEAS spiegels verhogen, alsmede het verband tussen insuline en DHEAS beïnvloeden *(2.2)*. Ten slotte bleek dat verhoogde concentraties van lever enzymen verband hielden met toegenomen insulineresistentie, onafhankelijk van geslacht, leeftijd, mate van overgewicht, vetverdeling en alcoholgebruik. Deze resultaten suggereren dat gestoorde leverfunctie een karakteristiek is van insulineresistentie *(2.3)*.

Verschillende onderzoekers hebben aangetoond dat insulineresistentie verband houdt met een aantal cardiovasculaire risicofactoren, zoals overgewicht, afwijkende bloedvetspiegels en verhoogde bloeddruk. Deze clustering van risicofactoren wordt het 'insulineresistentie syndroom' genoemd. *Hoofdstuk 3* beschrijft een aantal onderzoeken naar deze clustering. De nuchtere serumconcentraties van insuline, glucose, HDL-cholesterol, triglyceriden, en de Quetelet-index, taille/heup- ratio en systolische bloeddruk bleken onderling sterk samen te hangen. Dit geeft aan dat de componenten van het cluster bij ouderen hetzelfde zijn als bij personen van middelbare leeftijd. Bovendien is de nuchtere insuline spiegel waarschijnlijk de beste indicator van dit syndroom *(3.1)*. Het is bekend dat anti-hypertensieve medicatie diabetogeen kan werken, wat de verbanden van het glucose metabolisme met hypertensie kan beïnvloeden. Personen die anti-hypertensieve medicijnen gebruiken bleken inderdaad hogere insulineconcentraties te hebben, ook na correctie voor bloeddruk. Verhoogde bloeddruk ging samen met verhoogde insulinespiegels, en het verminderde de leeftijdsafhankelijke toename van insulineresistentie. Het effect van anti-hypertensieve medicatie op de insulineresistentie was onafhankelijk van de leeftijd *(3.2)*. De gegevens van een 11,5 jaar durend vervolgonderzoek onder de bevolking van Zoetermeer laten zien dat personen met hypertensie een verhoogd risico hebben op het ontstaan van suikerziekte. Dit is een indirecte ondersteuning voor de aanwezigheid van insulineresistentie bij personen met hypertensie *(3.3)*.

Het ERGO onderzoek beslaat vier terreinen van chronische ziekten: hart- en vaatziekten, oogziekten, neurogeriatrische ziekten en osteoporose. De relaties van insulineresistentie met deze ziekten komen aan de orde in *Hoofdstuk 4*. De mate van atherosclerose werd bepaald door middel van de enkel/arm index en het aantal plaques in de halsslagaders. Symptomatische hart- en vaatziekte werd gedefinieerd als een doorgemaakt hartinfarct of beroerte, of de aanwezigheid van angina pectoris of claudicatio intermittens. Insulineresistentie was marginaal verhoogd in vrouwen

met atherosclerose. Onder zowel mannen als vrouwen was insulineresistentie en de aanwezigheid van suikerziekte meer frequent bij personen met symptomatische hart- en vaatziekte. Dit ondersteunt de hypothese dat de verhoogde insulinespiegels bij personen met hart- en vaatziekten voornamelijk een uiting is van de verhoogde cardiovasculaire risicofactoren, verenigd in het insulineresistentie syndroom. Insuline zelf zou echter nauwelijks effect hebben op het ontstaan van atherosclerose *(4.1)*. Verhoogde glucose- en fructosaminespiegels, maar niet insulineresistentie, hielden verband met de aanwezigheid en ernst van diabetische netvliesaandoeningen, bepaald aan de hand van fundusfoto's. Deze verbanden waren hetzelfde in personen met en zonder suikerziekte, hetgeen aangeeft dat het risico van een verhoogde bloedsuikerspiegel al aanwezig is bij niet-diabetische waarden *(4.2)*. De 'Mini Mental State Examination (MMSE)' werd gebruikt voor het bepalen van de cognitieve functie. Een verhoogde insulinespiegel was geassocieerd met een verminderde cognitieve functie in vrouwen, maar niet in mannen. Dit verband was onafhankelijk van de mate van overgewicht en de systolische bloeddruk. Deze resultaten passen meer bij een direct effect van insuline op de hersenen dan bij een effect door verhoogde cardiovasculaire risicofactoren *(4.3)*. Ten slotte bleken de insuline en glucose spiegel geassocieerd te zijn met een verhoogde botdichtheid. Dit wijst erop dat, in tegenstelling tot andere chronische ziekten, insulineresistentie gerelateerd is aan een verminderd risico op osteoporose, wat wordt verklaard door een direct effect van insuline op de botvorming. Inderdaad is de insulinespiegel lager bij personen die in de afgelopen vijf jaar een bot gebroken hadden *(4.4)*.

De rol van insulineresistentie als maat voor biologische leeftijd komt aan de orde in *Hoofdstuk 5*. Ten eerste is insulineresistentie als risicofactor voor verschillende ziekten onderzocht, gebaseerd op gegevens van het ERGO onderzoek en de literatuur. Insulineresistentie is geassocieerd met een clustering van een aantal chronische ziekten. Bovendien hebben personen die meer medicijnen gebruiken een hogere insulineresistentie. Deze comorbiditeit zou een maat kunnen zijn voor biologische leeftijd. Verder kunnen de mate van beperkingen in het dagelijks funktioneren en de ervaren gezondheid gebruikt worden voor het meten van biologische leeftijd. Insulineresistentie houdt verband met elk van deze maten. Daarom zou insulineresistentie gebruikt kunnen worden als indicator voor biologische leeftijd, en voor de evaluatie van behandelingen (zowel in onderzoeksprojecten als patiëntenzorg), gericht op toename van de levensverwachting en uitstel van chronische ziekten bij ouderen.

Nawoord

Nu het werk gedaan is wil ik tenslotte jullie bedanken zonder wie ik dit boekje nooit had kunnen en willen maken.

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Curriculum vitae

The writer of this thesis was born on November 8th, 1963 in Rotterdam. In 1982 he graduated secondary school (Chr. Scholengemeenschap 'Westland-Zuid' in Vlaardingen) and started his medical study at the Erasmus University Medical School. He combined his study with several other student activities; worth mentioning are the membership of the board of the Medical School (one year), and a stay in Colombia (three months). He obtained his medical degree in 1989.

In this year he commenced his training in epidemiology at the Department of Epidemiology & Biostatistics of the same university (chair: Prof. A. Hofman) under the guidance of Prof. D.E. Grobbee. He explored the cardiovascular epidemiology with a grant from the Netherlands Heart Foundation.

The investigations for this thesis started in 1991 and were supported by the Netherlands Diabetes Fund. Most studies were conducted in collaboration with the Department of Internal Medicine III of the Academic Hospital 'Dijkzigt' (Prof. S.W.J. Lamberts, Prof. F.H. de Jong, Dr. H.A.P. Pols). In addition to the activities related to the project, he coordinated the scientific field work of the Rotterdam Study during one year. Specific knowledge in diabetes epidemiology was obtained as visiting fellow at the Diabetes Research Center of the Graduate School of Public Health in Pittsburgh (Prof. T.J. Orchard and Prof. R.E. LaPorte). Moreover, he participated in the 'Fifth Cambridge Seminar on the Epidemiology and Public Health aspects of Diabetes Mellitus'.

Currently he is secretary of the Netherlands Diabetes Epidemiology Study Group and faculty member of the Erasmus Summer Programme. He obtained a post-doc position at the Department of Epidemiology & Biostatistics, and will be involved in several projects related to diabetes mellitus and insulin resistance.

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