RELATIONSHIP BETWEEN HYPERINSULINEMIA AND ANGIOGRAPHICALLY DEFINED CORONARY ATHEROSCLEROSIS IN NON-DIABETIC MEN

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SUMMARY - Background: Our aim was to estimate the relationship between hyperinsulinemia and angiographically validated coronary atherosclerosis.

Methods: 102 consecutive non-diabetic male subjects (age 48.9 ± 9.6 years) with a body mass index 25.9 ± 2.8 kg/m² referred to coronary angiography in our centre were studied. A 75-g oral glucose tolerance test (OGTT) was performed and the areas under the curve (AUC) of serum insulin and glucose were calculated.

Results: Discriminant analysis with stepwise forward variable selection revealed that in younger patients (< 50 years), the following parameters affected positively the number of significant coronary lumen reductions: age (F = 8.5, p = 0.005), lnAUCinsulin (F = 5.8, p = 0.02), low HDL cholesterol (F = 4.4, p = 0.04), the smoking habit (F = 4.1, p = 0.05). In those ≥ 50 years of age, exclusively age (F = 5.8, p = 0.02) and hyperuricemia (F = 3.8, p = 0.06) entered the final model.

Conclusion: Our results indicate that in non-diabetic male subjects the association of hyperinsulinemia with the severity of coronary atherosclerosis is only seen in younger patients.

Key-words: coronary artery disease, insulin, oral glucose tolerance rest.

RéSUMÉ - Relation entre hyperinsulinisme et athérosclérose coronaire définie sur l’angiographie chez l’homme non-diabétique.

But : Le but de cette étude est d’évaluer la relation entre l’hyperinsulini- mie et l’athérosclérose mesurée lors de coronographies.

Méthodes : Cent-deux hommes non diabétiques (âge 48.9 ± 9.6) avec un IMC de 25.9 ± 2.8 kg/m² référés pour une angiographie coronarienne ont été étudiés dans notre centre. Un test oral de tolérance au glucose (75 g) a été effectué et la tolérance au glucose et l’hyperinsulinisme ont été évalués par le calcul de la surface sous la courbe.

Résultats : Une analyse discriminative par étape a révélé que, chez les jeunes patients (< 50 ans), les paramètres suivants ont influencé de ma- nière significative le nombre de sténoses coronariennes : l’âge (F = 8.5, p = 0.005), la réponse insulinique (F = 5.8, p = 0.02), un HDL cholestérol diminué (F = 4.4, p = 0.04), le tabagisme (F = 4.1, p = 0.05). Chez les pa- tients de ≥ 50 ans, seuls l’âge (F = 5.8, p = 0.02) et l’hyperuricémie (F = 3.8, p = 0.06) ont une influence significative sur le nombre de sténoses coro- nariennes.

Conclusion : Nos résultats indiquent que chez les hommes non diabéti- ques, l’association de l’hyperinsulinémie avec la sévérité de l’athérosclé- rose coronarienne est uniquement relevée chez les jeunes patients de moins de 50 ans.

Mots-clés : maladie coronarienne, insuline, hyperglycéémie provoquée.
Disturbances of carbohydrate metabolism have been demonstrated to be significantly more frequent in the patients with angiographically confirmed atherosclerotic coronary heart disease. Not only is the incidence of diabetes and of impaired glucose tolerance higher in this condition [1], but also the patients with normal glucose tolerance exhibit hyperinsulinemia and insulin resistance [1-5]. It is a well established phenomenon that at least a part of the association of insulin resistance/hyperinsulinemia with coronary atherosclerosis results from coexistence with multiple atherosclerotic risk factors [3, 6-8]. However even after adjustment for other variables hyperinsulinemia was shown to be independently related to elevated risk of the development of major coronary events during follow-up [9, 10].

Nevertheless, a recent meta-analysis [11] of the studies dealing with this problem revealed that the magnitude of this effect was different in various races, depended on the type of insulin assay involved as well as there was remarkable interstudy variety in the type of confounding variables for which adjustment was made. By analogy to these findings, multivariate analysis of factors affecting the presence and/or severity of coronary [2, 3, 12-14] or carotid artery [15, 16] atherosclerosis yielded contradictory results with regard to an independent effect of hyperinsulinemia. Additionally, even in a simple univariate analysis, in some studies hyperinsulinemia was shown to be unrelated to the presence of significant coronary artery narrowings [17, 18] or even that of diffuse coronary atherosclerosis in non-diabetics [19]. It is also noteworthy that relations of hyperinsulinemia with either cardiovascular risk [11] or carotid atherosclerosis [15, 16] differed in various ethnic groups. Finally, the European Fat Distribution Study have shown that relations of fasting insulinemia with serum lipids and blood pressure differed between European centres [20].

The aim of the present study was to estimate the relationship between hyperinsulinemic response to an oral glucose tolerance test and angiographically validated coronary atherosclerosis in nondiabetic male patients.

## MATERIALS AND METHODS

### Patients

We studied 102 consecutive male subjects referred to coronary angiography and hospitalised in our centre. Exclusion criteria were as follow: diabetes mellitus [diagnosed according to the results of a 75-g oral glucose tolerance test (OGTT) interpreted according to the World Health Organisation (WHO) criteria [21]], body mass index (BMI) > 35 kg/m², myocardial infarction or unstable angina within previous 6 months, congestive heart failure, clinical evidence of renal failure, diseases of the liver, thyroid gland, lungs and other coexistent relevant disorders or abnormalities. Cholesterol, triglycerides and uric acid levels were determined with standard methods.

The patients were divided into two groups on the basis of the severity of coronary atherosclerosis in angiography – moderate coronopathy (n = 57) was defined as one or no significant (≥ 75%) coronary artery lumen reductions, whereas those with severe coronopathy (n = 45) had more than one such narrowing.

### Oral glucose tolerance test (OGTT)

A 75-g OGTT was performed in the fasting state, blood samples for insulin and glucose assay were taken 30, 60 and 120 min after glucose intake, serum was separated and frozen until assayed. Glucose was determined with the enzymatic method and immunoassay. The magnitudes of glucose and insulin output during the OGTT were computed as the areas under the curve of levels of glucose (AUCglucose) and insulin (AUCinsulin), plotted against time, respectively. In addition, insulin/glucose ratio was calculated as AUCinsulin/AUCglucose, by analogy to the approach of Fujiwara et al. [3].

Since preliminary comparisons of insulinemia and glycemia between the patients with moderate and severe coronopathy brought different results in younger and older subjects, further analysis was also performed separately in the men below 50 years of age (n = 52) and the older (n = 50). In agreement with this approach biochemical variables related to carbohydrate metabolism were calculated in four subgroups of patients: younger men with moderate coronopathy (n = 35), younger men with severe coronopathy (n = 17), older men with moderate coronopathy (n = 22) and older men with severe coronopathy (n = 28).

### Statistical analysis

The intergroup comparisons were performed by a Student’s t-test. Due to a skewed pattern of distribution, fasting insulinemia, AUCinsulin and AUCinsulin/AUCglucose were natural logarithmically (ln)-transformed prior to any calculations in order to obtain the normal distribution (tested by the Shapiro-Wilk’s W test). Homogeneity of variances was checked by the Levene’s test.

In search for variables independently affecting the severity of coronary atherosclerosis, a discriminant analysis with stepwise forward variable selection was performed. In addition to fasting insulinemia (ln), fasting glucose, lnAUCinsulin, AUCglucose and lnAUCinsulin/AUCglucose the selected variables were taken into consideration as potential determi-
RESULTS

Mean patients’ age was 48.9 ± 9.6 years, BMI 25.9 ± 2.8 kg/m², total cholesterol concentration 5.5 ± 1.1 mmol/l, triglycerides levels 1.9 ± 0.8 mmol/l. Time from the first diagnosis of coronary artery disease was 4.0 ± 6.4 years. From among the analysed risk factors smoking habit was the most frequent being reported in 76% of the study group. 29% of the patients exhibited impaired glucose tolerance, smoking habit, hypertension, and a positive family history of premature coronary artery disease. The value of F to enter a variable to the model was set at 2.0. Results of discriminant analysis have been shown as follows: an independent variable which was entered, the step number, F to enter this variable, p value for that F value, overall Wilks’ λ after the respective step, F value associated with that λ, p level for that F value. Finally, ANOVA for the whole study group was performed to estimate the relationship between the number of the above mentioned risk factors in an individual patient and AUCinsulin. The statistical significance was defined at a probability levels of below 0.05.

For the study population as a whole there were no significant differences between groups with moderate and severe coronopathy, AUCinsulin and the AUCinsulin/AUCglucose ratio were significantly higher in those with severe coronary artery disease (p = 0.018 and p = 0.014 for AUCinsulin and AUCinsulin/AUCglucose, respectively) (Table I). When analysing variables concerning carbohydrate metabolism in the older group, no significant differences were found, yet there were tendencies for lower AUCinsulin/AUCglucose ratio (p = 0.06) and AUCinsulin (p = 0.07) in the subgroup with severe coronary artery disease (Table I).

Discriminant analysis revealed that in the study population as a whole, age was the only factor who influenced the number of critical coronary narrowings (F = 18.2, p < 0.0001, λ = 0.85). Within the younger group treated separately, the following variables affected positively variability of the number of significant coronary lumen reductions: age, AUCinsulin, smoking habit, hypercholesterolemia, low HDL cholesterol, impaired glucose tolerance (Table II). Within the older group, age and hyperuricemia entered the final model discriminating between the subgroups with moderate and severe coronary artery disease [age, step 1, F = 5.8 (p = 0.02), λ = 0.89, F = 5.8 (p = 0.02); hyperuricemia, step 2, F = 3.8 (p = 0.06), λ = 0.83, F = 5.0 (p = 0.01)].

ANOVA has confirmed the association of higher AUCinsulin with increasing number of analysed risk factors for atherosclerosis (p < 0.03).

DISCUSSION

Our results indicate the superiority of insulinemia on glucose challenge over fasting insulin levels with regard to the relationship with coronary atherosclerosis. The lack of significant differences in fasting insulinemia between the groups exhibiting more or less than one coronary narrowing exceeding 75% is con-

### Table I. Biochemical parameters in younger, older men and both groups together with moderate and severe coronopathy.

<table>
<thead>
<tr>
<th>Coranopathy</th>
<th>Moderate</th>
<th>Severe</th>
<th>Moderate</th>
<th>Severe</th>
<th>Whole group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>35</td>
<td>17</td>
<td>22</td>
<td>28</td>
<td>57</td>
</tr>
<tr>
<td>Fasting insulin, µU/ml</td>
<td>23.4 ± 4.3</td>
<td>24.5 ± 8.5</td>
<td>25.2 ± 7.1</td>
<td>20.4 ± 3.5</td>
<td>24.1 ± 3.8</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>4.2 ± 0.7</td>
<td>4.4 ± 0.9</td>
<td>4.2 ± 0.7</td>
<td>4.5 ± 1.0</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>lnAUCinsulin, (µU x min)/ml</td>
<td>9.3 ± 0.5</td>
<td>9.6 ± 0.5*</td>
<td>9.6 ± 0.5</td>
<td>9.4 ± 0.4</td>
<td>9.4 ± 0.5</td>
</tr>
<tr>
<td>AUCglucose mmol/l</td>
<td>992 ± 230</td>
<td>1033 ± 240</td>
<td>1120 ± 260</td>
<td>1151 ± 282</td>
<td>1042 ± 248</td>
</tr>
<tr>
<td>AUCinsulin/AUCglucose, mU/mmol</td>
<td>11.9 ± 4.2</td>
<td>16.4 ± 7.0*</td>
<td>15.9 ± 9.1</td>
<td>11.6 ± 4.4</td>
<td>13.4 ± 6.8</td>
</tr>
</tbody>
</table>

*p < 0.02 between severe and moderate coronopathy
contrary to the results by Negri et al. [14] who found both univariate and multivariate correlations between fasting insulinemia and the coronary atherosclerosis severity score, in 64 non-diabetic/non-obese men. However our data are partially consistent with those by Spallarossa et al. [2] who have revealed, using a comparable statistical approach. The age and insulinemia at 2 h of an OGTT were the only parameters entering the stepwise analysis discriminating men with no previous myocardial infarction on the basis of the presence of significant coronary narrowings. Moreover, Fujiwara et al. [3] have reported a relationship of the presence of significant coronary atherosclerosis with insulinemia on glucose challenge with no respective effect of fasting insulinemia in non-diabetic men as well as no intergroup differences in glycemia, similarly to our results. Several other authors have also described no association between angiographically validated coronary atherosclerosis and fasting insulin levels in non-diabetics [17, 19]. Moreover, in both Paris Prospective Study [22] and Helsinki Policemen Study [10] insulinemia during an OGTT but not fasting insulinemia were independently associated with cardiovascular risk.

A potential explanation for these [3, 10, 17, 19, 22] and for our findings has been proposed by Yen and Komshian et al. [23] from the Reaven’s group. In this recent study, reported in 490 healthy subjects, insulin resistance measured by the steady-state plasma glucose technique is more closely correlated with the total integrated insulin response to a 75-g oral glucose than with fasting insulin concentration. Accordingly, since it has been demonstrated by the Insulin Resistance Atherosclerosis Study (IRAS) investigators [15] that insulin resistance but not hyperinsulinemia was related to increased intimal-medial thickness of the carotid artery wall in non-Hispanic white non-diabetics, it might suggest that hyperinsulinemia might not promote atherosclerosis directly being rather only a hallmark of the insulin resistance syndrome. This concept might explain the relationship between glucose-stimulated but not fasting insulinemia in the present study.

In search for mechanisms responsible for the differences in determinants of the severity of coronary atherosclerosis between the two age groups, it is much easier to comment those found in the younger subgroup. In fact, clustering of insulin resistance and hyperinsulinemia with cardiovascular disease risk factors, previously shown to be more strongly expressed in lean versus obese subjects [8], was also detected in our data. In the subjects below 50 years of age, impaired glucose tolerance, hypercholesterolemia and low HDL cholesterol, a part of the insulin resistance-dependent dyslipidemia [6], remained predictors of severe coronopathy even after adjustment for insulinemia on glucose challenge.

It is to be pointed out that the described relationship between coronary atherosclerotic changes and insulinemia was limited to the men younger than 50 years. Neither insulinemia nor glycemia exhibited any relation to atherosclerotic lesions in older men. In addition, there was an insignificant tendency to lower AUCInsulin/AUCGlucose ratio and AUCInsulin in those with two and more coronary narrowings. Such findings seem to suggest that age determines the kind of association between insulin resistance and atherosclerosis. The opposite character of this association in

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Step number</th>
<th>F to enter the variable</th>
<th>p</th>
<th>overall Wilks' λ after the respective step</th>
<th>F associated with the λ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td>8.5</td>
<td>0.005</td>
<td>0.85</td>
<td>8.5</td>
<td>0.005</td>
</tr>
<tr>
<td>lnAUCinsulin</td>
<td>2</td>
<td>5.8</td>
<td>0.02</td>
<td>0.76</td>
<td>7.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Low HDL-cholesterol</td>
<td>3</td>
<td>4.4</td>
<td>0.04</td>
<td>0.61</td>
<td>5.9</td>
<td>0.00027</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>4</td>
<td>4.1</td>
<td>0.05</td>
<td>0.70</td>
<td>6.7</td>
<td>0.00070</td>
</tr>
<tr>
<td>AUCglucose</td>
<td>5</td>
<td>3.0</td>
<td>0.09</td>
<td>0.57</td>
<td>5.6</td>
<td>0.00019</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6</td>
<td>2.6</td>
<td>0.11</td>
<td>0.67</td>
<td>5.8</td>
<td>0.00066</td>
</tr>
</tbody>
</table>

TABLE II. Summary of stepwise discriminant analysis in younger patients; dependent variable: severity of coronopathy.
the two age groups could lead to the lack of the relationship between insulinemia and coronary atherosclerosis in our study group as a whole. The presence of these effects counterbalancing each other might also provide an explanation why in some studies there was no coincidence of coronary atherosclerosis with hyperinsulinemia even on glucose challenge [18, 19]. Moreover, this concept is not contradictory to the results of the Honolulu Heart Program [24] which revealed no significant association of hyperinsulinemia with coronary heart disease in 2,801 elderly non-diabetics. Another likely explanation for the differential association with age is a “diabetes bias”. After the age of 50 years old, a proportion of the hyperinsulinemic subjects have progressed to diabetes. Therefore, the exclusion of diabetic subjects from the study may have removed the more hyperinsulinemic subjects preferentially from the older group.

According to a hypothesis put forward by Burchfiel et al. [24], it might be speculated that men with coronary heart disease who survived might have suffered from a less-severe coronary heart disease than those who had died previously. Assuming that hyperinsulinemic subjects had also increased risk of death, this could have weakened the relationship between hyperinsulinemia and coronary atherosclerosis also in our study. That such a “survival bias” might take place, is in agreement with the results of the Paris Prospective Study [22] and of the Helsinki Policemen Study [10] where the associations of hyperinsulinemia with the increased age-adjusted hazard ratios for a major coronary event and for cardiovascular death were reduced with lengthening follow-up time.

In conclusion, our results indicate that in the selected subpopulation of non-diabetic male subjects the association of hyperinsulinemia with the severity of coronary atherosclerosis is independent of its relations with routinely assessed variables exclusively in men below 50 years of age.

REFERENCES


