Relationship of Plasma Insulin Levels to the Incidence of Myocardial Infarction and Coronary Heart Disease Mortality in a Middle-aged Population

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Summary. The possible role of plasma insulin levels as a risk factor of coronary heart disease has been studied in a population of 7246 non diabetic, working men, aged 43–54 years, initially free from heart disease, and followed for 63 months on average. 128 new coronary heart disease events (non fatal myocardial infarction and coronary related deaths) were detected during this period. The annual risk is analysed by a multivariate model including age, serum cholesterol and triglycerides, blood pressure, smoking, obesity, plasma glucose and insulin fasting and 2 hours after a 75 g oral glucose load. It is shown that the fasting plasma insulin level and the fasting insulin-glucose ratio are positively associated with risk independent of the other factors. The same variables, 2 hours after the glucose load are also positively associated with risk but their contributions are not significant in the multivariate analysis. It is concluded that high insulin levels may constitute an independent risk factor for coronary heart disease complications in middle aged non diabetic men.

Key words: Epidemiology, coronary heart disease risk factors, oral glucose tolerance test, plasma insulin.

It has been long recognized that diabetes mellitus leads to various clinical manifestations of atherosclerosis including coronary heart disease (CHD). Many retrospective and prospective studies [1] have confirmed this. Investigation of a possible link between so-called chemical diabetes as defined by glucose intolerance alone and the development of CHD have been extensive. However, the results remain unclear and controversial mainly due to the lack of adequate incidence data [2].

The hypothesis that high circulating insulin levels may be associated with the development of CHD has been supported in the past by experimental and clinical evidence. Stout [3, 4, 5] suggested two possible non exclusive mechanisms: a direct effect on arterial metabolism or through an effect on lipid metabolism.

The relationships of plasma insulin levels, fasting and two hours post oral glucose load to the onset of myocardial infarction and CHD related deaths as observed in the non diabetic middle aged males of the Paris Prospective Study have been investigated in the present work. In assessing these relationships, multivariate adjustment of classical CHD risk factors, particularly plasma lipids, has been systematically carried out.

Material and Methods

The general aims, methodology and first results of the Paris Prospective Study have been published [6] and only relevant data are given here.

One year after entry into the study, 7535 men aged 43–54 years, working in the Paris Civil Service attended the first annual follow-up session (1968–1973). This included a cardiovascular clinical examination, medical history questionnaire and biological measurements. At that time, 150 cases of coronary heart disease were diagnosed which represented 2% of the population. These cases have been excluded from the follow-up analysis.

Systolic blood pressure was measured in a sitting position during the clinical examination by a standard cuff technique. A body mass index (BMI) was computed from height (H) in metres and weight (W) in kg as BMI $= 1000 [\log_{10}(W/H^2)-1]$ [7]. Cigarette consumption was measured by the mean daily consumption during the preceeding 5 years estimated from a questionnaire about smoking habits during the life of the subject. After an overnight fast venous blood was taken for analysis of cholesterol [8] and triglycerides [9], plasma glucose [10] (G_0) and insulin [11] (I_0). A 75 g oral glucose tolerance test was performed [12] with measurement of plasma glucose (G_2) and insulin (I_2) two hours after the load.

Among the 7385 subjects initially free from CHD, followed-up for 63 months on average, 73 cases of myocardial infarction ascer-

Table 1. Coronary heart disease risk factors and plasma glucose and insulin in 7246 non diabetic men. Results are stated as mean, and mean ± 2 SD

Age (years)	Cholesterol (mg/dl)	Systolic BP (mm Hg)	Cigarettes (n/d)	Body mass index[a]	Triglycerides[b] (mg/dl)	Plasma glucose[b] (mg/dl)		Plasma insulin[b] (mU/ml)	
						0	2 h	0	2 h
49 (45–53)	217 (133–301)	144 (101–186)	9 (0–28)	410 (302–518)	109 (38–314)	101 (71–144)	99 (50–194)	10 (3–38)	32 (6–172)

0 = at fasting, 2 h = 2 h post oral glucose load
[a] Body mass index is expressed by 1000 [$\log_{10}$ (weight kg/height^{2}m)-1]
[b] m and m ± 2SD are computed in natural logarithm values, then transformed into original units
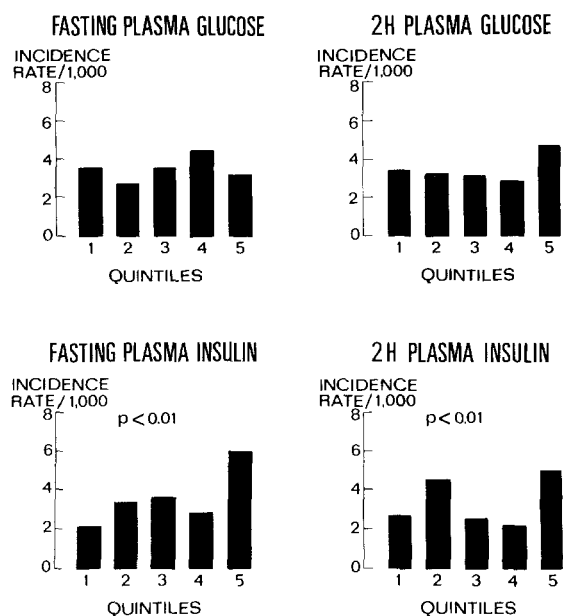

Fig. 1. Mean annual incidenve of CHD complications by quintiles of plasma glucose (G) and insulin (I) levels, fasting (0) and 2 hours after glucose load (2). Quintiles limits are defined as follows:
G_0 mg/dl: ≤ 92, 93–98, 99–103, 104–110, ≥ 111;
G_2 mg/dl: ≤ 77, 78–90, 91–105, 106–125, ≥ 126;
I_0 mU/l: ≤ 4, 5–7, 8–10, 11–15, ≥ 16;
I_2 mU/l: ≤ 16, 17–27, 28–41, 42–61, ≥ 62.
Comparison of observed and expected number of events among quintiles (chi-square test)

tained by hospital data and 59 CHD deaths, mostly sudden deaths were detected as of 1st January 1977. The mean annual incidence of overall CHD complications is 3.4/1000. Among 139 men with overt diabetes as defined by existing treatment for diabetes by drugs or diet, 4 new CHD cases were observed. All diabetic cases have been excluded from the following results.

Statistical Techniques

Multivariate analysis of the relationship between annual CHD incidence rates and different variables was performed by an exponential survival model with covariates which allows for unequal follow-up durations [13, 14]. The outline of the model is given in the Appendix.

Results

The mean and the mean ± 2SD interval for each variable analysed are given Table 1. Taking into account the "heavy tailed" distribution of serum triglycerides, plasma glucose and insulin values, these data were transformed by natural logarithms prior to analysis.

The annual incidence per 1000 of CHD complications is shown in Figure 1 according to quintiles of glucose and insulin levels, both fasting and two hours after glucose load. No clear-cut relationship of the CHD risk with any of the four variables is evident. The comparison (by a chi-square test) of observed and expected numbers of complications in each group showed no significant difference among G_0 and G_2 quintiles. No significant higher CHD incidence was detected for individuals with blood glucose above the 95th percentile (120 mg/100 ml for G_0 and 162 mg/100 ml for G_2) as the mean annual incidence rate was respectively 4.7/1000 (8 events) and 3.3/1000 (7 events) in these groups. However, significant differences of incidence were observed among I_0 and I_2 quintiles (p < 0.01). In both cases the highest rate was observed in the top quintile.

The estimate of CHD complications risk was based on classical factors which included the variables cholesterol, systolic BP and cigarette consumption as significant predictors (Table 2, left side). Taking into account the narrow range of age in the population, its coefficient was not significantly different from 0. Coefficients for triglycerides (taken as ln triglycerides) and body mass index were not significant when added to the previous variables as shown in Table 2 (right hand side).

Table 2. Coronary heart disease multivariate risk function including the four classical risk factors (left side) and then body mass index and triglycerides level (right side)

Variable	Coeff. b[a]	t value	P	Coeff. b[a]	t value	P
Age (years)	0.039	0.78	NS	0.041	0.80	NS
Cholesterol (mg/dl)	0.004	3.51	< 0.001	0.004	2.81	< 0.01
Systolic BP (mm Hg)	0.016	4.82	< 0.001	0.015	4.27	< 0.001
Cigarettes (n/d)	0.040	5.56	< 0.001	0.041	5.66	< 0.001
Body mass index				0.000	0.15	NS
Triglycerides[b] (mg/dl)				0.208	1.23	NS

[a] Coefficient of each variable in the multivariate expression of risk (see Appendix)
[b] ln value

Table 3. Coronary heart disease multivariate risk function including successively fasting (left side) and 2 h post load (right side) plasma glucose (G) and insulin (I) levels

Variable	Coeff. b[a]	t value	P	Coeff. b[a]	t value	P
Cholesterol (mg/dl)	0.005	3.52	< 0.001	0.004	3.30	< 0.001
Systolic BP (mm Hg)	0.015	4.12	< 0.001	0.015	4.32	< 0.001
Cigarettes (n/d)	0.042	5.77	< 0.001	0.044	5.90	< 0.001
G_0[b] (mg/dl)	−1.291	−1.56	NS			
I_0[b] (mU/l)	0.379	2.58	< 0.01			
G_2[b] (mg/dl)				−0.234	−0.67	NS
I_2[b] (mU/l)				0.182	1.32	NS

[a] Coefficient of each variable in the multivariate expression of risk (see Appendix)
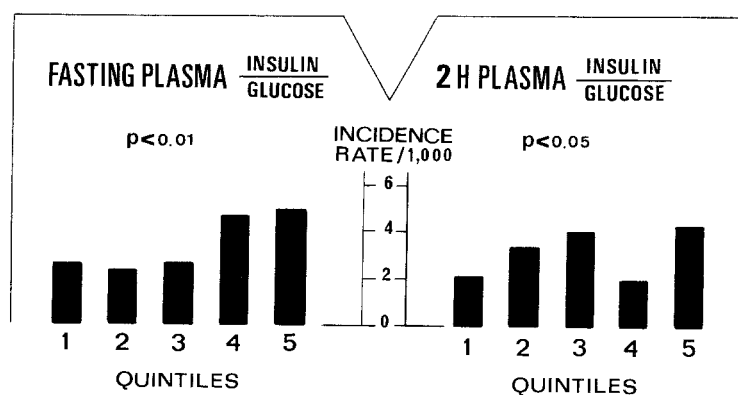[b] ln value

Fig. 2. Mean annual incidence of CHD complications according to quintiles of fasting (0) and 2 h after glucose load (2) plasma insulin/glucose ratios (I/G). Quintiles limits are defined as follows:
I_0/G_0 mU/g: ≤ 6, 7–9, 10–12, 13–17, ≥ 18;
I_2/G_2 mU/g: ≤ 16, 17–24, 25–34, 35–53, ≥ 54.
Comparison of observed and expected number of events among quintiles (chi-square test)

As a first step, fasting and two hour post glucose load insulin and glucose parameters were introduced independently in the multivariate model for CHD incidence (Table 3). In both cases, positive coefficients for insulin and negative coefficients for glucose were obtained, but only the I_0 coefficient reached statistical significance (p < 0.01) while the coefficients for the other risk factors did not show much change. These results suggest that the insulin/glucose ratios may be more strongly associated with risk than insulin values alone, at least when assuming the exponential model for risk dependence.

The annual incidence rate of CHD complications according to quintiles of I_0/G_0 and I_2/G_2 is depicted in Figure 2. It shows a higher CHD risk (multiplied by 1.9) for the last two quintiles of I_0/G_0 (≥ 13 mU/g) without any evident trend below this level. A pattern of consistent increase is not obtained with I_2/G_2 but incidence levels among quintiles are significantly different for both variables. Table 4 shows that in the multivariate analysis I_0/G_0 brought a significant independent contribution to the CHD risk estimate (left side). On the other hand, the coefficient of I_2/G_2 introduced in the same condition was not significant.

Table 4. Coronary heart diesease multivariate risk function including successively fasting (left side) and 2 h post load (right side) plasma insulin/glucose ratios (I/G)

Variable	Coeff. b[a]	t value	P	Coeff. b[a]	t value	P
Cholesterol (mg/dl)	0.004	3.36	< 0.001	0.004	3.28	< 0.001
Systolic BP (mm Hg)	0.014	4.09	< 0.001	0.015	4.51	< 0.001
Cigarettes (n/d)	0.042	5.77	< 0.001	0.043	5.90	< 0.001
I_0/G_0[b] (mU/g)	0.331	2.39	< 0.02			
I_2/G_2[b] (mU/g)				0.183	1.39	NS

[a] Coefficient of each variable in the multivariate expression of risk (see Appendix)
[b] In value

In these multivariate analyses, inclusion of body mass index and triglycerides levels, which were positively correlated with glucose and insulin levels in this population [12], did not cause any change in the results.

Discussion

The relationship of diabetes to the development of coronary heart disease has been a subject of major interest during the last fifteen years: a huge body of facts has been gathered, mostly from retrospective studies comparing diabetics, or patients with CHD, to controls.

The assessment of a relationship between parameters of the glucose metabolism and subsequent CHD complications in asymptomatic populations was an alternative approach. As far as impairment of glucose tolerance in otherwise asymptomatic people may represent "chemical diabetes", could it be considered as an aetiologic factor for CHD? In some studies an excess of CHD risk seems to be present in persons with borderline fasting blood glucose levels and/or glucose intolerance [15, 16], but there is no convincing evidence whether or not hyperglycaemia is an independent risk factor.

Recent data from fifteen population studies from the International Collaborative Group [17] failed to indicate a clear-cut continuous independent association of glucose level (fasting or post load) with CHD mortality. The hypothesis of a threshold effect with higher CHD mortality in the extreme 2 or 3% of the 2 h post load glucose distribution was raised mainly from data of the Whitehall Study [18] which was the only one to include an adequate number of men in this subgroup. In the preliminary results of the Paris Prospective Study [19], only one CHD death was observed in the 2.5th percentile and no conclusion could be drawn. However, the present results do not show a significant increase of risk of myocardial infarction and CHD death which would be confined to the extreme 5% of the G_0 or G_2 distribution.

The possibility that hyperinsulinaemia would be involved in the development of the atherosclerotic process and CHD complications in humans was set forth particularly by Stout [5] who presented a series of convincing experimental and clinical arguments. The recent results of the Helsinki Policemen Study [20, 21], the Busselton Study [22], and those of the present work produce the first prospective bulk of evidence for this association in asymptomatic middle aged men.

As early as 1965, plasma insulin levels during an oral glucose tolerance test were found to be higher in non diabetic myocardial infarction survivors than in controls [23, 24]. From that time, numerous retrospective studies have globally verified this fact but detailed results are often at variance, as discussed in [5].

Our finding that a high fasting insulin level (I_0) is more closely associated with CHD risk than corresponding values two hours after a glucose load (I_2) is not in accordance with the results of Pyorälä [21]. He found that fasting, 1 h and 2 h plasma insulin levels were associated with a 5 year incidence of CHD death and non fatal myocardial infarction. The 2 h level appeared, however, a better predictor than the fasting level, and was kept in the multivariate analysis of risk. Welborn [22] observed a significant association of 1 h insulin level with CHD incidence and CHD mortality in men and, in this last case, it persisted when other risk factors were taken into account. However, the same association was not found among women.

When the insulin and other risk factor levels are kept constant in the multivariate analysis, the negative coefficient for glucose, though non significant, is a striking feature of the present results. This may suggest that in non diabetic middle aged men an excess of insulin for a given glucose level, classically called "insulin resistance" [25], is the key variable for predicting CHD complications. Pyorälä [22] found a non significant but positive coefficient for 2 h blood glucose when 2 h insulin was taken into account. The insulin/glucose ratio has been proposed [26, 27] as a

measure of insulin resistance and, accordingly, this variable has been conventionally introduced in the analysis. However, Sorge [28] has already found a higher $\Sigma I/\Sigma G$ ratio in subjects with myocardial infarction than in controls (with I and G measured 1 and 2 hours after glucose load), and Santen [29] a higher fasting I/G ratio in diabetics with atherosclerosis compared to diabetics without atherosclerosis.

The inter-relationship of insulinaemia with obesity and hyperlipidaemia in diabetics and non diabetics has been extensively studied [1]. This discussion is beyond the scope of the present work; but whatever mechanisms are involved, highly significant positive correlations have been generally observed between these variables and particularly triglycerides [12]. In each case where a possible link between insulinaemia and CHD has been evoked, a key role for hypertriglyceridaemia has been suggested. However, there is no consistent epidemiological evidence [30] for the relationship of triglycerides to CHD risk in men when the cholesterol level is controlled. Negative results, as in this study, were obtained in Framingham [31], Göteborg [32] and Helsinki [21]. On the contrary a clear-cut positive relationship was published by Carlson [33] and most evidence cited relies on this work alone, the methodology of which has been criticized [34]. From the present results, it seems unlikely that the CHD risk increase with the insulin level is mediated by corpulence and/or triglycerides. No data is available, however, on the role of other forms of circulating lipids particularly HDL cholesterol, whose importance has recently been stressed once again. At least in women over fifty years of age, diabetes but not triglycerides remained a CHD risk factor independently of HDL cholesterol in the Framingham Study [32]. Further studies are required to elucidate the role of insulin in the aetiology of CHD in man, but the hypothesis of a direct effect on the atherosclerosis process may be considered from the present evidence.

Appendix

Exponential Survival Model with Covariates

During follow-up (5 years on average) the hazard rate for CHD is supposed to be constant (r) for each subject. This assumption is equivalent to stating that the probability that the subject will suffer or die from CHD before the instant t is $1-e^{-rt}$, the classical exponential survival model. The hazard rate r is a function of the covariates $x_1, x_2 \ldots$ (age, cholesterol ...) chosen as:

$$r = r_0 \exp (b_1 x_1 + \ldots + b_k x_k)$$

If N is the total number of subjects, n the number of new CHD events, t_i their time delay before the occurence of the CHD complications and the follow-up duration for the other subjects, then the ln likelihood of the observations is:

$$L = n \ln r_0 + \sum_{i=1}^{i=n} (b_1 x_1^i + \ldots + b_k x_k^i)$$

$$- r_0 \sum_{i=1}^{i=N} t_i \exp (b_1 x_1^i + \ldots + b_k x_k^i)$$

Maximizing this quantity by an iterative technique gives the estimates and standard errors of r_0 and b_j's, providing a test of significance of the b_j's by the Student t test.

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