Clinical research
Prevention and epidemiology

Circulating concentrations of insulin markers and coronary heart disease: a quantitative review of 19 Western prospective studies

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Aims It is uncertain whether there are associations between circulating levels of insulin markers and coronary heart disease (CHD) risk. We report an updated meta-analysis of studies of circulating levels of three insulin markers (fasting insulin, non-fasting insulin, and pro-insulin) and CHD risk.

Methods and results Prospective studies based in Western populations that reported on associations between levels of fasting insulin, non-fasting insulin, and pro-insulin and incident CHD [defined as non-fatal myocardial infarction (MI) or coronary death] were identified by computer-based searches and by manual searches of the relevant literature. Nineteen relevant population-based studies were identified, of which 14 reported on fasting insulin levels involving 2649 CHD cases, eight reported on non-fasting insulin levels involving 1980 CHD cases and three reported on pro-insulin levels involving 413 CHD cases. In a comparison of individuals who had circulating levels of each of these markers in the top third with those in the bottom third of the population, the odds ratio for CHD was 1.12 [95% confidence interval (CI): 0.98–1.28] for raised fasting insulin, 1.35 (1.14–1.60) for raised non-fasting insulin, and 2.23 (1.65–3.00) for raised pro-insulin. There was no good evidence of heterogeneity in these estimates attributable to the several study characteristics recorded, including sex, assay methods used, or degree of adjustment of risk estimates, but the available data in many of these sub-groups, particularly by sex, are sparse.

Conclusion Associations between CHD risk and fasting or non-fasting insulin levels are likely to be more modest than previously suspected. Preliminary data suggest that pro-insulin levels may be more strongly associated with CHD risk than are insulin levels, and this possibility should be evaluated in larger and more rigorous studies.

KEYWORDS
Insulin; Pro-insulin; Coronary disease; Meta-analysis

Introduction

Epidemiological studies have consistently demonstrated that individuals with type 2 diabetes have around a two-fold increased risk of coronary heart disease (CHD) compared with individuals free from diabetes, but the cause of this excess remains uncertain.1 It has been suggested that elevated circulating levels of insulin (an indirect marker of insulin resistance) or its precursor pro-insulin (which may act as a more direct agent of vascular damage and better reflect impaired β cell function3) might be mediators of the association between type 2 diabetes and CHD risk.3,4 Two previous reviews have attempted to quantify associations between such insulin markers and subsequent risk of CHD by synthesizing available data from prospective studies. The first was a meta-analysis reported in 1998 of published data from 12 prospective studies involving a total of 800 CHD endpoints.4 It reported relative risks of 1.17 (95% CI 1.09–1.26) and 1.16 (1.06–1.27) for a 50 and 250 pmol/L increase in fasting and non-fasting insulin levels (approximately equivalent to the inter-quartile range for each), respectively. It also suggested that the risks might vary importantly by ethnic background and by insulin assay methods.4 The validity of these observations was, however, limited by the relatively few CHD cases available for analysis, by possible heterogeneity in CHD outcomes used and by combination of results from Western and non-Western populations (which, due to substantially different insulin concentrations among different ethnic groups, could complicate interpretation). A more recent, non-overlapping meta-analysis1 that involved individual data from 11 prospective studies, comprising a total of about 400 vascular deaths in Western European populations, reported a relative risk of 1.54 (1.16–2.03) in men and 2.66 (1.45–4.90) in women in a comparison of individuals in the top quarter compared with those in the bottom quarter of circulating fasting insulin concentrations.

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[with corresponding relative risks of 0.85 (0.60–1.21) in men and 1.36 (0.53–3.45) in women for non-fasting insulin concentrations]. Although the second review focused exclusively on Western populations and involved re-analysis of primary data, it was potentially limited by inclusion of only a few hundred disease endpoints and by the use of the broad outcome of total vascular mortality.

The present updated literature-based meta-analysis differs in several important ways from these previous reviews. First, its definition of coronary disease is less heterogeneous, defined as non-fatal MI according to the World Health Organisation or similar criteria or coronary death. Second, it involves about three times as many cases as in both earlier reviews combined, involving data from 19 prospective studies2–23 involving a total of about 3600 incident CHD cases. Third, it is restricted to studies in North American and Western European populations, which should reduce ethnic heterogeneity. Fourth, it considers evidence on three different insulin parameters: concentrations of insulin in fasting and in non-fasting participants, as well as of pro-insulin, circulating levels of which are thought to increase, perhaps even more steeply than those of insulin, as the glucose tolerance of individuals deteriorates from normal glucose tolerance to impaired glucose tolerance to type 2 diabetes.2 Finally, it has recorded potentially relevant study characteristics (e.g. features related to assay and population sampling methods) to enable a more detailed exploration of possible sources of heterogeneity than previously reported.

Methods

Prospective cohort studies and ‘nested’ case-control studies published before January 2006 with over a year’s follow up in approximately general populations (i.e. in cohorts not selected on the basis of pre-existing disease) that excluded all participants with diabetes at baseline were sought using MEDLINE searches, not limited to the English language, scanning of relevant reference lists, and hand searching of relevant journals. Computer searches used free search terms and combinations of key words related to insulin [e.g. insulin, pro-insulin, hyperinsulin(a)emia, metabolic] and to CHD [e.g. coronary heart disease, cardiovascular diseases, ischaemic disease, myocardial infarction, atherosclerosis]. As shown in Figure 1, the initial search identified 2022 articles that were reduced to 87 potentially relevant articles following screening of published abstracts. Nineteen eligible studies were included in the present report after application of the exclusion criteria (i.e. eligible studies had to involve a prospective design in an essentially general population, record non-fatal MI or coronary death, exclude participants with diabetes at entry and report estimates of effects of insulin levels on coronary risk: see Figure 2 legend). The following information was abstracted in duplicate from each article using a standardized abstraction form: study size, study population (including population source and the sampling method employed), age range of participants at baseline, mean age at baseline, mean duration of follow-up, inclusion and exclusion criteria applied, percentage of male participants, whether blood samples used were fresh or frozen (and the temperature at which they were stored), the source of blood (whether plasma or serum), assay methods used, mean levels of insulin markers in controls (where available), reported risks associated with CHD (recorded separately where available for men and women), and the degree of adjustment for any potential confounders. The present meta-analysis is based only on within-study comparisons (i.e. cases have been compared directly only with controls within the same study), thereby avoiding possible biases that may be caused by methodological differences between studies. Reported results were converted to a standardized format (for the analyses of the risk of CHD among individuals in the top third of the distribution vs. those in the bottom third) using methods previously described.24 Results of studies were combined using inverse variance weighted averages of log risk ratios using a fixed effect model (Stata Corporation, USA). To make some allowance for multiple comparisons, 99% confidence limits were used for individual studies with 95% confidence limits reserved for combined estimates. Heterogeneity was assessed by standard χ² tests and the I²-statistic, which describes the percentage of variation in the log odds ratios that is attributable to genuine differences across studies rather than random error.25 The presence of publication bias was formally tested using the Begg and the Egger tests. Study size, mean duration of follow-up, geographical location, exclusion of participants with pre-existing CHD at baseline, sex differences, source of blood (i.e. plasma or serum), assay specificity, assay method, degree of adjustment of risk estimates, and whether insulin measurements were made after a 2 h oral glucose tolerance test were pre-specified as characteristics for assessment of heterogeneity, but other potentially relevant subgroup analyses (such as by age and by different levels of circulating insulin concentrations or any continuous associations) could not be reliably investigated, since individual participant data were not available.

Results

Nineteen relevant studies were identified5–23 of which 145,7,8,10–17,19,20,22 reported on fasting insulin levels (total of 2649 CHD cases; weighted mean follow-up 9.1 years); eight5,6,9,12,14,18,21,22 reported on non-fasting insulin levels (total of 1980 CHD cases; weighted mean follow-up 13.7 years); and three10,15,19 reported on pro-insulin levels (total of 413 CHD cases; weighted mean follow-up 9.5 years) (Table 1). There was no clear evidence of publication bias in any of the three markers (Begg and Egger tests P > 0.1 for each). There was some evidence of heterogeneity among the findings of the eight published studies of fasting insulin (χ² = 36.6; P = 0.001; I² = 59% (95% CI 29–76%)), but not much of it was explained by study size (χ² = 0.2; P = 0.62), mean duration of follow-up (χ² = 1.5; P = 0.22), geographical location (χ² = 0.01; P = 0.91), sex (χ² = 3.2; P = 0.20), blood source (χ² = 0.7; P = 0.40), assay specificity (χ² = 0.06; P = 0.81), or assay type (χ² = 0.64; P = 0.42), although there was a statistically significant difference between studies that excluded individuals with prevalent CHD at baseline compared with studies that did not (χ² = 6.2; P = 0.01), with stronger association with risk in the former group. There was no discernible difference in the magnitude of the association when studies were grouped according to the degree of adjustment made for possible confounding factors, including, notably, studies that adjusted risk estimates for body mass index (χ² = 1.5; P = 0.47). Using only within-study comparisons, a combined analysis of these 14 studies yielded a risk ratio of 1.12 (0.98–1.28) in a comparison of extreme thirds of fasting insulin values (Figures 2 and 3). There was no clear evidence of heterogeneity among the findings of the eight published studies of non-fasting insulin (χ² = 17.0; P = 0.05; I² = 47% (95% CI 0–74%)), with little of it due to study size (χ² = 0.4; P = 0.55), mean duration of follow-up (χ² = 0.5; P = 0.49), geographical location (χ² = 1.9; P = 0.17), sex (χ² = 0.9; P = 0.64), exclusion of individuals with prevalent CHD at baseline (χ² = 0.1; P = 0.98), blood source (χ² = 1.6; P = 0.20), assay specificity (χ² = 0.2; P = 0.64), assay type (χ² = 0.2;
$P = 0.64$), the timing of insulin measurement (i.e., whether insulin levels were measured following a 2 hr oral glucose tolerance test) ($\chi^2 = 0.5; P = 0.48$), or in studies grouped according to the degree of adjustment of risk estimates ($\chi^2 = 5.7; P = 0.06$). A combined analysis of these eight studies yielded a risk ratio of 1.35 (1.14–1.60) in a comparison of extreme thirds of non-fasting insulin values (Figures 2 and 3). There was no evidence of heterogeneity among the findings of the three published studies of pro-insulin ($\chi^2 = 0.2; P = 0.89; I^2 = 0\% \ (95\% \ CI \ 0–90\%)$), and a combined analysis of them yielded a risk ratio of 2.23 (1.65–3.00) in a comparison of extreme thirds (Figure 2).

**Discussion**

Previously reported meta-analyses have not been able to quantify precisely associations between circulating levels of fasting and non-fasting insulin and CHD risk.$^{3,4}$ The present updated meta-analysis, which involves 19 prospective studies based in essentially general Western populations with information on over 3600 incident cases of non-fatal MI or CHD death, provides a more comprehensive assessment than previously possible of associations between circulating levels of different markers of insulin and CHD. It suggests that associations between CHD risk and fasting or non-fasting insulin levels are likely to be modest and do not appear to vary strongly by the laboratory characteristics shown in Figure 3, although further data are needed to test this finding. Circulating levels of pro-insulin, the precursor of insulin, may be more strongly associated with CHD risk than circulating insulin levels, but the evidence is still preliminary and comprises three prospective studies involving a total of only about 400 cases.$^{10,15,19}$ Although there are plausible mechanisms by which insulin and pro-insulin levels might directly promote vascular diseases$^2$ (reviewed previously$^{3,4}$), the discussion below focuses on the quantitative and methodological uncertainties that need to be addressed in the interpretation of existing and future epidemiological data to help improve knowledge about any associations.

In the present meta-analysis, cases were compared directly only with controls within the same studies, thereby avoiding any bias due to the use of different laboratory methods or population characteristics. Even so, limitations of the present meta-analysis merit careful consideration. The comparability of insulin distributions between the different studies could not accurately be assessed, as most studies did not report appropriate measures of distribution for this highly skewed analyte (e.g., medians and inter-quartile ranges). Available assay methods for the measurement of insulin or pro-insulin levels are not standardized, underscoring the need to use, as in the current review, only within-study comparisons. Reports typically contained insufficiently detailed information on the duration between blood collection and blood separation (as degradation of insulin molecules by proteases released by red blood cells may occur if separation is not performed with suitable rapidity, ideally
Table 1  Characteristics of published prospective studies of insulin measurements and coronary heart disease in Western populations

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Population source/sampling method</th>
<th>Time of baseline survey</th>
<th>Total number of participants</th>
<th>Number of CHD cases</th>
<th>Age range (years)</th>
<th>Male (%)</th>
<th>Mean follow-up (years)</th>
<th>Mean (SD) values in controls at baseline (pmol/L)</th>
<th>Insulin assay</th>
<th>Source</th>
<th>Specific assay</th>
<th>Sample state at analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malmo</td>
<td>Sweden</td>
<td>Population register/complete</td>
<td>1974–84</td>
<td>6074</td>
<td>677</td>
<td>25–63</td>
<td>100</td>
<td>19</td>
<td>48.0 (53.4) b</td>
<td>150.0 (238.8) b</td>
<td>NS</td>
<td>No</td>
<td>NS</td>
</tr>
<tr>
<td>BRHS</td>
<td>UK</td>
<td>GP registers/random</td>
<td>1978–80</td>
<td>5550</td>
<td>621</td>
<td>40–59</td>
<td>100</td>
<td>11.5</td>
<td>81.8 (36.6–127.0) c</td>
<td>No</td>
<td>NS</td>
<td>No</td>
<td>Frozen (–20°C)</td>
</tr>
<tr>
<td>PRIIME</td>
<td>France/NI</td>
<td>Occupational register/complete</td>
<td>1991–94</td>
<td>10 592</td>
<td>294</td>
<td>50–59</td>
<td>100</td>
<td>5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>No</td>
<td>Frozen (–80°C)</td>
</tr>
<tr>
<td>Busselton</td>
<td>Australia</td>
<td>Electoral rolls/complete</td>
<td>1966</td>
<td>2179</td>
<td>231</td>
<td>&gt;20</td>
<td>47</td>
<td>23</td>
<td>-- Males: 238.9 (12.0) c; females: 300.7 (9.5) c</td>
<td>Sanofi</td>
<td>No</td>
<td>No</td>
<td>Frozen (NS)</td>
</tr>
<tr>
<td>Upsala</td>
<td>Sweden</td>
<td>Population register/complete</td>
<td>1970</td>
<td>874</td>
<td>219</td>
<td>50</td>
<td>100</td>
<td>26</td>
<td>48.2 (34.9) d</td>
<td>3.0 (3.2) d</td>
<td>Sanofi</td>
<td>Yes</td>
<td>Frozen (–70°C)</td>
</tr>
<tr>
<td>MRFIT</td>
<td>USA</td>
<td>Occupational register/complete</td>
<td>1973–76</td>
<td>12 866</td>
<td>208</td>
<td>35–57</td>
<td>100</td>
<td>7</td>
<td>97.9 (54.8)</td>
<td>NS</td>
<td>No</td>
<td>No</td>
<td>Frozen (–70°C)</td>
</tr>
<tr>
<td>Paris</td>
<td>France</td>
<td>Occupational register/complete</td>
<td>1968–73</td>
<td>6903</td>
<td>174</td>
<td>42–53</td>
<td>100</td>
<td>15</td>
<td>71.0 (13.8)</td>
<td>NS</td>
<td>No</td>
<td>No</td>
<td>NS</td>
</tr>
<tr>
<td>Goteborg</td>
<td>Sweden</td>
<td>Birth cohort/complete</td>
<td>1980</td>
<td>833</td>
<td>161</td>
<td>57–67</td>
<td>100</td>
<td>13</td>
<td>75.6 (51.6) c</td>
<td>NR</td>
<td>--</td>
<td>NS</td>
<td>Phadebas (NS)</td>
</tr>
<tr>
<td>Lempiänen et al.</td>
<td>Finland</td>
<td>Population register/complete</td>
<td>1986–88</td>
<td>1069</td>
<td>151</td>
<td>65–74</td>
<td>37</td>
<td>7</td>
<td>8.0 (1.8)c; females: 91.8 (1.8) c</td>
<td>NR</td>
<td>--</td>
<td>NS</td>
<td>Phadebas (NS)</td>
</tr>
<tr>
<td>Caerphilly</td>
<td>UK</td>
<td>Electoral rolls/Random</td>
<td>1979–83</td>
<td>1056</td>
<td>127</td>
<td>45–59</td>
<td>100</td>
<td>12</td>
<td>26.4 (18.4–39.4) c</td>
<td>NR</td>
<td>--</td>
<td>NS</td>
<td>Novo (–80°C)</td>
</tr>
<tr>
<td>Kuopio</td>
<td>Finland</td>
<td>Population register/complete</td>
<td>1984–89</td>
<td>1521</td>
<td>110</td>
<td>42–60</td>
<td>100</td>
<td>9</td>
<td>77 (47)c</td>
<td>NR</td>
<td>--</td>
<td>NS</td>
<td>Novo (–80°C)</td>
</tr>
<tr>
<td>Quebec</td>
<td>Canada</td>
<td>Population register/complete</td>
<td>1985</td>
<td>2103</td>
<td>91</td>
<td>45–76</td>
<td>100</td>
<td>5</td>
<td>78.2 (28.8)</td>
<td>Linco</td>
<td>Yes</td>
<td>Frozen (NS)</td>
<td></td>
</tr>
<tr>
<td>Helsinki Policemen</td>
<td>Finland</td>
<td>Occupational register/complete</td>
<td>1971–72</td>
<td>970</td>
<td>80</td>
<td>34–64</td>
<td>100</td>
<td>22</td>
<td>NR</td>
<td>NS</td>
<td>No</td>
<td>No</td>
<td>NS</td>
</tr>
<tr>
<td>Northern Sweden</td>
<td>Sweden</td>
<td>Population register/complete</td>
<td>1985</td>
<td>32 400</td>
<td>67</td>
<td>25–64</td>
<td>82</td>
<td>9</td>
<td>22.8 (NS)</td>
<td>NS</td>
<td>Yes</td>
<td>Frozen (NS)</td>
<td></td>
</tr>
<tr>
<td>Glostrup</td>
<td>Denmark</td>
<td>Birth cohort/complete</td>
<td>1976</td>
<td>1052</td>
<td>54</td>
<td>40</td>
<td>48</td>
<td>17</td>
<td>60.0 (NS)</td>
<td>NS</td>
<td>No</td>
<td>No</td>
<td>Frozen (–20°C)</td>
</tr>
<tr>
<td>Rancho Bernardo</td>
<td>USA</td>
<td>Population register/complete</td>
<td>1984–87</td>
<td>1243</td>
<td>42</td>
<td>50–89</td>
<td>43</td>
<td>5</td>
<td>Males: 353.4 (NS)c; females: 418.2 (NS)d</td>
<td>NS</td>
<td>No</td>
<td>No</td>
<td>NS</td>
</tr>
<tr>
<td>Barilla</td>
<td>Italy</td>
<td>Occupational/complete</td>
<td>1981</td>
<td>647</td>
<td>23</td>
<td>NS</td>
<td>64</td>
<td>12</td>
<td>38.4 (2.4)d</td>
<td>NS</td>
<td>No</td>
<td>No</td>
<td>NS</td>
</tr>
<tr>
<td>Hargreaves et al.</td>
<td>Scotland</td>
<td>Population register/complete</td>
<td>1976</td>
<td>107</td>
<td>11</td>
<td>40</td>
<td>100</td>
<td>12</td>
<td>NS</td>
<td>NS</td>
<td>No</td>
<td>No</td>
<td>NS</td>
</tr>
</tbody>
</table>

–, not known to be measured; CHD, coronary heart disease; BRHS, British Regional Heart Study; ARIC, Atherosclerosis Risk in Communities Study; PRIIME, Prospective Epidemiological Study of Myocardial Infarction; MRFIT, Multiple Risk Factor Intervention Trial; NS, not stated; NR, although measurements were made in these studies, results were not reported; NI, Northern Ireland.

aInsulin-specific assays are reported to have little or no cross-reactivity with other insulin-like molecules (such as pro-insulin), in contrast to non-specific assays.

bMedian (standard deviation).

cResults reported as nested-case–control studies.

dReported for cases and controls combined.

eMedian (inter-quartile range).
A similar lack of detail in reports made it impossible to explore the potential impact of pre-analytical factors, such as whether participants were instructed to limit heavy exercise, smoking, or alcohol consumption in the 24 h preceding venepuncture. It was not possible to assess reliably sex-specific associations, as the data on women comprised only about 150 cases. Neither could the impact of adjustment for possible confounding factors be reliably investigated because the present review was based on variably adjusted data reported in the published literature rather than on individual participant data (although grouping studies by degree of reported adjustment did not indicate major differences, this was not a sensitive assessment given the available numbers: Figure 3). For similar reasons, it was not possible to characterize in the current analyses the shape of any dose–response relationships, leaving open the possibility that a re-analysis of individual data (or generation of new larger-scale data) could reveal stronger associations based on non-linear threshold effects. Analysis of primary data would also be required to assess whether the magnitude of associations change with duration of follow-up (although grouping studies by the mean duration of follow-up did not suggest any important differences; Figure 3). It was not possible to correct risk estimates for within-individual fluctuations over time (i.e. regression dilution bias) in insulin and pro-insulin levels because reliable reproducibility data involving serial measurements have not yet been reported; lack of such correction would tend to underestimate any associations. Conversely, due to the relatively widespread availability of assays for the measurement of circulating insulin levels, it may be that studies with less striking findings have been less likely to be reported; lack of such correction would tend to underestimate any associations. Conversely, due to the relatively widespread availability of assays for the measurement of circulating insulin levels, it may be that studies with less striking findings have been less likely to be reported; lack of such correction would tend to underestimate any associations. Conversely, due to the relatively widespread availability of assays for the measurement of circulating insulin levels, it may be that studies with less striking findings have been less likely to be reported; lack of such correction would tend to underestimate any associations.

Figure 2  Prospective studies of concentrations of circulating insulin markers and coronary heart disease in Western populations. Two studies\textsuperscript{27,28} included in a previously reported meta-analysis\textsuperscript{4} (involving a total of 36 cases) were not included in the current report as they reported on the risk associated with electrocardiogram abnormalities (rather than non-fatal myocardial infarction or coronary heart disease death) and were not based in Western populations. Eight studies included in a previously reported meta-analysis of individual data\textsuperscript{3} (involving a total of 328 cases) were not included in the current report as they reported on the risk associated with any cardiovascular mortality (rather than non-fatal myocardial infarction or coronary heart disease death). BRHS British Regional Heart Study; ARIC Atherosclerosis Risk in Communities Study; PRIME, Prospective Epidemiological Study of Myocardial Infarction; MRFIT, Multiple Risk Factor Intervention Trial. Asterisk indicates that 99% confidence limits are used for individual studies with 95% confidence limits reserved for pooled estimates.
Studies of the risk of coronary heart disease in individuals in the top third vs. bottom third of circulating fasting and non-fasting insulin levels, grouped according to several study characteristics. Asterisk indicates that circulating insulin levels were measured 2 h after the ingestion of a 75 g load of glucose.

**Conclusion**

Associations between CHD risk and circulating levels of insulin are likely to be more modest than previously suspected. The sparse data available suggest that pro-insulin levels may be more strongly associated with CHD risk than are insulin levels, and this possibility should be evaluated in larger and more rigorous studies.

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