Short report

Proinsulin and insulin responses to a mixed meal in hypertriglyceridaemic men

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Abstract

Aim—To investigate the contribution of proinsulin to the “hyperinsulinaemia” of hypertriglyceridaemia.

Methods—Plasma glucose, triglyceride, immunoreactive insulin, and intact proinsulin concentrations were measured before and after a mixed meal in 11 hypertriglyceridaemic men and six healthy normotriglyceridaemic male controls.

Results—Hypertriglyceridaemic subjects had greater fasting ($101 \text{ v } 50 \text{ pmol/l}$) and integrated ($139 \text{ v } 81 \times 0^{-3} \text{ pmol/l/h}$) insulin concentrations than controls. Fasting and integrated glucose and proinsulin concentrations were similar in the two groups.

Conclusions—Proinsulin does not contribute to the hyperinsulinaemia observed in hypertriglyceridaemic subjects and is therefore unlikely to contribute to the increased cardiovascular risk associated with hypertriglyceridaemia.

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Keywords: hypertriglyceridaemia; insulin; proinsulin

Hypertriglyceridaemia and postprandial lipaemia are risk factors in the development of coronary artery disease.¹ Hypertriglyceridaemia is characterised by hyperinsulinaemia which until recently has been defined by insulin assays using polyclonal antisera in which lower biological activity proinsulin and partially processed proinsulin cross react.² It has been shown that apparently hyperinsulinaemic patients with type 2 (non-insulin-dependent) diabetes mellitus are insulin deficient but hyperproinsulinaemic.³ It is therefore possible that proinsulin could contribute to the hyperinsulinaemia of hypertriglyceridaemia. This may be of importance since hyperproinsulinaemia has been associated with increased cardiovascular risk⁴⁻⁶ and may partly explain the increased cardiovascular morbidity reported in hypertriglyceridaemia. There are, however, few data on plasma proinsulin levels in hypertriglyceridaemic subjects with normal glucose tolerance; in particular the contribution of proinsulin to the postprandial hyperinsulinaemia of hypertriglyceridaemia has not been studied before. We therefore measured plasma glucose, triglyceride, insulin, and proinsulin concentrations before and after a mixed meal for four hours in 11 hypertriglyceridaemic men and in six healthy normotriglyceridaemic male controls.

Methods

Eleven hypertriglyceridaemic men and six normotriglyceridaemic men were studied. Fasting serum triglycerides were more than 2.8 mmol/litre in hypertriglyceridaemic subjects and less than 2.1 mmol/litre in controls. All were non-obese (body mass index (BMI) less than 30 kg/m²), without clinical or biochemical evidence of diabetes mellitus or any other disease and with no family history of diabetes mellitus. All consented to participate in the study, which was approved by the South West Surrey District ethics committee.

After a 14 hour overnight fast, subjects consumed a 1129 kcal meal consisting of 53.7 g of fat, 154 g of carbohydrate, and 18.2 g of protein. Blood was collected from an indwelling venous cannula before the meal and then every 30 minutes for four hours. Plasma was separated and either analysed for glucose and triglycerides or stored at -20°C until analysed for insulin and proinsulin.

Glucose, triglycerides, and cholesterol were measured by automated standard enzymatic methods. Insulin and proinsulin were measured by radioimmunoassay.^{7,8} The insulin assay has 100% cross reactivity with proinsulin. The proinsulin assay does not cross react (< 0.01%) with insulin or C peptide, less than 5% with 31/32 split proinsulin, and 92% with 65/66 split proinsulin. Respective interassay and intra-assay coefficients of variation were for glucose 2.0% and 1.6%; for triglycerides 2.2% and 1.2%; for insulin 7.4% and 5.4%; and for proinsulin 8.2% and 7.4%. Respective detection limits for insulin and proinsulin were 19 pmol/l and 4 pmol/l.

Results are expressed as medians with 95% confidence limits unless otherwise stated. Since the data were not normally distributed, the Mann-Whitney U test was used to compare the significance of difference between groups. Integrated responses (total area under the curve) were calculated using the linear trapezoid rule. Spearman's rank correlation (*r*) was used to measure the significance of association between variables.

Results

Age, BMI, and serum cholesterol were similar in both groups (table 1). Subjects with hypertriglyceridaemia had greater fasting ($p = 0.030$) and integrated ($p = 0.020$) insulin concentrations than controls (table 1 and fig 1). Fasting and integrated glucose and proinsulin were similar in the two groups (table 1 and fig 1).

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Table 1 Characteristics of hypertriglyceridaemic men and male controls, and their plasma triglyceride, glucose, insulin, and proinsulin responses during a mixed meal

Variable	Controls	Hypertriglyceridaemic men	p Value
n	6	11	
Age (years)	36.8 (26.3 to 47.4)	42.0 (35.3 to 48.7)	0.301
BMI (kg/m ²)	27.1 (24.7 to 28.8)	27.0 (25.7 to 28.1)	0.850
Fasting plasma cholesterol (mmol/litre)	6.1 (4.4 to 8.0)	7.0 (5.9 to 7.8)	0.350
Fasting plasma triglyceride (mmol/litre)	1.3 (0.8 to 1.8)	5.5 (3.9 to 9.3)	< 0.001
Integrated triglycerides (mmol/litre/h)	399 (160 to 709)	1506 (1123 to 2286)	< 0.001
Fasting glucose (mmol/litre)	4.8 (4.0 to 5.4)	5.2 (4.9 to 5.4)	0.078
Integrated glucose (mmol/litre/h)	1332 (1198 to 1677)	1568 (1472 to 1791)	0.149
Fasting insulin (pmol/litre)	50 (25 to 73)	101 (56 to 235)	0.030
Integrated insulin $\times 10^{-3}$ (pmol/litre/h)	81 (52 to 102)	139 (100 to 222)	0.020
Fasting proinsulin (pmol/litre)	15 (5 to 27)	14 (10 to 19)	0.961
Integrated proinsulin $\times 10^{-3}$ (pmol/litre/h)	9.9 (2.4 to 19.8)	10.3 (5.7 to 19.7)	0.999

Values are medians (95% confidence intervals).
BMI, body mass index.

Fasting and integrated triglycerides correlated with fasting insulin ($r = 0.62$, $p = 0.009$ and $r = 0.63$, $p = 0.006$, respectively), and with integrated insulin ($r = 0.54$, $p = 0.026$ and $r = 0.50$, $p = 0.41$, respectively). Fasting glucose correlated with age ($r = 0.66$, $p = 0.004$) and fasting triglycerides ($r = 0.57$, $p = 0.017$). Integrated glucose correlated with age ($r = 0.66$, $p = 0.004$), integrated triglycerides ($r = 0.49$, $p = 0.045$), integrated insulin ($r = 0.68$, $p = 0.003$), fasting proinsulin ($r = 0.69$, $p = 0.002$), and integrated proinsulin ($r = 0.57$, $p = 0.017$). Integrated insulin correlated with integrated proinsulin ($r = 0.64$, $p = 0.006$). There were no other correlations.

Figure 1 Mean triglyceride (A), glucose (B), insulin (C), and proinsulin (D) responses to a mixed meal in 11 hypertriglyceridaemic men (■) and six male control subjects (●). Error bars = SEM. * $p < 0.05$.

Discussion

We have confirmed that hypertriglyceridaemic subjects have fasting and stimulated hyperinsulinaemia. The similar proinsulin concentrations in hypertriglyceridaemic subjects and their controls suggest that proinsulin hypersecretion does not contribute to their fasting or postprandial hyperinsulinaemia. It is possible that 31/32 split proinsulin (which does not cross react in this proinsulin assay) could contribute to the hyperinsulinaemia but this is unlikely as raised 31/32 split concentrations are usually associated with hyperproinsulinaemia.⁹ Although hyperproinsulinaemia has been reported in hypertriglyceridaemic subjects with type 2 diabetes mellitus,¹⁰ this is probably related to their glucose intolerance³ rather than their hypertriglyceridaemia.² Since hypertriglyceridaemic subjects with normal glucose tolerance have normoproinsulinaemia it is unlikely that proinsulin contributes to the increased cardiovascular morbidity of hypertriglyceridaemia.

In summary proinsulin does not contribute to the hyperinsulinaemia observed in hypertriglyceridaemic subjects and therefore the increased cardiovascular morbidity associated with hypertriglyceridaemia is unlikely to be caused by altered proinsulin concentrations.

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