The role of insulin in clustering of serum lipids and blood pressure in children and adolescents

The Cardiovascular Risk in Young Finns Study

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Summary In adults hyperinsulinaemia is associated with an atherogenic risk profile including obesity, low levels of HDL-cholesterol, high levels of triglycerides and elevated blood pressure. To examine these associations in the young we studied the cross-sectional relationships of insulin with obesity indices (body mass index, subscapular skinfold thickness), serum lipids and blood pressure in 1,865 children, adolescents and young adults aged 6–24 years. We also used longitudinal data to study the value of a single insulin measurement to predict high risk factor levels and clustering of multiple risk factors after a 6-year follow-up. In cross-sectional analyses the levels of triglycerides, HDL-cholesterol, systolic blood pressure and obesity indices were usually significantly different across the quartiles of fasting insulin in both sexes among children, adolescents and young adults. In general, no associations were seen with total cholesterol or LDL-cholesterol. In prospective analysis elevated baseline insulin was related to the incidence of hypertriglyceridaemia ($\geq$ 95th percentile) at the follow-up. This relationship persisted even after adjustments for baseline obesity or 6-year change in obesity status. Moreover, baseline insulin concentration was higher in subjects who subsequently showed clustering of high triglycerides, low HDL-cholesterol and high systolic blood pressure levels at the follow-up. We conclude that high fasting insulin levels measured in children and adolescents predict the development of hypertriglyceridaemia years later. In addition, high insulin levels seem to precede the development of a potentially atherogenic risk factor profile including low HDL-cholesterol, high triglycerides and high systolic blood pressure. [Diabetologia (1995) 38: 1042–1050]

Key words Insulin, longitudinal, clustering, children, adolescents, serum lipoproteins, blood pressure.

Dyslipidaemia and high blood pressure often co-exist within the same individual [1–5]. Both of these disorders have been related to obesity and increased plasma insulin levels [6–9]. Lipid abnormalities, elevated blood pressure and high insulin levels are all risk factors for coronary heart disease [10–15]; therefore this cluster of disorders has attracted much atten-

tion. Recently a hypothesis has been suggested that hyperinsulinaemia and/or insulin resistance may be the key factor that underlies the association of serum lipids and blood pressure [16]. However, this view has also been challenged [17].

While the above-mentioned associations are recognized in adults, only a few studies have been done in children and young adults, none of which have been prospective studies [18]. The available prospective studies in adults have been carried out in two populations: among adults in the San Antonio Heart Study [19, 20], and among elderly subjects in eastern Finland [21, 22]. These studies have shown that serum insulin level predicts the development of multiple metabolic disorders including hypertriglyceridaemia, low (HDL)-cholesterol concen-

tration, hypertension, and non-insulin-dependent diabetes.

The present analysis was carried out to study the associations of insulin with serum lipids, blood pressure and obesity index variables in a representative cohort of children and young adults. We will describe the cross-sectional associations of insulin with these risk indicators, and show results from prospective analysis aimed at testing the hypothesis that high insulin levels precede the changes in lipids and blood pressure.

Subjects and methods

Population. The Cardiovascular Risk in Young Finns Study is an ongoing large multicentre study on the risk factors of coronary heart disease and their determinants in children, adolescents and young adults. A baseline cross-sectional study was done in 1980. The initial study population consisted of 4,320 children and adolescents aged 3, 6, 9, 12, 15, and 18 years. Of those invited, 3,596 subjects (83.2%) participated. Details of the baseline study in 1980 have been published previously [23]. Follow-up studies were carried out in 1983 and 1986 with the same protocol [24]. The loss of subjects was approximately 20% and 30% after 3 and 6 years, respectively. Complete data on serum fasting insulin, LDL-cholesterol, total cholesterol, HDL-cholesterol, triglycerides and blood pressure variables from 1980 and 1986 were available on 1,865 subjects, who comprise the cohort in this analysis (age at baseline 6–18 years). Children 3 years of age at baseline were not included due to differences in methodology of blood pressure measurements. The study protocol was approved by the Ethics Committees of each five participating universities (medical schools of Helsinki, Kuopio, Oulu, Tampere, and Turku).

Obesity and blood pressure measurements. Height was measured by a Seca anthropometer (Vogel & Halke, Hamburg, Germany) and weight by a Seca weighing scale. Body mass index (BMI) was calculated from the formula: weight kg/height m^2. Subscapular skinfolds, expressed as mm, were measured in triplicate from the nondominating arm using a Harpenden skinfolds caliper (Holtain and Bull, British Indicators Ltd., Luton, Beds., UK). Errors in subscapular skinfold measurements were studied according to standard methods [25]. The mean intraobserver error (coefficient of variation) in practice was 4.9%, and the interobserver error during the field study was 14.8%. Measurement errors were of similar magnitude as in other studies [25]. The unadjusted Pearson's correlation coefficient between BMI and subscapular skinfold thickness was 0.76 in 1980, and 0.72 in 1986 (both, $p < 0.001$).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a standard mercury sphygmomanometer in 1980, and in 1986 a random zero sphygmomanometer was used. Readings to the nearest even number of mm Hg were performed three times on each subject. The mean value of three measurements was used in statistical analysis. Korotkoff's fifth phase was used as the sign of DBP. Details of the methods describing the measuring of blood pressure and anthropometric variables have been presented elsewhere [26, 27].

Blood samples. For the determination of serum lipoprotein levels, venous blood samples were taken after an overnight fast. Serum samples were stored frozen for no more than 6 months until analysed. All lipid determinations were done in duplicate and in the same laboratory having a reference system with the World Health Organization (WHO) Central Laboratory (Prague, Czech Republic). Standard enzymatic methods were used for serum cholesterol (Boehringer CHOD-PAP kits, Boehringer Mannheim GmbH, Mannheim, Germany) and triglycerides (Boehringer Mannheim GmbH). Serum HDL-cholesterol concentration was measured from the serum supernatant after precipitation of (VLDL) and LDL with dextran sulphate and $MgCl_2$ [28]. Details of the methods have been presented elsewhere [29, 30]. The concentration of LDL-cholesterol was calculated using the Friedewald formula [31]. Serum samples for the determination of insulin were taken after an overnight fast. Sera were separated and stored at −20 °C until assayed. Insulin was measured using a modification of the immunoassay method of Herbert et al. [32].

Statistical analysis

The associations of insulin level with obesity indices, serum lipids and blood pressure were tested with linear regression models. The cross-sectional analyses were carried out after stratification of the study population by age and sex. In 1980, two age groups were formed: children (6–12 years) and adolescents (15–18 years). In 1986, three age groups were formed: children (12 years), adolescents (15–18 years) and young adults (21–24 years). Clustering of risk factors was studied by comparing the observed number of subjects with multiple risk factors with the expected number. The expected number was calculated from the assumption of independence, i.e. assuming that risk factors do not occur simultaneously more often than based on their independent occurrences. Clustering was defined as having simultaneously high triglycerides, high SBP and low HDL-cholesterol ($\geq$ 75th age- and sex-specific percentile for triglycerides and SBP, $\leq$ 25th percentile for HDL-cholesterol). The significance of the difference between the observed and expected rates was tested with the chi-square test. The independent effects of insulin and obesity levels on clustering were studied by logistic regression models, in which insulin and obesity indices were independent variables. In these analyses BMI and subscapular skinfold thickness, as well as insulin, were modelled as four-class variables (age- and sex-specific quartiles).

Longitudinal data were used to study whether baseline insulin level would predict the incidence of extreme serum lipid or blood pressure values, or the incidence of clustering at the follow-up. Risk factor values were defined as extreme, if they were at or above the age- and sex-specific 95th percentile at the follow-up ($\leq$ 5th percentile for HDL-cholesterol). The definition of clustering was similar as in the cross-sectional analysis, i.e. simultaneous occurrence of high triglycerides, high SBP and low HDL-cholesterol (at their extreme quartiles: highest for triglycerides and SBP, and lowest for HDL-cholesterol). Only subjects free of these particular risk factors at baseline were included in the analysis. The effect of baseline insulin level on these end-points was tested by logistic regression models adjusted for baseline BMI and the 6-year change in BMI. All statistical tests were performed with Statistical Analysis System, SAS [33]. Data are shown as mean ± SD.

Table 1. Cross-sectional associations of insulin with obesity indices at baseline

Mean insulin concentration (mU/l) in each quartile	Sex	Age (years)	Insulin quartiles				p-value[a]
			I	II	III	IV	
	Females	6–12	4.0	7.1	10.0	15.2	
		15–18	7.3	11.3	14.6	21.1	
	Males	6–12	3.2	5.6	8.0	12.3	
		15–18	5.8	9.6	12.7	18.4	
BMI (kg/m^2)	Females	6–12	16.0 ± 1.8	16.2 ± 2.3	16.8 ± 2.3	18.0 ± 1.8	< 0.001
		15–18	20.0 ± 1.8	20.1 ± 2.5	20.5 ± 2.6	21.4 ± 2.3	< 0.001
	Males	6–12	15.8 ± 2.0	16.2 ± 1.7	16.7 ± 2.1	17.5 ± 2.8	< 0.001
		15–18	20.5 ± 2.0	20.4 ± 2.5	20.2 ± 2.3	21.7 ± 3.6	< 0.05
Subscapular skinfold (mm)	Females	6–12	7.2 ± 2.9	7.4 ± 3.6	8.4 ± 4.3	10.6 ± 6.3	< 0.001
		15–18	11.6 ± 3.8	12.5 ± 5.5	12.7 ± 4.9	15.1 ± 6.1	< 0.001
	Males	6–12	5.4 ± 1.6	5.8 ± 1.7	6.9 ± 3.9	7.7 ± 5.0	< 0.001
		15–18	8.8 ± 4.1	8.7 ± 3.2	9.3 ± 3.7	11.8 ± 7.5	< 0.001

Data are mean ± SD.
[a] Linear trend by multiple regression analysis.
No. of subjects by quartiles of insulin: Females, age-group 6–12 years: I = 148; II = 180; III = 156; IV = 190.

Females, age-group 15–18 years: I = 74; II = 84; III = 86; IV = 86. Males, age-group 6–12 years: I = 134; II = 145; III = 153; IV = 167. Males, age-group 15–18 years: I = 63; II = 62; III = 64; IV = 73

Results

Cross-sectional associations with obesity indices, serum lipids and blood pressure. Table 1 shows the mean values of BMI and subscapular skinfold thickness across the age- and sex-specific quartiles of fasting insulin level at baseline (1980). These obesity indices were strongly and directly associated with insulin level in both sexes, and in both age groups, i.e. children (6–12 years) and adolescents (15–18 years). Similar cross-sectional associations between obesity indices and insulin were seen at follow-up (1986) (Table 2) in all age groups, i.e. children (12 years), adolescents (15–18 years) and young adults (21–24 years).

The associations of fasting insulin with serum lipids and blood pressure at baseline (1980) are shown in Table 3. In general, in both sexes among children and adolescents, the levels of triglycerides and SBP became linearly greater from lowest to highest insulin quartile. However, an inverse association was usually seen with HDL-cholesterol levels. No associations were seen between LDL-cholesterol or DBP and insulin levels. In young males (6–12 years), a weak, but significant ($p < 0.05$) inverse association was seen between total cholesterol concentration and insulin level.

The cross-sectional associations of insulin and other risk markers at the follow-up (1986) are shown in Table 4. Again, among both sexes and in nearly all age groups, the levels of triglycerides and SBP were directly associated with the level of insulin, whereas an inverse association was seen between HDL-cholesterol and insulin levels. In addition, a direct association was seen between DBP and insulin level among females in two age groups (15–18 and 21–24 years), and among males in the youngest age group (6–12 years).

Since triglycerides, HDL-cholesterol and blood pressure levels are known to associate with obesity indices [34], the p-values shown in Tables 3 and 4 were adjusted for BMI (the results were identical after adjustment for subscapular skinfold thickness). When all age groups where pooled together, the results remained essentially the same both at the baseline and at the follow-up: in both sexes triglycerides and SBP were directly, and HDL-cholesterol inversely, associated with the insulin level.

Clustering of risk factors. According to data in Tables 3 and 4 increased insulin concentrations were most consistently related to high triglycerides and low HDL-cholesterol levels, as well as to elevated SBP. Next we assessed whether these three factors showed significant clustering in the study population. At baseline, there were 75 subjects (4.0 %) having all these three risk factors in the extreme age- and sex-specific quartiles ($\geq$ 75th percentile for triglycerides and SBP, $\leq$ 25th percentile for HDL-cholesterol). The expected proportion would have been 1.6 % (= 0.25^3), i.e. when assuming no clustering between the study variables. Chi-square test between the observed and expected rates did not support the assumption of independence ($\chi^2 = 20.9$, $df = 1$, $p < 0.001$), and thus indicated significant clustering between these three variables. Clustering was of similar magnitude among females and males, and at various ages, data not shown.

The effect of insulin level on clustering of these three risk factors in 1980 is shown in Figure 1. The number of subjects with all three risk factors became greater across the quartiles of insulin. The independent effect of insulin level on clustering was tested with a logistic regression model adjusted for BMI and subscapular skinfold thickness. According to the

Table 2. Cross-sectional associations of insulin with obesity indices at follow-up

Mean insulin concentration (mU/l) in each quartile	Sex	Age (years)	Insulin quartiles				p-value[a]
			I	II	III	IV	
	Females	12	5.5	9.3	12.3	18.9	
		15–18	6.1	9.2	11.9	18.1	
		21–24	4.4	7.0	9.2	14.4	
	Males	12	4.9	7.2	9.5	14.5	
		15–18	5.7	8.7	11.3	16.8	
		21–24	4.4	6.9	8.8	14.0	
BMI (kg/m²)	Females	12	17.1 ± 2.3	17.7 ± 1.7	19.5 ± 2.9	20.3 ± 3.1	< 0.001
		15–18	19.8 ± 2.3	20.8 ± 2.3	21.0 ± 2.3	22.3 ± 3.7	< 0.001
		21–24	21.0 ± 2.1	21.5 ± 2.5	21.8 ± 2.8	23.0 ± 3.2	< 0.001
	Males	12	16.9 ± 1.8	17.5 ± 2.1	18.4 ± 2.2	20.5 ± 4.2	< 0.001
		15–18	19.7 ± 2.2	20.4 ± 2.3	20.9 ± 2.6	22.1 ± 3.6	< 0.001
		21–24	22.2 ± 2.2	22.4 ± 2.2	22.8 ± 2.5	24.2 ± 3.7	< 0.001
Subscapular skinfold (mm)	Females	12	8.0 ± 3.7	8.0 ± 2.6	10.4 ± 5.7	12.2 ± 5.9	< 0.001
		15–18	10.8 ± 4.0	12.2 ± 5.6	12.6 ± 4.7	15.3 ± 4.5	< 0.001
		21–24	12.1 ± 5.1	13.8 ± 5.9	15.3 ± 7.2	16.4 ± 7.1	< 0.001
	Males	12	6.0 ± 2.8	7.0 ± 3.6	8.3 ± 5.6	11.6 ± 8.9	< 0.001
		15–18	7.2 ± 2.0	8.4 ± 3.6	8.5 ± 2.8	11.1 ± 6.6	< 0.001
		21–24	10.5 ± 3.8	10.7 ± 3.3	11.8 ± 4.5	14.4 ± 7.3	< 0.001

Data are mean ± SD.
[a] Linear trend by multiple regression analysis.
No. of subjects by quartiles of insulin: Females, age-group 12 years: I = 53; II = 58; III = 58; IV = 62. Females, age-group 15–18 years: I = 104; II = 107; III = 114; IV = 118. Females, age-group 21–24 years: I = 74; II = 79; III = 94; IV = 83. Males, age-group 12 years: I = 51; II = 48; III = 59; IV = 58. Males, age-group 15–18 years: I = 89; II = 89; III = 99; IV = 106. Males, age-group 21–24 years: I = 57; II = 67; III = 67; IV = 71

Table 3. Cross-sectional associations of insulin with serum lipids and blood pressure at baseline

	Sex	Age (years)	Insulin quartiles				p-value[a]
			I	II	III	IV	
Total cholesterol (mmol/l)	Females	6–12	5.38 ± 0.84	5.30 ± 0.88	5.41 ± 1.13	5.12 ± 0.77	NS
		15–18	5.00 ± 0.83	5.06 ± 0.82	4.97 ± 0.79	4.89 ± 0.79	NS
	Males	6–12	5.28 ± 0.73	5.26 ± 0.79	5.19 ± 0.83	5.13 ± 0.82	< 0.05
		15–18	4.61 ± 0.62	4.80 ± 0.71	4.70 ± 0.82	4.78 ± 0.73	NS
LDL-cholesterol (mmol/l)	Females	6–12	3.52 ± 0.77	3.47 ± 0.79	3.54 ± 1.09	3.31 ± 0.71	NS
		15–18	3.10 ± 0.73	3.16 ± 0.72	3.07 ± 0.76	3.03 ± 0.67	NS
	Males	6–12	3.33 ± 0.66	3.39 ± 0.72	3.31 ± 0.71	3.27 ± 0.77	NS
		15–18	2.93 ± 0.55	3.07 ± 0.68	2.94 ± 0.72	3.03 ± 0.67	NS
HDL-cholesterol (mmol/l)	Females	6–12	1.57 ± 0.27	1.51 ± 0.27	1.52 ± 0.26	1.45 ± 0.27	< 0.05
		15–18	1.55 ± 0.34	1.52 ± 0.29	1.52 ± 0.25	1.45 ± 0.27	= 0.08
	Males	6–12	1.67 ± 0.29	1.57 ± 0.28	1.56 ± 0.29	1.51 ± 0.29	< 0.001
		15–18	1.35 ± 0.24	1.36 ± 0.27	1.39 ± 0.25	1.34 ± 0.27	NS
Triglycerides (mmol/l)	Females	6–12	0.64 ± 0.16	0.72 ± 0.24	0.77 ± 0.25	0.83 ± 0.31	< 0.001
		15–18	0.75 ± 0.21	0.84 ± 0.31	0.82 ± 0.27	0.92 ± 0.42	< 0.05
	Males	6–12	0.61 ± 0.22	0.66 ± 0.24	0.70 ± 0.23	0.78 ± 0.29	< 0.001
		15–18	0.72 ± 0.26	0.83 ± 0.28	0.81 ± 0.30	0.88 ± 0.32	< 0.05
SBP (mmHG)	Females	6–12	107.3 ± 9.9	108.8 ± 9.9	108.9 ± 9.2	113.8 ± 9.6	< 0.001
		15–18	117.2 ± 9.9	118.0 ± 10.5	118.7 ± 9.5	118.6 ± 9.9	NS
	Males	6–12	107.8 ± 8.1	107.7 ± 8.3	110.1 ± 10.2	111.4 ± 9.5	< 0.01
		15–18	119.4 ± 10.7	123.6 ± 12.6	122.7 ± 10.4	125.3 ± 13.1	< 0.05
DBP (mmHG)	Females	6–12	67.4 ± 9.8	67.0 ± 8.6	66.0 ± 8.8	68.2 ± 9.6	NS
		15–18	70.2 ± 9.1	68.4 ± 10.3	70.2 ± 8.8	71.0 ± 8.5	NS
	Males	6–12	68.0 ± 9.9	68.0 ± 8.9	67.7 ± 9.3	67.5 ± 10.2	NS
		15–18	69.0 ± 9.7	69.8 ± 10.6	69.8 ± 10.3	72.3 ± 10.5	= 0.09

Data are mean ± SD.
[a] Linear trend by multiple regression analysis adjusted for BMI.
No. of subjects by quartiles of insulin: Females, age-group 6–12 years: I = 148; II = 180; III = 156; IV = 190. Females, age-group 15–18 years: I = 74; II = 84; III = 86; IV = 86. Males, age-group 6–12 years: I = 134; II = 145; III = 153; IV = 167. Males, age-group 15–18 years: I = 63; II = 62; III = 64; IV = 73

Table 4. Cross-sectional associations of insulin with serum lipids and blood pressure at follow up

	Sex	Age (years)	Insulin quartiles				p-value[a]
			I	II	III	IV	
Total cholesterol (mmol/l)	Females	12	5.03 ± 0.72	4.79 ± 0.68	4.75 ± 0.79	4.91 ± 1.25	NS
		15–18	4.87 ± 0.94	4.75 ± 0.79	4.75 ± 1.01	4.87 ± 1.04	NS
		21–24	5.11 ± 0.89	4.85 ± 0.82	5.07 ± 0.84	4.83 ± 0.91	NS
	Males	12	4.97 ± 0.88	4.89 ± 0.72	4.74 ± 0.88	5.00 ± 0.78	NS
		15–18	4.32 ± 0.67	4.29 ± 0.95	4.39 ± 0.90	4.53 ± 0.94	NS
		21–24	4.73 ± 0.84	4.75 ± 0.74	4.78 ± 0.95	4.86 ± 0.95	NS
LDL-cholesterol (mmol/l)	Females	12	3.20 ± 0.67	2.98 ± 0.64	2.85 ± 0.71	3.06 ± 1.15	NS
		15–18	2.99 ± 0.80	2.91 ± 0.72	2.91 ± 0.94	2.95 ± 1.02	NS
		21–24	3.16 ± 0.68	2.94 ± 0.70	3.06 ± 0.78	2.90 ± 0.82	< 0.05
	Males	12	3.15 ± 0.83	3.07 ± 0.65	2.91 ± 0.76	3.13 ± 0.73	NS
		15–18	2.61 ± 0.64	2.63 ± 0.90	2.68 ± 0.83	2.80 ± 0.91	NS
		21–24	2.95 ± 0.80	3.00 ± 0.72	2.94 ± 0.85	3.03 ± 0.91	NS
HDL-cholesterol (mmol/l)	Females	12	1.48 ± 0.24	1.45 ± 0.25	1.49 ± 0.23	1.37 ± 0.21	NS
		15–18	1.53 ± 0.29	1.46 ± 0.25	1.44 ± 0.24	1.45 ± 0.24	= 0.09
		21–24	1.60 ± 0.41	1.52 ± 0.27	1.60 ± 0.27	1.48 ± 0.28	< 0.05
	Males	12	1.54 ± 0.27	1.51 ± 0.26	1.49 ± 0.31	1.44 ± 0.29	NS
		15–18	1.36 ± 0.25	1.27 ± 0.21	1.32 ± 0.20	1.27 ± 0.21	< 0.001
		21–24	1.41 ± 0.25	1.34 ± 0.24	1.38 ± 0.29	1.27 ± 0.23	< 0.05
Triglycerides (mmol/l)	Females	12	0.78 ± 0.25	0.79 ± 0.25	0.90 ± 0.30	1.06 ± 0.41	< 0.001
		15–18	0.77 ± 0.27	0.85 ± 0.35	0.89 ± 0.31	1.02 ± 0.46	< 0.001
		21–24	0.77 ± 0.25	0.85 ± 0.31	0.90 ± 0.31	1.00 ± 0.41	< 0.001
	Males	12	0.63 ± 0.17	0.70 ± 0.23	0.74 ± 0.24	0.95 ± 0.29	< 0.001
		15–18	0.77 ± 0.33	0.85 ± 0.42	0.84 ± 0.27	1.01 ± 0.41	< 0.01
		21–24	0.83 ± 0.31	0.89 ± 0.40	1.01 ± 0.59	1.22 ± 0.75	< 0.001
SBP (mmHG)	Females	12	106.7 ± 9.2	108.0 ± 8.4	109.1 ± 8.3	112.5 ± 9.9	NS
		15–18	109.3 ± 9.5	113.7 ± 9.7	114.3 ± 10.1	117.3 ± 10.3	< 0.001
		21–24	115.0 ± 10.4	116.1 ± 10.2	117.8 ± 10.2	118.5 ± 13.1	= 0.05
	Males	12	104.3 ± 8.1	105.0 ± 8.2	107.6 ± 6.2	112.0 ± 10.9	< 0.01
		15–18	118.3 ± 11.8	118.6 ± 10.3	122.4 ± 12.4	125.1 ± 10.6	< 0.01
		21–24	126.0 ± 12.6	126.6 ± 9.7	127.7 ± 9.2	130.5 ± 10.2	< 0.05
DBP (mmHG)	Females	12	61.0 ± 9.1	62.7 ± 8.1	60.5 ± 10.4	64.3 ± 7.7	NS
		15–18	63.5 ± 9.3	67.0 ± 7.9	65.7 ± 8.3	68.4 ± 9.1	< 0.01
		21–24	68.0 ± 9.0	68.7 ± 9.8	70.2 ± 9.1	71.9 ± 8.7	< 0.01
	Males	12	56.5 ± 9.6	58.0 ± 9.9	60.7 ± 8.7	62.4 ± 8.0	< 0.01
		15–18	66.0 ± 9.5	66.2 ± 9.8	67.3 ± 9.3	68.9 ± 9.1	NS
		21–24	72.2 ± 11.4	72.3 ± 10.3	72.5 ± 9.7	74.2 ± 10.5	NS

Data are mean ± SD.
[a] Linear trend by multiple regression analysis adjusted for BMI
Number of subjects by quartiles of insulin: Females, age-group 12 years: I = 53; II = 58; III = 58; IV = 62.
Females, age-group 15–18 years: I = 104; II = 107; III = 114; IV = 118. Females, age-group 21–24 years: I = 74; II = 79; III = 94; IV = 83. Males, age-group 12 years: I = 51; II = 48; III = 59; IV = 58. Males, age-group 15–18 years: I = 89; II = 89; III = 99; IV = 106. Males, age-group 21–24 years: I = 57; II = 67; III = 67; IV = 71

logistic model insulin and subscapular skinfold thickness levels were both independently associated with clustering, but the effect of subscapular skinfold thickness became non-significant when BMI was also introduced to the model. In this logistic model with insulin, BMI and subscapular skinfold thickness as independent variables the odds ratio for trend for the effect of insulin was 1.60 (95 % confidence interval 1.24–2.06; $p < 0.001$), for the effect of BMI: 1.79 (1.29–2.47; $p < 0.001$), and for the effect of subscapular skinfold thickness: 1.10 (0.81–1.47; NS).

When clustering was studied at the follow-up the results were essentially similar. There were 71 subjects (3.8 %) having all three risk factors in 1986 (observed vs expected: $\chi^2 = 20.9$, $df = 1$: $p < 0.001$). In

the logistic model adjusted for both obesity indices, the odds ratio for trend for the effect of insulin was 1.72 (1.30–2.26; $p < 0.001$), for the effect of BMI: 2.36 (1.65–3.38; $p < 0.001$), and for the effect of subscapular skinfold thickness 0.98 (0.73–1.33; NS).

Prospective analyses. The relationships of baseline insulin level to the incidence of various end-points are shown in Table 5. The proportion of subjects who developed hypertriglyceridaemia at the follow-up (triglyceride concentration ≥ 95th percentile) became greater across baseline quartiles of insulin. Furthermore, subjects presenting clustering of risk factors at the follow-up had higher baseline insulin levels. After adjustments for baseline BMI and 6-year
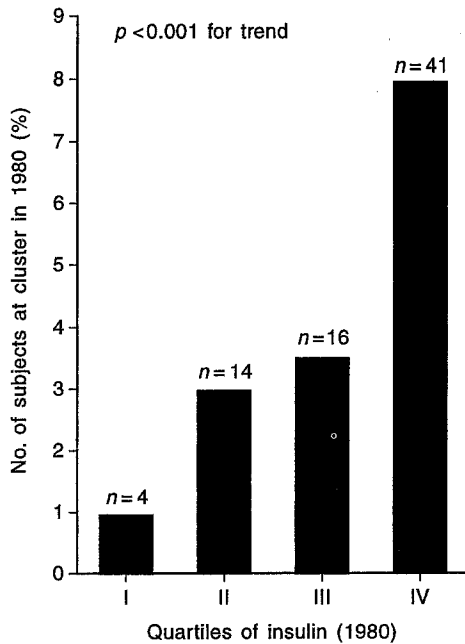
Fig.1. Proportion (%) of subjects at cluster in 1980 (baseline), i.e. with high triglycerides ($\geq$ 75th percentile), low HDL-cholesterol ($\leq$ 25th percentile) and high SBP ($\geq$ 75th percentile) across quartiles of insulin. Odds ratio for trend for the effect of insulin: 1.60 (95% confidence interval 1.24 to 2.06; $p < 0.001$), adjusted for BMI and subscapular skinfold thickness

change in BMI, high baseline insulin level remained significantly associated with the incidence of hypertriglyceridaemia and clustering at the follow-up. When the adjustments were made, in addition to BMI, also with subscapular skinfold thickness and the 6-year change in subscapular skinfold thickness, the effects of insulin on the incidence of hypertriglyceridaemia and clustering remained statistically significant (both $p < 0.05$).

We also looked at the parallel models to see if the baseline triglyceride or obesity levels would predict the incidence of hyperinsulinaemia (i.e. testing for causality in the opposite direction). The incidence of hyperinsulinaemia at the follow-up did not differ significantly across quartiles of baseline triglyceride level (data not shown). On the contrary, the incidence of hyperinsulinaemia became greater across the quartiles of baseline obesity levels. The incidence of hyperinsulinaemia across quartiles of baseline BMI was: I = 3.5%, II = 3.1%, III = 4.7%, and IV = 8.3%. Odds ratio for trend was 1.42 (1.16–1.73; $p < 0.001$). The incidence of hyperinsulinaemia across quartiles of baseline subscapular skinfold thickness was: I = 4.4%, II = 3.6%, III = 3.9%, and IV = 7.5%. Odds ratio for trend was 1.24 (1.02–1.51; $p < 0.05$). When both obesity indices where included in the same logistic regression model, the effect of BMI remained significant ($p < 0.01$), but the effect of subscapular skinfold thickness became insignificant ($p = 0.89$).

Discussion

The existence of an insulin resistance syndrome requires that the components of this syndrome occur together more often than would be expected by chance alone, and that a common aetiologic factor is associated with this clustering. We found that high insulin concentration was associated with high levels of triglycerides, low levels of HDL-cholesterol and elevated SBP, and that these three conditions showed significant clustering in the study population. Moreover, this clustering was associated with increased insulin levels independent of obesity. Thus, our results support the idea that a specific syndrome with multiple metabolic disorders exists already in the young. Furthermore, our data show that the associations between insulin, triglycerides, HDL-cholesterol and SBP are not only limited to high levels of these factors, but are in fact present within the normal ranges of serum lipid, blood pressure and insulin levels. This implies that the relationship of insulin concentration with serum lipid and blood pressure profile are fundamental metabolic functions. Therefore, the insulin resistance syndrome may reflect one end of a continuum of normal physiological links rather than a clear-cut syndrome [9]. In the present study high insulin concentration was not associated with high total cholesterol or high LDL-cholesterol concentrations. This result is in concert with previous findings suggesting that hypercholesterolaemia is not an integral factor of the metabolic syndrome [19, 35].

Most of the studies linking hyperinsulinaemia and/or insulin resistance to changes in serum lipids and blood pressure have been cross-sectional and conducted in adult populations [6, 9, 16, 36]. Our group has previously studied the cross-sectional relationships between insulin and other risk factors in this population by correlation analyses [37, 38]. The cross-sectional results in this study are in line with these previous findings. In this study we also used longitudinal data that had been gathered during the 6-year follow-up. Earlier prospective analyses of the insulin resistance syndrome are few and none of them have included children or adolescents. At present it is still not certain whether hyperinsulinaemia is causally associated with worsening of the lipid or blood pressure profile [39]. We found that high insulin level measured 6 years earlier in children and adolescents was predictive of hypertriglyceridaemia at the follow-up. Furthermore, the opposite did not occur, i.e. baseline triglyceride level was not associated with later hyperinsulinaemia. This finding supports the idea that insulin has a direct regulatory effect on triglyceride concentrations. Earlier studies showing that lowering of triglyceride concentration with drugs does not improve insulin sensitivity also suggest that hypertriglyceridaemia seems to be a consequence rather than a cause of insulin resistance [40, 41].

Table 5. Incidence (%) of various end-points according to age- and sex-specific quartiles of insulin at baseline

End-point at follow-up	Incidence % (incident cases/others[c])	Baseline insulin quartile				Odds ratio[d] [95 % CI]	Odds ratio[e] [95 % CI]	Odds ratio[f] [95 % CI]
		I	II	III	IV			
High BMI	3.0 (53/1723)	2.6	1.9	3.2	4.2	1.23 [0.96–1.58]	–	–
High Subscapular skin fold	3.9 (70/1707)	3.4	3.3	3.6	5.5	1.20 [0.97–1.50]	–	–
High Total-C	3.5 (62/1715)	4.5	3.4	2.5	3.6	0.92 [0.73–1.15]	–	–
High LDL-C	3.4 (60/1716)	4.3	3.2	2.3	3.8	0.95 [0.76–1.19]	–	–
Low HDL-C	4.4 (78/1701)	2.9	5.4	4.1	5.0	1.13 [0.92–1.38]	–	–
High TG	4.8 (85/1694)	3.9	2.9	4.4	7.7	1.36[b] [1.11–1.67]	1.33[b] [1.08–1.63]	1.27[a] [1.02–1.56]
High SBP	4.3 (77/1710)	4.2	3.7	4.3	5.0	1.01 [0.82–1.25]	–	–
High DBP	5.1 (91/1701)	5.8	4.2	4.5	5.9	1.03 [0.85–1.24]	–	–
Clustering[g]	3.5 (63/1727)	1.9	2.8	3.4	5.7	1.44[b] [1.14–1.84]	1.38[b] [1.08–1.76]	1.30[a] [1.02–1.66]

[a] $p < 0.05$; [b] $p < 0.01$.
[c] Subjects free of the particular risk factor at baseline. [d] Odds ratio for trend, non-adjusted. [e] Odds ratio for trend adjusted for baseline BMI. [f] Odds ratio for trend adjusted for baseline

BMI and 6-year change in BMI. [g] Three risk factors (HDL-cholesterol, triglycerides, SBP) at or above the age- and sex-specific 75th percentile (lowest quartile used for HDL-cholesterol)

Although insulin did not predict the incidence of low levels of HDL-cholesterol or high blood pressure, it was significantly associated with clustering of low HDL-cholesterol, high triglycerides and high SBP at follow-up (all three risk factors at their extreme quartiles). Therefore, our results suggest that hyperinsulinaemia may be causally related with the deterioration of lipid and blood pressure profile. Earlier prospective evidence about the role of insulin in the development of multiple metabolic disorders comes from two adult population, namely the San Antonio Heart Study and the study of elderly subjects in eastern Finland. These data have shown that elevation in insulin concentration precedes the development of high levels of triglycerides and low levels of HDL-cholesterol [19–22]. The evidence that hyperinsulinaemia would precede the development of elevated blood pressure is not so consistent. In the above-mentioned analyses from the San Antonio Heart Study the association of insulin and blood pressure elevation was only seen in certain subpopulations, such as in lean subjects [19] or subjects not on antihypertensive medication [20], but not in the whole study population.

There are several mechanisms that would explain the cause-and-effect relationship of insulin and other risk factors. Hyperinsulinaemia is associated with increased visceral fat and abundant availability of non-esterified fatty acids from fat tissue may lead to increased production of VLDL by the liver [42]. In addition, insulin itself may enhance the synthesis of hepatic VLDL and thereby contribute to high levels of triglycerides [16, 43]. Low HDL-cholesterol concentration may result from increased rate of apolipoprotein AI/HDL degradation seen in hyperinsulinaemia and insulin resistance [44], or alternatively it may be secondary to elevations in triglyceride concentration [45]. Hyperinsulinaemia or insulin resistance may raise blood pressure by increasing the activity of the sympathetic nervous system [46], or by promoting

the renal reabsorption of sodium [47]. Also, cellular deficiency of insulin may alter calcium metabolism which leads to the increase in vascular smooth muscle tone [48]. However, contradictory data also exist: possible mechanisms have been suggested that consider elevations in VLDL-triglycerides or blood pressure as causes of hyperinsulinaemia. Infusion of lipids has been shown to impair insulin action and thereby stimulate insulin secretion [49]. Julius et al. [50] have suggested that blood pressure elevation may induce loss of capillary vessels in skeletal muscle, and that this would promote local insulin resistance and subsequently lead to compensatory hyperinsulinaemia [50]. Thus, the specific mechanisms involved in the pathogenesis of the metabolic syndrome are not established.

One limitation of this study is that we measured only fasting insulin levels which are only a crude measure of insulin resistance. Therefore, fasting insulin levels may underestimate the strength of association between insulin resistance and other risk factors. Another limitation to our study is that we did not have a validated measure, such as waist to hip ratio, to indicate unfavourable fat distribution. Subscapular skinfold thickness was included in this study, since it has been used earlier as a measure of truncal or centralized fat distribution in children of the Bogalusa Heart Study [51]. In our study, subscapular skinfold thickness was cross-sectionally associated with a clustering of risk factors. Furthermore, it was prospectively associated with the incidence of hyperinsulinaemia suggesting that obesity estimated by measuring the subscapular skinfold thickness is a risk factor for the development of hyperinsulinaemia. However, these effects disappeared after BMI was also included in the statistical models, i.e. BMI was a stronger predictor of clustering or the incidence of hyperinsulinaemia compared to subscapular skinfold thickness.

In summary, bearing the limitation in mind that even prospective data cannot prove causality, these

data demonstrate that high fasting insulin concentrations measured in children and adolescents are predictive of the development of hypertriglyceridaemia years later. Furthermore, our results suggest that hyperinsulinaemia precedes unfavourable changes in the lipid and blood pressure profile including clustering of high triglycerides, low HDL-cholesterol and high SBP.

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