

Short communication

APOE/intrauterine undernutrition interaction and hypercholesterolemia in children.

Running title: Low birth weight and genetic caused hypercholesterolemia.

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Summary

The inconsistency of data regarding intrauterine programming of cardiovascular risk factors may be largely caused by genetic predisposition and later lifestyle. We analyzed whether low birth weight and apolipoprotein E polymorphism participate in the onset of hypercholesterolemia in children. Our approach was based on hypothesis that genetically enhanced susceptibility of different individuals may influence the effects of intrauterine programming. Two groups were selected from 2000 children at the beginning of an ongoing study: high cholesterol group (HCG, n=67) and low cholesterol group as a control (LCG, n=72). Both groups were divided into tertiles according to birth weight and we compared birth weight and apo E gene polymorphism between and within groups. The birth weight in HCG was 0.3 kg lower than the controls ($p < 0.001$). The frequency of apoE4 was 31% in HCG and only 10% in LCG. The frequency of apoE4+ genotypes was not significantly different between tertiles based on birth weight in HCG.

We suppose that intrauterine undernutrition, demonstrated by a lower birth weight, participates in the development of hypercholesterolemia already in childhood. The effects of low birth weight and the candidate gene - apoE, are synergic.

Key words: low birth weight, hypercholesterolemia, apolipoprotein E

Introduction

Atherogenesis is a process affected by a variety of different mechanisms, for which we have no complete explanation. The idea that the pathogenesis of coronary heart disease (CHD) started already in childhood is generally accepted. It is also generally recognized that dietary manipulation in the early life of individuals modulates cholesterol metabolism in adulthood (Hahn et al. 1989).

Low birth weight (BW) has been proved as a risk factor for CHD. The concept of an intrauterine effect on later function is based on the extensive body of data emerging from epidemiological studies. So-called programming theory proposed that susceptibility to CHD is determined still in utero (Barker et al. 1995). The strong association between low BW, high blood pressure (Law et al. 1993, Whincup et al. 1995), development of insulin resistance (Barker et al. 1993a) and non-insulin dependent diabetes (Phillips et al. 1994) was described. The effect on the development of hypercholesterolemia in later life is still uncertain (Barker et al. 1993b, Stanner et al. 1997). Hypothesis was supported by some of experimental models (Sztányi et al. 2000). The primary cause for all of these risks manifesting themselves in adulthood could be intrauterine undernutrition leading to long-term morphological and metabolic defects, but the inconsistency of results from variously designed studies (Stanner et al. 1997, Lumen et al. 1997) has led to criticism of this programming theory (Jones et al. 1998).

The reason may be that the effect of undernutrition depends on the genetic background of each individual. To assess the effect of genetic predisposition and low BW, we retrospectively analyzed the birth weights and apo E polymorphism of a group of hypercholesterolemic children and those of a control group.

Patients and methods

Two groups of probands were compared in the study. A high cholesterol group (HCG, 95-100 percentile) and a low cholesterol group (LCG, 5-10 percentile) had been selected from 2000 children (10-11 years old, of complete biological families in Prague). The HCG consists of 93 children with cholesterolemia exceeding the 95th percentile of the distribution curve (>5.5 mmol/l), the LCG of 92 children with cholesterolemia between the 5th and 10th percentile (a study desirable range). There were no differences in actual BMI, insulin and thyroxin concentrations or in recent diet between the two groups (analysed by 3-days questionnaire), whereas LDL-C concentration was by definition (Table) almost twice as high in the HCG group (Pistulková et al. 1991, Poledne et al. 1994).

A venous blood sample for lipoprotein analysis had been drawn after an overnight fasting (12 hours). Total cholesterol (TC) triglycerides (TG) and cholesterol in all fractions had been determined on Hoffman-LaRoche COBAS MIRA autoanalyzers with enzymatic Boehringer Mannheim kits. HDL-C was analyzed in the supernatant after apoB-containing particles were precipitated by the phosphotungstate method. LDL-C was analyzed after fractional ultracentrifugation in fractions between 1.009 and 1.063. BW was obtained from questionnaires completed by the children's mothers and was confirmed by data from hospital records. Only one family displayed familial hypercholesterolemia (FH, one of parents with TC >8 mmol/l and history of premature CHD before 55 years of age) all others had polygenous hypercholesterolemia (PHC) or familial combined hyperlipoproteinemia (FCHL). Criteria for FCHL were TC > 6.5 mmol/l + TG > 2.2 mmol/l at least in one of parents. PHC was characterized by TC >6.5 mmol/l at least in one of parents.

For this study, 25 HCG children and 20 LCG children were excluded because it was

impossible to confirm BW data from hospital records leaving 67 and 72 children in these groups, respectively.

The apoE genotype was determined by PCR-RFLP method (Hixson et al. 1990).

Data were expressed as mean (\pm SD) and analyzed by unpaired Student's t test and χ^2 test.

Results

Of the 13 polymorphisms in 11 genes analyzed, some differed between these groups (apoE, LPL, HMG-CoA reductase, apoAII, LDL-receptor) (Hubacek et al. 2004). But, all of these, the effect of apoE gene was by far the most important (Poledne et al. 1994). The mean BW of HCG was significantly lower than that of LCG ($p < 0.001$) (Table). The distribution of BW in the HCG was skewed towards lower values (Figure). Six out of the HCG children and none from the LCG children had a BW under 2.5 kg, the usual borderline of hypotrophic children ($p < 0.05$). Almost 40% of the HCG had a BW under 3.0 kg. To determine the relationship between intrauterine undernutrition (demonstrated by a low BW) and the direct effect of the most influential candidate gene, analysis of subgroups was performed. Both groups were divided into tertiles according to BW, starting from the lowest BW in the 1st tertile. The mean value of LDL-C tended to increase from the 1st tertile to 3rd tertile in LCG and oppositely to decrease in HCG. However, these differences did not reach statistical significance. The frequency of "disadvantageous" genotypes having the E4 allele was 31% in the HCG but only 10% in the LCG ($p < 0.01$). The distribution of "disadvantageous" apoE4+ genotypes was low and similar in all LCG tertiles. The distribution of these genotypes in the HCG subgroups was 9, 7 and 5 respectively. This trend did not reach statistical significance by the χ^2 test (Table).

Discussion

Intrauterine growth retardation manifested as a lower BW, may imprint pathological defects (Barker et al. 1995) in metabolism and organ functions. In such a situation hypertension (Law et al. 1993, O'Sullivan et al. 2002), insulin resistance (Phillips et al. 1994) and probably also hyperlipoproteinemia (Barker et al. 1993b) might appear after exposure to the Western life-style for several decades.

Two groups of children (HCG and LCG) with assumed different sensitivity to the diet were compared. No differences in dietary records were found and total fat consumption was 40% of the total energy intake with 80% of the fat of animal origin in both groups (typical Czech diet in the time of study – high fat, high sodium, low fiber intake, with low proportion of fresh vegetable and fruit, high red meat, practically no fish intake).

The genetic background of this different sensitivity was proved by differences in genotype frequencies (Hubacek et al. 2004), of which the apoE gene was the most important.

In our retrospective study HCG children have a lower BW than LCG children and the number of children with a BW under 2.5 kg, which is usual borderline of hypotrophy was six in HCG and none in LCG. These facts imply that intrauterine undernutrition also participates, with hypercholesterolemia onset in HCG children even as young as 10-11 years.

It was shown (Infante-Rivard et al. 2003), that babies born with intrauterine growth retardation has less often transmitted protective apoE2 allele, which is supporting the hypothesis of Barker. Unfortunately in the study, levels of lipid parameters were not determined as a clinical linkage (Infante-Rivard et al. 2003). Another two studies dealt with high TC, IUGR and apoE gene polymorphism has confirmed the data

about association of high TC and low birth weight. Akisu et al. (2004) did not find differences in the distribution of apoE gene polymorphism between the IUGR and control groups. Garces et al. (2002) concluded greater effect of the apoE polymorphism on cholesterolemia in children with BW in the lowest tertiles.

So far are our data in agreement with two studies (Akisu et al. 2004, Garces et al. 2002) documenting relationship of low BW and high cholesterol concentration in children and support Garces observation of greater effect of apoE genotype in individuals with BW in the lowest tertile.

ApoE gene and intrauterine undernutrition, might affect the development of higher cholesterol concentration either independently or simultaneously. If the effect of intrauterine undernutrition would be dominant and independent, then the frequency of disadvantage apoE4+ genotype in HCG group would increase from the lowest BW tertile (where low BW is mostly expressed) to the highest one (with normal BW). Since it is not so (on the contrary a slight opposite trend was documented) we conclude that the effect of both factors is rather synergic.

We suggest that our data were affected by rather strong environmental effects of the diet consumed in the Czech society at the time of this study. The inconsistency of results regarding intrauterine programming to high TC by undernutrition may be caused by differences in individual genetic-environmental interactions. We would like to hypothesize that individuals less sensitive to environmental effects on TC concentration are also less sensitive to intrauterine undernutrition, whereas in our group of selected hypercholesterolemic individuals the effect was manifested. Intrauterine undernutrition seems to play a more important role in children of parents with PHC or with positive CHD history.

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References

- AKISU M, BALIM Z, CETIN H, KOSOVA B, YALAZ M, TOPCUOGLU N, KULTURSAY N: The role of angiotensin-converting enzyme and apolipoprotein-E gene polymorphism on lipid compositions in newborn infants with intrauterine growth restriction. *Early Hum Dev* **78**: 95-103, 2004.
- BARKER DJP, HALES CN, FALL CHD, OSMOND C, PHIPPS K, CLARK PMS: Type 2 (non-insulin dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrom X): relation to reduced fetal growth. *Diabetologia* **36**: 62-67, 1993a.
- BARKER DJP, MARTYN CN, OSMOND C, HALES CN, FALL CHD: Growth in utero and serum cholesterol concentrations in adult life. *BMJ* **307**: 1524-1527, 1993b.
- BARKER DJP: Fetal origins of coronary heart disease. *BMJ* **311**: 171-174, 1995.
- GARCES C, BENAVENTE M, ORTEGA H, RUBIO R, LASUNCION MA, RODRIGUEZ ARTALEJO F, FERNANDEZ PARDO J, DE OYA M: Influence of birth weight on the apo E determinants of plasma lipid levels in children. *Pediatr Res* **52**: 873-8, 2002.
- HAHN P: Late effects on early nutrition. In: *Atherosclerosis: a Pediatric Perspective*, M.T.R. SUBBIAH (ed.), CRC Press, Boca Raton, 1989, pp. 155-164.
- HIXSON JE, VERNIER D: Restriction isotyping of human apoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* **31**: 545-8, 1990.
- HUBACEK JA, PISTULKOVA H, SKODOVA Z, LANSKA V, POLEDNE R. Antagonistic effect of insertion/deletion polymorphisms (HpaI) in the regulatory

part of the apolipoprotein C1 gene in Children with high and low plasma cholesterol levels. *Cas Lek Cesk* **143**: 94-6, 2004.

- JONES SE, NYENGAARD JR: Low birth weight and cardiovascular disease: myth or reality. *Curr Opin Lipidol* **9**: 309-12, 1998.
- LAW CM, DE SWIET M, OSMOND C, FAYERS PM, BARKER DJP, CRUDDAS AM, FALL CHD: Initiation of hypertension in utero and its amplification throughout life. *BMJ* **306**: 24-7, 1993.
- LUMEN L, STEIN A: Offspring birth weight after maternal intrauterine undernutrition: a comparison within sibships. *Am J Epidemiol* **146**: 801-9, 1997.
- O'SULLIVAN J, WRIGHT C, PEARCE MS: The influence of age and gender on the relationship between birth weight and blood pressure in childhood. a study using 24-hour and casual blood pressure. *Eur J Pediatr* **161**: 423-7, 2002.
- PHILLIPS DIW, BARKER DJP, HALES CN, HIRST S, OSMOND C: Thinness at birth and insulin resistance in adult life. *Diabetologia* **37**: 150-4, 1994.
- PISTULKOVÁ H, POLEDNE R, KAUCKÁ J, ŠKODOVÁ Z, PETRŽÍLKOVÁ Z, PACLT M, VALENTA Z, GRAFNETTER D, PÍŠA Z: Cholesterolemia in school-age children and hypercholesterolemia aggregation in the family. *CorVasa* **33**: 139-49, 1991.
- POLEDNE R, HUBÁČEK J, PÍŠA Z, PISTULKOVÁ H, VALENTA Z: Genetic markers in hypercholesterolemic and normocholesterolemic Czech children. *Clin Genet* **16**: 88-91, 1994.
- STANNER SA, BULMER K, ANDRES C, LANTSEVA OE, BORODINA V, PATEEN W, YUDKIN JS: Does malnutrition in utero determine diabetes and

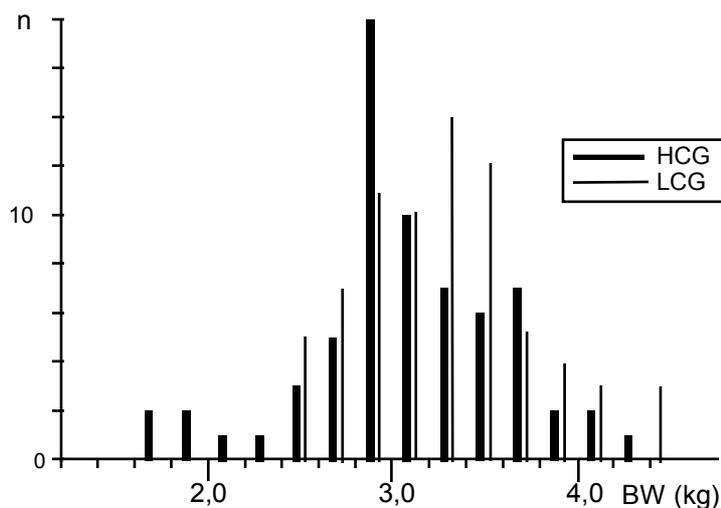
coronary heart disease in adulthood? Results from the Leningrad Siege Study, a cross sectional study. *BMJ* **315**: 1342-9, 1997.

- SZITÁNYI P, HANZLOVÁ J, POLEDNE R: Influence of intrauterine undernutrition on the development of hypercholesterolemia in an animal model. *Physiol Res* **49**: 721-4, 2000.
- WHINCUP P, COOK D, PAPCOSTA D, WALKER M: Birth weight and blood pressure: cross sectional and longitudinal relations in children. *BMJ* **311**: 773-9, 1995.

Table: Mean birth weight (kg), lipoprotein concentrations (mmol/l) and apoE4 genotype frequencies in tertiles according the birth weight of LCG and HCG and in whole groups (*, #, \$, § p<0.01, SD in brackets).

Parameter	Group	1 st tertile	2 nd tertile	3 rd tertile	Whole group
birth weight (kg)	LCG	3.00 (0.23)	3.52 (0.14)	3.94 (0.21)	3.48 (0.43)*
	HCG	2.67 (0.40)	3.20 (0.13)	3.78 (0.26)	3.21 (0.54)*
LDL-C (mmol/l)	LCG	2.18 (0.54)	2.32 (0.42)	2.45 (0.61)	2.30 (0.54) [#]
	HCG	4.07 (0.56)	3.99 (0.72)	3.83 (0.58)	3.97 (0.63) [#]
HDL-C (mmol/l)	LCG	1.23 (0.26)	1.36 (0.28)	1.19 (0.27)	1.25 (0.28) ^{\$}
	HCG	1.43 (0.56)	1.46 (0.32)	1.45 (0.37)	1.44 (0.33) ^{\$}
apoE4+	LCG	3/24	2/24	2/24	7/72 [§]
	HCG	9/23	7/22	5/22	21/67 [§]

Figure: Distribution curve of birth weight (BW, kg) in high cholesterol group (HCG) and low cholesterol group (LCG)



Dear Sirs,

enclosed you will find our manuscript „APOE/intrauterine undernutrition interaction and hypercholesterolemia in children“.

We have analyzed a possible genetic influence to the relation between intrauterine undernutrition and cardiovascular risk and I believe in your understanding of our ambition.

With best regards.

Sincerely,

Peter Sztányi, MD, PhD

PS

An original of agreement of all authors with submitting of the manuscript „APOE/intrauterine undernutrition interaction and hypercholesterolemia in children“ is enclosed.

I agree with submission of the manuscript „APOE/INTRAUTERINE UNDERNUTRITION INTERACTION AND HYPERCHOLESTEROLEMIA IN CHILDREN“ to the Physiological Research.

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