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RAPID COMMUNICATION

Influence of Intrauterine Undernutrition on the Development of Hypercholesterolemia in an Animal Model

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Summary

A low birth weight is a new risk factor for the development of premature atherosclerosis. The effect of intrauterine undernutrition on hypercholesterolemia in later life was studied in an experimental model using the Prague Hereditary Hypercholesterolemic (PHHC) rat. Compared to animals in the control group (Wistar rats), animals with an increased sensitivity to high-cholesterol diet (PHHC rats) display hypercholesterolemia. Only in PHHC animals, individuals undernourished in their intrauterine life (hypotrophic group, HG) had a significantly higher total cholesterol, compared with individuals without food restriction in pregnancy (eutrophic group, EG). Restricted food intake in pregnancy led to smaller nests and a decreased number of pups in each litter. We found no significant diferences in birth weight between HG and EG. In spite of similar birth weights in PHHC and Wistar rats, intrauterine undernutrition caused an increase in cholesterolemia in the HG group of the PHHC rats. The effect of intrauterine undernutrition on the development of hypercholesterolemia will most likely play a role in individuals with geneticaly determined increased susceptibility to a high-cholesterol diet. The use of this model of intrauterine undernutrition for the study of hypercholesterolemia has proved to be feasible.

Key words

Rat • Intrauterine undernutrition • Hypercholesterolemia

There is a strong correlation between elevated plasma cholesterol levels and coronary heart disease (CHD). The idea that the pathogenesis of CHD can be traced back to childhood is generally accepted and early prevention of atherogenesis should be shifted into early childhood. It is generally recognized that dietary cholesterol manipulation in the early life of individuals modulates cholesterol metabolism in adulthood (Hahn 1989). A low birth weight seems to be a newly discovered potential cause of the development of some risk factors for atherosclerosis. The theory was put forth by a team headed by Prof. Barker proposing that susceptibility to CHD is determined still *in utero* (Barker *et al.* 1996). The Barker group demonstrated a strong

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association between the incidence of hypercholesterolemia (Barker *et al.* 1993), high blood pressure (Law *et al.* 1993, Martyn *et al.* 1995), non-insulin dependent diabetes (Phillips *et al.* 1994), and a low birth weight. Recent epidemiological data published by the above group were obtained by examining sexa- and septagenarians whose birth weights and anthropometric parameters at birth were known. Barker hypothesized that the primary cause for all of these risks manifesting themselves in adulthood is intrauterine malnutrition leading to long-term morphological and metabolic defects.

Some animal models and designs have been used to study development of hypercholesterolemia (Hahn 1976, Poledne 1998). By using an experimental model of intrauterine undernutrition in an inbred strain of the Prague Hereditary Hypercholesterolemic (PHHC) rats the effect of intrauterine undernutrition on the development of hypercholesterolemia in adulthood was analyzed. The PHHC strain was obtained in our laboratory by selective cross-breeding of Wistar rats with elevated plasma cholesterol levels (Poledne 1986). PHHC rat is characterized by its increased basal cholesterolemia with a prominent response to dietary cholesterol. The distribution of cholesterol in lipoprotein fractions is similar to the pattern found in humans (Poledne 1986). The aim of the present study was to introduce a new experimental model of intrauterine undernutrition and to evaluate the effect of intrauterine growth retardation on cholesterolemia in sensitive individuals.

Two groups of adult pregnant females of the PHHC strain, and two groups of Wistar strain as controls were used in this experiment. In the first group, hypotrophic group (HG), intrauterine undernutrition was induced by reducing the food intake of pregnant females by 30 % during the 2nd and 3rd weeks of pregnancy. In the first week of pregnancy, normal food consumption was determined in order to obtain baseline data to calculate the amount of food representing a restricted diet. During the last week of pregnancy the animals were returned to a normal diet. A group of females with unrestricted food intake during pregnancy served as the control eutrophic group (EG).

The dietary cholesterol mixture used in this study contained a standard diet supplemented with 2 % crystalline cholesterol dissolved in 5 % beef tallow. Feeding was finished by midnight and the rats were restricted food for 8 hours prior to blood sampling. Blood samples were taken from the tail vein under ether anesthesia.

The total serum cholesterol in the young rats, aged 6 weeks, was determined in both groups. Cholesterol concentration was analyzed enzymatically (Boehringer Mannheim, Germany).

The restriction of food intake led to a reduction in the number of pups in each litter, and a lower total weight of the nest (Table 1). The individual birth weight of the pups was not affected by food restriction of their mother. We did not find any significant difference in birth weight between HG and EG pups. Generally, there was a negative correlation between the number of fetuses and birth weight. A restriction of food higher than 30 % could result in abortion. Restriction of food intake in pregnancy is a factor influencing intrauterine development of the fetus. This is clearly demonstrated by the significant differences in nest weight and by the reduced number of pups in litter (Table 1).

Table 1. Effect of reduction of food intake in pregnancy on birth weight of pups

	Eutrophic group	Hypotrophic group	p=
Weight of nest	50.25±7.96	33.62±11.28	0.001
Number of rats in litter	9.50±2.69	6.38±2.53	0.02
Birth weight	5.53±0.94	5.43±0.86	n.s.

(means ± S.D., p=p-value by Student's t-test, n.s. = not significant)

We found a significantly higher cholesterolemia in PHHC rats, animals predisposed to develop hypercholesterolemia by a high-cholesterol diet, compared to Wistar rats (Table 2). We have demonstrated the higher sensitivity of PHHC rats to a high-cholesterol diet in the early period of life. In the PHHC rats the total serum cholesterol concentration in HG group was significantly higher 5.51±1.03 mmol/l (mean±S.D.), compared to the

EG group 4.34±0.88 mmol/l ($p<0.01$). There was no significant difference in the control Wistar rats between HG and EG group (Table 2).

When males and females were compared separately, a significant difference was observed in both sex groups. However, females were significantly more susceptible to a high-cholesterol diet then males (Table 2).

Our data support the theory of intrauterine determination and programming of defects in adulthood. Since we found no significant differences in birth weight between HG and EG groups, there should to be another unknown mechanism mediating the effects of intrauterine undernutrition on the development of hypercholesterolemia in later life.

The model used in the present study allows us to monitor the concomitant effect of genetic determination and intrauterine undernutrition on the development of hypercholesterolemia.

The verification of the results, which showed an effect of intrauterine undernutrition on the development of hypercholesterolemia in later life, seems to require a larger number of lots using nests with identical numbers of pups in the litter.

It can be concluded that 1) the experimental model used in our study clearly demonstrated the effect of the intrauterine undernutrition on the development of hypercholesterolemia in sensitive individuals, 2) the effect of the high-cholesterol diet on cholesterolemia in rats is stronger in females compared to males, and 3) the intrauterine undernutrition led to enhanced sensitivity to a high-cholesterol diet in later life, and resulted in hypercholesterolemia only in sensitive individuals, i.e. in PHHC rats.

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Table 2. Cholesterolemia in hypo- and eutrophic groups of PHHC and Wistar rats

	Males	Females	All rats
PHHC			
HG	5.39±1.34 (n=6)	5.63±0.54§ (n=6)	5.51±1.03* (n=12)
EG	3.95±0.76 (n=42)	4.9±0.88§ (n=42)	4.34±0.88* (n=74)
Wistar			
HG	2.09±0.16 (n=11)	2.35±0.13$^{\#}$ (n=9)	2.24±0.2 (n=20)
EG	2.08±0.15 (n=9)	2.16±0.12$^{\#}$ (n=10)	2.12±0.14 (n=19)

*(mean ± S.D. *=$p<0.01$. $^{\#}$ and §=$p<0.05$)*

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