

## Arterial stiffness in 10-year-old children: current and early determinants

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It has been suggested that CVD has its origins in early life. An impairment of fetal growth and early postnatal nutrition may have programming effects on cardiovascular physiology. In addition, traditional risk factors for CVD may initiate the atherosclerotic process during childhood. We explored the effect of fat intake, physical activity and lipid profile in childhood, and birth weight, growth during infancy and breast-feeding on arterial stiffness in a cohort study of ninety-three 10-year-old children followed during infancy and re-examined at the age of 10 years. Arterial stiffness in two arterial segments (aorto-radial and aorto-femoral) was measured as pulse wave velocity. Arterial stiffness was inversely associated with physical activity (a regression coefficient in cm/s (95 % CI) of  $-6.8$  ( $-11.2$ ,  $-2.4$ ) and  $-3.9$  ( $-6.9$ ,  $-0.8$ ) per h of high physical activity/d in the aorto-radial and aorto-femoral segments, respectively). Arterial stiffness was also positively associated with dietary fat energy percentage ( $3.1$  (95 % CI  $0.9$ ,  $5.2$ ) and  $1.8$  (95 % CI  $0.2$ ,  $3.2$ ) per fat energy percentage in the aorto-radial and aorto-femoral segments, respectively) but was not related to body composition, insulin resistance or lipid profile. Arterial stiffness was also positively associated with duration of breast-feeding for the aorto-femoral segment only ( $2.1$  (95 % CI  $0.4$ ,  $3.7$ ) per month) but was not associated with growth in early life. In conclusion, patterns of physical activity and diet, and history of breast-feeding in infancy, have an influence on the stiffness of the large arteries in children. The long-term effects of this are unknown.

**Arterial stiffness: Pulse wave velocity: Fat intake: Physical activity: Breast-feeding: Birth weight: Growth: Programming: Lipid profile: Insulin**

Most studies have shown that low birth weight is associated with an increased risk of CVD, and it has also become evident that a low weight gain during infancy followed by an accelerated weight gain in childhood further increases the risk of CVD. This is now called the ‘developmental origins hypothesis’ (Barker 2004). Early nutrition may also have long-term effects on the risk of CVD since breast-feeding is associated with a small reduction in blood pressure (Owen *et al.* 2003) and serum cholesterol in later life (Owen *et al.* 2002). Furthermore, pathological studies have shown that the atherosclerotic process starts during childhood (Stary, 2000), and from the late teens associations between the early stages of atherosclerotic lesions and CVD risk factors have been seen (McGill *et al.* 2000). This suggests that traditional CVD risk factors may be acting several decades before CVD becomes manifest. Additionally, obesity in childhood is associated with insulin resistance, increased blood pressure and a more adverse lipid profile (Lobstein *et al.* 2004).

Arterial stiffness (AS) is determined by the arterial wall structure and is, in adults, associated with numerous well-established risk factors for CVD (Arnett *et al.* 1994) and increased in patients with atherosclerotic disease (van Popele *et al.* 2001). Furthermore, AS has been shown to be a predictor of cardiovascular

mortality in certain patient groups (Laurent *et al.* 2001; Blacher *et al.* 2003) and in the elderly (Meaume *et al.* 2001). AS is also affected by the mean arterial blood pressure and smooth muscle tone, which are in turn influenced by the endothelial function, sympathetic neuronal activity and eicosanoids (Mottram *et al.* 1999). Only a few studies have measured AS in children, showing a positive association between serum cholesterol and AS in a population-based study (Leeson *et al.* 2000), and increased AS in children with hypercholesterolaemia (Aggoun *et al.* 2000) and severe obesity (Tounian *et al.* 2001).

Three types of AS can be considered – systemic, regional and local – describing the overall stiffness of the arterial system, the stiffness in a specific arterial segment and the stiffness at a local site, respectively (Pannier *et al.* 2002). Regional AS can be measured indirectly by measuring pulse wave velocity (PWV) over the segment of interest and is calculated as the distance in meters divided by the transit time in seconds (Van Bortel *et al.* 2002). PWV is one of many indices for AS, none of which has proven superior, and all have their weaknesses (O’Rourke *et al.* 2002). Other often-used indices are compliance and distensibility, which are the absolute or relative change in arterial diameter and pressure at a local point, respectively (O’Rourke *et al.* 2002).

PWV is inversely related to the distensibility (D) by the Bramwell-Hill formula:

$$\text{PWV} = \sqrt{(1/\rho \times D)}$$

where  $\rho$  represents blood density (Van Bortel *et al.* 2002).

The aim of the present study was to explore the effect of the current lifestyle factors of fat intake and physical activity, and the early-life factors of breast-feeding and growth in early life, on AS measured as PWV in 10-year-old healthy Danish children.

## Subjects and methods

### *Copenhagen Cohort Study on Infant Nutrition and Growth*

The Copenhagen Cohort Study on Infant Nutrition and Growth was an observational study in which a random sample of infants born at Hvidovre Hospital from October 1987 to February 1988 was followed from birth to 12 months of age. Hvidovre Hospital is the main hospital in the municipality of Copenhagen, and the participating families were comparable with those in the remaining area with respect to the number with low income and poor housing. Two hundred and fifty-one infants out of 560 infants born during 109 predetermined days fulfilled the following inclusion criteria: parents of Danish origin, singleton birth, gestational age between 37 and 42 weeks, birth weight for gestational age between the 10th and 90th percentiles, no neonatal diseases resulting in admission to the neonatal department and no severe malformations, and mother and infant admitted to the hospital for at least 3 d.

The infants were randomised to a study group ( $n$  139) who were examined eight times during infancy, or a control group ( $n$  112) who were examined only at the age of 9 months. The control group was not contacted until 8 months after delivery. In the study group, ninety-one families agreed to participate, and eighty-four completed the study. In the control group, fifty-nine families agreed to participate. Based on information available from hospital records, participants and non-participants were compared with respect to maternal age, weight, height, BMI and several birth variables, the only difference being that non-participants spent approximately 0.4 d less on the maternity ward.

To test whether participating in the study group, with the frequent contact with the investigators, influenced dietary pattern and growth, a number of dietary and growth characteristics at 9 months were compared between the study and the control group. No major differences were seen between the groups, implying that participation in the study did not have any significant impact on the nutrition or growth of the infants. A more detailed description has been published elsewhere (Michaelsen, 1997).

### *Follow-up*

Of the 143 children participating in the original study, 136 were invited to the 10-year follow-up examination (October 1997 to February 1998). Two were not invited because they lived outside Denmark, and no addresses could be found for the remaining five children at the Central Person Registry in Denmark. One hundred and six children (fifty-one boys) agreed to participate. Children participating in the follow-up examination were compared with children not attending the follow-up examination with respect to duration of breast-feeding, size and growth in infancy (weight, length and skin folds at birth and at 9 months of age). No significant differences were seen (data not shown).

Furthermore, maternal social group at birth did not differ between children participating and children not participating in the follow-up examination.

### *Ethical considerations*

The study was performed in agreement with the Declaration of Helsinki (1964) and approved by the local ethical committee (Journal no. KF-01-226/97).

### *Infancy*

Weight at birth was available from medical records, and at 9 months of age the infant was measured naked on an electronic balance to the nearest gram. Crown–heel length was measured on the fourth or fifth day of life and at 9 months of age, with the mother holding the head of the infant, using a wooden measuring board with a fixed headboard and a movable footboard. At 9 months of age, triceps and subscapular skinfolds were measured using the Harpenden skinfold caliper (Chasmors Ltd, London, UK) according to standard procedures (Tanner & Whitehouse 1975).

Breast-feeding status was reported monthly for the study group but only at the 9-month examination for the control group. Information collected at the 9-month examination on duration of breast-feeding measured in whole months was therefore used for both groups, and 9 months therefore also became the maximum duration even though 34% of the infants in the study group were still being partially breast-fed at this time. Breast-feeding was defined as exclusive or partial. Exclusive breast-feeding allowed supplements of water or camomile tea with no sugar or milk (commonly used for infants in Denmark). Infants were classified as partially breast-fed as long as they were breast-fed at least once a day. In this study, the total duration of breast-feeding, calculated as the sum of exclusive and partial breast-feeding, was used.

### *Follow-up examination*

Height was measured to the nearest 1 mm using a wall-mounted digital stadiometer (FORCE Institute, Copenhagen, Denmark) and weight was measured to the nearest 0.1 kg using an electronic digital scale (Model 707, Seca, COMACO A/S, Hammel, Denmark). Subjects wore only underpants and a cotton T-shirt when weighed. BMI was calculated as  $\text{kg/m}^2$ . Body fat percentage was determined by dual-energy X-ray absorptiometry using a Hologic 1000/W (Hologic, Waltham, MA, USA). For analysis, software version 5.61 was used. Stages of puberty were assessed using the method of Tanner (1962). Breast development in girls and pubic hair development in boys were used to assess pubertal stage.

Venous blood samples were collected after an overnight fast. Concentrations of total cholesterol and triacylglycerol were measured by an enzymatic assay (Boehringer Mannheim, Mannheim, Germany). HDL-cholesterol was measured by a homogenous method after the specific binding of LDL and VLDL cholesterol by  $\alpha$ -cyclodextrin (Boehringer Mannheim, Mannheim, Germany). LDL-cholesterol was calculated by the Friedewald formula:

$$\text{LDL-cholesterol} = \text{total cholesterol} - (\text{HDL cholesterol} + \text{triacylglycerols}/2.2)$$

Glucose was measured by the hexokinase method (Boehringer Mannheim) after haemolysis (Merck KgaA, Germany). Insulin

was measured by a solid phase, two-site fluorometric assay (Wallac Oy, Turku, Finland). The homeostasis model assessment, which has been proposed as a method of assessing insulin resistance from fasting glucose and insulin concentrations, was calculated as (Matthews *et al.* 1985):

$$\text{insulin } (\mu\text{U/ml}) \times \text{glucose (mmol/l)} / 22.5$$

Blood pressure and pulse were measured with an electronic instrument (Omron HEM-705CP; Omron Corporation, Japan). The measurement was performed after 10 min of rest in the supine position both before and after measuring PWV in the aorto-radial (AR) segment, and after measuring PWV in the aorto-femoral (AF) segment.

The participants (and their parents) kept a 7 d food record with pre-printed response categories supplemented with open-ended alternatives, using household measures, similar to the methods of the national dietary survey (Andersen *et al.* 1996). Before parents completed the questionnaire, the description and reporting of serving size and frequency of food intake was explained to them with the aid of food portions and common household measures. After school, the children reported to the parents what foods, snacks and beverages they had eaten at school. The importance of maintaining the habitual dietary intake during the 7 d period was emphasised to both children and parents. Average daily intakes of energy and fat were calculated for each subject by using the national food database of the Danish Food Agency.

Physical activity was recorded using a Danish version of a 24 h recall questionnaire (Habitual Activity Estimation Scale; Boucher *et al.* 1997). The day was divided into four periods: before breakfast, breakfast to lunch, lunch to dinner, and dinner to bedtime. In each of these periods, records were made of the amount of time (%) spent in activity levels corresponding to the following activities: (I) lying, (II) sitting, (III) walking, (IV) playing or sport that affected breathing. From this, a total time duration in each activity level during the whole day was calculated. In a Danish validation study with children, h/d spent in the highest activity level was strongly associated with data from Caltrac activity monitors (Model C100, Caltrac Activity Monitor, Caltronics Div., Hemokinetics Inc., Vienna, Virginia, USA), which was not the case for the other activity levels (Mølgaard, 1996). The highest activity level was therefore used as an estimate of physical activity level in this study. Physical activity was registered for the day before the examination by filling out the form on the day they were examined; this could therefore have been any day from Sunday to Thursday.

AS was determined as PWV by a non-invasive optical method. It was measured in two arterial segments from the heart to the radial artery at the wrist, and from the heart to the femoral artery at the level of the inguinal ligament. An electrocardiogram belt, with an electrode on each side of the heart, was placed around the chest. The pulse at the wrist or groin was localised and an infrared transducer (MRC Environmental Epidemiology Unit, Southampton University, UK) placed over it. The position of the transducer was adjusted until a clear pulse curve with a sharp increase was found. Pulse and electrocardiogram were measured with the programme Pulse, version 4.0 (MRC Environmental Epidemiology Unit, Southampton University, UK). The electrocardiogram and pulse curve were then measured over 20 s, and transit time was calculated as time from the R peak in the electrocardiogram to the transducer registered the beginning of the pulse curve. The analysis program used was Pulse Analysis

version 97.1.1 (MRC Environmental Epidemiology Unit, Southampton University, UK), which normally automatically calculates the transit time, although in some cases it was necessary to identify the R peak and the beginning of the pulse curve manually.

The distance between the heart (sternal notch) and the transducer was measured to the nearest 0.5 cm using a tape measure. PWV was then calculated as the distance travelled divided by the transit time. The method has recently been shown to correlate well with results obtained using Doppler ultrasound and intra-arterial measurements (Loukogeorgakis *et al.* 2002).

#### Statistical analyses

Results are expressed as means and standard deviations unless otherwise stated. Differences between boys and girls were determined by two-sided Student's *t* test or Mann-Whitney test. Determinants of PWV were explored in multiple linear regression analyses. The level of significance was set to 0.05. All analyses were performed with SPSS (version 10; SPSS Inc., Chicago, IL, USA).

To adjust fat intake for total energy intake, the method suggested by Willet & Stampfer (1998) was used. Briefly, fat intake (g/d) was regressed on energy intake (MJ/d). Mean fat intake was then added to the residuals from this model, providing an estimate for fat intake unrelated to total energy intake.

## Results

### Basic characteristics at 10 years of age

Owing to technical problems, PWV was not measured in either of the two arterial segments in five children, only in the AR segment in four children, and only in the AF segment in three children, leaving ninety-three children (forty-four boys) with both measurements. The majority of the children (forty-one boys, thirty-two girls) showed no signs of pubertal development (Tanner stage I), whereas two boys and fifteen girls were in Tanner stage II, and only two girls were in Tanner stage III. Pubertal development was not assessed in one boy.

There were no significant differences between boys and girls in terms of age, height, weight and BMI (Table 1), whereas girls had a significantly higher body fat percentage than boys. According to international BMI cut-offs (Cole *et al.* 2000), four girls and four boys were overweight, and none was obese. LDL- and HDL-cholesterol levels did not differ significantly between boys and girls, but triacylglycerol levels and the homeostasis model assessment index were significantly higher in girls than in boys. Both LDL- and HDL-cholesterol were close to values found in American children (Olson, 2000). Boys had a significantly higher energy intake than girls, whereas the fat energy percentage, fat composition of the diet and physical activity did not show any gender differences. Energy and fat intakes were close to Danish reference values (Andersen *et al.* 1996).

Results from the measurements of the cardiovascular variables are shown in Table 2; these did not differ significantly between girls and boys except for pulse rate, which was significantly higher in girls than in boys.

### Basic characteristics in infancy

Boys and girls did not differ significantly in size at birth (Table 3), whereas boys were significantly heavier and longer at 9 months of

**Table 1.** Basic characteristics at the age of 10 years

	Girls			Boys		
	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
Age (years)	49	9.97	0.08	44	9.96	0.10
Height (cm)	49	141.2	5.8	44	143.2	5.3
Weight (kg)	49	34.0	4.9	44	35.0	4.9
BMI (kg/m <sup>2</sup> )	49	17.0	2.1	44	17.0	1.8
Body fat percentage	49	22.7	3.9	44	19.0***	4.0
Triacylglycerol (mmol/l)	48	0.75	0.34	41	0.57**	0.21
LDL-cholesterol (mmol/l)	48	2.46	0.56	42	2.27	0.48
HDL-cholesterol (mmol/l)	48	1.43	0.27	42	1.44	0.25
HOMA (index)	45	1.15	0.66	37	0.85*	0.33
Energy intake (kJ/d)	47	9078	1357	42	10	1702
Dietary fat (energy %)	47	35.2	3.7	42	35.7	4.3
Dietary saturated fat (energy %)	47	15.6	2.1	42	15.7	2.1
Dietary MUFA (energy %)	47	10.1	1.2	42	10.1	1.6
Dietary PUFA (energy %)	47	4.3	0.8	42	1.3	0.7
Physical activity (h/d)†	49	3.3	2.0	43	4.1	1.8

HOMA, homeostasis model assessment.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , for girls compared with boys.

† At the highest activity level; for further details see p. 1006.

age. Duration of breast-feeding showed no significant difference between boys and girls.

#### Correlations between the variables studied

Correlation coefficients adjusted for gender are shown in Table 4. PWV in the AR and AF segment was positively correlated ( $r = 0.57$ ,  $P < 0.001$ ), and both were positively correlated with systolic blood pressure, and negatively correlated with physical activity. In addition, PWV in the AR segment was positively correlated with diastolic blood pressure, BMI and body fat percentage, whereas PWV in the AF segment was positively correlated with the duration of breast-feeding.

#### Determinants of pulse wave velocity

All models included a constant and were adjusted for gender, height, weight, body fat percentage and blood pressure. Adjusting for blood pressure is necessary since AS is directly related to the blood pressure (London, 1995). In the initial model, the influence of cardiovascular variables, lipid profile and homeostasis model assessment were explored: none was significantly associated with PWV (Table 5). An analysis, including the LDL:HDL-cholesterol ratio

**Table 2.** Basic characteristics at the age of 10 years

	Girls			Boys		
	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
Pulse (beat/min)	46	80	10	44	75**	8
Systolic blood pressure (mmHg)	49	109	7	44	109	8
Diastolic blood pressure (mmHg)	49	61	5	44	60	5
PWV (aorto-radial) (m/s)	49	3.9	0.5	44	3.8	0.4
PWV (aorto-femoral) (m/s)	49	2.6	0.3	44	2.6	0.2

PWV, pulse wave velocity.

\*\*  $P < 0.01$  for girls compared with boys (Mann-Whitney test).

instead of LDL- and HDL-cholesterol but otherwise the same variables as in Table 5, showed that the cholesterol ratio also had no influence on PWV (data not shown).

In the second model, energy intake, dietary fat intake, physical activity and duration of breast-feeding were included. Fat energy percentage was positively, and physical activity negatively, associated with PWV in both segments, whereas energy intake was only associated with PWV in the AR segment (Table 6). Furthermore, duration of breast-feeding was positively associated with PWV, but significantly only in the AF segment. Including maternal social class at birth in the model did not change any of the estimates (data not shown). Adding fat composition, expressed as the ratio of saturated fatty acid energy percentage to MUFA energy percentage or saturated fatty acid energy percentage to PUFA energy percentage, to the model shown in Table 6 showed that it was not associated with PWV (data not shown). We also tried to include fat intake adjusted for total energy intake. In these models, which were otherwise identical to the models shown in Table 6 and also included total energy intake, fat intake was also positively associated with PWV in the AR segment (1.4 (SD 0.5) cm/s per fat energy percentage;  $P = 0.004$ ) and almost significant in the AF segment (0.7 (SD 0.4) cm/s per fat energy percentage;  $P = 0.051$ ).

In the third model, variables relating to infancy were added. Size at birth (weight, height, ponderal index) and size at 9 months of age (weight, height, ponderal index) were added singly or in combination, for example weight at birth and weight at 9 months of age, thus also testing the influence of growth during the first 9 months (Cole *et al.* 2001). None of the anthropometric measurements was significantly associated with PWV in any of the arterial segments (data not shown).

Gender, weight, height and body fat were not significantly associated with PWV in any of the multivariate analyses.

#### Discussion

In this study of 10-year-old children, we found that the stiffness of two arterial segments was increased in children with low levels of physical activity and with high dietary fat intakes. Stiffness of the

**Table 3.** Basic characteristics in infancy

	Girls			Boys		
	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
Birth weight (g)	49	3424	300	44	3534	406
Birth length (cm)	49	52.0	1.8	44	52.2	2.0
Ponderal index at birth (kg/m <sup>3</sup> )	49	24.4	2.1	44	24.8	2.2
Weight at 9 months (g)	49	8582	884	42	9489***	1001
Length at 9 months (cm)	49	70.6	2.2	42	72.8***	2.3
Ponderal index at 9 months (kg/m <sup>3</sup> )	49	24.4	2.1	42	24.6	2.2
Breast-feeding (months)†	48	6.5	(0–9)	44	5.5	(0–9)

\*\*\*  $P < 0.001$ .

† Data are median (range) owing to a deviation from normal distribution.

**Table 4.** Correlations adjusted for gender (*n*)

	PWV (aorto-radial)	PWV (aorto-femoral)
Systolic blood pressure	0.33*** (93)	0.27** (93)
Diastolic blood pressure	0.37*** (93)	0.18 (93)
Pulse	0.13 (90)	0.16 (90)
Height	0.02 (93)	0.16 (93)
Weight	0.18 (93)	0.10 (93)
BMI	0.21* (93)	-0.01 (93)
Body fat %	0.23* (93)	-0.02 (93)
LDL-cholesterol	0.20 (90)	0.08 (90)
HDL-cholesterol	-0.06 (90)	-0.07 (90)
Triacylglycerol	0.13 (89)	0.05 (89)
HOMA	0.11 (82)	0.13 (82)
Physical activity	-0.26* (92)	-0.26* (92)
Diet fat energy percentage	0.12 (89)	0.11 (86)
Birth weight	0.04 (93)	0.09 (93)
Breast-feeding	0.10 (92)	0.23* (92)

PWV, pulse wave velocity; HOMA, homeostasis model assessment.

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  (Significance of the individual  $\beta$ -coefficients).

AF arterial segment, but not the AR segment, was greater in those who had been breast-fed for longer. Together, these factors explained 32% and 20% of the variation in AS in the AR and AF segments, respectively.

The strengths of the study population are that it is a well-characterised random sample of healthy Danish children followed up at the same age, and that the information on breast-feeding was collected in infancy. The prevalence of overweight was 9% in

our study, not very different from the 13% found among schoolchildren examined during the same years (Pearson *et al.* 2005). Overweight children might be more reluctant to participate in the follow-up, which could explain the difference. However, we did not find that this was an important limitation as we were studying biological phenomena within the group. PWV is an accepted and useful index of AS, which can be measured both accurately and reproducibly (Lehmann, 2000) and is found to correlate well with intra-arterial measurements (Loukogeorgakis *et al.* 2002). A weakness of the method is that the long-term consequences of increased AS in childhood are not known, but studies of certain adult patient groups (Laurent *et al.* 2001; Blacher *et al.* 2003) and the elderly (Meaume *et al.* 2001) suggest that AS is associated with an increased risk of CVD.

The adverse effect of a high fat intake on AS in childhood seen in our study has not been reported before but is in agreement with an intervention study in obese adults showing an improvement in AS after 4 weeks with a low fat intake (Nestel *et al.* 1997). This fast change in AS suggests that structural changes in the vessel wall could not be the mechanism. Furthermore, a fatty meal has an acute effect on endothelial function (Vogel *et al.* 1997; Gokce *et al.* 2001), which could be mediated by a systemic change in basal arterial tone (Gokce *et al.* 2001) that will also affect AS. However, the mechanisms explaining an association between daily fat intake and the acute effect of a fatty meal on AS could be different. Fat composition was not associated with AS in the present study, whereas a high PUFA intake in an intervention studies of obese adults was associated with an improved

**Table 5.** Pulse wave velocity (PWV) and lipid profile (Results from a multiple linear regression with PWV as the dependent variable and all the variables in the table included in the same model†)

	PWV (aorto-radial) in cm/s ( <i>n</i> 79, adjusted $R^2 = 0.03$ )			PWV (aorto-femoral) in cm/s ( <i>n</i> 79, adjusted $R^2 = -0.05$ )		
	$\beta$	95% CI	Partial <i>r</i>	$\beta$	95% CI	Partial <i>r</i>
Body fat (%)	2.4	-1.5, 6.3	0.15	0.6	-1.8, 2.9	0.06
Pulse (beat/min)	-0.7	-1.8, 0.5	-0.14	-0.2	-0.9, 0.5	-0.07
Systolic blood pressure (mm Hg)	1.3	-0.4, 2.9	0.19	0.9	-0.1, 1.9	0.21
Diastolic blood pressure (mm Hg)	1.3	-1.8, 4.3	0.10	-0.8	-2.6, 1.1	-0.10
Triacylglycerol (mmol/l)	14.5	-23.0, 51.9	0.09	-3.3	-26.3, 19.7	-0.04
HDL-cholesterol (mmol/l)	0.8	-40.2, 41.8	0.01	-0.3	-25.5, 24.9	0.00
LDL-cholesterol (mmol/l)	6.1	-13.4, 25.6	0.08	2.5	-9.5, 14.5	0.05
HOMA (index)	-5.3	-27.0, 16.4	-0.06	6.7	-6.6, 20.1	0.12

HOMA, homeostasis model assessment.

† Adjusted for gender, height and weight.

**Table 6.** Pulse wave velocity (PWV), fat intake, physical activity and breast-feeding (Results from a multiple linear regression with PWV as the dependent variable and all the variables in the table included in the same model†)

	PWV (aorto-radial) in cm/s (n 87, adjusted $R^2 = 0.32$ )			PWV (aorto-femoral) in cm/s (n 87, adjusted $R^2 = 0.20$ )		
	$\beta$	95% CI	Partial $r$	$\beta$	95% CI	Partial $r$
Body fat (%)	1.4	-1.9, 4.6	0.09	-1.7	-3.9, 0.5	-0.17
Systolic blood pressure (mm Hg)	1.2	-0.1, 2.5	0.21	0.7	-0.2, 1.6	0.17
Diastolic blood pressure (mm Hg)	2.4	-0.1, 4.8	0.22	1.2	-0.4, 2.9	0.17
Energy (MJ/d)	-6.4*	-11.7, -0.8	-0.25	-1.8	-5.6, 2.0	-0.11
Fat energy percentage (%)	3.1**	0.9, 5.2	0.31	1.8*	0.2, 3.2	0.27
Physical activity (h/d)	-6.8**	-11.2, -2.4	-0.34	-3.9*	-6.9, -0.8	-0.28
Breast-feeding (months)	1.6	-0.8, 4.0	0.15	2.1*	0.4, 3.7	0.27

\* $P < 0.05$ , \*\* $P < 0.01$  (Significance of the individual  $\beta$ -coefficients).

† Adjusted for gender, height and weight.

AS (McVeigh *et al.* 1994; Nestel *et al.* 1997). It may be that the effect of PUFA on AS is only seen at levels of intake higher than those in our study, where there was also a low variation in intake.

In all analyses, physical activity, measured as h/d at the highest activity level, was inversely associated with PWV. Since physical activity was based on only 1 d of registration, we were surprised to see so consistent an association, which might even be an underestimation of the true association seen if a more detailed registration of physical activity were performed. As far as we know, this has not been explored in children before, but both observational (Kupari *et al.* 1994; Kakiyama *et al.* 1998; Tanaka *et al.* 1998, 2000) and intervention studies of adults support the association (Cameron & Dart 1994; Tanaka *et al.* 2000). Animal studies have shown that extended physical exercise increased the elastin content and decreased the calcium content in the aorta (Matsuda *et al.* 1993), but since an immediate and momentary effect of physical activity on AS was seen after 30 min of physical exercise (Kingwell *et al.* 1997), other mechanisms must also be involved. Furthermore, a period of physical training of only one arm caused restored flow-dependent vasodilatation in patients with chronic heart failure, but only in the exercised arm, indicating a local mechanism that could be an enhanced release of NO (Hornig *et al.* 1996). However, different mechanisms could be responsible for the effect of aerobic training and training of specific muscle groups on AS.

In some of our analyses, energy intake was inversely associated with PWV, which is difficult to explain. An explanation could be residual confounding owing to children with a high energy intake also having a high physical activity level, which might not be completely adjusted because of the simple way in which it was measured.

In our regression analysis, there were no significant effects of body size, blood lipids and homeostasis model assessment index. Increased AS has been found in severely obese children (thirty-four) and in children with hypercholesterolaemia, but only few children in our study were overweight and none had hypercholesterolaemia. However, associations between total and LDL-cholesterol and AS have been seen in a population-based study of 9–11-year-old children (Leeson *et al.* 2000), although this disagreement with our result may be due to a slightly higher mean level of LDL-cholesterol in that study.

A proposed mechanism behind the developmental hypothesis is that fetal growth restriction leads to a reduced elastin content in the vessel walls, resulting in increased AS (Martyn & Greenwald,

1997). Studies of the association between birth weight and AS have, however, been inconsistent (te Velde *et al.* 2004), and our study of children with normal birth weight and born at term did not suggest an effect of birth weight or early growth on AS. Cheung *et al.* (2004) have recently shown that children born pre-term and small for gestational age had increased arterial stiffness compared with children born preterm and at term with normal weight for gestational age.

The finding in the AF segment that a longer duration of breast-feeding was associated with increased AS supports an earlier study (Leeson *et al.* 2001). A possible mechanism is that exposure of the vessel wall to the high level of serum cholesterol during infancy in breast-fed compared with formula-fed infants results in the early formation of arterial fatty streaks (Leeson *et al.* 2001). That would imply a higher risk of CVD in breast-fed infants. This is, however, not supported by a current meta-analysis (Martin *et al.* 2004), and, in addition, other meta-analyses suggest that a longer duration of breast-feeding is associated with lower serum cholesterol (Owen *et al.* 2002) and a small reduction in blood pressure (Owen *et al.* 2003) in adulthood. Furthermore, studies of children suggest that breast-feeding is associated with a decreased risk of obesity (Arenz *et al.* 2004), but it is not known whether this association persists into adulthood.

In our study, the association between breast-feeding and AS was significant only in the AF segment, but the association in the AR segment was in the same direction. The difference could also be due to different susceptibilities in the two arteries. The brachial artery is a muscular artery whose compliance would be altered by smooth muscle activity, whereas the central artery from the heart to the groin is a more elastic artery whose stiffness is dependent largely on its scleroprotein content. However, for the other explored variables associated with PWV, i.e. fat intake and physical activity, no differences were seen between the arteries, and the PWV measured in the two arteries were significantly correlated.

Our finding that a low fat intake and a high physical activity might have a beneficial effect on the arterial wall in this sample of healthy 10-year-old children is in line with the general concept of lifestyle factors that will prevent CVD. The study is, however, based on a small number of children, and our findings should be confirmed in other studies. However, even though there is no firm evidence on the long-term effects of AS values measured in childhood, we believe that the present data support the importance of a healthy lifestyle during childhood, with a low fat intake and high

physical activity. Additionally, our study tends to support a previous study of an adverse effect of breast-feeding on AS, but since the literature suggests that breast-feeding has beneficial effects on blood pressure, lipid profile and risk of obesity, the present findings should not be used to advise against breast-feeding. In addition to possible positive effects on other risk factors of CVD, breast-feeding has many important beneficial effects, including protection against infectious diseases, long-term effects on the occurrence of certain non-communicable diseases and, most likely, a positive effect on cognitive function reaching into adulthood (Mortensen *et al.* 2002).

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