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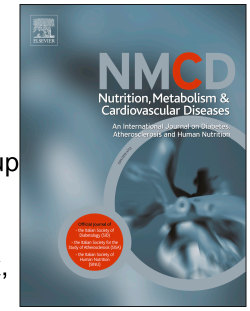

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Association of early protein intake and pre-peritoneal fat at five years of age: follow-up of a randomized clinical trial

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Abstract

Background and aims: The double-blind randomized European Childhood Obesity Project (CHOP) demonstrated that reduced protein content in infant formula leads to a lower body mass index (BMI) up to six years of age. Here we aimed at assessing pre-peritoneal fat, a marker of visceral fat, in children participating in the CHOP trial.

Methods and results: Healthy term formula-fed infants in five European countries were randomized either to higher (n = 550) or lower (n = 540) protein formulas in the first year of life. Infants who were exclusively breastfed for at least three months (n = 588) were enrolled as an observational (non randomized) group. At age 5 years, subcutaneous fat (SC) and pre-peritoneal fat (PP) were measured by ultrasound in a subgroup of 275 children. The PP fat layer was thicker in the higher compared to the lower protein group (adjusted estimated difference: 0.058 cm, 95%CI 0.002; 0.115; p=0.043), while SC fat was not different. Girls showed a thicker SC fat layer than boys.

Conclusions: Higher protein intake in formula-fed infants appears to enhance pre-peritoneal fat tissue accumulation at the age of 5 years, but not of subcutaneous fat, which may trigger adverse metabolic and health consequences.

Introduction

Obesity is an increasing health problem in both adults and children, because obese children tend to be overweight or obese in adulthood.[1] Although a few studies in the last decade report some symptoms of stability in obesity prevalence, they are not representative of all populations and age groups.[2] Obesity is an important risk factor for insulin resistance, abnormal metabolic response and dyslipidemia, and cardiovascular disease.[3] There is growing evidence that not only the total amount of fat, but in particular visceral body fat, determines risks for metabolic and cardiovascular disease. Health risks are associated primarily with the excess central fat compartment, consisting of visceral adipose tissue and subcutaneous adipose tissue.[4]

The mechanisms of the association between visceral fat and disease risk are not clearly established. Visceral fat produces several factors with endocrine functions, including adipokines and cytokines, that affect insulin sensitivity, lipid metabolism, and inflammation.[5] Visceral fat is also a major site for metabolizing sex steroids and glucocorticoids.[6]

CT scanning, a gold standard in the direct evaluation of visceral adiposity, is unsuitable for epidemiological or clinical studies in healthy children because of considerable radiation exposure.[7] Magnetic resonance imaging can also provide details of fat distribution and is radiation-free, but it requires children to lie still for considerable periods, is time-consuming and expensive.[8] Sonography is noninvasive, radiation-free, of limited cost and easily accessible. It can be performed within several minutes, even in very young children, is safe and does not present any detectable health risk. Using ultrasound, subcutaneous fat (SC) as well as pre-peritoneal fat (PP) can be easily measured.[9]

Pre-peritoneal fat estimated by ultrasound is a marker of visceral fat measurement by CT, since several studies show a high correlation between the two methods, indicating that ultrasound can be used to assess abdominal fat distribution in adults and children. [10 9]

The European Childhood Obesity Project (CHOP) has demonstrated that a lower protein content in infant formula reduces body mass index (BMI) at two and at six years of age.[11 12] Moreover, the children fed with formula with a higher protein content had also higher levels of insulin-like growth factor-1 (IGF-1) at 6 months of age,[13] which was shown to preferentially stimulate differentiation of visceral fat preadipocytes in children.[14]

Since the main objective of the CHOP study is to determine the relationship between early nutrition and later obesity, as well as its consequences, the assessment of body fat mass and fat distribution as a possible key driver of later cardiovascular risk factors is of interest. The aims of this secondary analysis are to obtain further insights into the effect of early nutritional intervention - different protein intake in infancy - on the amount of pre-peritoneal (PP) and subcutaneous (SC) fat measured by ultrasound at the age of five years.

Methods

Study design

The design of the CHOP study has been reported previously.[11] Briefly, CHOP is a randomized double-blind intervention study. Infants were enrolled during the first eight weeks of life at centers in five countries. The whole cohort is followed-up with regular anthropometrical measurements and additional examinations and interviews. The Medical Ethics Committees of all study centers approved the study protocol and written informed parental consent was obtained for each infant.

Intervention

Healthy, full-term singleton infants born between 1/10/2002 and 31/7/2004 were recruited in the eleven sites in Germany (Munich and Nuremberg), Belgium (Liege and

Brussels), Italy (4 sites in Milan), Poland (Warsaw) and Spain (Reus and Tarragona). At recruitment, breastfeeding was encouraged and supported. If parents expressed the wish to formula feed, their infant was randomized to one of the intervention groups. Infants were enrolled in the breastfed observational group if parents expressed the intention to exclusively breastfeed for at least three months. Breastfed children who stopped breastfeeding within the first 3 months were excluded. The intervention consisted of two sets of infant and follow-on formulae with either conventional (2.05 and 3.2 g/dl; HP) or reduced (1.25 and 1.6 g/dl; LP) protein but equal energy contents. These were given for the duration of the first year of life. All formulae complied with the 1991 EU Directive on Infant and Follow-on Formulae.[15] Identical energy density was achieved by adapting the difference in energy caused by differential protein content with the fat content.

Study population and data collection

A total of 1,678 infants (distributed as 540 LP, 550 HP, and 588 BF) were enrolled ; the median age at the baseline visit was 16 days. Clinical details of the participants have been published previously.[11] Ultrasound measurements were offered to all study participants (n=655) who were taking part in the 5 year examination.

The children's length and weight at birth came from hospital records. All anthropometric measures were taken by trained professional study personnel. Methods were based on the WHO Multicentre Growth Reference Study.[16] Waist circumference was measured at the uppermost lateral border of the hip crest (ilium).

Data on the parents' educational levels, type of delivery, maternal behavior during pregnancy, family medical history, and the children's physical activity (including time with computers and TV) was collected using questionnaires.

Ultrasound measurement of pre-peritoneal and subcutaneous fat

The method of abdominal fat measurement by ultrasound has been previously described by Suzuki et al [17] and validated in infants by Holzhauer et al [18] as well as in 9 ½ years old children by Mook-Kanamori et al.[9] Briefly, pre-peritoneal fat thickness was measured by a trained person in each dedicated center with a linear transducer, placed perpendicular to the skin surface on the median upper abdomen. Scans were performed longitudinally from just below the xiphoid process to the navel, along the midline, at the end of a spontaneous inspiration. All measurements were performed off-line. The pre-peritoneal fat was measured as the distance between the linea alba and the peritoneum on top of the liver. Subcutaneous fat was measured as the distance between the surface of subcutaneous tissue and the linea alba. The fat mass areas were measured as areas of 1-cm length along the midline starting from the maximum pre-peritoneal distance in the direction of the navel. **Figure 1**

Images were saved and transferred to the Children's Memorial Health Institute in Warsaw, Poland. Blinded measurements with a manual cursor placement method were performed by one trained physician (K.G.). Three measurements were performed on each picture and mean values for PP and SC fat were calculated.

Statistical analysis

Symmetric continuous data is presented as mean ± standard deviation (SD), and skewed continuous and count data as median and inter-quartile range [IQR]. Comparisons between the feeding groups were performed using Student's t-test, Wilcoxon rank sum tests or X^2-tests, respectively.

A linear regression model was applied to estimate the gender and study center -adjusted difference between the two randomized formula groups (minimally adjusted model). To examine confounding, we additionally adjusted the model for parameters which differed either between the randomized groups, or between the original study cohort and the measured subgroup: parental education level, smoking in pregnancy and baseline BMI z-score, as a marker of both birthweight and birth length (fully adjusted model). Residual plots and leverage values were checked for model specification.

Data management was done with SAS version 9.3 (SAS Institute Inc, Cary, NC) and statistical analysis with R 3.1.1 (The R Foundation for Statistical Computing).

Results

Subject characteristics

Fifty percent (n=550) of the randomized 1090 formula-fed children were either excluded for non-compliance during the first year of life or lost to follow-up, or excluded for illness or medication until the age of 5 years. Dropout numbers and reasons were not significantly different between the two randomized groups. Loss to follow-up and non-compliance was higher in the observational breastfed group (57%, n=341). **Figure 2**.

All children taking part in the visit at five years of age were invited to an additional visit to explore abdominal fat. Forty-five percent (n=297) of the families of these 655 children agreed to attend the study center for the additional ultrasound examination. Images of adequate quality were obtained in 275 of these cases. This subsample had a slightly higher birth weight and birth length, higher parental education level and lower number of mothers smoking during pregnancy compared to the original study population. Detailed clinical characteristics of this subgroup are listed in **Table 1**. The two groups of formula-fed infants (HP and LP) differed in birthweight, father's education, and maternal smoking in pregnancy. Breastfed children differed from the formula-fed in terms of socioeconomic status of the family, and prevalence of smoking in pregnancy.

Early protein intake and fat distribution

In children from the HP compared to the LP group the crude, non-adjusted results showed significantly higher values of PP fat layer - median 0.501(IQR 0.377;0.637) vs. 0.432(IQR 0.330;0.553); p=0.038 and PP fat area - median 0.427(IQR 0.322;0.540) vs. 0.360(IQR

0.273; 0.470); p=0.020. The PP fat layer tended to be thicker after minimal adjustment for a study center and gender (adjusted estimated difference: 0.052 cm, 95%CI -0.003; 0.106; p=0.062), and was significantly thicker if additionally adjusted for parental education, maternal pregnancy smoking and baseline BMI z-score (adjusted estimated difference: 0.058 cm, 95%CI 0.002; 0.115; p=0.043). The PP fat area was larger in HP than in LP formula-fed children in both models, the minimally adjusted and the fully adjusted. **Table 2**. In contrast, no differences in SC fat layer thickness or in SC fat area were detected between the formula-fed groups. Waist circumference and BMI at 5 years of age did not significantly differ in our sample between the two intervention groups.

Comparison with breastfed and gender differences

Since the breastfed observational group differed in several parameters from the randomized groups and confounding effects may be an issue, we consider this comparison as explorative. Comparison of fat layers and areas between all formula-fed children and breastfed children showed no significant differences. However, significantly higher SC fat layer and area was observed in the HP group, comparing to breastfed children in a linear regression model including all three feeding groups. **Table 3**. The SC fat measures were also higher in children of mothers smoking during pregnancy.

We looked for gender differences in the whole study sample and observed a significantly thicker SC fat layer and larger area in girls compared to boys: median for layer 0.280 (IQR 0.188;0.437) vs. 0.195 (IQR 0.117;0.316); p<0.0001 and median for area 0.285 (IQR 0.174;0.437) vs. 0.201(IQR 0.119;0.319); p<0.0001, respectively. The SC fat also differed after adjustment for a study center and feeding group – layer: estimated difference 0.072 cm; 95% CI 0.022; 0.123; p=0.005, area: estimated difference 0.069 cm^2; 95% CI 0.019;

0.120; p=0.007. No differences were observed in PP fat. There was no statistical significant interaction effect between feeding type and gender in any of the analyses.

Discussion

Here we demonstrate for the first time that modification of the protein content of infant formula fed during the first year of life may affect the central fat distribution in healthy prepubertal children. This is in line with previous publications from the CHOP study which have shown the effect of higher protein intake in infancy on early weight gain[11] and later BMI and obesity risk.[12] The long-term effect of this early nutritional intervention on later abdominal fat distribution supports the hypothesis that a high early protein intake may increase the risk for later cardiovascular diseases.

A number of epidemiological studies have shown that visceral fat plays an important role in the pathogenesis of several metabolic and cardiovascular diseases in adults and in children.[4 19] Adolescents with a prevalent visceral fat distribution have a five times higher risk of developing metabolic syndrome than those with subcutaneous distribution.[20] Therefore, early risk factors need to be studied in more detail.

To our knowledge, this is the first study based on a clinical trial evaluating effects of early protein intervention on measures of central fat deposition in children. However, abdominal fat has already been used as an outcome in studies exploring effects of other dietary variables. In a randomized trial with provision of n-3 long chain polyunsaturated fatty acids (LCPUFA) or control during pregnancy and lactation, Hauner et al.[21] found no differences in skinfold thickness and abdominal fat measured by ultrasound at 1 year of age.

We used a validated method of ultrasound measurement of abdominal fat. Although, according to existing literature, waist circumference is also a good single predictor of visceral fat in children,[22] in our sample we have not demonstrated a significant difference in waist circumference between formula-fed groups, while the HP group showed increased PP fat.

The mechanisms behind the relationship of early protein intake and fat distribution are not clear. Nutrition and, in particular protein intake, can affect growth hormone secretion, which is the primary determinant of IGF-1 production in the liver.[23] Evidence suggests that protein restriction results in low IGF-I concentrations in healthy children.[24] Furthermore, protein intake from cow's milk was positively associated with IGF-I concentrations in children.[25] Higher IGF-1 levels were also demonstrated in HP compared to LP group in our own CHOP cohort at the age of 6 months.[13]

It has been suggested that the IGF-I axis may be programmed by diet during infancy through a resetting of the pituitary control of growth hormone on IGF-1 levels as a response to high IGF-1 in early childhood.[26] Larnkjaer et al. demonstrated a negative correlation between IGF-1 levels at 9 months and 17 years, indicating a long-term programming effect.[27]

We speculate that a protein-induced stimulation of insulin-like growth factor axis, may play a role in an adipocyte differentiation and proliferation. Grohmann et al. described regional differences in the effects of IGF-1 on adipogenesis.[14] Exogenous IGF-1 upregulated the adipogenic potential of primary cell cultures of subcutaneous and visceral preadipocytes from normal prepubertal children. It stimulated differentiation more effectively in cells derived from the visceral fat depots. It is possible that there may be a difference in a receptor distribution, which could lead to differences in overall adipose tissue sensitivity.[28]

The reasons for a difference in SC fat between the HP group and BF children remain not fully explained. Although breastfeeding is associated with lower IGF-I concentrations in infancy,[13 29] there are probably more factors than early protein intake influencing the pre-peritoneal and subcutaneous fat distribution in the breastfed children. One of them could be leptin, as higher leptin serum levels were observed in the first four months of life in breastfed than in formula-fed infants, due to the presence of leptin in the mother's milk. [30] It is not clear if leptin could provide a link between breastfeeding and later child's fat mass distribution. We hypothesize that there is a complex mechanism probably involving both IGF-1 axis and leptin. The latter was recently shown in children between 2 and 6 years of age to predict subcutaneous fat mass gain over time better than in other locations. [31]

Although variations in the accumulation and distribution of fat between boys and girls are well-documented in adolescents,[32] little data are available on sex differences during early childhood and the results are not consistent.[33] Our findings are in accordance with studies by Goran et al. and Herd et al., who showed more subcutaneous fat in pre-pubertal girls than boys.[34] [35] We also confirm the results of Liem et al. demonstrating sex differences in the ratio between visceral to subcutaneous adipose tissue measured by ultrasound in 6- to 7-year-old children.[36]

Another interesting observation from our study was a significantly higher SC fat layer and area in children of mothers smoking during pregnancy. A meta-analysis of 17 studies found that children of mothers who smoked being pregnant had an increased risk of obesity at the age of 9 years.[37] Durmus et al. demonstrated increased obesity and both SC and PP fat in 6 years old children from Generation R, whose mothers smoked during pregnancy, but they did not report any selective increase in SC fat, as in our sample of children.[38] This observation needs to be confirmed in other studies.

Strengths and limitations

Our study was a follow-up of a double-blind early nutritional intervention trial with randomization. We recruited a large, homogenous group of healthy children from different European populations. The distribution of children among countries was not equal, due to local possibilities and the availability of qualified personnel. The highest number of children was evaluated in Italy and Poland, but no significant differences in the distribution between the HP vs. LP group was observed within these study centers.

However, the study had more limitations. The long follow-up time was associated with significant attrition. The lost to follow-up was similar to other cohorts, and was suggested as acceptable in the context of long-term follow-up studies in healthy children.[39] Based on available data the subgroup participating in this analysis differs from the original CHOP cohort in respect of higher baseline BMI, higher birthweight and birth length, better education and less smoking in pregnancy in mothers of children included in the evaluation. . A potential influence of each of these parameters on the results obtained was analyzed with linear regression models. There was only a borderline difference of PP fat layer between the LP and HP group in the minimally adjusted model but was significant in the case of PP fat area. It may be due to the influence of other covariates on the effect. However, similarly to our experience, Holzhauer et al., in a study performed in infants, also found slightly better reproducibility of an ultrasound abdominal fat area measurement over a simple distance measurement. [18]

There are several advantages of using ultrasound for assessing body fat, but interpretation of ultrasound images requires some technical skill and practice. PP fat provides a good approximation of visceral fat, but a recent study by Koot et al. demonstrated that the correlation of ultrasound results with visceral adiposity quantified by MRI was not satisfactory in severely obese children and adolescents.[40] This is not in the case of our study, as the prevalence of obesity in our sample of children was 5.1%, (16% overweight), which is comparable to other reported European cohorts.[41]

According to Bazzocchi et al. potential limitations associated with ultrasound abdominal fat imaging can be related to technique or methodological issues. Reproducibility was more stable if special attention was paid to the phase of respiration, when the measurement was performed, and quality of equipment used for measurements.[42] In our study common training sessions for sonographers from all study centers were organized and all final measurements from saved scans were performed by one trained person. In order to improve the reproducibility we used a standardized protocol and high-quality ultrasound equipment in all centers. Although different devices were used in each center, including one using a transducer with lower frequency (5-10 MHz, Brussels), all of them were adequate for the purpose of the study measurements.

Because of a long distance between the study centers, no standard inter-center variability analysis was possible to perform. Despite detailed standard operating procedures and common training sessions for sonographers, the authors are aware of a bias during image acquisition. Because of a random distribution of children to high and low protein groups and adjustment for potential confounding, the risk of a false positive result is low, but still it cannot be excluded, that the study may be underpowered to detect some other smaller effects.

Conclusions

Higher protein intake in formula-fed infants appears to enhance pre-peritoneal fat accumulation in the preschool age, but not of subcutaneous fat tissue. This observation supports the hypothesis of programming effects of early nutrition on mechanisms responsible for central fat distribution. The effect of breastfeeding on abdominal fat distribution is not consistent.

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Conflict of Interest

The authors declare no potential conflicts of interest.

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Tables:

- Table 1. Study groups characteristics
- Table 2. Abdominal fat ultrasound measurement results from a linear regression model including HP and LP groups (without BF).
- Table 3. Effect of feeding type and of other covariates on abdominal fat measures. Results from a linear regression model including all three feeding groups, with additional adjustment for a study center.

Figure titles:

- Figure 1. Abdominal fat ultrasound measurement
- Figure 2. Randomization, allocation and study participation

Table 1. Characteristics of the study sample with measured abdominal fat.

	Lower protein (LP)		Higher protein (HP)		Breastfed (BF)	
	N	Mean ± SD; Median [IQR]; N (%)	N	Mean ± SD; Median [IQR]; N (%)	N	Mean ± SD; Median [IQR]; N (%)
Country	97		86		92	
• Germany		9(9.3)		5(5.8)		6(6.5)
• Belgium; Liege/ Brussels		8(8.2); 7/1		3(3.5); 1/2		13(14.1); 11/2 ¶
• Italy		46(47.4)		45(52.3)		48(52.2)
• Poland		30(30.9)		31(36.0)		24(26.1)
• Spain		4(4.1)		2(2.3)		1(1.1) #
Parents:						
Mother's pre-pregnancy BMI (Kg/m2)	93	23.62±4.39	84	24.44±5.09	82	23.07±4.60
Father's BMI (Kg/m2)	97	25.83±4.09	86	26.40±3.62	91	25.70±3.58
Education – mother (ISCED)	96		86		92	
• No/low		20(20.8)		14(16.3)		7(7.6) # ¶
• Middle		61(63.5)		54(62.8)		52(56.5)
• High		15(15.6)		18(20.9)		33(35.9) # ¶
Education – father (ISCED)	96		86		92	
• No/low		29(30.2)		11(12.8)*		13(14.1) #
• Middle		52(54.2)		59(68.6)		54(58.7)
• High		15(15.6		16(18.6)		25(27.2)
Pregnancy and birth:						
Cesarean section	97	33(34)	86	24(27.9)	92	17(18) #
Gestational age (weeks)	97	39.67±1.22	86	39.72±1.10	91	39.69±1.16
Smoking during pregnancy	96	39(40.6)	86	21(24.4)*	92	12(13) # ¶
Birth weight (Kg)	97	3.285±0.332	86	3.392±0.375 *	92	3.383±0.341
Birth length (cm)	96	51.07±2.64	86	51.81±2.79	92	51.13±2.46 ¶
Child at 5 years:						
Weight (Kg)	97	19.60[17.90-20.55]	86	19.25[17.35-21.55]	92	19.14[17.47-20.75]
Height (cm)	97	111.19±4.49	86	110.51±4.76	92	110.27±4.85
BMI (Kg/m2)	97	15.81[14.88-16.44]	86	15.65[15.01-17.20]	92	15.53[14.73-16.58]
Waist circumference (cm)	97	54.20[51.90-56.55]	86	54.75[51.90-58.15]	92	53.40[51.50-56.00]
Overweight/obese	97	14(14.4)/4(4.1)	86	18(20.9)/8(9.3)	92	12(13)/2(2.2) ¶
TV/computer (h/day)	78	1.00[0.94-2.00]	72	1.50[1.00-2.00]	79	1.50[0.50-2.00]

* p<0.05 for HP vs. LP; # p<0.05 LP vs. breastfed; ¶ p<0.05 HP vs. breastfed

ISCED - International Standard Classification of Education

	Intervention formula groups		Comparison of HP vs. LP				Observational group
	Lower Protein N = 97	Higher Protein N = 86	Multiple linear regression				Breastfed N = 92
	Median [IQR]		minimally adjusted estimated difference (95% CI)*	P-value	fully adjusted estimated difference (95% CI) #	P-value	Median [IQR]
SC fat layer (cm)	0.258 [0.148; 0.397]	0.245 [0.177; 0.410]	0.036 (-0.032; 0.104)	0.292	0.047 (-0.023; 0.117)	0.185	0.225 [0.125; 0.332]
PP fat layer (cm)	0.432 [0.330; 0.553]	0.501 [0.377; 0.637]	0.052 (-0.003; 0.106)	0.062	**0.058 (0.002; 0.115)**	**0.043**	0.485 [0.389; 0.590]
SC fat area (cm^2)	0.242 [0.144; 0.373]	0.244 [0.166; 0.396]	0.037 (-0.031; 0.104)	0.288	0.048 (-0.021; 0.118)	0.173	0.211 [0.137; 0.327]
PP fat area (cm^2)	0.360 [0.273; 0.470]	0.427 [0.322; 0.540]	**0.052 (0.002; 0.102)**	**0.041**	**0.060 (0.008; 0.112)**	**0.024**	0.410 [0.316; 0.514]
SC/PP fat area	0.692 [0.397; 1.080]	0.665 [0.416; 1.000]	-0.018 (-0.159; 0.124)	0.807	-0.014 (-0.158; 0.131)	0.851	0.548 [0.311; 0.873]
Waist circum. (cm)	54.200 [51.900;56.550]	54.750 [51.875;58.187]	0.643 (-0.887; 2.173)	0.408	0.961 (-0.590; 2.513)	0.223	53.400 [51.500;56.000]
BMI (Kg/m^2)	15.814 [14.883;16.446]	15.650 [14.997;17.222]	0.321 (-0.259; 0.901)	0.276	0.376 (-0.214; 0.967)	0.210	15.534 [14.718;16.599]

Table 2. Abdominal fat ultrasound measurement results from a linear regression model including HP and LP groups (without BF).

* adjusted for study center and gender; # adjusted for study center, gender, baseline BMI, parental education level and smoking in pregnancy (observational BF group – not included in the model)

		SC fat layer (cm)		PP fat layer (cm)		SC fat area (cm^2)		PP fat area (cm^2)	
		estimated difference (95% CI)	p	estimated difference (95% CI)	p	estimated difference (95% CI)	p	estimated difference (95% CI)	p
Intervention formula groups compared to breastfed (reference) (N=92)	Lower Protein (N=97)	0.023 (-0.040; 0.086)	0.472	-0.037 (-0.088; 0.014)	0.157	0.017 (-0.045; 0.080)	0.587	-0.041 (-0.089; 0.006)	0.089
	Higher Protein (N=86)	**0.0680 (0.005; 0.131)**	**0.034**	0.020 (-0.032; 0.072)	0.449	**0.064 (0.001; 0.127)**	**0.047**	0.018 (-0.030; 0.066)	0.465
Parental education level according to ISCED# (reference low/no) (N=17)	Middle (N=168)	0.024 (-0.082; 0.130)	0.653	-0.023 (-0.110; 0.063)	0.595	0.025 (-0.081; 0.130)	0.645	-0.028 (-0.108; 0.053)	0.500
	High (N=89)	-0.009 (-0.120; 0.103)	0.879	-0.033 (-0.125; 0.058)	0.473	-0.013 (-0.125; 0.099)	0.816	-0.035 (-0.120; 0.050)	0.413
Smoking in pregnancy (reference no) (N=202)	Yes (N=72)	**0.076 (0.016; 0.137)**	**0.013**	0.014 (-0.035; 0.063)	0.573	**0.078 (0.018; 0.138)**	**0.011**	0.020 (-0.025; 0.066)	0.377
Baseline z-score BMI (N=274)		0.005 (-0.025; 0.034)	0.751	-0.007 (-0.032; 0.017)	0.556	0.003 (-0.026; 0.033)	0.833	-0.009 (-0.032; 0.013)	0.417
Gender (reference Male) (N=138)	Female (N=137)	**0.065 (0.014; 0.116)**	**0.011**	0.013 (-0.029; 0.055)	0.551	**0.063 (0.012; 0.114)**	**0.015**	0.021 (-0.018; 0.059)	0.293

Table 3. Effect of feeding type and of other covariates on abdominal fat measures. Results from a linear regression model including all three feeding groups, with additional adjustment for a study center.

*bold estimates are significant with $p < 0.05$

International Standard Classification of Education (ISCED)

Figure 1. Abdominal fat ultrasound measurement

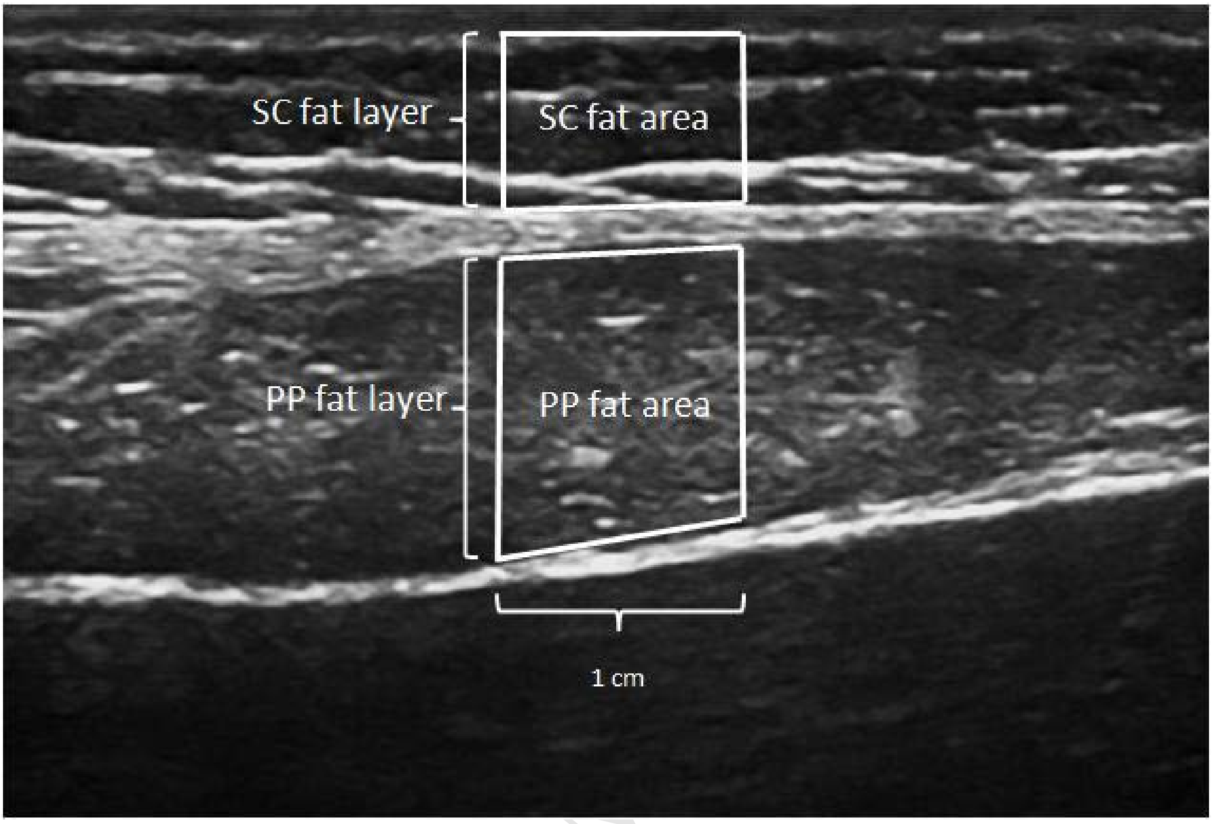
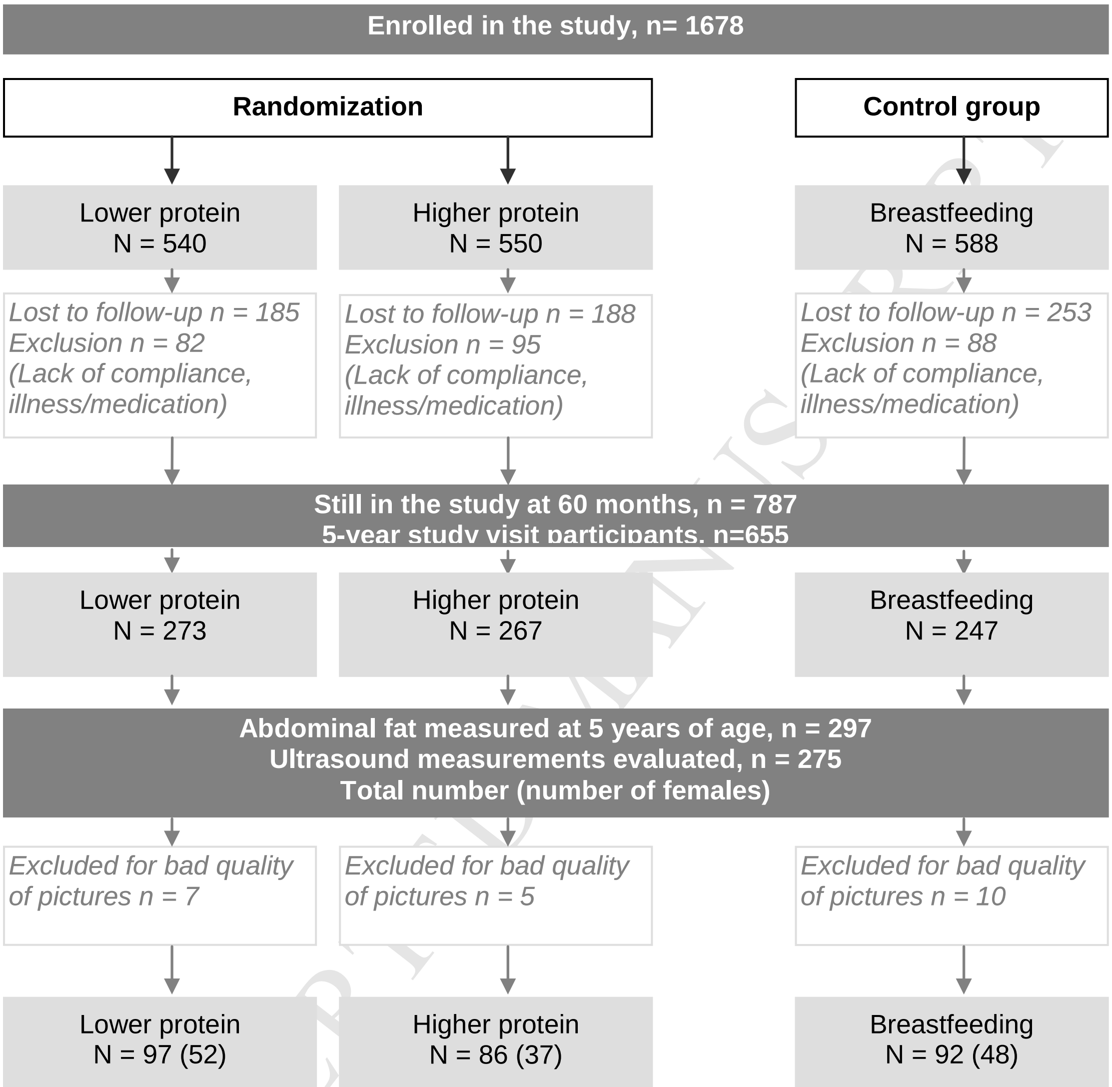


Figure 2. Randomization, allocation and study participation



Association of early protein intake and pre-peritoneal fat at five years of age: results from a randomized clinical trial

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Highlights:

- Higher protein intake in formula-fed infants appears to enhance pre-peritoneal fat tissue accumulation in preschool children