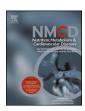


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Association of early protein intake and pre-peritoneal fat at five years of age: Follow-up of a randomized clinical trial



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KEYWORDS

Infant formula; Protein; Dietary proteins; Intra-abdominal fat; Subcutaneous fat; Child **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 preperitoneal 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.

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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 preperitoneal (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 1678 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.

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