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## Short communication

# Inverse association between body mass and frequency of milk consumption in children

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Recent studies have shown an inverse association between the level of dietary Ca, particularly from dairy sources, and body weight in adults; there is, however, a paucity of data regarding this relationship in children. We therefore investigated this issue in 1087 children who underwent body weight and height measurement during a survey on childhood obesity. Lifestyle and dietary habits were investigated by a questionnaire. After excluding children who were following a dietary regimen for any reason, 884 children (M:F 451:433; age 7.5 (SD 2.1) years) were selected. Milk consumption was pooled into four frequency categories: poor ( $\leq 1$ /week; *n* 125), moderate (>1 but  $\leq 5-6$ /week; *n* 133), regular (1/d; *n* 408) and high ( $\geq 2/d$ ; *n* 218). The frequency of consumption of milk was inversely and significantly associated (t=-2.964, P=0.003) with age- and sex-specific BMI *z*-scores by linear regression analysis, controlling for sex, age, physical activity, birth weight and parental overweight and education. The statistical association remained significant (t=-2.831, P=0.005) after the inclusion of children consuming only skimmed milk (*n* 91). Milk consumption was still significantly and inversely associated with BMI *z* score (t=-2.791, P=0.005) in the whole-milk consumers when controlling for age and the frequency of consumption of various foods; this association was no longer significant (P=0.21) when children consuming skimmed milk were included in the analysis. This is the first report showing a significant inverse association between frequency of milk consumption and body mass in children. Regardless of the mechanisms involved, our results might encourage further research on this issue and might have important implications for the prevention of obesity.

Milk: Childhood: Obesity: Prevention: School

Almost 20 years ago, in a study on the relationship between nutrient intake and blood pressure, McCarron *et al.* (1984) described an inverse association between Ca intake and body weight. A few years later, Trevisan *et al.* (1988), while evaluating the relationships between dietary Ca and blood pressure, incidentally observed that BMI was inversely associated with the frequency of consumption of milk, but not of cheese, in Italian male adults. Statistical evidence of an inverse association between dietary Ca and body weight has also emerged from other epidemiological studies (see Zemel, 2002; Heaney, 2003; Teergarden, 2003; for a review of the data).

Zemel *et al.* (2000) and Xue *et al.* (2001) recently showed that intracellular Ca levels modulate lipolytic activity in human adipocytes, thus providing a plausible biological mechanism by which dietary Ca could influence body weight and energy expenditure. Further studies were prompted, mainly reanalyses of data previously collected to evaluate the effects of Ca intake on bone density in postmenopausal women. Most reports confirmed the inverse relationship between dietary Ca and body weight (Davies *et al.* 2000; Pereira *et al.* 2002; Parikh & Yanovski, 2003), whereas randomised trials of Ca supplementation on body mass showed little or no effect (see Davies *et al.* 2000; Barr, 2003; Parikh & Yanovski, 2003; Zemel, 2002; for review). Taken together, these data suggest that the inverse association with body fat could be due, at least in part, to additional components of dairy food. This could be the case for milk, for example, which is rich in bioactive molecules that may act independently of Ca to reduce body fat accumulation (Shah, 2000).

Although milk is an important component of children's diet, population studies to date have not explored the association between milk consumption and body mass in children. To the best of our knowledge, only one study, performed in a small sample of pre-school children, explored the role of dietary Ca in modulating body composition in children and showed a significant inverse association between dietary Ca (or servings of dairy products) and percentage body fat (Carruth & Skinner, 2001). We thus investigated the relationship between frequency of milk consumption and body mass in a large sample of school children in Southern Italy.

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## Methods

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## Study population

Children from three primary schools in the Avellino district of Southern Italy (*n* 1467) were invited to participate in a survey of anthropometry, life-style and dietary habits (the BRAVO Project and the Gabbiano Study). The schools were located either in urban and suburban areas or in the countryside. Between April 2000 and June 2002, 1087 children (74% of the entire population; age 7.7 (sD 3.5) years, range 3–11; BMI 19.01 (sD 3.69) kg/m<sup>2</sup>, range 11.1–36.7; boys 51%) were examined. Several *ad hoc* meetings with the families were held within the schools to explain the purposes and the in-the-field procedures of the project in order to obtain written informed consent from the parents. No children who refused to participate in the study were screened, even if the parents provided their consent.

For the purposes of the present analysis, 884 children were selected after excluding those whose data set was incomplete (n 61), those following a specific dietary regimen for any reason (n 51) and those reporting that they consumed only skimmed or partially skimmed milk (n 91). This last group was excluded because skimmed milk consumption is unusual among children living in the geographical area under investigation. Moreover, they were older and heavier (age 8.5 (SE 0.13) v. 7.5 (SE 0.08) years; age-adjusted BMI 19.7 (SE 0.38) v. 18.6 (SE 0.13) kg/m<sup>2</sup>; P < 0.001): it could be hypothesised that children consuming only skimmed milk in our sample would not be on a free diet, which could bias the interpretation of the results. Since, however, this assumption is questionable, statistical analysis was also performed on the larger sample including children consuming skimmed milk (n 975). The characteristics of the groups investigated are reported in Table 1.

*Measurement of outcome*. The methods have been previously described in detail (Barba *et al.*, 2001). In summary, body weight and height were measured on a standard beam balance scale (Seca, Vogel & Halke GmbH & Co., Hamburg, Germany) with an attached ruler, with children wearing light clothes without shoes. Measurements were performed on the school premises, in the presence of a teacher or the child's parent, if possible. The BMI was calculated as weight in kilograms divided by the square of the height in meters. For statistical analysis purposes,

Table 1. Characteristics of the study population

	Mean	SD	Range
Whole-milk group (n 884)			
Age (years)	7.5	2.1	3–11
Boys:girls (%)	51.5:49.5	49.5	
BMI (kg/m <sup>2</sup> )	18.7	3.4	11.8-31.5
% Overweight	47.4		
Birth weight (kg)	3.337	0.530	1.100-7.000
% With at least one	76.9		
overweight parent			
Whole + skimmed-milk gr	oup ( <i>n</i> 975)		
Age (years)	7.9	2.0	3–11
Boys:girls (%)	51.4:49.6		
BMI (kg/m <sup>2</sup> )	19.0	3.5	11.1-36.7
% Overweight	47.6		
Birth weight (kg)	3.223	0.534	1.000-7.000
% With at least one	77.4		
overweight parent			

BMI values were converted to age- and sex-specific z-scores, which were calculated by standard formula (z score = (BMI –  $\mu_{BMI}$ )/ $\sigma_{BMI}$ ) from data collected in a larger population (n 3007; M:F 1519:1488; age 8.5 (sD 1.7) years, range 3–11; BMI 19.5 (sD 3.9) kg/m<sup>2</sup>, range 11.3–42.3) living in the same geographical area and screened for body weight and height adopting the same procedures as the present study. Further information about this reference population is available on request.

*Measurement of exposure*. Parents were asked to fill in a 120item questionnaire that investigated:

- 1. dietary habits (1-year food frequency);
- 2. medical history and life-style of the participating children;
- 3. the parents' medical history.

Age was calculated as the difference between the date of the visit and the date of birth and expressed in years as a continuous variable. Reported birth weight was expressed in kilograms. Children regularly practising sport for at least 1 h three times per week were considered physically active. Self-reported body weight and height of the parents were recorded and used to calculate their BMI; parental overweight was defined as the number of parents (0, 1 or 2) having a BMI of over 25 kg/m<sup>2</sup>. The education level of each parent was scored from 0 (primary school) to 3 (degree); the sum of the scores of the father and the mother (range 0-6) was used as an index of parental education level.

With regard to the dietary questionnaire, closed answers were allowed to evaluate the frequency of consumption of specific foods (never,  $\leq 1/\text{month}$ , 2–3/month, 1/week, 2/week, 3–4/ week, 5–6/week, 1/d,  $\geq$ 2/d). Food items were grouped in the following categories: milk; dairy foods; fish; cereals (including pasta); meat; fruit; vegetables; sweet beverages; snacks. Milk consumption was pooled into four frequency categories: poor ( $\leq$  1/week); moderate (>1/week and  $\leq$ 5–6/week); regular (1/d); high ( $\geq$ 2/d). Similarly, the frequencies of consumption of the other food groups were pooled into four main categories (rare, moderate, frequent and high).

The prevalence of overweight was estimated according to the standard definition criteria proposed by Cole *et al.* (2000) and adopted by the International Childhood Obesity Task Force (ILSI Europe Overweight & Obesity in Children Task Force, 2000), which are based on age- and sex-specific cut-off values obtained from centile curves leading to a BMI of  $25 \text{ kg/m}^2$  at age 18 years.

Statistical analysis. Data were expressed as mean and standard deviation or as mean and 95% CI, as indicated. Statistical analysis was performed by ANOVA, analysis of covariance (trend analysis model), multiple linear analysis, logistic regression analysis or chi-square statistics, as appropriate. In the linear regression models, BMI *z*-score was used as the dependent variable to allow for linear adjustment by age and to adjust for the increase in relative weight with age; demographic indices and food consumption categories were used as independent variables. Two-sided *P* values of <0.01 were considered statistically significant. Statistical analysis was performed by SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

## Results

Children in the four categories of frequency of whole-milk consumption showed significant differences for age and BMI (Poor: n 125, age 8.0 (sD 2.1) years, BMI 20.0 (sD 3.5) kg/m<sup>2</sup>; Moderate: *n* 133, age 8·4 (sD 1·9) years, BMI 19·4 (sD 4) kg/m<sup>2</sup>; Regular: *n* 408, age 8·1 (sD 1·9) years, BMI 18·9 (sD 3·4) kg/m<sup>2</sup>; High: *n* 218, age 7·1 (sD 2·1) years, BMI 18·2 (sD 3) kg/m<sup>2</sup>; P < 0.0001 by ANOVA). Differences in BMI between groups were still significant after adjusting for age (analysis of covariance; *P* for trend=0.0001).

Age-adjusted ANOVA is, however, unlikely to be adequate to investigate the association between the outcome and the main exposure because the relationship between BMI and age is unlikely to be linear within this age range, due to BMI changes with growth. For this reason, and to take into account the effect of other possible confounders, multiple regression analysis models were conducted, using sex- and age-specific BMI z scores as dependent variables and milk consumption as an independent variable. Model 1 included age, birth weight, parental overweight/obesity, parental education level and physical activity as supplemental independent variables, whereas model 2 included the frequency of consumption of the other groups of foods (dairy foods, fish, cereals, meat, fruit, vegetables, sweet beverages, snacks). As shown in Table 2 (wholemilk group), parental overweight/obesity and frequency of milk consumption were the sole significant predictors of BMI in both models.

Multiple regression analysis was repeated in the larger cohort, including children consuming skimmed milk (Table 2, whole + skimmed-milk group): in model 1, milk consumption retained a significant, independent, effect on BMI z score, whereas in model 2, the frequency of consumption of milk was no longer significant.

The prevalence of overweight was inversely associated with the frequency of consumption of whole milk in comparison to the other frequency categories (60.5%, 51.2%, 45.9% and 40.7%, respectively, from poor to high milk consumption; chi-square = 13.2, *P*=0.004; pooled data for both sex and age). These results were confirmed by logistic regression analysis in which the same independent variables of model 1 of the linear regression analysis were included: the risk of being overweight was significantly higher in those with poor whole milk consumption (relative risk 2.18, 95% CI 1.30–3.66). This association was,

however, no longer apparent in the larger cohort including children consuming skimmed milk.

#### Discussion

The present study demonstrates, for the first time, evidence of a statistically significant inverse association between frequency of milk consumption and body mass in a large sample of children. This association is independent of other possible determinants of body mass, such as age, birth weight, parental overweight, education of parents, physical activity and reported dietary habits. In our population, the prevalence of overweight is significantly lower in children consuming whole milk daily than in those who consumed milk less frequently.

In principle, we decided to exclude from the statistical analyses children consuming skimmed milk by including them in the group of those following a dietary regimen for any reason, whose inclusion could have represented a possible source of bias in the interpretation of the results. Skimmed milk consumption is unusual in Southern Italy in the age range studied, unless prescribed for medical reasons. In fact, in our population, children consuming skimmed milk were older and heavier than those consuming whole milk. However, we analysed data with and without these children. As shown in the results section, linear regression in model 1 remained unchanged after the inclusion of children consuming skimmed milk. In model 2 (the 'food' model), and in the logistic regression analysis, inclusion of these children was associated with a lower (and no longer significant) weight of milk consumption in the association observed, perhaps because of overall changes in dietary habit in children habitually consuming skimmed milk.

Our data are consistent with previous reports on the relevance of dietary Ca/dairy products in the regulation of body weight in other age classes (McCarron *et al.* 1984; Trevisan *et al.* 1988; Davies *et al.* 2000; Carruth & Skinner, 2001; Pereira *et al.* 2002; Zemel, 2002; Heaney, 2003; Parikh & Yanovski, 2003; Teergarden, 2003). The cross-sectional nature of the study does not allow for the detection of any cause-and-effect relationship

Table 2. Stepwise multivariate	regression analysis	with age- and	sex-specific BMI	z scores as the dependent
variable				

Variable	В	95 % CI of B	t	P value
Whole-milk group (n 884)				
Model 1. Independent variab	les: age, sex, birth w	eight, parental overweight, ph	ysical activity, parer	ntal education,
frequency of milk consumption	on			
Parental overweight	0.266	0.112, 0.421	3.379	0.001
Milk consumption	-0.101	-0·167, -0·034	-2.964	0.003
Model 2. Independent variab	les: age, sex, birth w	veight, parental overweight, ph	ysical activity, parer	ntal education,
frequency of consumption of	milk, dairy foods, fish	n, cereals, meat, fruit, vegetab	les, sweet beverages	s, snacks
Parental overweight	0.384	0.206, 0.561	4.243	0.001
Milk consumption	-0.112	-0.190, -0.033	-2.791	0.005
Whole + skimmed-milk group	o ( <i>n</i> 957)			
Model 1. Independent variab	les: age, sex, birth w	veight, parental overweight, ph	ysical activity, parer	ntal education,
frequency of milk consumption	on			
Parental overweight	0.337	0.195, 0.479	4.658	0.001
Milk consumption	-0.080	<i>−</i> 0·154, <i>−</i> 0·010	-2.831	0.005
•	•	veight, parental overweight, ph n, cereals, meat, fruit, vegetab		
Parental overweight	0.398	0.234, 0.562	4.764	0.001

B, regression coefficient; t, value of t-statistic for co-efficient B.

Entered variables are reported.

in the association observed, but several mechanisms to explain this association have been suggested.

Dietary Ca intake is inversely associated with intracellular Ca levels, a phenomenon known as the 'calcium paradox' (Zemel *et al.* 2002). It has been shown in cultured human and mice adipocytes that a low level of intracellular Ca (associated with a high dietary Ca intake) promotes lipolysis, whereas a high level of intracellular Ca (associated with a low Ca intake) stimulates lipogenesis and fat accumulation. It has also been proposed that calcitrophic hormones, such as parathyroid hormone and 1,25 dihydroxyvitamin D, might influence lipid metabolism in the adipocyte, independently of dietary Ca (Zemel *et al.* 2000; Shi *et al.* 2001; Xue *et al.* 2001,). Finally, supplemental dietary Ca has been shown to influence dietary fat absorption (Shahkhalili, 2001; Papakostantinou *et al.* 2003).

In the present study, however, we specifically referred to milk consumption, and other plausible mechanisms should be taken into account. It has recently been suggested that milk is rich in bioactive compounds that may also act independently of Ca in modulating body fat accumulation (Pihlanto-Leppala et al. 2000; Shaah, 2000), including vitamin D (the role of which has previously been discussed) and angiotensin-converting enzyme inhibitory peptides. The latter limit angiotensin II production and thereby the angiotensin II stimulation of adipocyte lipogenesis. Interestingly, Strazzullo et al. (2003) recently reported, in a large sample of an adult male population, that carriers of the DD variant of the angiotensin-converting enzyme gene (associated with higher plasma levels of angiotensin-converting enzyme activity) showed a higher incidence of overweight. Milk also promotes insulin-like growth factor production (Nagashima et al. 1990), thus potentially influencing body fat accumulation (Frick et al. 2002), again independently of dietary Ca. We cannot, however, exclude the possibility that milk consumption could be a marker of other factors either not measured (or mismeasured) in the present study or as yet unidentified.

Our analysis has limitations. The use of self-reported food-frequency questionnaires might have introduced misclassification in our primary exposure. However, the large sample size and the high level of significance of the results reduces the likelihood that the observed outcomes were due to chance alone. Moreover, in the present study, energy intake was not measured, thus not allowing for the control of a relevant confounder. In principle, however, it is unlikely that reporting the frequency of consumption of milk would be biased with regard to the total energy intake. Finally, socio-economic status was not investigated in detail, and only data about the education level of the parents were considered in the statistical analyses.

Regardless of the mechanisms, our findings may encourage further research on the mechanisms of the milk-mediated regulation of body mass and might have important implications for the prevention of both childhood and adult obesity.

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