

## CLINICAL STUDY

# Children whose diet contained olive oil had a lower likelihood of increasing their body mass index Z-score over 1 year

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## Abstract

**Objective:** Changes in eating habits may be influential in the ever-increasing rate of childhood obesity. Our aim was to determine whether those children who consume olive oil have a lower risk of weight gain compared with children who consume other oils.

**Design and methods:** The study included 18 girls and 74 boys, all aged 13–166 months. A survey was completed for each subject about eating habits and physical activity. A sample of subcutaneous adipose tissue was also obtained for cellular study. Data were recorded on the mean size of the adipocytes, the number of preadipocytes, and the concentration of particular fatty acids. The weight and height of the children were measured 13 months later.

**Results:** The likelihood that after 1 year the children would have increased their body mass index (BMI) Z-score above the initial score was less in the children who consumed only olive oil (odds ratio (OR) = 0.22; 95% confidence interval (CI): 0.08–0.63;  $P=0.005$ ). These results remained after adjusting for age, physical activity and BMI (OR = 0.19; 95% CI: 0.06–0.61;  $P=0.005$ ) and after adjusting for age, physical activity and adipocyte volume (OR = 0.15; 95% CI: 0.04–0.52;  $P=0.003$ ).

**Conclusions:** Diets with mono unsaturated fatty acid (MUFA)-rich olive oil could reduce the risk of obesity in childhood.

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## Introduction

Childhood obesity is a growing public health problem. A sedentary lifestyle, changes in eating habits and a genetic predisposition are considered to be factors determining the increase in obesity. Obesity is caused by overgrowth of adipose tissue due to increase in the size and number of adipocytes, which may be influenced by environmental factors such as diet.

The number of adipocytes in the body is set in childhood and adolescence, remaining constant in adulthood (1). Therefore, childhood and adolescence are a critical period for the development of adipose tissue. The increase in adipocyte number is due to the differentiation of preadipocytes into adipocytes (2). The capacity of preadipocytes for cell replication and differentiation can be modulated by numerous endogenous and exogenous factors, and Ailhaud *et al.* (3) recently reviewed the importance of the dietary *n*-6 fatty acid composition as a determinant of childhood obesity.

In many Mediterranean countries, particularly those in Europe, there have been very important lifestyle changes, especially concerning eating habits and

physical activity. All these changes are associated with a marked increase in the prevalence of obesity, and it has been proposed that this increased prevalence is related to the abandoning of the traditional Mediterranean diet (4).

Several studies have shown an inverse relationship between Mediterranean diet and obesity (5). The large cohort of the SUN project (6) showed that a high intake of olive oil is not related to a significantly higher risk of developing obesity. Our group has shown that those adults who consume olive oil have a lower likelihood of being obese and that this lower incidence is independent of physical activity and calorie intake (7).

In this study, we attempted to determine whether children who consume olive oil have a lower risk of weight gain and, if so, the relationship of this with certain characteristics of adipose tissue in childhood.

## Subjects and methods

Children aged from 13–166 months (18 girls and 74 boys) were studied during the course of inguinal hernia surgery. Children with any other disorder were excluded

from the study. After obtaining consent from the parents, a sample of subcutaneous fat, weighing 40–120 mg, was taken during the operation from the area of the inguinal incision. The sample was studied fresh.

All the children underwent an anthropometric study and questionnaires were completed by their parents on nutrition and physical activity. All children were introduced to their diet containing all food groups and vegetable oil. The follow-up was done 1 year later in all children studied at baseline. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethics committee of the Hospital Universitario Carlos Haya of Malaga, Spain. Written consent was obtained from all parents or legal tutors of subjects.

### Procedures

All procedures are related to basal study; during follow-up, only weight and height were recorded.

The weight and height were measured using standardised procedures. Body mass index (BMI) was calculated ( $\text{weight}/\text{height}^2$ ,  $\text{kg}/\text{m}^2$ ) and standardised for age and sex (Z-scores) based on recently published weight, height and BMI tables for Spanish children (8).

The nutritional study was done by the same dietician for all the children, using a questionnaire that collected information on frequency of consumption of the main foods in Spain. Data were also recorded on the time spent sleeping, resting, lying down, sitting, walking and running.

Adipose tissue fatty acid measurement was done by extraction of the total fat in the tissue (9) and lipid separation by thin-layer chromatography (hexane-diethyl ether-acetic acid, 80:20:2). Methyl-ester fatty acids were obtained and separated by gas chromatography in a chromatograph Agilent6850 (Agilent Technologies, Barcelona, Spain, column Agilent 122–2212E,  $15 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ ).

The adipocytes from the subcutaneous adipose tissue were isolated by enzyme digestion for 45 min in KRBHA medium with collagenase, according to the method of Robdell, and the cell volume was calculated using the Goldrick equation (10).

The number of preadipocytes per milligram of tissue was determined according to the protocol of Sengenés *et al.* (11). Briefly, the stromal fraction isolated by enzyme digestion was incubated with anti-CD34 and anti-CD31 MAB labelled with fluorescence (BD Biosciences, San José, CA, USA). Tubes with a known amount of beads coated with the proteins CD31 and CD34 were used as an internal standard (TrueCount, BD Biosciences). Using flow cytometry, the preadipocyte population was then identified as that stained for  $\text{CD34}^+/\text{CD31}^-$  using an FACSCalibur flow cytometer and the CellQuest Pro Software (BD Biosciences).

### Statistical analysis

The data are expressed as mean  $\pm$  s.d. or proportions. The hypothesis contrast for continuous variables was done with age- and sex-adjusted ANOVA models. The association between variables was evaluated by calculating the odds ratio (OR) from logistic regression models. The correlation between continuous variables was done by calculating the Pearson *r*.

### Results

Food was prepared solely with olive oil for 71.1% of the children and with sunflower oil or a mixture of olive and sunflower oils for 28.9%. Both groups of children had similar age and sex ratios (Table 1).

At baseline, 51.2% of the children had a BMI above the 50th percentile for their age and sex. This proportion did not change significantly during the follow-up (48.9%).

There were no differences in baseline BMI Z-score or adipocyte volume according to the type of oil consumed (Table 1).

The fatty acid composition of adipose tissue varied according to the type of oil consumed (Table 1). Children who consumed olive oil had a higher proportion of oleic acid in adipose tissue, whereas children taking sunflower oil had a higher proportion of linoleic acid. There were no differences in other fatty acids (Table 1).

**Table 1** Main variables according to olive oil intake. Data are presented as mean  $\pm$  s.d. or as percentages. Group 1, children who consumed only olive oil in salads and cooked foods; Group 2, children who consumed a mixture or other types of oil in salads and cooked foods (usually sunflower oil).

	Group 1	Group 2	P (age adjusted)
<i>n</i>	66	26	
Age (years)	4.8 $\pm$ 2.7	5.8 $\pm$ 3.3	0.4
Sex (% male)	80.3	80.8	0.7
BMI Z-score			
Baseline	0.17 $\pm$ 0.95	0.18 $\pm$ 0.91	0.9
Follow-up	–0.11 $\pm$ 1.07	0.50 $\pm$ 0.81	0.02
Adipocyte volume (pl)	309.7 $\pm$ 160.0	326.5 $\pm$ 204.4	0.6
Preadipocyte number*	44.26 $\pm$ 44.78	33.54 $\pm$ 35.06	0.4
Adipose tissue fatty acid composition (%)			
Myristic acid	4.00 $\pm$ 1.16	3.81 $\pm$ 1.10	0.8
Palmitic acid	23.74 $\pm$ 2.53	24.12 $\pm$ 2.02	0.5
Palmitoleic acid	3.49 $\pm$ 1.12	3.30 $\pm$ 0.94	0.5
Stearic acid	6.08 $\pm$ 1.97	6.45 $\pm$ 1.86	0.5
Oleic acid	47.72 $\pm$ 4.48	45.01 $\pm$ 4.08	0.03
Linoleic acid	14.27 $\pm$ 3.28	16.69 $\pm$ 2.69	0.01
Linolenic acid	0.43 $\pm$ 0.22	0.35 $\pm$ 0.20	0.3
Arachidonic acid	0.19 $\pm$ 0.10	0.22 $\pm$ 0.10	0.5
EPA	0.01 $\pm$ 0.07	0.01 $\pm$ 0.02	0.8
DHA	0.07 $\pm$ 0.07	0.04 $\pm$ 0.04	0.09

\* $\times 10\ 000$  cells/mg tissue. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

**Table 2** Food frequency intake according to olive oil. Data are presented as % (95% CI). Group 1, children who consumed only olive oil in salads and cooked foods; Group 2, children who consumed a mixture or other types of oil in salads and cooked foods (usually sunflower oil).

Foodstuff	Serving	Group 1	Group 2	P (age adjusted)
Cereals	≥ 2 s/day	69.7 (58.6–80.8)	73.1 (55.9–90.2)	0.7
Pastries	> 1 s/day	51.5 (39.4–63.6)	57.7 (38.6–76.8)	0.5
Sweets	> 1 s/day	22.7 (12.6–32.8)	19.2 (4.0–34.4)	0.7
Dairy products	> 3 s/day	80.3 (70.6–89.9)	76.9 (60.6–83.2)	0.9
Eggs	> 2 s/week	63.6 (52.0–75.3)	65.4 (47.1–83.8)	0.7
Potatoes	> 1 s/day	25.8 (15.2–36.4)	26.9 (9.8–44.0)	0.8
Vegetables	> 1 s/day	56.1 (44.1–68.1)	57.7 (38.6–76.8)	0.6
Fresh fruit	> 1 s/day	59.1 (47.2–71.0)	53.8 (34.6–73.0)	0.9
Juices	> 2 s/day	48.5 (36.4–60.6)	61.5 (42.7–80.3)	0.4
Legumes	> 3 s/week	31.8 (20.5–43.1)	23.1 (6.8–39.4)	0.5
Meat	> 1 s/day	37.9 (26.2–44.7)	65.4 (47.1–83.8)	0.02
Fish	> 1 s/day	89.4 (82.0–96.9)	69.2 (51.4–87.0)	0.04
Butter	> 1 s/month	24.2 (38.1–34.6)	30.8 (13.0–48.6)	0.6
Margarine	> 1 s/month	47.0 (34.9–59.1)	69.2 (51.4–87.0)	0.1

Dietary habits differed between the two groups; the children who consumed only olive oil were more likely to consume fish and less likely to eat meat. No significant differences were seen according to the type of oil used for cooking in the other foods evaluated (Table 2).

The age correlated directly and significantly with the adipocyte volume ( $r=0.55$ ;  $P<0.001$ ).

The likelihood that at the end of 1 year a child would have increased the BMI Z-score above the baseline Z-score was lower in the children who consumed only olive oil (OR=0.22; 95% confidence interval (CI): 0.08–0.63;  $P=0.005$ ). The strength of association remained after adjusting the model for age, BMI at the start of the study or physical activity estimated by the number of hours the child remained seated daily (Table 3, model 1). The introduction into the model of adipocyte volume contributed significantly to the explanation of the evolution of the BMI Z-score (Table 3, model 2). The contribution of the type of oil consumed to the risk of increasing the BMI Z-score was not affected by the incorporation into the analysis of both variables simultaneously (baseline BMI and adipocyte volume). The introduction into the model of the different food groups whose consumption was associated with the intake of olive oil did not significantly change the strength of the association between the follow-up BMI Z-score and the intake of olive oil (data not shown).

## Discussion

The main finding in this study was that children whose diet contained olive oil as the sole source of cooking oil had a lower likelihood of increasing their BMI Z-score over 1 year than children who consumed sunflower oil or a mixture of olive and sunflower oils, and this result

is not affected when physical activity is introduced in the model.

Previous studies determined that the triglyceride composition of adipose tissue varies depending on the diet (12). In our study, the children who consumed only olive oil had higher levels of oleic acid and lower levels of linoleic acid in the triglycerides than the other children. These two fatty acids are of particular interest as linoleic acid is an essential dietary fatty acid that is not synthesised by the organism and oleic acid is the fatty acid mainly present in olive oil, consumption of which defines the Mediterranean diet.

The role of dietary fats in the genesis of obesity is not fully understood. Some studies have found a weak positive association between intake of animal fats and

**Table 3** Predictors of body mass index (BMI) Z-score variation in the follow-up. The dependent variable is the variation of BMI Z-score in two categories: <0 (BMI Z-score decreased) versus >0 (BMI Z-score increased). Logistic regression models: model 1 is adjusted by age, time seated and BMI; model 2 is adjusted by age, time seated and adipocyte volume. Group 1, children who consumed only olive oil in salads and cooked foods; Group 2, children who consumed a mixture or other types of oil in salads and cooked foods (usually sunflower oil).

	OR	95% CI		P
		Lower	Upper	
<b>Model 1</b>				
Type of oil consumed*	0.19	0.06	0.61	0.005
Age (months)	1.001	0.985	1.018	0.9
Time seated (>4 vs <4 h/day)	0.28	0.06	1.36	0.1
BMI (kg/m <sup>2</sup> )	1.37	1.03	1.83	0.03
<b>Model 2</b>				
Type of oil consumed*	0.15	0.04	0.52	0.003
Age (months)	0.99	0.98	1.01	0.4
Time seated (>4 vs <4 h/day)	0.43	0.08	2.20	0.3
Adipocyte volume (pl)	1.006	1.002	1.011	0.006

\*Group 1 vs Group 2.

weight gain (13). Most studies have examined the role of saturated and *n*-6 and *n*-3 poly unsaturated fatty acid (PUFA) in obesity development, with the role of mono unsaturated fatty acid (MUFA) in body weight being less well known (14).

MUFAs are able to intervene actively in the regulation of body weight, as demonstrated by several studies suggesting that MUFAs act on the regulation of appetite (15), on the intestinal absorption of fat (16), on the lipolytic activity of the adipocyte (17) and on thermogenesis (18), among other functions. These experimental observations are beginning to be contrasted in epidemiological studies (7, 14) and support the results found in this study. In diet, oleic acid replaces linoleic acid when children take olive oil. This increase in MUFA may inhibit lipogenesis either directly by increasing the lipolysis or other effects (19) or by decreasing the availability of *n*-6 fatty acids, which have a proved adipogenic ability (3). On the other hand, MUFAs appear to be powerful stimulators of GLP1 secretion in animal models and in patients that had taken an olive oil-enriched meal, getting better the insulin sensibility (20). Furthermore, olive oil maintains its healthy properties, even after cooked due to its thermal stability and safety, being able to reduce the glycaemic load of a meal decreasing carbohydrate absorption rate, insulin secretion and lipogenesis (21). Taken together, these considerations point to the MUFAs as inhibitors or regulators of adipose tissue growth.

In a previous study, we showed that changes in adipocyte size during the first 2 years of life correlate with the concentration of linoleic acid in the adipose tissue (22). Fatty acids can affect cell multiplication in adipose tissue and the number of fat cells could begin to increase when the cells reach a certain size.

In rats, a diet in which the ratio of linoleic acid to linolenic acid was 59/1 had a greater adipogenic effect than a diet in which their ratio was 2/1 (23). *In vitro* studies have shown that linoleic acid and/or arachidonic acid favour prostacyclin synthesis and release in preadipocytes, and this could be the mechanism of their adipogenic effect. Oleic and linoleic acid compete between each other and those persons with a greater intake of MUFA have a lower intake of *n*-6 PUFA (24), an effect that was also seen in the fatty acid composition of the adipose tissue triglycerides.

This study has several strengths and limitations. The strong points include its prospective nature, confirmation of the precision of the nutritional survey by measuring the adipose tissue fatty acids, the simultaneous measurement of adipocyte volume and the number of preadipocytes, and the coherence of the results with those recently reported in an adult population from the same geographical area (7). On the other hand, although some of the confounding variables, such as physical activity or the intake of other foods, were controlled for, other confounding variables may be present, such as the cultural level of the parents,

not included in this study. Quantitative nutritional data are not available. However, we have data about the fatty acid composition of adipose tissue that reflects the predominant oil in the diet, and, as far as we know, this composition is not modified by the energy intake. Besides, in a study about adults from the same geographic area, the persons who consumed mainly sunflower oil had a similar intake of energy and macronutrients than those who consumed olive oil but a higher weight (5).

Only subcutaneous adipose tissue was available; therefore, we cannot rule out that changes in BMI Z-score are related to the properties of visceral adipose tissue too. Although visceral adipose tissue is metabolically more active and is more related to metabolic diseases in adulthood (25), high intake of food causes hypertrophy of the subcutaneous tissue, which may be the source of metabolic impairment of other tissues, including visceral adipose tissue (26).

Our results support the hypothesis proposed by Ailhaud *et al.* (3) that the increase in the prevalence of childhood obesity may partly be explained by the increased consumption of fat rich in *n*-6 PUFA (sunflower oil for example), which would favour the continuous development of adipose tissue during childhood. The conclusion is that enriching the diet with *n*-9 MUFA could have the opposite effect, thus reducing the risk of increasing fat mass and consequently obesity.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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