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Relationship of Docosahexaenoic Acid Supplementation and Insuline Resistance in Obese Children

Scientific Area: MED/38

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ABSTRACT

BACKGROUND: obesity may be associated with a later onset of chronic disorders and clinical complications. Insulin resistance, glucose intolerance and hyperinsulinemia are major components of the metabolic syndrome, which is highly prevalent among children and adolescents with severe obesity. Low levels of LCPUFAs, especially docosahexaenoic acid (C22:6 n-3, [DHA]) and a high n-6/n-3 LCPUFA ratio in skeletal muscle membrane phospholipids have been associated with insulin resistance in adults. Recent data suggest that the synthesis of DHA differs between obese children and normal weight children. In particular, in obese children, the highest quartile of BMI z-score was associated with higher plasma levels of the n-6/n-3 LCPUFA ratio. Given the high prevalence of insulin resistance in childhood obesity, we asked whether supplementation with DHA would be more effective than diet and physical activity alone in reducing this metabolic alteration.

AIM: to determine whether DHA supplementation, in addition to adequate diet and lifestyle, may reduce insulin resistance compared with diet and physical activity only, in obese children. Secondary aims were to evaluate whether may exist an association of the change of steatosis degree after the intervention with DHA supplementation..

SUBJECTS AND METHODS: this is a multicenter, longitudinal, double-blind, randomized, placebo-controlled trial that started on January 01, 2010. Up to September 30, 2012, thirty (14 boys, 16 girls, mean [SD] age, 11.4 [1.29] years, range 8-13), were recruited. All obese children consecutively admitted to the Department of Paediatrics, San Paolo Hospital, Milan, and to the Department of Paediatrics, Federico II Hospital, Naples, for routine examinations were assessed for eligibility. The study protocol scheduled daily oral supplementation of either an intervention “product”, that is two capsule of purified DHA (500 mg) or two capsule of wheat germ oil (500 mg). A nutritional-behavioural intervention was additionally recommended in all recruited children promoting a normocaloric balanced diet and an active lifestyle based on the Italian guidelines for treatment of childhood obesity. Additional recommendations were given to engage in a moderate daily exercise program (30-45 minutes/day aerobic physical exercise), tailored to individual preferences. Children were visited at the care centers within 3±1 days (baseline) after enrolment, and at 6 months after starting intervention. Evaluations included anthropometrical measurements, nutritional, metabolic assessment and liver ultrasonography.

Children were randomly assigned to the intervention or control group based on a computer generated, blocked randomization list by each center. A block size of four was used, stratified according to gender. The investigator who generated the randomization sequence was independent of the research staff and unaware of children.

RESULTS: at baseline there was no significant difference between groups for any anthropometrical (minimum $P \leq 0.806$) or dietary (minimum $P \leq 0.318$) or biochemical (minimum $P \leq 0.539$) variable.

No significant difference among groups occurred for daily intake of energy or any macronutrient and the end of the study (minimum P=0.111).

At the end of the intervention a significant reduction of plasma fasting glucose (DHA group $P=0.046$; placebo group $P=0.048$), insulin (DHA group $P=0.001$; placebo group $P=0.048$) and HOMA (DHA group $P=0.001$; placebo group $P=0.050$) in both groups was observed. A higher percentage variation of plasma fasting insulin ($P=0.0046$) and HOMA ($P=0.0045$) in DHA than placebo group was showed.

There was a difference between groups for percentage reduction of liver steatosis: in DHA group from 14 to 7 % ($P=0.655$), in placebo group from 20 to 13 % ($P=0.275$).

0. PRELIMINARY REMARKS AND EPIDEMIOLOGY

In the last two decades overweight and obesity prevalence showed an exponential increase throughout the world, in the population in general and specifically at paediatric age. For this reason it is now defined as one of the most important health and medical issues of our times. The neologism "*globesity*" was created to indicate the worldwide diffusion of this phenomenon (Ebbeling CB et al., 2002).

In many European countries, in the last 30 years obesity levels trebled: 25 - 79% of adults are considered overweight and 5 – 30% obese, depending on the specific country of origin. Youth obesity specifically is currently ten times higher than in the 1970s (Skelton JA et al., 2009): 20% of European children are overweight with peaks of 33-34% for the age between 6 and 9, a third of these children are obese (the World Health Organization [WHO], 2012).

In Italy the 2009 ISTAT survey "Aspetti della vita Quotidiana" (Aspects of everyday life) showed that during the period 2001-2009 overweight and obesity diffusion had an increase. Specifically, there was a shift from the 33.9% of 2001 to the 36.1% in 2009 of people overweight, and from the 8.5% of 2001 to the 10.3% of 2009 of people considered obese. As far as childhood obesity in Italy is concerned, an epidemiological survey carried out by the Ministry of Health in 2008 and in 2010 to promote health and healthy development for primary school children "Okkio alla Salute" (Okkio to health) which involved over 42.000 children from the third class of primary schools (age 8-9), showed that overweight and obesity prevalence is of 22.9% for overweight and of 11.1% for obesity, thus confirming the European data (Istituto Superiore di Sanità- Rapporti ISTISAN: 09/24- Roma 2009). Furthermore the survey allowed verifying an important geographical variability, with overweight-obesity percentages which lower in the Northern regions in comparison to those of the South. The trend proved to be the same for children and adults alike (Figures 1 and 2).

FIGURE 1. Overweight-obesity percentages of children from the third class of primary schools (age 8-9) in the Northern and South regions of Italy (2008-

2009). Source: “Okkio alla Salute”, 2008-2009 (Ministry of Health, Rome, Italy)

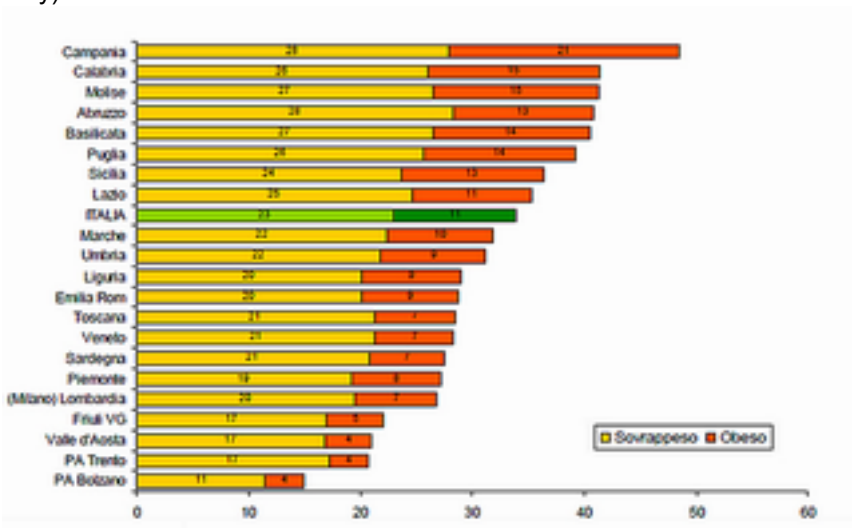
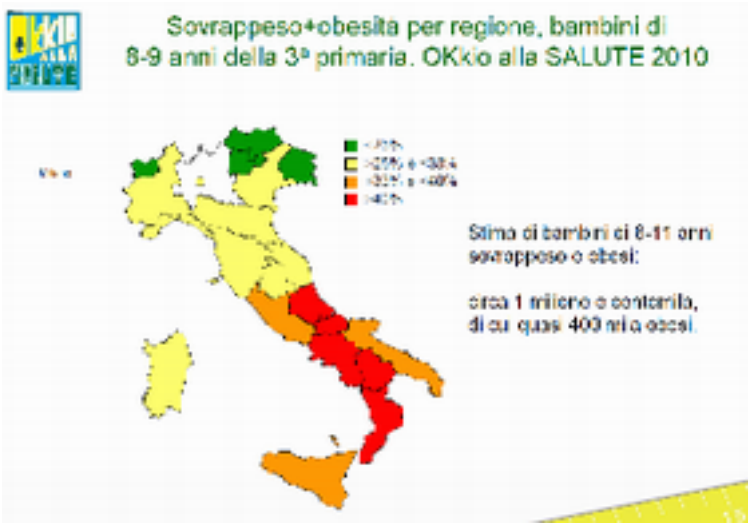


FIGURE 2. Overweight-obesity percentages of children from the third class of primary schools (age 8-9) in the Northern and South regions of Italy (2010). Source: “Okkio alla Salute”, 2010 (Ministry of Health, Rome, Italy)



The interest concerning the pathology, besides the prevalence issue, is connected to the renowned link between obesity and other important risk

factors for cardiovascular pathologies such as arterial hypertension (AHT), glucose intolerance or insulin-resistance and dyslipidemia. Indeed the latter disorders constitute the clinical picture defined metabolic syndrome which is the clinical picture recognized as being the main mortality cause in industrialized countries (Lau DC et al., 2007; Park MH et al., 2012).
For paediatric age too, scientific literature highlights the risks for future pathologies, the negative effects on psychological and relational development. Finally it was also pointed out how the presence of overweight and obesity, of an adiposity rebound at around the age of five and an obese parent represent risk factors for a persistent obesity in the future (Kuhl ES et al., 2012).
Nowadays, obesity is still one of the most complex and lesser understood diseases, so frequent yet so refractory to treatment (Ebbeling CB et al., 2002); indeed there are no effective medical-surgical therapies to treat obesity at paediatric age and weight-reducing diets and pharmacotherapy used for adults are greatly debated.
The limited successes of therapies and the severe complications connected to excess weight and obesity require a pre-emptive intervention on the population. Therefore prevention strategies and medical education to reduce nutritional excesses, to improve food-related habits and to foster a more adequate life style, for children as well as for whole families, gain primary importance.
These interventions prove to be more worthwhile when carried out at paediatric age, thus justifying the commitment to create prevention programs concerning over weight and obesity since infancy (Drake KM et al., 2012).

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1. DEFINITION

Obesity is a clinical condition characterized by an excess of body fat, caused by a disorder of energy metabolism, which is marked by an imbalance between the intake and the expenditure of energy, with an accumulation of excess calories as triglycerides in the adypocites. Such imbalance can derive from an excessive caloric intake, from reduced energy expenditure, or from an alteration of the mechanisms regulating energy balance. The tool currently in use to define the condition of overweight and of obesity, for the child as well as for the adult, is the Body Mass Index (BMI) (Krebs NF & Jacobson MS, 2003) which is calculated dividing the weight (kg) by the squared height (m^2). For adulthood there are thresholds values, within which the levels of overweight and of obesity are defined, as shown in table 1. It is not possible to apply the same threshold values for childhood due to the continuous variation of height during growth (Ludwig DS, 2012).

To diagnose over weight and obesity at paediatric age the tools available are:

Subjects up to 24 months:

BMI according to the WHO curve, especially if breast fed: overweight can be identified for BMI values over 2 SD (standard deviation), also including obesity (BMI > +3DS) (Figures 3 and 4).

FIGURE 3. BMI according to the WHO curve for the girls up to 24 months

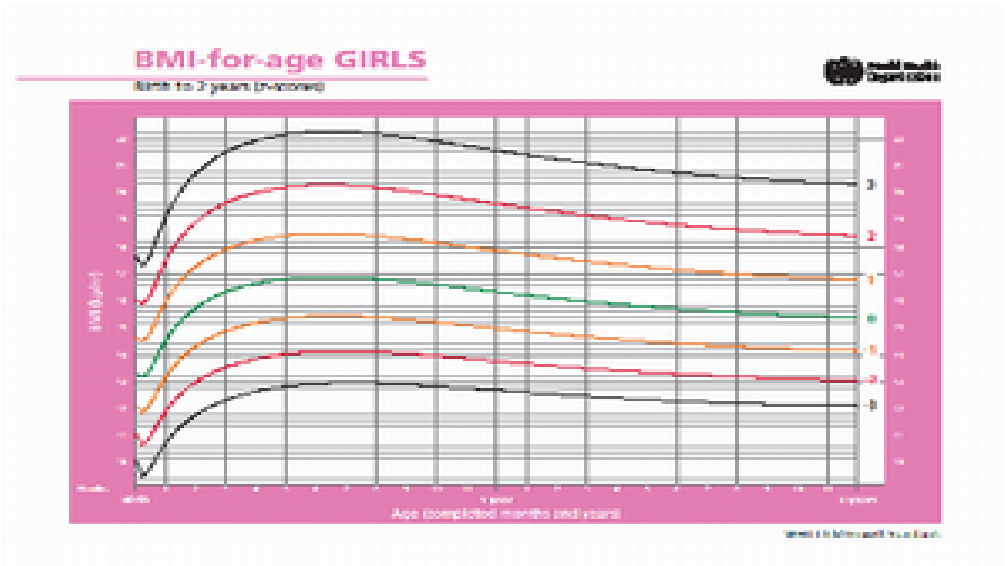
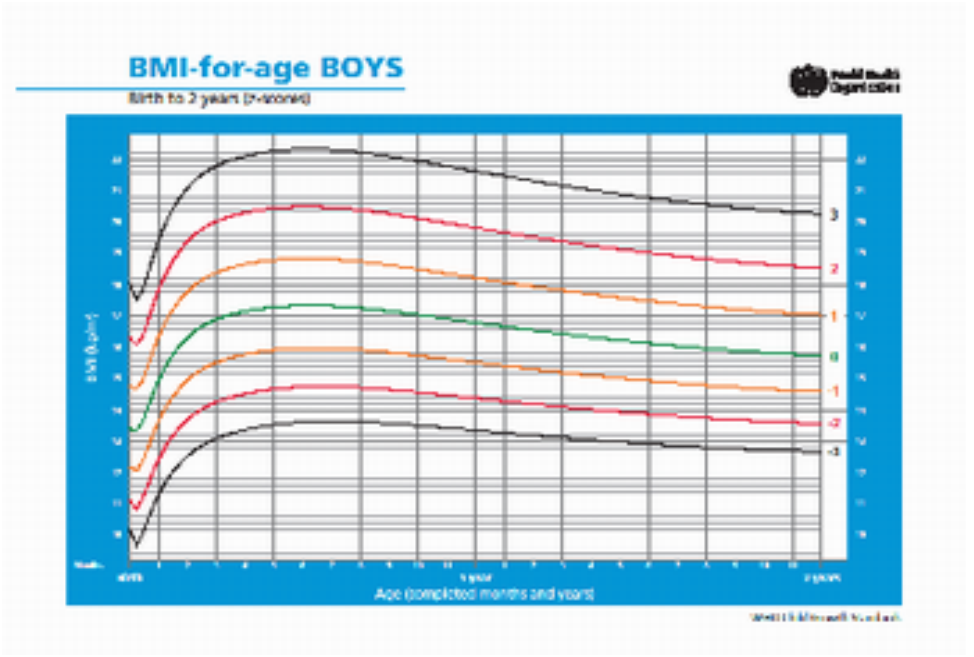


FIGURE 4. BMI according to the WHO curve for the boys up to 24 months



Additional information can be retrieved by referring to the weight/length relationship according to the reference table published in 2000 by the Center for Disease Control and prevention (CDC) in Atlanta (Center for Disease Control and Prevention [CDC], 2000) (Ogden CL et al., 2002). Cut-off value: 85th percentile for overweight and 95th percentile for obesity (Figures 5 and 6).

FIGURE 5. Weight/length curve, girls, 2000 CDC

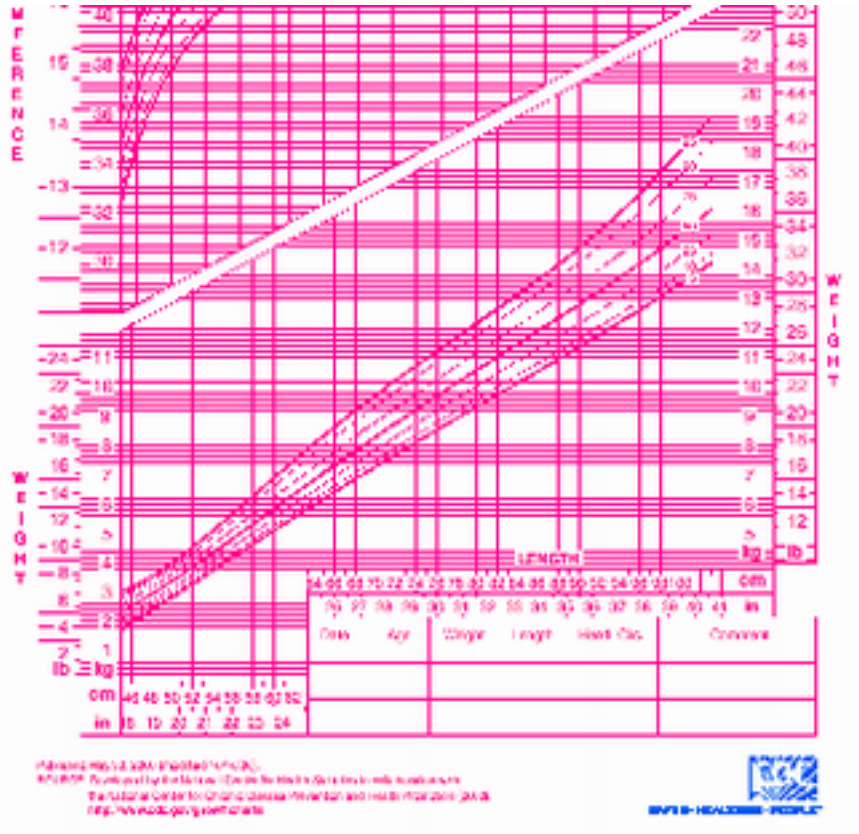
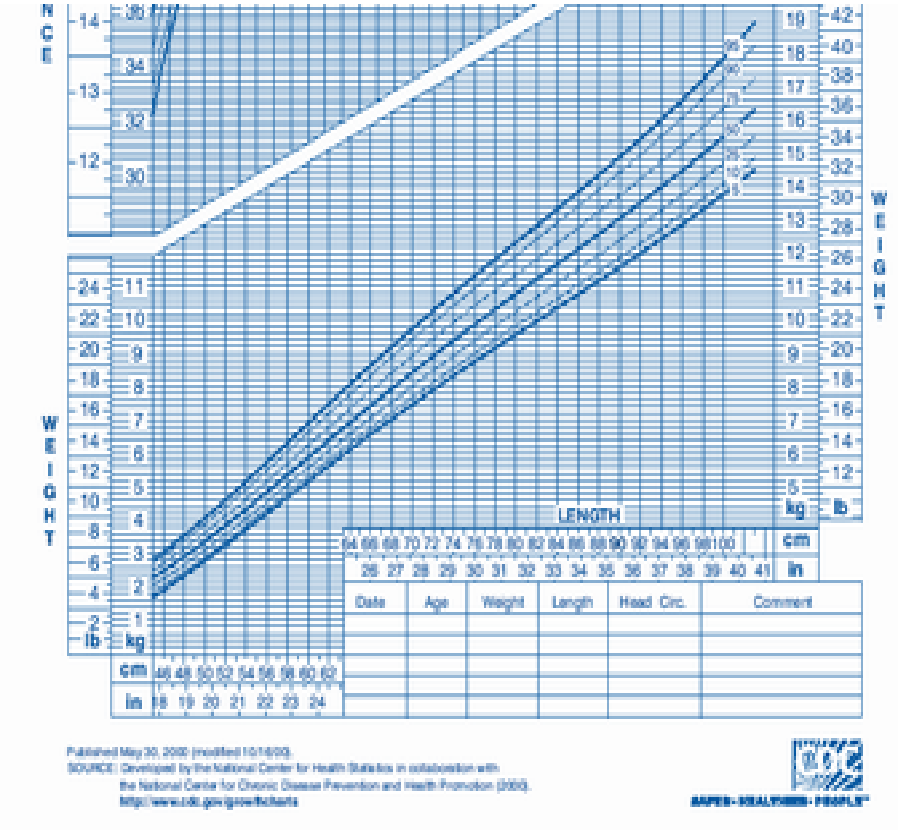


FIGURE 6. Weight/length curve, boys, 2000 CDC



Subjects older than 24 months:

BMI according to Cole: BMI values for different ages are related 25-29 values and equal or above 30 for adults, which are the known limits for overweight and obesity respectively. The charts were devised by processing the anthropometric data collected from the studies carried out in six different nations (Table 1).

TABLE 1. BMI values for different ages in according to Cole

	Overweight B.M.I. 25 kg/m^2		Obesity B.M.I. 30 kg/m^2	
Ages	**Boys**	**Girls**	**Boys**	**Girls**
2	18.41	18.02	20.09	19.81
2.5	18.13	17.76	19.80	19.55
3	17.89	17.56	19.57	19.36
3.5	17.69	17.40	19.39	19.23
4	17.55	17.28	19.29	19.15
4.5	17.47	17.19	19.26	19.12
5	17.42	17.15	19.30	19.17
5.5	17.45	17.20	19.47	19.34
6	17.55	17.34	19.78	19.65
6.5	17.71	17.53	20.23	20.08
7	17.92	17.75	20.63	20.51
7.5	18.16	18.03	21.09	21.01
8	18.44	18.35	21.60	21.57
8.5	18.76	18.69	22.17	22.18
9	19.10	19.07	22.77	22.81
9.5	19.46	19.45	23.39	23.46
10	19.84	19.86	24.00	24.11
10.5	20.20	20.29	24.57	24.77
11	20.55	20.74	25.10	25.42
11.5	20.89	21.20	25.58	26.05
12	21.22	21.68	26.02	26.67
12.5	21.56	22.14	26.43	27.24
13	21.91	22.58	26.84	27.76
13.5	22.27	22.98	27.25	28.20
14	22.62	23.34	27.63	28.57
14.5	22.96	23.66	27.98	28.87
15	23.29	23.94	28.30	29.11
15.5	23.60	24.17	28.60	29.29
16	23.90	24.37	28.88	29.43
16.5	24.19	24.54	29.14	29.56
17	24.46	24.70	29.41	29.69
17.5	24.73	24.85	29.70	29.84

The International Obesity Task Force (IOTF) recommends using the BMI charts devised by T. Cole. These charts express BMI variations according to age up to a BMI value equal 25 to 29.9 kg/m^2 or ≥30 kg/m^2 at the age of 18

for overweight or obesity, respectively (Cole TJ & Lobstein T, 2012; Couper JJ et al., 2009) (Figures 7 and 8).

FIGURE 7. BMI according to the Cole curve, girls

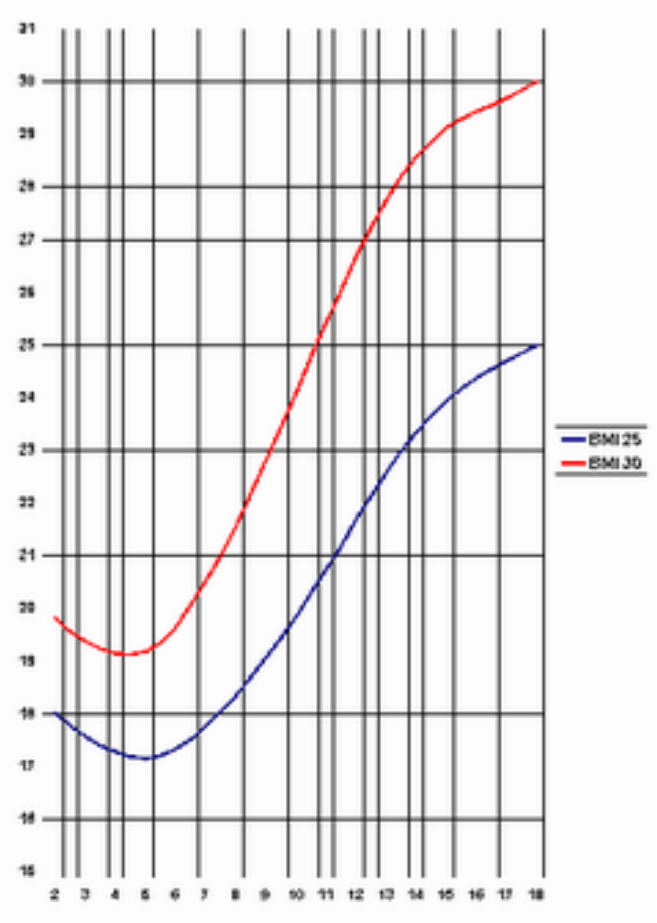
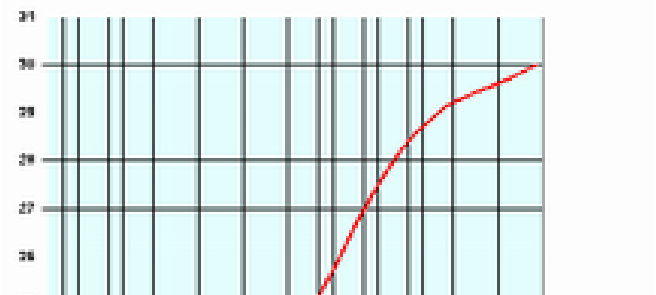


FIGURE 8. BMI according to the Cole curve, boys



The calculation of the BMI value is recommended at every clinical check-up. Moreover in case of overweight and of obesity, to better quantify fat mass, body composition can be estimated with anthropometric methods (by measuring the arm, the waist, the hips, the skinfolds of the triceps, the bicep, and the subscapular and suprailiac areas) or more precise method devices such as DEXA's. The triceps skinfold in particular, can be used to diagnose the excess of weight according to the reference chart by Barlow & Diets (Barlow SE & Dietz WH, 1998). Cut-Off value: between 85th and 95th percentile for overweight and 95th percentile for obesity.

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2. ETHIOPATOGENESIS

Weight excess appears when the intake of calories is higher than the daily expenditure of energy. The cause for excess body fat is thus connected to an excess of food in comparison to energy expenditure. The human body is made by a group of tissues whose main components are: water, lean body mass represented by muscular mass and adipose tissue, with proportions that vary according to age. Each individual's body composition is closely related to daily energy expenditure and it is also influenced by diet. The quality of energy that we use every day is divided in 3 components: Basal Metabolic Rate (BMR), thermogenesis and physical activity. The latter component of energy expenditure can be modified by personal will, as opposed to the former factors which are genetically predetermined.

The Basal Metabolic Rate represents the highest share of daily energy expenditure and besides being influenced by age and sex it is also determined by lean body mass (thus by muscles) whilst water and adipose tissue basically live "*without energy-expenditure*".

An active life style and reducing sedentary occasions are the most effective strategies to maintain a correct balance between weight and height. Daily physical activity of the aerobic medium kind, allows the development of muscular mass which determines high daily energy expenditure. The more muscular mass the higher will be the body consumption of energy. This proves the importance of adequate physical activity; indeed a few minutes (45-60 minutes) of physical activity per day are enough.

Essential obesity is a chronic disease with a multi-factorial origin whose main genetic and environmental components (nutrition, physical activity) can mutually influence each other with different modalities, mechanisms and percentages.

2.1 Environmental factors related to obesity risks

Many socio-economic and environmental factor both inside and outside the family contribute to food intake and to a sedentary life style. Due to a genetic predisposition, such factors were able to trigger the progressive increase of average adiposity of child population (Dorosty AR et al., 2000). The relative risk for an obese child to grow up to be an obese adult increases with age and it is directly proportioned to the overweight. Obese children at pre-school age have the 26-41% risk of developing obesity during adulthood; for school age children the risk is from 42 to 63%; overall the risk for obese children to be obese as adults ranges between 2 to 6.5 times in comparison to children who are not obese. The risk percentage rises to 70% for obese teenagers. Having one or both parents obese is the most important risk factor for the manifestation of obesity in a child. Further environmental risks involved in the

development of obesity include:

- geographical region (south>north);
- density of population (city>countryside);
- socio-economic level which is inversely proportional to obesity prevalence. The level of education of the family, the type of working activity of the parents and the economic incomes of the family are somehow associated to obesity in about 60% of cases;
- nutritional condition during pregnancy: foetus malnutrition especially in the first two trimesters seems to be associated with the subsequent development of obesity and with an increased risk of hypertension and of type 2 diabetes, independently from familiarity or heredity. The most widely accepted hypothesis states that the condition of malnutrition provokes metabolic and physiological responses in foetuses which are meant to the help the child survive in future situations. However if in the following stages of life, food becomes more abundant, the same adaptation responses can lead to develop hypertension, cardiovascular pathologies and glucose intolerance (risk factor for diabetes). If the condition of malnutrition affecting the foetus occurs in the third trimester or immediately after birth, obesity and related risk percentages will be much lower;
- family life standards and food-related habits: these factors heavily influence the nutritional habits of the child (Wardle J & Cooke L, 2008); sedentary parents who have a high calories intake diet will more probably have lazy and obese children. Within the family nucleus mothers' wet-nurse behaviour responding to any manifestation of the child by offering food, is considered to be the reason why even in the years to follow, the child might use food as a way to find comfort (Maffeis C et al., 1998);
- loneliness of children who spend many hours at home in front of the TV, videogames and the computer with a subsequent increase of sedentary activities and a reduction of energy expenditure (Collison KS et al., 2010);
- toxic nutritional environment: easy access to high-energy-dense food, at low cost, heavily advertised and with a good taste (Veldhuis L et al., 2012).

2.2 Anthropometric factors related to obesity risks

- *Different ponderal increase for breastfed and infant formula fed children*

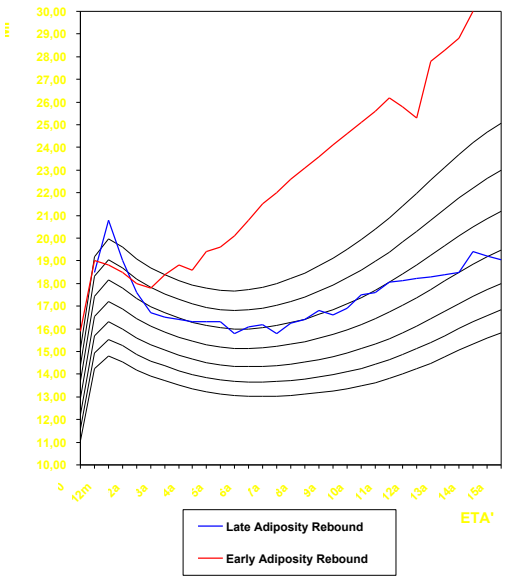
The first three-four months of life have been defined as the critical moment, indeed a quick and precocious growth seems to expose to an increased risk of obesity in the following stages of life. This has led to think that the permanent structuring of the individual's physiology occurs during the first weeks of life and that in this phase lay the foundations of future chronic pathologies. Therefore acting on growth during the first year of life could be a key strategy to prevent obesity and metabolic syndrome in adulthood.
Nutrition plays a fundamental role in controlling the speed of growth during the earliest stages of life. Many studies have associated infant formula feeding with a more rapid growth. At the same time, breastfeeding was defined a protective factor against obesity and over weight in adulthood (Dewey KG, 2003). The anthropometric indexes collected confirm that there is a different growth pattern between breast-fed and formula-fed infants. More specifically, the breast-fed child grows more rapidly during the first 2-3 months of life, than between 6 and 12 months he or she will have a lower weight and length than formula-fed children (De Onis M et al., 2009). The higher ponderal growth level of formula-fed children as opposed to breast-fed children could be explained by the different intake of metabolizing substrates, specifically of proteins (Koletzko B et al., 2009): in formula-fed infants the protein intake for weight unit is 55-80% higher in comparison to breast-fed children. Indeed it is important to remember that during breastfeeding the concentration of proteins in breast milk is progressively reduced from 1.6 g/dl to 0.8-0.9 g/dl providing during the first year of life 7-8% of total daily energy. Instead artificial formulas provide between 1.2 to 1.9 g/dl of proteins. Moreover nutritional investigations prove that, at weaning or when cow milk is introduced in the infant's diet, the protein intake increases up to 3-5g/Kg, even though the protein requirement tends to decrease as months go by, (1.887 g/Kg at 12 months). Recently, two systematic reviews restored the connection between rapid ponderal growth levels in the first years of life and the subsequent development of obesity (Baird J et al., 2005). Rapid ponderal increase would then be an early anthropometric marker of obesity development during adolescence and adulthood, and thus of the related metabolic consequences (type 2 diabetes), with an even greater forecasting quality than weight at birth (Socha P et al., 2011; Li R et al., 2012).

- *Adiposity rebound*

Another widely studied aspect, connected with the development of obesity during childhood is adiposity rebound (Dorosty AR et al., 2000; Reilly JJ et al., 2005). By observing the BMI trend in the general paediatric age

population, one should notice that in the first year of life there is a very rapid increase of BMI reflecting the remarkable deposition of the fat stored during the first months of life. After the age of one, BMI values diminish to then stabilize and increase again generally only after the age of 5-6. The age when the body reaches the minimum value of BMI before the physiological increase is called adiposity rebound and generally it corresponds to the age 5-6. An increase of BMI values before the age of 5 (early adiposity rebound) is recognized as a marker of precocious risk of developing obesity (Figure 9). Therefore the anamnesis (of the pregnancy, the family, the nutrition and the life style) and the evaluation of statural-ponderal growth and of BMI are the means that allow to identify children more at risk of developing obesity before that the actual disorder begins (Dorosty AR et al., 2000; Scaglioni S et al 2000; Rolland-Cachera MF et al, 2006).

FIGURE 9. Adiposity rebound



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3. APPROACH TO THE OBESE CHILD AND CLINICAL EVALUATUION

To be able to intervene with an efficient system and to devise the most adequate nutritional education program, the Paediatrician needs, starting from the first meeting, to gain as much information as possible on the child, on the family and on all elements that may have an influence. Furthermore, devising an adequate anamnesis and an objective exam is a way to gain the patient's trust and willingness to undertake a new life style. Indeed if the latter were not to be understood and recognized in its importance it may be perceived as an imposition from the parents and therefore rejected.

In 2006 the Italian Society of Paediatricians published a consensus of prevention, diagnosis and therapy of essential obesity for the child and for the teenager, to be used as reference when dealing with an obese child (Consensus Società Italiana di Nutrizione Pediatrica SINUPE, 2001; Società Italiana di Pediatria, 2006).

3.1 Anamnesis

In addition to a general complete and detailed anamnesis it is necessary to collect information to verify the risk, within the family, for chronic-degenerative diseases and obesity, environmental and family factors exposing to obesity, possible symptoms that may turn into a non essential obesity, nutritional factors and life style.

- Family anamnesis
  - Weight and height (BMI) of parents and siblings;
  - Familiarity condition concerning chronic-degenerative diseases in relatives with first and second degree of kinship
  - Ponderal increase during pregnancy;
  - Family attitude towards weight:
  - Any possible alterations concerning the relationship with food within the family;
  - Family awareness and expectations concerning body weight.
- Physiological anamnesis
  - Weight at birth;
  - Feeding modality during infancy;
  - Statural and ponderal growth and BMI curve;
  - Psychomotor development and school performance;
  - Presence of first pubertal signs;
  - Age of menarche and features of menstrual periods.
- Pathologic anamnesis
  - Period when overweight appeared and ponderal increase/year;

- Any previous attempts to reduce weight and patient's expectations concerning ponderal decrease;
- Possible presence of food-related behaviour disorders (EDNOS).

• Nutritional Anamnesis

Collecting precise information on eating habits entails a number of difficulties: especially in obese subjects it is very difficult to assess the exact calories intake, because either consciously or not there is a general underrating of food intake and the consumption of food and drinks tends not to be precisely communicated. It was proved that nutritional anamnesis, whatever the instrument used, may represent an educational way that allows the patient and the family to focus the attention on their habits. Furthermore during follow-ups it will allow monitoring whether variations have actually taken place and thus assess the efficacy of the intervention that will be devised. For this purpose Paediatricians can use: the Food Frequency Questionnaire (FFQ), the diet journal of 3 or 7 days or the dietary history.

- Life habits
  - Weekly hours of physical activity and sport;
  - Interests;
  - Hours of sedentary activities (TV, computer, music, studying); it has been estimated that 15% of children at pre-schooling age spend more than 5 hours per day in front of the TV (Kuhl ES et al., 2012).
- Further information
  - Relationship with family members;
  - Relationship with school friends;
  - Reasons to request medical advice;
  - Beliefs concerning the topic of obesity;
  - Self-evaluation of excess weight;
  - Patient's and family expectations in terms of weight loss;
  - Patient and family willingness to change food and life habits.

3.2 Clinical evaluation

The clinical exam of an overweight child or teenager, in the majority of cases allows to diagnose an essential obesity (or a primitive one) or at least to suspect an endocrinal or genetic cause.

The child affected by essential obesity has very precise features:

- Normal psychomotor development
- Obesity developed gradually, with highest incidence for school age and adolescence;
- The distribution of fat is even on the face, the trunk and on limbs;

- Average or above average height, with an even growing curve;
- If analyzed, bones maturation will result accelerated and corresponding to the statural age;
- Pubertal maturation is generally accelerated;
- Pseudo-hypogenitalism: the genitals of the obese child usually appear to have a reduced size due to the ample presence of fat which hides the genitalia, however an accurate exam will reveal that they have a normal size;
- Pseudo-gynecomastia: a condition which usually worries young patients as well as their families, when actually is only excessive fat;
- Possible furunculosis and intertrigo in skinfolds as well as stretch marks on the skin of the abdomen, hips, sides, thighs, buttocks, breasts and at times on the arms and legs. Marks are usually whitish or red, mostly caused by mechanical-type factors;
- In case of severe obesity there is a chance of finding acanthosis nigricans, which is a lesion marked out by hyper-pigmented areas of skin, with a velvety, verrucose surface localized at the bottom of the neck, in the armpit, in the groin and in the folding areas of the limbs. This is an important sign of an altered carbohydrates and insulin metabolism (Figures 10 and 11).

FIGURE 10. Acanthosis nigricans

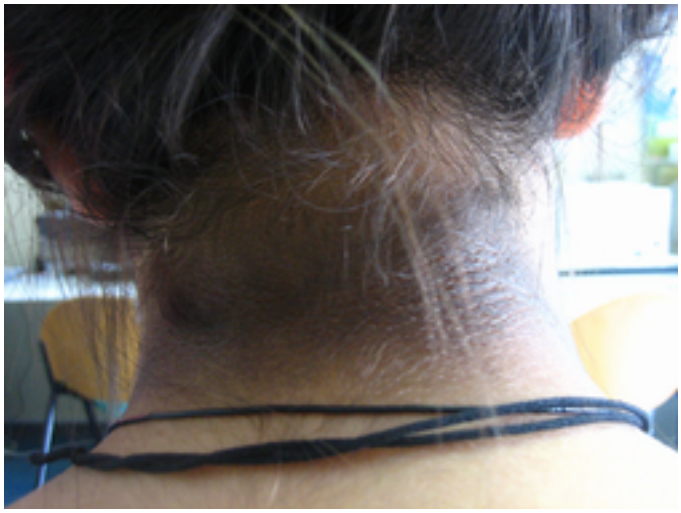
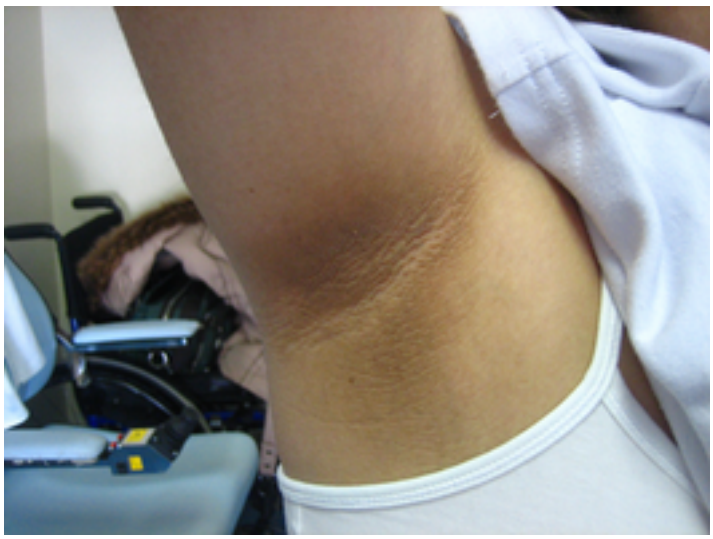


FIGURE 11. Acanthosis nigricans



- Genu valgum and flat feet are also frequently present in the obese child. These are caused by the stress on the joints by the high ponderal load. Pain in the hips and in the lower limbs are also frequent and they are caused by a coxa vara or by a slipped capital femoral epiphysis;
- Normal performance at school;
- Absence of stigmata malformation;

The full objective general examination is characterized by the collection of the following:

- Height and, when possible, the retracing of the growth curve;
- Weight and the retracing of the ponderal growth curve through the evaluation of previous weights, to assess the speed of ponderal increase;
- Estimate of the ideal weight in relation to height and to the measurement of the overweight percentage;
- measurement of BMI;
- body circumferences (arm, waist, hips). Body circumferences reveal the cross-sectional sizes of the body segments and indicate the current nutritional condition and the distribution of body fat as well as

growth.

The most paediatric relevant circumferences to assess the nutritional condition of the child are three: circumference of the waist, the hips and the arm. At paediatric age, waist circumference alone can be considered a marker of cardiovascular risk in overweight subjects. McCarthy report cut-off values (90th percentile) referring to minimum waist circumference (collected with the procedure indicated by OMS (McCarthy HD et al., 2001).

Furthermore it was recently suggested that a ratio waist circumference (cm)/height (cm) >0.5 is associated to a higher risk to develop cardiovascular pathologies, therefore during diagnosis the said ratio should be carefully assessed (Mokha JS et al., 2010).

The ratio between waist and hips circumferences (WHR, waist hip ratio) is instead an anthropometric index that can be used to evaluate the distribution of body fat. More specifically, abdominal or “android” fat distribution, indicated by WHR values higher than 1 for men and higher than 0.8 for women, is considered to expose to a higher risk.

The most appropriate techniques to study visceral fat in a paediatric context are: DEXA, bioelectrical impedance analysis, nuclear magnetic resonance (NMR) and computerized tomography. Due to costs and/or invasiveness such methods should be restricted to a limited number of patients.

- Skinfolds (triceps, bicep, subscapular, suprailiac).

  Skinfolds testing is the most widely used method to measure body fat. By measuring subcutaneous fat, with the calliper, it is possible to estimate, using appropriate equations, the overall content of body fat, assuming that subcutaneous fat represents a constant fraction of the total. Skinfolds can be measured in a number of areas of the body, however the most frequently tested ones are the biceps, the triceps, the subscapular and the suprailiac. The triceps skinfold can be used as an additional method to diagnose ponderal excess and to monitor diet therapy for obesity and for slimness. The said skinfold is closely linked to both total and percentage body fat, but it is less correlated to blood pressure. The biceps skinfold, combined with the triceps skinfold, can be useful to estimate total body fat. The subscapular skinfold is an important index of the nutritional condition and it is also useful to measure the total mass of body fat. The suprailiac skinfold is an index of body fat and its assessment is important in relation to the risk of metabolic syndromes.
- Pubertal stage;

- Size of the thyroid gland and search for possible hyperthyroidism symptoms;
- Blood pressure. In overweight subjects, who are more at risk to develop hypertensions, blood pressure should always be measured by using appropriate bracelets (the bracelet should be placed =40% arm circumference) for each child.
  The American Academy of Pediatrics (AAP) indicated hypertension reference values according to sex, age and height. To be defined as "Normal blood pressure" values should be lower than 90th percentile, "hypertension condition" if values are between 90th and 95th percentile, "arterial hypertension" if values are above 95th percentile;
- Presence of possible gynecomastia;
- Possible presence of stretch marks or acanthosis nigricans;
- Orthopaedic alterations

3.3 Laboratory and instrumental investigations

Laboratory investigations shall be pursued in relation to the level of obesity and to the familiarity with cardiovascular risk factors (diabetes mellitus, hypertension, dyslipidemia, cardiovascular diseases). Moreover, tests should try to identify in advance, possible markers of a secondary metabolic syndrome in the overweight condition of the child. Screening must include:

- Fasting blood glucose (reference value < 100 mg/dl or 5.6 mmol/l; fasting glucose intolerance: 100-125 mg/dl or 5.6-6.9 mmol/l; diabetes: ≥ 126 mg/dl or 7 mmol/l). For fasting blood glucose >100mg/dl which is often confirmed, there is a curve indicating the oral intake of glucose (0-120 minutes). Blood sugar values between 140 and 200 mg/dl after 120 minutes from glucose administration, tends to indicate a glucose intolerance, whilst a value above 200 mg/dl clearly indicates a diabetes mellitus type 2;
- Fasting insulinaemia: according to Consensus guidelines for prevention, diagnosis and therapy of obesity in the child and in the adolescent, basal insulin values above 15 (> 15 μU/ml) should be compatible with insulin resistance regardless of sex and age. However the Consensus publication on insulin-resistance at paediatric age states that basal insulin dosage alone is not sufficient. In 2009 the Italian Society of Paediatric Endocrinology and Diabetes (ISPED) set the normality cut-offs for insulin sensitivity, insulin secretion by pancreas β-cells and insulin-resistance according to sex and pubertal stages based on a population of healthy Italian children and teenagers using index such as: QUICKI Index (Quantitative

Insulin-Sensitivity Check Index), HOMA-β% (HOMA of percent of β-cell function) HOMA-IR (Homeostasis Model Assessment of Insulin-Resistance Index) (Katz A et al., 2000; D'Annunzio G et al., 2009).

$$\text{HOMA-IR} = \frac{[\text{fasting insulinaemia } (\mu U/l) \times \text{basal blood sugar } (mmol/l)]}{22.5}$$

$$\text{QUICKI} = \frac{1}{(\log_{10} \text{basal insulin in } mU/l + \log_{10} \text{blood sugar in } mg/dl)}$$

$$\text{HOMA-}\beta\% = \frac{(20 \times \text{basal insulin in } mU/l)}{(\text{blood sugar in } mmol/l - 3.5)}$$

- lipid profile: triglycerides (standard values: <95th percentile according to age and sex), total cholesterol (reference value < 180 mg/dl), HDL cholesterol (reference value > 40 mg/dl), LDL cholesterol (reference value < 130 mg/dl);
- transaminases: ALT (reference value < 40 UI/L), AST (reference value < 35) . In case hepatomegaly and/or hypertransaminasemia are detected close examinations of liver function and liver ultrasound should be carried out;

3.4 Evaluation of the risk for related cardiovascular diseases

- Obesity is a cardiovascular risk factor
- Waist circumference alone can be considered a marker for cardiovascular risks in overweight subjects.
- Hypertension: is defined: 1) “normal blood pressure” if values are below 90th percentile; 2) “hypertension condition” if values range between 90th and 95th percentile; 3) “arterial hypertension” if values are above 95th percentile.

An in-depth diagnostic examination should be carried out in overweight patients showing hypertension based on:

- Cardiologic medical examination – ECG – echocardiography
- Evaluation of the possibly related Metabolic Syndrome
- Blood creatinine and kalaemia
- Standard urine test
- Microalbuminuria

3.5 Evaluation of the risk for related endocrine-metabolic diseases

In an obese child it is important to carry out a screening for the diagnosis of metabolic Syndromes even though so far there is no shared criterion for paediatric age or for adult age (NCEP ATP III). The International Diabetes Federation (IDF) recently published the first Consensus on the definition of metabolic syndrome which divides the paediatric population in three ranges (Table 2).

TABLE 2. The IDF consensus definition of metabolic syndrome in children and Adolescents

Age group (years)	Obesity* (WC)	Triglycerides	HDL-C	Blood pressure	Glucose (mmol/L) or known T2DM

6–<10	≥90 th percentile	Metabolic syndrome cannot be diagnosed, but further measurements should be made if there is a family history of metabolic syndrome, T2DM, dyslipidemia, cardiovascular disease, hypertension and/or obesity.			
10–<16 Metabolic syndrome	≥90th percentile or adult cut-off if lower	≥1.7mmol/L (≥150 mg/dL)	<1.03mmol/L (<40 mg/dL)	Systolic ≥130/ diastolic ≥85 mm Hg	≥5.6 mmol/L (100 mg/dL) (If ≥5.6 mmol/L [or known T2DM] recommend an OGTT)
16+ Metabolic syndrome	Use existing IDF criteria for adults, i.e: Central obesity (defined as waist circumference ≥ 94cm for Europid men and ≥ 80cm for Europid women, with ethnicity specific values for other groups*) plus any two of the following four factors: • raised triglycerides: ≥ 1.7mmol/L • reduced HDL-cholesterol: <1.03mmol/L (<40 mg/dL) in boys and <1.29mmol/L (<50 mg/dL) in girls , or specific treatment for these lipid abnormalities • raised blood pressure: systolic BP ≥130 or diastolic BP ≥85mm Hg, or treatment of previously diagnosed hypertension • impaired fasting glycemia (IFG): fasting plasma glucose (FPG) ≥5.6 mmol/ L (≥100 mg/dL), or previously diagnosed type 2 diabetes				

WC: waist circumference; HDL-C: high-density lipoprotein cholesterol; T2DM: type 2 diabetes mellitus; OGTT: oral glucose tolerance test.
*The IDF Consensus group recognises that there are ethnic, gender and age differences but research is still needed on outcomes to establish risk.

3.6 Evaluation of the risk for related gastroenterological diseases

In an obese child the risk for steatosis should always be taken into consideration. In children with ALT values confirmed above 40 U/L it is advised to carry out a closer diagnostic examination based on:

- Blood gamma glutamyltransferase
- Liver ultrasonography
- Hepatitis differential diagnosis

3.7 Evaluation of the risk for related respiratory and otorhinolaryngology diseases

In presence of respiratory symptoms such as bronchospasm, snoring at night and sleep apnoea, the patients should undergo the following:

- otorhinolaryngoiatric evaluation
- Respiratory function examination (spirometry)
- Possible polysomnographic examination.

3.8 References

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4. TYPICAL FEATURES OF AN OBESE CHILD DIET

Diet has definitely a key role in the development of essential obesity. Indeed it is important to bear in mind that even children and teenagers with normal weight make daily nutritional mistakes. Besides the most common mistakes of the whole paediatric population, obese subjects present additional erroneous nutritional behaviours that further jeopardize the difficult balance between calories intake and energy expenditure. More specifically, the diet features of the obese child are partially related to those committed by the vast majority of the paediatric population in general, as well as being peculiar to this category of patients.

The main diet mistakes in an obese patient are:

- Excessive food intake in comparison to caloric expenditure (a very sedentary lifestyle)
- Skipping breakfast intake
- Obese children who do have breakfast, do however tend to eat very little in the morning
- Tendency to eat preferably in the afternoon and in the evening, and often outside main meals; tendency to eat a lot of snacks in the afternoon and in the evening.
- Scarce intake of whole grains, pulses, fish, fibre, seasonal vegetables and fruit.
- High intake of sugars with high glucose index (potatoes, bread, pastries, refined grains) cold cuts, cheese and meat
- Predilection for liquid foods (i.e. fruit juice) or particularly creamy ones that do not require chewing (i.e. cakes, puddings, muffins).

5. OBESITY COMPLICATIONS

At the same age, mortality is generally higher in obese adults than in non-obese ones. For the obese child there is no actual risk of death. However, considering the high persistency (30-60%) of paediatric obesity during adulthood, it is reasonable to think that precocious metabolic modifications are related to morbidity and mortality in the medium to long term. Mortality during adulthood is mainly caused by cardiovascular diseases, and the main factors for this are arterial hypertension, arteriosclerosis, dysplidemia and diabetes mellitus (Park MH et al., 2012).

All the aforementioned metabolic alterations are generally associated with obesity and usually, at paediatric age, they do regress by improving the condition of overweight and of obesity. Many of the metabolic and cardiovascular complications (insulin-resistance, diabetes mellitus type 2, metabolic syndrome, hypertension) that until a few years ago were considered as peculiar problems of adulthood, have now become relevant pathologies for paediatric age too. For this reason the later consequences of paediatric obesity include both the persistency during adulthood of the obesity and morbidity originated during childhood and cardiovascular disorders. The latter are especially arteriosclerotic based ones, such as an increased risk for gout and for a number of tumours (colorectal) for the man and for arthritis and menstrual disorders for the woman, as well as an increase of cardiovascular mortality due to general causes.

5.1 Insulin-resistance

Insulin-resistance is a risk factor for many pathologies, among which diabetes mellitus type 2 is the predominant one. In the child as well as in the adult it is determined by the combination of insulin-resistance with an insufficient secretion from beta cells. Obesity is one of the main causes for altered insulin sensitivity and thus for insulin-resistance; indeed the majority of patients suffering from diabetes mellitus type 2 are obese.

Ethnical and geographical differences in the incidence of insulin-resistance indicate that this alteration is an extremely complex metabolic disorder, with a heterogeneous aetiology. The disorder is indeed determined by environmental and social risks that unmask individual underlying susceptibility.

The role plaid by genetic factors is evident when considering the prevalence differences of such disorders among different ethnic groups. The Afro-American population in particular, resulted as the most insulin-resistant one (Arslanian S, 2002). Environmental factors which were proved to be involved in the development of insulin-resistance include, besides the aforementioned

obesity, a positive energetic balance, a sedentary lifestyle, a diet with a lot of high glucose index food, high in fat and high in proteins.

Insulin-resistance is a condition characterized by the inability of insulin to adequately stimulate the access of glucose inside cells. It is determined by a number of causes, but whichever the origin may be, the consequence is a diminished use of glucose, that accumulates in the circulation and further stimulates insulin secretion. At the first stage, the hyperinsulnaemia created fosters the cellular collection of glucose, which initially is able to overcome insulin-resistance and to maintain euglycemia. At a second stage, glycemic levels become progressively higher with a further increase of insulin secretion: at this stage glucose intolerance is manifested. Postprandial glucose values appear high (> 126 mg/dl) and they do not allow an adequate pancreatic response, but on the contrary they reduce it and cause the onset of diabetes mellitus type 2.

The circulation of free fatty acids plays a key role in determining the alteration of carbohydrate metabolism in the obese subject. High adipose mass causes a greater turnover of fats due to an increased hydrolysis and a higher re-esterification of triglycerides. The Free Fatty Acids (FFAs) , being more available are oxidized at liver and muscle level causing an increase of cellular production of Acetyl-Coa that stimulates gluconeogenesis at hepatic level and inhibits the oxidation of glucose at muscular level. The lower peripheral use of glucose causes a receptors down-regulation, with a reduction of the number and of the activity of insulin receptors and a lower turnover of liver glycogen. Furthermore the high concentrations of FFAs are able to interfere with the binding between insulin and its receptor and in some of the post-receptor processes with the transduction of the signal.

In the last decade, the adipose system has been considered as a proper endocrine tissue, secreting hormonal substances, “fat hormones”, which were recently renamed “adipocytokines”, which mainly function to regulate energy homeostasis. Recent scientific evidences prove that adypokines can play an important role for the genesis of obesity and of related pathologies. Among all these hormonal substances, adiponectin is one of the most studied adipocytokines of the recent years. Normal size adypocites physiologically secrete “insulin sensitive” hormones including adiponectin. However, in situations of adipocyte hypertrophy caused by hyper-caloric diets, there is a decrease of production and secretion of “insulin-sensitizing” hormones, and an increase in “insulin-resistant ones”, with the result of fostering a condition of increased adiposity and of insulin-resistance. Adiponectin acts by reducing lipid synthesis and glucose production by the liver, causing a drop of blood free fatty acids and of glucose synthesized through gluconeogenesis.

Therefore the production of triglycerides by the liver also results reduced. In fact adiponectin also acts by increasing the oxidation of fats by muscular tissue and thus the consumption of energy, probably by regulating the synthesis and the activity of proteins related to triglycerides metabolism (CD 36, Acetyl-Coa oxidase, PPARα).
Insulin-resistance is responsible for the increased risk of cardiovascular pathologies through a number of mechanisms:

- loss of the endothelial protecting effects of insulin such as vasodilatation, reduction of the antioxidant condition, anti-inflammatory action, antithrombotic, profibrinolytic, antiatherosclerotic;
- inhibition of lipid metabolism and thus induction of dyslipidemia that contributes to the induction of endothelial malfunction;
- stimulation of the sympathetic nervous system with increased levels of norepinephrine, a vasoconstrictor agent inducing higher blood pressure;
- sodium retention action of insulin.

The above data account for the use of the intima-media thickness (IMT) measure of the carotid artery as a precocious marker of cardiovascular risk.

5.2 Fatty Liver Disease (FLD)

Fatty Liver Disease (FLD) represents one of the earliest complications linked to obesity and insulin-resistance, and it is the most frequent cause for chronic hepatopathy at paediatric age. The term NAFLD (Non Alcoholic Fatty Liver Disease) describes a wide range of clinical-pathological entities, starting from simple fatty liver moving to non-alcoholic steatohepatitis, up to cirrhosis and terminal stage hepatic disease.
An autopsy study carried out in the United States on children who died for accidental causes, indicated that 9.6% of the American population between the age of 2 and 19 presents NAFLD and the value tends to increase up to 38% for obsess children (Schwimmer JB et al., 2006). Steatohepatitis defines the accumulation of lipids inside the hepatocyte, considered pathological only when values are above 5% of the liver's weight. Lipid accumulation generally includes triglycerides, more rarely sphingolipids or cholesterol esters. There are two fundamental histological variations of fatty liver: macrovescicular steatosis whereby a voluminous lipid vacuole displaces the nucleus laterally, o microvescicular steatosis whereby the central nucleus of hepatocyte is surrounded by lipid drops.
Macrovescicular steatosis (the most frequent histological type) is generally caused by a number of alterations of the lipid metabolism, of toxic,

dysmetabolic, nutritional or hereditary nature. This type of steatosis is frequently found in cases of chronic alcoholism, obesity, insulin-resistance, diabetes, cachexia, drug-related hepatopathy, hereditary metabolic disorders, HCV and CMV hepatitis, autoimmune hepatitis, Wilson's disease and in cystic fibrosis.

The pathogenesis of macrovescicular steatosis is caused by the shift of lipolysis towards lipogensis, with a subsequent intrahepatic accumulation of lipids.

Microvescicular steatosis is found instead in conditions of acute alterations of mitochondrial and ribosomal functions. FFA transported to the liver derive from the plasma hydrolysis of chylomicrons after a meal or from the triglycerides of fatty tissue during fasting. Inside the liver acid fats can be oxidized in the mitochondria (β-oxidation) or they can be used for the synthesis of triglycerides, phospholipids or cholesterol esters.

An increase of FFA uptake at hepatic level can be secondary to:

- an increase of lipids or carbohydrates in the diet
- an increase of circulating FFA levels (obesity, dyslipidemia)
- greater mobilization of triglycerides in presence of a reduced sensitivity of tissue receptors to insulin actions (insulin-resistance)

At paediatric age the prevalence of NAFLD increases at the same time of the diffusion of obesity, therefore it was essential to retrieve diagnostic standards that could be used by paediatricians and hepatologists.

The diagnostic gold standard of NAFLD is histological examination. The said method is not a suitable screening procedure due to its invasiveness and for the excessive costs, therefore it was necessary for research to find hepatopathy markers. Currently, to assess the level of fibrosis and steatosis as well as the risk for the progression to terminal a stage of hepatopathy further methods are being used: imaging through hepatic ultrasound and/or magnetic resonance, serum markers for function and hepatic fibrosis.

Despite some of the aforementioned markers are generally used for the diagnostic appraisal of a patient suspected of NAFLD, none of these seem to have the high specificity and sensitivity required to certainly rule out other underlying hepatic diseases.

In clinical practice the diagnosis of NAFLD is generally suggested by results showing hepatobiliary enzymes (especially ALT and GGT) above the normal range and/or by positivity to the ultrasound evaluation. In overweight patients above the age of 3, with high waist circumference and NAFLD positive familiarity, the first diagnostic examination should consist of abdominal ultrasonography, the evaluation of hepatic function markers together

with anamnesis and clinical data, to exclude other possible causes for hepatopathy.
A NAFLD activity score (NAS) (Brunt EM et al., 2009) was suggested in the attempt to standardize the diagnostic histological criteria for NAFLD.
The score is based on the weighted sum for each of the following lesions:

- steatosis (0-3)
- lobular inflammation (0-3)
- ballooning de generation of hepatocytes (0-2).

A score > 5 strongly suggests the presence of NASH (Non Alcoholic Steatohepatitis), whereas a score < 3 strongly indicates the absence of NASH.
However the histological features of NASH at paediatric age are different to those retrieved in adults. In a study conducted by Schwimmer et al (Schwimmer JB et al., 2005) 100 obese or overweight children underwent hepatic biopsies.
Three distinct pathological types were derived from the results hereby obtained:
TYPE 1: presents the same features found in the adult (ballooning degeneration, lobular inflammation, with/without perisinusoidal fibrosis and without portal inflammation (17%);
TYPE 2: predominant one (51% of the survey), is distinguished by the presence of macrovescicular steatosis with portal inflammation, with/without portal inflammation in absence of ballooning degeneration and perisinusoidal fibrosis;
TYPE 3: overlapping of the other two types.
According to recent indications, hepatic biopsy is recommended:

- to rule out any other curable diseases,
- in case of clinical suspect for a hepatic disease at advanced stage,
- before starting a pharmacological/surgical treatment,
- as part of the protocol or a research trial.

Considering the limitations of the said procedure, there has been a thorough study of alternative methods that could replace biopsy evaluation for the diagnosis and the monitoring of NAFLD.
The most significant include:

- Hepatic ultrasound: it is the most commonly used imaging method for the screening of NAFLD, as it is safe, widely available and scarcely expensive. In case of steatosis the liver appears hyperechogenic. The level of lipid infiltration is assessed on the basis of the extent of echogenicity. This technique presents sensitivity values ranging from 60 to 96% and a specificity between 84 and 100%.

- CAT (computed axial tomography) without contrast agent: presents sensitivity and specificity values similar to those of the ultrasound. However it exposes the patient to radiations, reason why this technique is not frequently used, especially at paediatric age.
- Magnetic Resonance Imaging (MRI): it is able to measure in a reliable way the fat infiltration in the liver. This technique, is growingly receiving more interest especially at paediatric age as it is not invasive and it does not expose to radiations.
  An emerging imaging modality for the quantitative evaluation of hepatic steatosis is spectroscopy 1H-MR (1H-MRS). The diagnostic sensitivity and precision of such procedure varies between 87 and 100% and between 80 and 85% respectively.

5.3 Metabolic Syndrome

Metabolic Syndrome (MS) was defined for the first time by Reaven et al as a combination of cardiovascular risk factors such as obesity, insulin-resistance, glucose intolerance or diabetes mellitus type 2 (DMT2), arterial hypertension and dyslipidemia (intended as high density levels of lipoproteins, HDL reduced and high triglycerides values). If in adults MS is difficult to define it is even more complicated to do so in a paediatric context.

Recently (2007) the International Diabetes Federation suggested a new definition of MS for developmental age with the aim of making its identification easier and more practical, so to improve in speed and precision the detection of the syndrome in children and adolescents at risk of developing DMT2 (Table 2) (Zimmet P et al., 2007).

As far as dyslipidemia is concerned, it is known that the obese subject presents higher circulating levels of FFAs in comparison to a normal weight one, due to the inability of the insulin to inhibit their release in the circulations. Triglycerides are also higher for the greater availability of precursors at hepatic level (FFAs and glucose), with an increase in the production of VLDL (very low density lipoprotein) and a lower removal from the bloodstream due to the reduced activity of lipoprotein lipase. Therefore there is a high concentration of VLDL causing an increase of LDL synthesis. The lower activity of lipoprotein lipase also diminishes the synthesis of HDL. In the obese child total average cholesterol levels and triglycerides are not constantly higher than those of the non-obese child, whereas the HDL/LDL ratio is generally inferior, same as for adults.

Hypertension (blood pressure values above 95th percentile according to sex and age) is not frequent in the child with ponderal excess, even though average blood pressure values in the obese are significantly higher than

those of the control-population. High blood pressure values are matched with the level of adiposity and with the duration of the obesity. The mechanism whereby insulin contributes to the increase of systolic pressure values is triggered by its sodium-retentive effect.
All the metabolic complications of child obesity are reversible, completely or partially, with the loss of weight. Even a moderate loss of weight can bring significant metabolic improvement.

5.4 Hormonal modifications

In the obese subject the circadian rhythm of cortisol is preserved, however there can be evidences of high secretion levels of cortisol and of corticosterone metabolites. The increased clearance of cortisol provokes a stimulation of ACTH secretion, which causes an increase of andrenal androgens (dehydroepiandrosterone sulphate and testosterone), attested by increased levels of urinary 17-ketosteroids causing precocious adrenarche in these children.
The secretory response of the growth hormone (GH) both spontaneously and in relation to exercise, sleep, hypoglycaemia, arginine and to GHRH will be reduced in the obese child in comparison to normal weight control groups. On the contrary the insulin-like growth factor (IGF-1) is usually higher in the obese. Such modifications are reversible with a reduction of the adipose mass. Despite the reduced levels of GH, the child with essential obesity does not show growth deficit, actually during the pre-pubertal period or during the early pubertal one he or she will have height above the norm. Moderately high triiodothyronine values (T3), even within norm limits are frequent in obese children and adults alike. This is determined by a greater peripheral conversion of T_4 (thyroxine or tetraiodothyronine) in T3.
Gonadal activity in the obese child is essentially normal. Adrenarche is usually early, but sexual development is regular. In the female, early menarche is common, often followed by alteration of the menstrual cycle such as amenorrhea, dysmenorrhoea and dysfunctional uterine bleeding. The Polycystic ovary syndrome is relatively frequent, characterized by the presence of many (>10) ovary cists with a diameter of more than 4cm associated with amenorrhea, dysfunctional uterine bleeding, signs of hyperandrogenism (hirsutism, virilization). Such syndrome is frequently associated to hyperinsulinemia.

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6. OBESITY TREATMENT

Treating essential obesity at developmental age should tend to positively and persistently affect the child's diet, behaviour and physical activity thus therapeutic programs should intervene on all three levels (Kaneita Y et al., 2008).

As far as essential obesity dietary therapy is concerned, its main goals can be summarized as follows:

- *Reducing overweight* and finding a new balance between energy expenditure and caloric intake (by improving physical activity and by persistently modifying life style and nutritional habits), not reaching ideal weight;
- *conservation of lean body mass,* especially muscular mass which represents the metabolically active compartment of the body, which is able to positively affect basal metabolism, and consequently the energy expenditure;
- *reducing lean body mass;*
- *maintaining adequate growth rhythms;*
- *achieving a correct weight-stature ratio;*
- *appropriate nutrition with an adequate repartition of nutrients as well as choosing food that induces a high sense of satiety;*
- *preserving the statural-ponderal balance achieved;*
- *preventing obesity-related complications.*

When intervening on childhood obesity the aim is to regulate body weight and fat mass by acting adequately from an anthropometric as well as a psychological standpoint. To achieve a correct and lasting ratio between weight and height, the therapeutic program should be able to radically modify nutritional and life habits of the child and of the family with a proper educational action. Thus the involvement and collaboration of the whole family are essential conditions for the success of the therapy devised. Nutritional education is the cornerstone of dietary treatment for childhood essential obesity.

6.1 Diet

A balanced and normocaloric diet, matched with an improvement of physical activity aiming to obtain a reduction of overweight through safeguarding weight is an appropriate treatment for:

- ✓ Children below 8 years of age
- ✓ Children above 8 years of age old with: mild or moderate ponderal excess (on the basis of BMI evaluation) without any further complications or clearly hypercaloric nutritional habits

Normocaloric diet is based on the following principles:

- CALORIC INTAKE corresponding to the one indicated by RDI according to sex and statural age, divided in 4-5 meals with the following repartition of calories: breakfast + snack 20%, lunch 40%, snack 10%, dinner 30%.
- PROTEIN INTAKE: 10-12% of the energy intake (1:1 ratio between animal and vegetable proteins)
- CARBOHYDRATE INTAKE: 60-65% of the energy intake (with an amount of high-glucose-index sugars < 10% of total calories)
- LIPIDS INTAKE: no restriction up to the age of 2 years old. After the age of 2 progressive reduction from 30 to 25% of total calories with a saturated fat intake < 10% of the energy intake and cholesterol not exceeding 100 mg/1000 kcal
- FIBER INTAKE: the amount of grams can be calculated on the basis of the following formulas: between age +5 and age +10 or equal to 10 g/1000 kcal, or even equal to 0.5 g/kg of ideal weight
- MINERAL INTAKE as indicated by Recommended Dietary Intakes (RDI) guidelines.

The advised repartition of protein sources with a high biological value as well as of main nutrients is as follows:

- More times per day CEREALS: pasta, rice, barley or spelt for both lunch and dinner. Preferably wholemeal bread or type 1 flour for breakfast, lunch and dinner, being careful to avoid excesses
- At least 2 times per day VEGETABLES AND FRUIT
- 4 times per week FISH
- 4 times per week LEGUMES
- Not more than 3-4 times per week MEAT
- 1 time per week CHEESE
- 1 time per week EGGS

If the family collaborates, this type of approach is characterized by a good acceptation of variations, especially qualitative ones, without excessive restrictions and thus it allows a gradual and lasting reduction of overweight and the acquisition by the child and by the family of a correct nutritional education. Awareness of the nutritional habits of the overweight-obese child allows targeting nutritional interventions (Ferrie JE, et al., 2011; Wisor JP et al., 2011).

6.2 References

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7. FATTY ACIDS

7.1 Description

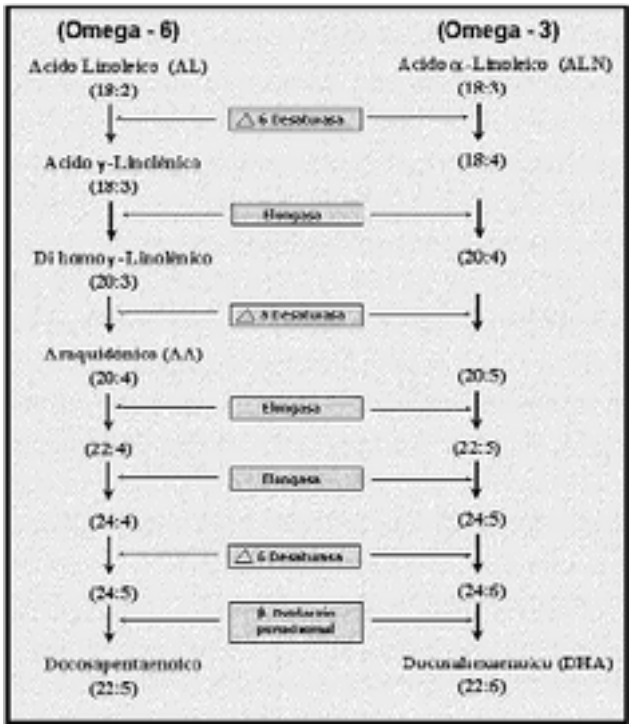
Fatty Acids are constituted by an alkyl chain with a carboxylic group at the end. The general formula is R-COOH, where the R group is represented by a linear chain shaped CH3 (CH2)n. The latter chain can have variable lengths between 2 and 30 atoms of carbon, however the most widespread and important fatty acids have between 12 and 22 atoms of carbon. Depending on the number of carbon atoms, fatty acids are distinguished for practical reasons between short chain fats (1-6 C), medium chain fats (8-12 C), long chain fats (14-20 C) and very long chain fats (> 22 C). Furthermore fatty acids are also grouped together according to the presence of double or treble bonds in two categories: SAURATED and UNSATURATED.

Saturated fatty acids, such as myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0) have simple chemical bonds and result more stable to heat and oxidation. These acids are contained in animal fats, such as butter, lard and meat.

Unsaturated fatty acids can contain one (monounsaturated) or more (polyunsaturated) “double or treble” bonds. They are more easily digested and absorbed than saturated ones, but they are more susceptible to oxidation. These are found in vegetable food (olive and seeds oil, hazelnuts and peanuts). The most important monounsaturated fatty acid is oleic acid (18:1 n-9), which is present in large quantities in olive oil. Double bonds make the structure of fatty more fickle; for this reason, cellular membranes with high unsaturation index present greater fluidity which is an essential condition especially for nervous structure, whereby more fluid membranes guarantee a quicker transmission of the nerve signal.

Unsaturated fatty acids are also grouped together in families according to the distance, in terms of atoms of carbon, of the first bond from the methyl end. Families range between n-1 and n-12, however the most important ones group together n-3, n-6 and n-9 fats. Fatty acids within each family are biosynthetically related, being interconvert by enzymatic processes of desaturation and elongation of the chain. The enzymes involved in these metabolic processes are however common to all three series, and for this reason, the essential fatty acids of the different families compete among themselves for the synthesis of long chain compounds when these are not adequately provided by the diet (Figure 12).

FIGURE 12. Metabolic transformation of essential fatty acids in LCPUFA



Among essential acids there is linoleic acid (18:2 n-6, LA), a very widespread fatty acids from the n-6 family. As this acid cannot be synthesized by the body, it has to be introduced through the diet. It can be found virtually in all vegetable, in greater quantities in seeds, nuts, grains and pulses. It is also available in lower quantities in animal fats and in fish oil.

Another essential fatty acid is α-linoleic acid (18:3 n-3, LNA) which belongs to the n-3 family. Like linoleic acid, this one too is available in significant quantities in the vegetable realm. It is found in green leaves, including phytoplankton and algae, in some seeds, nuts and pulses.

Essential fatty acids are vital to maintain the functioning and the integrity of cellular membranes. Furthermore these acids participate, with a regulating function, to metabolism and fats transportation and to the biosynthesis of prostaglandins, which are chemical substances regulating many bodily functions, including blood pressure and immune and inflammatory responses.

For biological purposes, derivates from the n-3 series (Cottin SC et al., 2011; Origin Trial Investigators, et al., 2012) have particular importance as they determine:

- Improvement for the metabolism of lipids and of lipoproteins (reduction of cholesterol and of triglycerides);
- Effects on blood pressure and on cardiac functions (antyarrhythmic effect);
- Effect on the endothelial function (increased production of NO) and on vascular reactivity (vasodilatation);
- Reduced production of cytokines by neutrophils and by monocytes;
- Strong anti-platelet effect (antithrombotic effect) and anti-inflammatory;
- Inhibition of the atherosclerosis process.

7.2 Long-Chain Polyunsaturated Fatty Acids (LC-PUFA)

Long-chain polyunsaturated fatty acids (LCPUFA) are the main components of cellular membranes, where they have an essential practical function as well as a structural one. Indeed they regulate the fluidity of the membranes as well as enzymatic, transport and receptor activity, and thus being the precursors of intra and intercellular mediators.

These fatty acids derive from n-3 and n-6 essential fatty acids, α-linoleic acid and linoleic acid, and they include the eicosapentaenoic acid (20:5 n-3, EPA), the docosahexaenoic acid (22:6 n-3, DHA) and the arachidonic acid (20:4 n-6, AA). The limiting stage for polyunsaturated fatty acids is caused by the action of delta-6 desaturase, the first enzyme operating on essential fatty acids from all series, for the synthesis of derivates (Figure12). It used to be believed that on the metabolic path leading to DHA synthesis there was a succession of three enzymatic activities called respectively delta 6, delta 5 and delta 4 desaturase, the latter in the final stage of DHA synthesis. However today there is reason to think that DHA synthesis occurs, if not univocally, at least mainly along a different path which is also more challenging from a biochemical standpoint. DHA synthesis is likely to involve two subsequent elongating stages, a new desaturation in Δ6 position and a β-oxidation in the peroxisomal see (Sprecher H, 2000). The direct proof of this mechanism was provided by congenital pathologies with the absence of peroxisomes, such as Zellweger Syndrome which are characterized by significant DHA deficits.

It is important highlighting that despite human beings can elongate the chain of the α-linoleic acid introduced through the diet, transforming it in an eicosapentaenoic acid and in a docosahexaenoic acid (n-3 long-chain

polyunsaturated fatty acids) the synthesis may not be sufficient to cater for the daily physiological need, and it is therefore recommended to introduce in the diet food containing these fatty acids.

Currently in western world diets, the predominant polyunsaturated fatty acids are the ones from the n-6 series, made of the linoleic acid and the arachidonic acid. In the last 100 years the proportion between n-3 and n-6 polyunsaturated fatty acids in western nutrition has deeply changed as these two families of polyunsaturated fatty acids share the same metabolic path, and this caused apprehension for potential health risks. However it is growingly clear how n-3 and n-6 have independent healthy effects on the body, and as the intake of n-6 is included in the recommended parameters, any possible preoccupations concerning the n-6:n-3 ratio are ascribable to a modest intake of n-3 instead of an excess of n-6. The said unbalance obviously affects the many molecules deriving from such precursors, bringing to a greater formation of metabolites deriving from AA (arachidonic acid).

Prostaglandins formation happens through a competition between AA and n-3, EPA in particular, at cyclooxygenase and lipoxygenase level. The increased intake of n-3 fatty acids reduces the incorporation of AA within cellular membranes fostering a sharp anti-inflammatory response. Generally eicosanoids deriving from arachidonic acid PGE2, LTB4, TBX2, have inflammatory effects, and in particular the 12-hydroxyeicosatetraenoic acid (12-HETE) has a positive correlation with carcinogenesis.

Eicosanoids deriving from EPA, series 3 prostaglandins and thromboxanes and series 5 prostaglandins and leukotrienes, reduce the production of AA and thus of AA-derived eicosanoids and they also increase their catabolism through the activity of peroxisomal enzymes. Furthermore they inhibit COX-2 activity. A diet providing an intake of n-3 in the human body leads to a decrease of inflammatory markers such as leukotrienes, prostaglandins, interleukins and TNF (Table 3).

TABLE 3. Effects of omega-3 fatty acid on inflammatory markers

Factor	Function	Effect of omega-3 fatty acid
Arachidonic acid	Eicosanoid precursor, aggregates platelets, stimulates white blood cells	↓
Thromboxane	Platelet aggregation, vasoconstriction, increase of intracellular Ca^{++}	↓
Prostacyclin ($PG_{3/3}$)	Prevent platelet aggregation, vasodilatation, increase cAMP	–
Leukotriene (LTB_4)	Neutrophil chemoattractant, increase of intracellular Ca^{++}	↓
Fibrinogen	A member of the acute phase response and a blood clotting factor	↓
Tissue plasminogen activator	Increase endogenous fibrinolysis	–
Platelet activating factor (PAF)	Activates platelets and white blood cells	↓
Platelet-derived growth factor (PDGF)	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Cellular damage, enhance LDL uptake via scavenger pathway, stimulate ARA metabolism	↓
Lipid hydroperoxides	Stimulate eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil 0_2 free radical formation, stimulate lymphocyte proliferation, stimulate PAF, express intercellular adhesion molecule-1 on endothelial cells, inhibit plasminogen activator, thus, procoagulants	↓
Interleukin-6	Stimulates the synthesys of all acute phase proteins involved in the inflammatory response: C-reative protein, serum amyloid A, fibrinogen, α_1-chymotrypsin and haptoglobin	↓

More specifically this leads to:

- A reduced production of prostaglandins E2 metabolites;
- Decrease of thromboxane A2, a strong vasoconstrictor and platelet aggregator and thus causing a thrombotic effect;
- Reduced formation of leukotrienes B4, a potent inducer of inflammation and chemotaxis and leucocytes adherence;
- An increase of thromboxane A3, a weak platelet aggregator and weak vasoconstrictor;
- An increase of prostacyclin PGI3, a vasodilator and platelet aggregation inhibitor;
- An increase of leukotrienes B5, a weak inflammation inducer and a weal chemotactic agent.

Recent research highlighted how the production of lipid mediators with an anti-inflammatory function is not a prerogative only of EPA but also of DHA.

In particular two new DHA deriving molecules were pointed out: docostrienes (10-17S docosatriene) and 17S resolvins (as they were identified during the resolution stage of acute inflammation). These molecules are found in the brain of the mouse, in human glial cells and in blood cells. They have an anti-inflammatory and an immunoregulatory effect for the role played regulating neutrophils migration, for cytokines expression as well as for their neuroprotective function. For these reasons they are also defined neuroprotectine.

Besides the anti-inflammatory effect, n-3 fatty acids also have anti-thrombotic, antiarrythmic and antiatherogenic functions. The stabilization of the cardiac tissue membrane determined by n-3 provides a potential antiarrythmic effect whilst the decrease of triglycerides is caused by the reduction and secretion of VLDL by the liver.

Contrary to EPA, in the brain and in the retina there are plentiful AA and DHA. Polyunsaturated long-chain fatty acids are built in the phospholipids structure of cellular membranes where besides their generic structural function for a greater or lower fluidity, they also work for a wide range of membrane functions. The most significant example is provided by DHA, which is available with a high concentration (30-50%) in the membranes of the external segments of rod cells in the retina, where it regulates the activity of rhodopsin which is a protein photopigment assigned to vision in conditions of low brightness.

The interaction between DHA and rhodopsin determines the hyper-polarization of the plasma membrane generating the necessary nervous response for an optimal vision. DHA is also present at cerebral level in the aminophospholipid of cellular membranes of neurons, in the plasma membranes of synaptosome and of synaptic vesicles, where it plays a preferential role for the mediation of biochemical activities allowing neurotransmission. Glial cells care for the synthesis of DHA which is released in extra-cellular space and thus stored in neuronal cells. Any process jeopardizing the ability of glial cells to synthesize DHA, may compromise the structure and the function of nerve cells. Examples of this are provided by severe genetic diseases, such as Zellweger syndrome and neonatal adrenoleukodystrophy which present scarce DHA plasma levels caused by the inefficiency of β-3-oxidation at the end of the synthesis. The aforementioned pathologies are characterized by cerebral demyelination, adrenal atrophy and accumulation of fatty acids in the white matter of the adrenal cortex. Their evolution is an alteration of CNS, blindness and precocious death.

The remarkable diffusion of LCPUFA in brain cells led to believe that the presence and the quantity of such elements in the diet can affect the cognitive function and behaviour. Although the research in the field is at the beginning stages, there are proofs, though minimal, suggesting an improvement of the cognitive function following the supplementation of fatty acids (Ryan AS et al., 2010). Indeed it was established that during pregnancy and breast-feeding, women should ensure a satisfying intake of n-3 long-chain polyunsaturated fatty acids, to foster the growth, the neurological development and the cognitive function of the child. n-6 polyunsaturated fatty acids are more plentiful in diets, thus meeting the required need is less problematic. For n-3s the situation is different.

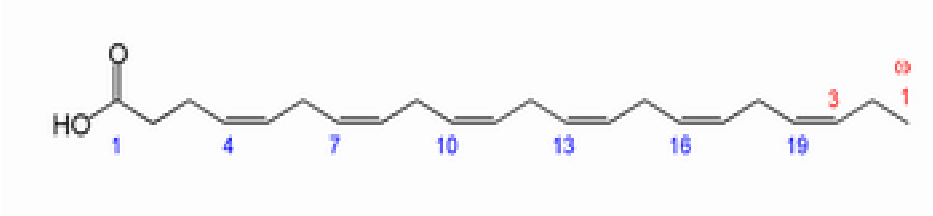
Furthermore, a hypothesis has been put forward connecting the intake of unsaturated fatty acids and the course of a number of diseases, even though evidences are not yet conclusive the hypothesis does not cease to raise interest. Unsaturated fatty acids could be associated to a reduced risk of developing certain types of cancer such as colorectal cancer, breast cancer and prostate cancer (Brasky TM et al., 2012; Moore MR et al., 2012). However at the moment the evidences supporting this connection are considered insufficient to justify nutritional recommendations from authoritative bodies. Many inflammatory conditions such as asthma, Crohn's disease and arthritis, in theory could be alleviated with diet changes.

The fatty acid composition of cellular membranes can be altered with the consumption of n-3 and n-6 polyunsaturated fatty acids, reducing the inflammatory activity. However it is not yet clear whether this effect goes together with a significant reduction of clinical symptoms.

7.3 Docosahexaenoic acid (DHA)

DHA (22:6 n-3 or docosahexaenoic acid) is an essential fatty acid of the n-3 series. The chemical structure is made of 22 atoms of carbon, with six double bonds spread along the structure, the first bond connects the third and the fourth carbon starting from the methyl section (ending section), from which derives the name ω3 (Figure 13).

FIGURE 13. DHA



DHA is available in reasonable quantities in fish, particularly in salmon, mackerel, sardines, herrings, tuna and anchovies (oily fish). DHA can be found in even greater percentages in the oils derived from these animals, and obviously it can also be found in some micro-algae eaten by fish. Besides these fish and algae, the food sources of DHA are particularly scarce, it can be found in small quantities in meat especially when the animal was fed with fish meal or linen seeds (in this case, it is also available in the eggs of oviparous animals such as chickens). Proving its essential role for the human body, DHA is also present in breast–milk whilst it is absent in cow milk, in milk derivates and in vegetable oils. This observation gave origin to the recent practice of integrating the diet of pregnant and breast-feeding women with DHA, to guarantee the correct development of cerebral tissue, visual sharpness and cognitive abilities of the foetus and of the infant. Many industries specialized in the production of milk add DHA to their products to make its composition closer to that of human milk.

On a nutritional level, the most renowned feature of docosahexaenoic acid concerns the proven capacity to reduce triglycerides in the blood. Therefore an adequate intake of DHA contributes to reducing the risk of cardiovascular disease, especially of thrombotic and atherosclerotic events. Low levels of DHA were related to neurological disease such as Alzheimer's disease, depression (low levels showed reduced percentages of serotonin at brain level) and attention deficit hyperactivity disorder (ADHD).

DHA is the main constituent of the phospholipids of the retina (it is used for the treatment of subjects affected by retinitis pigmentosa) and of brain synapses, and it is also concentrated in sperm cells and in the cardiac tissue. DHA guarantees the permeability and functionality of cellular membranes, contrasting the arachidonic acid (another polyunsaturated fatty acid that originates the so called "inflammatory cascade"). Docosahexaenoic acid was indeed ascribed anti-inflammatory properties, which justify its use to soothe the symptoms of rheumatoid arthritis and of ulcerative colitis.

From a metabolic standpoint, DHA can be considered a semi-essential fatty acid. It is indeed synthesized by the human body from the alpha-linoleic

acid (ALA), an essential fatty acid contained in fish, linen oil, hemp oil, colza oil, nuts and soy. However the enzymatic system operating this conversion may be inefficient in part, especially due to western life style and to its repercussions (diet rich of n-6 but poor of n-3, sedentary activities, excess of foods, fostering metabolic disease such as diabetes and obesity). The semi-essentiality or essentiality of DHA (depending on the authors) at nutritional level is thus based on these assumptions.

7.4 Long-chain fatty acids in essential obesity

In adults, the influence on metabolic syndrome, of fatty acids contained in the diet, has been repeatedly studied (Galgiani JE et al., 2008; U.S. Department of Agricolture and U.S. Department of Health and Human Services, 2010). Instead for paediatric age, the relationship between plasma polyunsaturated fatty acids and adiposity was scarcely investigated and it even produced contradictory results (Decsi T et al., 1998; Ailhaud G & Guesnet P, 2004; Okada T et al., 2005).

The importance of LCPUFA derives from evidence showing how their derivates, prostaglandins, play an essential role in adipocyte differentiation (Reginato MJ et al., 1998). Decsi et al. published a study on long-chain fatty acids on the paediatric obese population (Decsi T et al., 1998). The study compares a group made of 22 paediatric obese patients with a group of normal weight patients of the same age and it highlights a significant increase of plasma values of n-6 series LCPUFA in the obese population. This significant increase is connected to a greater activity of the delta-6 desaturase enzyme, and it is suggested that the high insulin levels registered for the obese population can stimulate the synthesis of n-6 LC-PUFA.

A further study carried out in 2005 by Okada (Okada T et al., 2005) on 59 obese children and on a control group made of 53 normal weight patients of the same age, accentuated a relationship between the plasma content of palmitoleic acid and abdominal obesity in obese children. This study suggests a possible connection between the profile modification of monounsaturated fatty acids (MUFA) and a hyper-activation of the Stearoyl-Coa desaturase enzyme, which is not sufficiently suppressed by leptin. In conclusion the study ascribes to endogenous lipogenesis an important role in the pathogenesis of obesity in children.

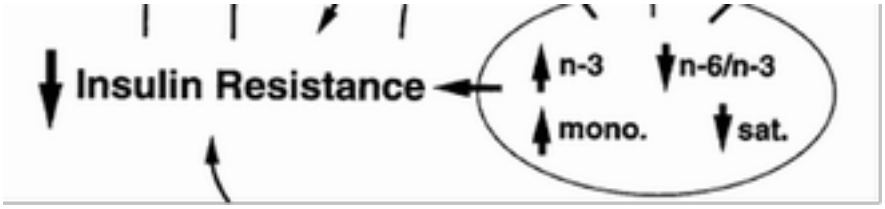
A recent study conducted in our Paediatric Clinic showed that obese children have lower plasma levels of LCPUFA in comparison to normal weight ones, even though they appear to have a higher daily intake of all fatty acids families through diet, PUFA included. In particular, obese children, in comparison to normal weight ones, showed a significant increase of n-6

series LCPUFA plasma levels and lower levels of DHA matched with higher levels precursors, alpha-linoleic (C18:3 n-3 or ALA) and EPA. Although this difference could be partially explained by the different intake of fatty acids through the diet, it cannot be ruled out that in obese children there is a metabolic alteration on n-3 series synthesis path. This in consideration of the fact that the ratio DHA/ALA (indicating n-3 series activity) resulted lower in obese children than in normal weight ones. Plasma levels of C18:2n-6 (linoelic acid) also resulted lower in obese children, whereas no differences were found between obese children and normal weight ones for what concerns C20:4 n-6(AA) values. Indeed like for other hypothesis it cannot be ruled out that lower plasma levels of C22:6n-3 (DHA) in obese children may mirror a greater contribution to the creation of hyperplastic tissues or a greater distribution in adipose tissue.
Furthermore the BMI z-score of the sample population resulted negatively connected to DHA levels and positively related to the plasma ratio of n-6/n-3 LCPUFA (AA/DHA). These results suggest that in obese children there may be both a relative PUFA deficit of n-6 series and an altered synthesis of DHA, in comparison to normal weight children. Indeed obese children with a higher BMI value have a different plasma pattern of fatty acids, to normal weight children and to obese ones with a lower BMI level. These further results suggest that the profile of plasma fatty acids could be directly correlated to the level of obesity at paediatric age (Scaglioni S et al, 2006). Furthermore, the study allowed understanding how an adequate nutritional intervention can positively affect the pattern of plasma fatty acids. After a year of nutritional education, there were evidences of increased levels of monounsaturated fatty acids, of n-6 andn-3 series PUFA, of C20:4N-6 (AA), of C22:6n-3(DHA), of the ratio C22:6n-3(DHA)/C20:4n-6(AA) and C22:6n-3(DHA)/C18:3n-3(ALA); and a reduction of saturated levels and of C20:3n-9.

7.5 LC-PUFA, insulin-resistance and liver steatosis

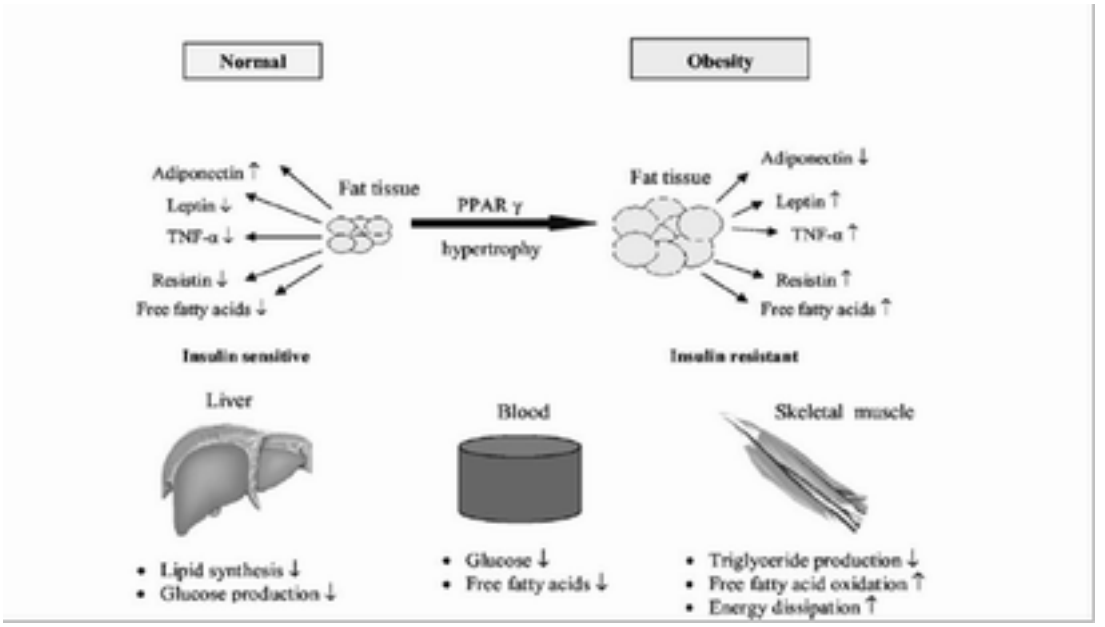
LCPUFA are important components of cellular membranes. Low levels of LCPUFA, especially of DHA, and a high intake of n-6/n-3 LCPUFA in the phospholipids of the membrane at skeletal muscle level were connected to the metabolic condition of insulin-resistance, which is one of the factors fostering the beginning of liver steatosis (Figure 14).

FIGURE 14. Possible associations between fatty acids and metabolic condition of insulin-resistance



Furthermore, it is important to consider the effects of LCPUFA in modulating the expression of a number of genes dedicated to the regulation of insulin-resistance and of lipid and carbohydrate metabolism. LCPUFA indeed would affect the modulation of the genetic expression of the PPARγ, which is a renowned factor of transcription involved in adypocyte differentiation, adipogenesis and insulin sensitivity, fostering the synthesis of adiponectin by the adipocyte (Figure 15).

FIGURE 15. Effects of LCPUFA on the modulation of the genetic expression of the PPARγ



More specifically, an imbalance of the n-3/n-6 LCPUFA ratio towards the n-6 series would lead to lower levels of adiponectin produced by the adipocytes, reducing the expression of the PPARγ gene, thus fostering the beginning of an insulin-resistance condition. As far as the hepatic tissue is concerned, a Chilean study of 2004 evaluated the pattern of LCPUFA in adults affected by liver steatosis and steatohepatitis registering a significant decrease of n-3 series LCPUFA levels in the adipose tissue and an increase of the n-6/n-3 LCPUFA ratio both in the phospholipids fraction at hepatic level and in the overall lipids of the adipose tissue. Moreover, according to this study, the low percentage of n-3 series LCPUFA would induce the down-regulation of the protein SREBP 1 expression, which lowers the stimulus for the oxidation of fatty acids, increasing the concentration at hepatocytes level. This mechanism together with a reduced release of triacylglycerol from hepatocytes, mediated by a diminished activation of PPARγ, which is itself induced by the reduction of n-3 series LCPUFA, could justify the transition to the condition of liver steatosis (Figure 15).

Currently, the evidence supporting the positive effect of n-3 series LCPUFA on insulin-resistance and on liver steatosis come from epidemiological and clinical studies carried out on adults. Indeed it was recently observed in two randomized studies conducted on overweight and obese young adults, that the intake of n-3 LCPUFA is connected to an improvement of insulin-resistance and of liver steatosis.
The effects of the supplementation of n-3 LCPUFA on metabolic complications retrieved in essential obesity at paediatric age have not yet been evaluated. Similarly, the metabolic consequences of the aforementioned supplementation, specifically the type of individual answer related to each specific genotype, remain unknown.
The pattern of plasma fatty acid also seems to be influenced by whether or not there is a condition of steatosis, as proved by the said Chilean study.

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8. STUDY

8.1 Objectives

Primary:

- To verify whether the supplementation of DHA, together with a dietary and behavioural intervention proves more efficient in improving the most frequent metabolic complication of childhood obesity, insulin-resistance, in comparison to a mere dietary and behavioural intervention.

Secondary:

- To reduce liver steatosis in the group supplemented with DHA.

8.2 Population

The present longitudinal double-blind randomized study recruited a total of 30 children, aged 8-13 years old, 14 boys (46.6%) and 16 girls (53.33%) affected by essential obesity, in the period between January 01, 2010 and September 30, 2012.

All obese children consecutively admitted to the Department of Paediatrics, San Paolo Hospital, Milan, and to the Department of Paediatrics, Federico II Hospital, Naples. The said subjects accepted to be hospitalized for 2 days, for the evaluation of nutritional and life habits, carbohydrates metabolism, lipid profile, body composition and for an initial nutritional education program for the patients and their parents. Subjects were defined obese according to the classification provided by the International Obesity Task Force (Cole TJ et al, 2000).

Children underwent a dietary-behavioural intervention and they were randomized in two groups:

1) Group A: subjects taking a DHA supplement, dosage 500 mg/diem
2) Group B: subjects taking wheat germ oil supplement (placebo).

The dosage of DHA was established so to provide 2.4 grams of DHA/1000 Kcal, which is ca. 2% of the daily caloric need.

The said children were observed over a period of 6 months.

Inclusion criteria are:

- Age between 8-13 years old
- Gestational age between 37 and 42 weeks
- Weight at birth ≥2500 gr and ≤ 4000 gr
- Absence of twins
- Caucasian race
- Obesity according to the International Obesity Task Force (IOFT)

- Insulin-resistance (fasting insulinaemia >15 uU/ml) according to the Italian Paediatrics Association (Società Italiana di Pediatria, 2006)
- Presence or absence of liver steatosis
- Normolipidemia

Exclusion criteria are:

- Secondary obesity
- Alteration of carbohydrate and insulin metabolism and/or fatty liver disease when not secondary to obesity.
- Intestinal malabsorption, chronic inflammatory pathologies of the alimentary canal

Children's parents received detailed information concerning the objectives pursued with the study and the evaluations that would have been carried out on children, for them to sign their consent to the study. The ethical committee of the University of Milan and of San Paolo Hospital in Milan approved the study's protocol.

8.3 References

Cole TJ et al., 2000, Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 320(7244):1240-1243.

Società Italiana di Pediatria, Istituto Scotti Bassani. Obesità de bambino e dell'adolescente: consensus su prevenzione, diagnosi e terapia. Argomenti di pediatria 1/06. Milano, Italia. ANCORA Arti Grafiche; 2006. http://www.ecog-obesity.eu/papers/Consensus_Italia.pdf

9. MATERIALS AND METHODS

For the present longitudinal double-blind randomized study, recruited subjects were evaluated for a time period of 6 months.

Before supplementation and after 6 months of observation, the evaluations were as follows:

1. Clinical examination and growth evaluation including weight and height, body circumference (arm, waist) as well as skinfolds (biceps and triceps, subscapular and suprailiac) measurement, evaluation of the general clinical conditions of the child through paediatric control and recording of arterial pressure with DINAMAP. Search for possible clinical signs of insulin-resistance (acanthosis nigricans).
2. Evaluation of nutritional habits, with the guidance of a dietician, according to patient's answers, with the help of a parent, to questionnaires on food frequency (for a semi-quantitative evaluation of the child's nutrition as well as of the variety of his/her diet).
3. Blood samples for a metabolic-nutritional evaluation and to assess the inflammatory condition:
    - Complete blood count (CBC)
    - Fasting glucose and insulinaemia
    - Glycated haemoglobin
    - Triglyceridemia
    - Cholesterolaemia (total, HDL, LDL)
    - Apoliprotein A and B levels
    - Hepatic functions (AST, ALT, GGT).

    Samples were taken on a blood level of ca. 10 ml and tests were carried out in the local laboratory.
4. Electrocardiogram (ECG).
5. Abdominal ultrasound to study the possible presence of, and the thus extent of, fatty liver disease and to assess visceral and abdominal fat.
6. Electrical bioimpedance (BIA) to analyze body composition.

Anthropometric measurements were carried out by two paediatric doctors as provided by standard procedure.

Weight was measured with a dedicated scale, either with bar and shifting weights one or with the electronic type. Accuracy is of 100g.

Height was measured with Harpenden stadiometer, with an accuracy of 0.1-0.5cm.

Tanner's tables were recommended for weight and height evaluation reference.

BMI was estimated by dividing the real weight of the child (in Kg) by the square of the stature in meters; the BMI z-score was calculated using Cole's LMS method (Cole TJ, 1990) and Italian reference data (Cacciari E et al., 2002).
A tape measure was used to take body circumferences. The positioning of the tape measure for every different circumference is very important as it affects the reliability and precision of the measurement.
Waist circumference was measured with the method indicated by WHO in 1995, in the middle point between the last rib and the iliac crest which corresponds to the narrowest part of the abdomen (minimum circumference). The measure thus obtained was round up to the closest 0.1 cm.
Skin-folds were evaluated according to standardized method, with Holtain Skinfold Calliper (Figure16).

FIGURE 16. Holtain Skinfold Calliper

The tool's quadrant is calibrated on 0.2 mm intervals, but measurement can be read up to 0.1mm.
The triceps skinfold was measured on the posterior surface of the left arm, above the triceps muscle, at the midpoint of a line traced from the acromial process of the scapula and the lower margin of the olecranon process of the ulna. The site for the measurement was determined with a tape measure indicating the distance between the lateral projections of the acromial process and the lower edge of the olecranon process of the ulna, with the

elbow bent at 90°. Once the midpoint was defined it was marked on the lateral surface of the arm. The operator lifted up the skinfold using the left thumb and the index, approximately 1 cm above the marked level and the extremities of the calliper were applied to it in correspondence of the marked level.

Skin-folds and circumferences were measured according to Lohman (Lohman TG et al 1997).

Parents' weight and height were collected to calculate BMI. Parents were defined affected by overweight or obesity if BMI resulted higher than 25 kg/ m^2 or 30 kg/m^2 respectively. Two paediatricians calculated pubertal stages conforming to Tanner's charts.

The arterial pressure of each child was measured using a DINAMAP electronic manometer, using appropriate bracelets according to the size of the arm and it was evaluated on the basis of cut off levels defined for age, sex and height by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents.

Body composition was estimated with the bioimpedance method (BIA) with a TANITA scale.

Blood sampling was performed in the morning, on an empty stomach. The evaluation considered lipid and carbohydrates profiles, hepatic functions, polyunsaturated fatty acids profile and ApoA and ApoB levels.

Insulin blood levels were determined using the electrochemiluminescence method and glucose levels with enzymatic method. In conformance to the National Consensus for the prevention, diagnosis and therapy of obesity in the child and the adolescent (Società Italiana di Pediatria, 2006) basal insulin values above 15 µU/mL were considered diagnosis of insulin-resistance.

Insulin-resistance and sensitivity were assessed using the HOMA-IR (Homeostasis Model Assessment of Insulin-Resistance Index) index calculation according to the following formula:

$$\text{HOMA-IR} = \frac{[\text{fasting insulinaemia } (\mu U/l) \times \text{basal blood sugar } (mmol/l)]}{22.5}$$

Children's nutritional habits were evaluated through a Frequency Food Questionnaire (FFQ) targeted according to age and made of 116 items (Block G et al., 1986). The analysis of questionnaires and the nutritional breakdown was carried out using a program previously developed by the Paediatric Clinic of San Paolo Hospital based on the "Food and Nutrient Data Base" – Istituto Nazionale di ricerca per gli alimenti e la Nutrizione – (National Institute of research on food and nutrition) (INRNA)(Morales AE & Rosenbloom AL, 2004).

To assess the presence and the extent of fatty liver disease an expert radiologist carried out a liver ultrasound with a Hitachi H21 (Hitachi High Technology Corporation Ltd., Tokyo, Japan) using a 3.5–MHz transducer. Longitudinal, subcostal, ascendant and oblique scans were performed. Liver echogenicity was assessed using a video recording, by three independent radiologists who did not know the examined subject and then established the result by consensus.

9.1 Test and statistical analysis

The present study is presented as a longitudinal, randomized, double-blind trial. Descriptive data is expressed in terms of average, standard deviation (SD), median and variability interval (continuous variables) or for the number of cases examined and percentage (discrete variables).
Tests performed:

1. Chi-square test on contingency tables
2. Mann-Whitney nonparametric test for two independent samples
3. Values of $p < 0.05$ were considered statistically significant; label "statistically significant tendency" referred to values between 0.10 and 0.05.
4. Statistical analysis was performed using the SPSS software version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

9.2 References

Block G et al., 1986, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol 124:453-469.

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Morales AE, Rosenbloom AL, 2004, Death caused by hyperglycemic hyperosmolar state at the onset of type 2 diabetes. J Pediatr 144: 270–279.

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10. RESULTS

10.1 Characteristics of the population at baseline

The present longitudinal, randomized, double-blinded study recruited 30 children, of which 14 boys (47%) and 16 girls (53%), aged between 8 and 13 years old (average age: 11 years and 4 months, SD 1.29) affected by essential obesity (Table 4).

TABLE 4. Age and sex of participants.

	Children (n=30)
Age (years)	
Mean	11.40
SD	1.2
Median	11.0
Minimum-maximum	8-13
Sex	Girls: 16 (53%) Boys: 14 (47%)

Average of anthropometric characteristics resulted as follows:

- height 1.55 m (SD 0.08)
- weight 72.57 kg (SD 13.50)
- BMI 29.89 kg/m^2 (SD 3.85)
- waist circumference 86.49 cm (SD 8.83)
- triceps skinfold 40.58 mm (SD 27.16)

All recruited subjects were at a pre-pubertal stage on the basis of the pubertal stage classification by Tanner (average stage 2.23, SD 0.89) (Table 5).

TABLE 5. Anthropometric characteristics of participants.

Children (n=30)			
	Mean (SD)	Median	Minimum-maximum
Height (m)	1.55 (0.088)	1.56	1.41-1.70
Weight(Kg)	72.57 (13.50)	75.55	48.70-94.00
BMI (Kg/m^2)	29.89 (3.85)	28.88	24,50-36.58
BMI z score	2.26 (0.47)	2.23	1.44-3.00
Waist circumference (cm)	86.49 (8.83)	88.20	68.50-99.00
Triceps skinfold (mm)	40.58 (27.16)	34.00	21.00-139.00
Tanner stage	2.23 (0.89)		1-4

The analysis of the data, derived from the breakdown of the food frequency questionnaire (FFQ) (Table 6) answered by examined subjects, shows an average caloric intake of 2735.46 kcal/die (SD 741.38), hereby divided for macronutrients classes:

- proteins: 14.79% (SD 1.64), 99.82 g (SD 21.83)
- lipids: 30.78% (SD 2.75), 96.76 g (SD 32.78)
- carbohydrates: 53.75% (SD 3.29), 390.06 g (SD 100.21)

As far as the lipids intake in particular is concerned:

- saturated: 8.96% (SD 3.94), 27.32 g (SD 13.07)
- monounsaturated: 11.19% (SD 2.59), 34.07 g (SD 11.38)
- polyunsaturated: 2.78% (SD 0.19), 8.68 g (SD 3.09)

TABLE 6. Total energy, macronutrients and fibre intake, overall glycemic index and glycemic load in participants.

Children (n=30)			
	Mean (SD)	Median	Minimum-maximum
Total energy (Kcal)	2735.46 (741.38)	2502.61	2036.26-
Total energy (KJ)	11453.48	10477.99	4355.09
Proteins (%)	(3103.09)	14.42	8518.82-
Proteins (g)	14.79 (1.64)	94.23	18229.83
Animal (g)	99.82 (21.83)	58.83	12.78-18.96
Vegetal (g)	65.29 (18.95)	38.15	73.41-139.14
Lipids (%)	35.11 (8.31)	30.50	48.21-98.38
Lipids(g)	30,78 (2,75)	84.56	24.64-48.58
Saturated (%)	96.76 (32.78)	7.61	26.83-36.02
Saturated (g)	8.96 (3.94)	20.69	64.97-165.13
Monounsaturated (%)	27.32 (13.07)	11.90	6.90-20.04
Monounsaturated (g)	11.19 (2.59)	29.29	16.45-58.48
Polyunsaturated (%)	34.07 (11.38)	2.71	7.27-16.16
Polyunsaturated (g)	2.78 (0.19)	7.51	17.73-57.76
Carbohydrates (%)	8.68 (3.09)	54.86	2.56-3.25
Carbohydrates (g)	53.75 (3.29)	348.15	5.81- 15.26
Simple (g)	390.06 (100.21)	116.61	47.72-58.41
Complex (g)	125.84 (50.98)	228.27	308.46-616.83
Fibre (g)	226.10 (58.59)	17.53	59.67-228.02
Overall glycemic index	16.86 (4.83)	72.46	160.36-340.77
Glycemic load	73.55 (16.30)	1111.56	8.14-23.10
	1215.75 (536.57)		43.26-93.04
			524.86-2299.55

Blood profile showed presence of insulin-resistance in 28 subjects out of 30 (93%) with average insulinaemia values equal to 21.82 μU/ml (SD 6.79).
As indicated in inclusion criteria, all recruited subjects presented a normal lipid profile at the beginning of the study.
Average levels of hepatic function indexes resulted as follows:

- ALT: 31.26 U/L (SD 19.03)
- AST: 27.20 U/L (SD 9.23)

- GGT: 13.40 U/L (SD 3.72)

Arterial pressure analysis showed average levels of systolic arterial pressure of 116.93 mmHg (DS 10.20) and of diastolic arterial pressure equal to 67.40 mmHg (SD 8.92) (Table 7).

TABLE 7. Blood profile and blood pressure in participants.

Children (n=30)			
	Mean (SD)	Median	Minimum-maximum
Glucose (mg/dl)	90.86 (17.32)	86.00	68-137
Insulin (μU/mL)	21.82 (6.79)	20.45	12.50-32.60
HOMA	4.70 (1.20)	4.52	2.57-6.85
Glycated haemoglobin (%)	5.44 (0.21)	5.45	5.10-5.80
Total cholesterol (mg/dL)	161.13	162.00	122-197
HDL (mg/dL)	(20.45)	44.50	35-58
LDL (mg/dL)	46.13 (7.74)	90.00	59-113
Triglycerides (mg/dL)	92.60 (15.30)	94.00	55-182
Total cholesterol/HDL	97.36 (33.94)	3.61	2.75-4.84
TG/HDL	3.55 (0.54)	2.21	1.05-3.95
ApoA (mg/dL)	2.19 (0.88)	104.00	93-142
ApoB (mg/dL)	107.96	66.00	53-81
ApoA/ApoB	(14.55)	1.63	1.35-1.92
ALT (U/l)	66.66 (6.68)	27.00	12-82
AST (U/l)	1.62 (0.16)	23.50	20-51
GGT (U/l)	31.26 (19.03)	12.00	8-19
C-reactive protein (mg/L)	27.20 (9.23)	1.00	0.33-15.00
Blood pressure (mmHg)	13.4 (3.72)		
Systolic	3.93 (4.97)	118.00	100-135
Diastolic		67.00	54-85
	116.93		
	(10.20)		
	67.40 (8.92)		

Ultrasonography evaluation showed the presence of liver steatosis in 5 subjects (17%), of which 3 patients also presented insulin-resistance (10%) (Table 8).

TABLE 8. Distribution of steatosis and acanthosis in participants.

Children (n=30)	
	n (%)
Steatosis (yes)	5 (17)
Acanthosis (yes)	20 (66)

The overall population was double-blind randomised in 2 groups:

1) Group A: subjects supplemented with DHA, dosage of 500 mg/die (2 pearls) for 6 months
2) Group B: subjects supplemented with wheat germ oil (placebo) 2 capsules/day for 6 months.

DHA dosage was established so to provide 2.4 grams of DHA/1000 Kcal, which is ca.2% of the needed daily caloric intake.
None of the subjects showed collateral effects caused by the administration of the substances prepared.

Distribution of steatosis and acanthosis, anthropometric data, dietary composition, and blood profile, at baseline and at the end of the study, are described in tables 9-16, in the two groups separately (A and B).

Group A (DHA)

TABLE 9. Distribution of steatosis and acanthosis in DHA children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)	T1 (n=15)	
	n (%)	n (%)	p§
Steatosis (yes)	2 (14)	1(7)	0.655
Acanthosis (yes)	10 (66)	1(7)	0.014*

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcoxon test)

*Statistically significant

TABLE 10. Anthropometric characteristics of DHA children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)			T1 (n=15)			
	Mean (SD)	Median	Min-Max	Mean (SD)	Median	Min-Max	P§
Height (m)	1.55 (0.08)	1.56	1.41-1.70	1.57 (0.08)	1.58	1.47-1.73	0.002*
Weight(Kg)	72.28 (13.95)	73.60	48.70-94.00	72.08 (14.64)	68.20	46.20-99.80	0.910
BMI (Kg/m^2)	29.89 (4.02)	28.60	24.50-36.58	28.95 (4.33)	27.90	22.00-35.79	0.088
BMI z score	2.25 (0.49)	2.17	1.44-3.00	1.90 (0.95)	1.93	0.04-3.02	0.073
Waist circumference (cm)	86.59 (9.07)	88.20	68.50-99.00	86.57 (9.50)	84.50	68.00-102.50	0.609
Triceps skinfold (mm)	40.62 (27.60)	34.40	21.00-139.00	31.84 (7.07)	3.00	21.00-40.00	0.245

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcoxon test)

*Statistically significant

TABLE 11. Dietary intake, overall glycemic index and glycemic load in DHA children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)			T1 (n=15)			
	Mean (SD)	Median	Min-Max	Mean (SD)	Median	Min-Max	p§
Total energy (Kcal)	2801.24 (825.55)	2502.61	2036.16-4355.09	2470.85 (418.62)	2450.67	1864.84-3260.61	0.507
Total energy (KJ)	11728.60 (3455.23)	10477.99	8518.82-18229.83	10343.97 (1754.71)	10261.95	7802.06-13655.46	0.507
Proteins (%)	14.68 (1.70)	14.42	12.78-18.96	20.23 (5.09)	18.98	14.27-33.01	0.007*
Proteins (g)	101.24 (23.75)	94.23	73.41-139.14	124.55 (35.01)	118.42	81.65-188.54	0.092
Animal (g)	66.51 (19.86)	60.50	48.21-98.38	90.77 (37.18)	85.79	35.58-159.48	0.059
Vegetal (g)	35.42 (8.87)	35.04	24.64-48.58	33.78 (7.57)	32.11	24.63-53.45	0.646
Lipids (%)	30.87 (2.84)	30.15	26.83-36.02	25.34 (4.32)	25.69	17.51-31.63	0.012*
Lipids(g)	99.42 (36.25)	84.56	64,97-165,13	72.28 (14.03)	68.22	53.23-101.24	0.022*
Saturated (%)	8.94 (3.93)	7.62	6.90-20.04	7.61 (1.98)	7.65	3.72-11.56	0.646
Saturated (g)	27.86 (13.20)	20.69	16.45-58.48	20.58 (4.95)	20.91	10.31-26.93	0.139
Monounsaturated (%)	10.94 (2.55)	10.89	7.27-16.16	8.38 (1.22)	8.38	5.73-9.90	0.028*
Monounsaturated (g)	34.04 (11.58)	29.17	17.73-57.76	22.79 (3.85)	23.30	15.90-28.10	0.022*
Polyunsaturated (%)	2.80 (0.21)	2.74	2.56-3.15	2.73 (0.77)	2.58	1.93-4.31	0.576
Polyunsaturated (g)	9.00 (3.44)	7.38	5.81- 15.26	7.62 (3.01)	7.52	4.43-14.37	0.444
Carbohydrates (%)	53.99 (3.22)	54.86	47.72-58.41	53.07 (6.86)	52.84	40.67-66.46	0.959
Carbohydrates (g)	400.97 (111.44)	348.15	308.46-616.83	350.97 (78.22)	360.17	23.02-459.69	0.386
Simple (g)	134.62 (53.42)	120.59	59.67-228.02	113.06 (43.47)	98.44	64.16-183.93	0.575
Complex (g)	229.44 (64.98)	210.72	160.36-340.77	195.06 (45.96)	193.25	115.64-255.17	0.284
Fibre (g)	16.68 (5.18)	17.53	8.14-23.10	21.20 (6.24)	21.76	7.85-33.62	0.059
Overall glycolic index	75.75 (16.74)	78.94	43.26-93.04	62.15 (29.17)	62.97	10.54-119.27	0.168
Glycolic load	1287.68 (585.48)	1193.58	524.86-2299.55	1080.43 (639.76)	923.88	423.02-2440.18	0.444

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcox on test). *Statistically significant

TABLE 12. Blood profile and blood pressure in DHA children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)			T1 (n=15)			
	Mean (SD)	Median	Min-Max	Mean (SD)	Median	Min-Max	p§
Glucose (mg/dl)	89.86 (18.04)	86.00	68.00-137.00	84.13 (9.91)	86.00	71.00-105.00	0.054
Insulin (μU/mL)	21.52 (6.85)	20.00	12.50-32.60	14.07 (4.58)	13.80	2.00-34.80	0.001*
HOMA	4.58 (1.21)	4.12	2.57-6.85	3.05 (1.06)	3.12	0.46-6.38	0.001*
Glycated haemoglobin (%)	5.42 (0.22)	5.40	5.10-5.80	5.36 (0.32)	5.4	4.80-5.90	0.422
Total cholesterol (mg/dL)	160.46 (20.86)	162.00	122.00-197.00	145.80 (22.15)	151.00	111.00-	0.031*
HDL (mg/dL)	45.26 (7.25)	44.00	35.00-58.00	45.73 (9.84)	45.00	187.00	0.777
LDL (mg/dL)	92.93 (15.62)	90.00	59.00-113.00	84.13 (16.89)	79.00	35.00-62.00	0.061*
Triglycerides (mg/dL)	97.46 (34.67)	94.00	55.00-182.00	70.60 (15.73)	69.00	65.00-122.00	0.002*
Total cholesterol /HDL	3.59 (0.53)	3.82	2.75-4.84	3.25 (0.50)	3.17	48.00-105.00	0.041*
TG/HDL	2.22 (0.88)	2.21	1.05-3.95	1.61 (0.46)	1.68	2.48-4.07	0.020*
ApoA (mg/dL)	106.88 (13.21)	104.00	93.00-142.00	107.00 (13.03)	101.00	0.79-2.28	0.623
ApoB (mg/dL)	66.20 (6.27)	66.00	53.00-81.00	65.66 (13.10)	62.00	94.00-132.00	0.220
ApoA/ApoB	1.61 (0.16)	1.63	1.35-1.92	1.66 (0.24)	1.68	55.00-108.00	0.078
ALT (U/l)	30.06 (16.53)	27.00	12.00-82.00	25.73 (9.68)	24.00	1.22-2.08	0.299
AST (U/l)	26.60 (8.95)	23.00	20.00-51.00	24.33 (6.42)	23.00	10.00-46.00	0.081
GGT (U/l)	13.26 (3.53)	12.00	8.00-19.00	13.73 (6.76)	11.00	17.00-38.00	0.693
C-reactive protein (mg/L)	4.39 (5.42)	1.00	0.33-15.0	3.20 (2.58)	4.00	9.00-36.00	0.649
Systolic blood pressure (mmHg)	115.73 (9.98)	111.00	100-135	115.53 (10.48)	114.00	0.33-6.60	0.806
Diastolic blood pressure (mmHg)	67.33 (8.07)	67.00	54-85	66.73 (11.43)	70.00	100-131 50-80	0.845

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcoxon test). *Statistically significant

Group B (placebo)

TABLE 13. Distribution of steatosis and acanthosis in placebo children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)	T1 (n=15)	
	n (%)	n (%)	P§
Steatosis	3 (20)	2 (13)	0.276
Acanthosis	9 (60)	3 (18)	0.025*

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcoxon test)

* Statistically significant

TABLE 14. Anthropometric characteristics of placebo children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)			T1 (n=15)			
	Mean (SD)	Median	Min-Max	Mean (SD)	Median	Min-Max	P§
Height (m)	1.55 (0.09)	1.57	1.41-1.70	1.57 (0.08)	1.59	1.45-1.73	0.003*
Weight(Kg)	72.86 (13.53)	77.50	48.70-94.0	73.54 (15.49)	74.80	46.20-99.80	0.649
BMI (Kg/m^2)	29.89 (3.81)	29.16	24.50-36.58	29.18 (4.47)	29.50	22.00-35.79	0.233
BMI zscore	2.27 (0.47)	2.29	1.44-3.00	1.95 (0.96)	2.16	0.04-3.02	0.198
Waist circumference (cm)	86.39 (8.89)	88.20	68.50-99.00	86.80 (9.76)	85.50	68.00-102.50	0.865
Triceps skinfold (mm)	40.54 (27.68)	34.40	21.00-139.00	31.54 (7.25)	33.00	21.00-40.00	0.140

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcoxon test)

*Statistically significant

TABLE 15. Dietary intake, overall glycemic index and glycemic load in placebo children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)			T1 (n=15)			
	Mean (SD)	Median	Min-Max	Mean (SD)	Median	Min-Max	p§
Total energy (Kcal)	2663.09 (673.09)	2502.61	2036.16-4355.09	2416.55 (474.84)	2406.33	1864.84-3260.61	0.374
Total energy (KJ)	11150.83 (2817.58)	10477.99	8518.82-18229.83	10116.36 (1990.54)	10076.52	7802.06-13655.46	0.374
Proteins (%)	14.90 (1.65)	14.60	12.78-18.96	20.73 (5.35)	20.29	14.27-33.01	0.011*
Proteins (g)	98.26 (20.66)	91.25	73.41-139.14	124.54 (36.95)	109.26	81.65-188.549	0.066
Animal (g)	63.94 (18.98)	58.83	48.21-98.38	91.02 (39.23)	78.64	35.58-159.48	0.038*
Vegetal (g)	34.76 (8.16)	38.15	24.64-48.58	33.51 (8.07)	31.62	24.63-53.45	0.678
Lipids (%)	30.69 (2.79)	30.27	26.83-36.02	25.90 (5.04)	26.13	17.51-31.63	0.050*
Lipids(g)	93.83 (30.16)	86.20	64.97-165.13	72.20 (14.81)	68.94	53.23-101.24	0.028*
Saturated (%)	8.97 (4.19)	7.61	6.90-20.04	7.71 (2.10)	8.08	3.72-11.56	0.859
Saturated (g)	26.72 (13.70)	19.62	16.45-58.48	20.30 (5.24)	19.54	10.31-26.93	0.260
Monounsaturated (%)	11.46 (2.75)	11.09	7.27-16.16	8.27 (1.22)	8.43	5.73-9,90	0.021*
Monounsaturated (g)	34.10 (11.84)	29.29	17.73-57.76	21.97 (4.12)	20.51	15.90-28.10	0.028*
Polyunsaturated (%)	2.75 (0.18)	2.70	2.56-3.15	2.78 (0.77)	2.45	1.93-4.31	0.859
Polyunsaturated (g)	8.33 (2.80)	7.51	5.81-15.26	7.64 (3.18)	7.44	4.43-14.37	0.441
Carbohydrates (%)	53.49 (3.51)	53.98	47.72-58.41	51.82 (7.51)	52.82	40.67-66.46	0.678
Carbohydrates (g)	378.06 (90.63)	341.53	308.46-616.83	336.38 (90.60)	354.27	238.02-459.69	0.314
Simple (g)	116.08 (49.35)	109.96	59.67-228.02	111.03 (48.47)	92.13	64.16-183.93	0.859
Complex (g)	222.39 (54.25)	228.27	160.36-340.77	182.06 (52.72)	191.89	115.64-255.17	0.213
Fibre (g)	16.94 (4.70)	17.89	8.14-23.10	20.73 (6.63)	19.80	7.85-33.62	0.066
Overall glycemic index	71.10 (16.42)	68.67	43.26-93.04	59.88 (31.17)	55.87	10.54-119.27	0.260
Glycemic load	1135.84 (498.51)	1035.12	524.86-2299.55	1054.27 (701.73)	795.62	423.02-2440.177	0.767

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcoxon test). *Statistically significant

TABLE 16. Blood profile and blood pressure in placebo children, at baseline (T=0) and at the end of the study (T=1)

	T0 (n=15)			T1 (n=15)			
	Mean (SD)	Median	Min-Max	Mean (SD)	Median	Min-Max	p§
Glucose (mg/dl)	91.86 (17.14)	86.00	68.00-137.00	85.86 (9.51)	88.00	71.00-105.00	0.059
Insulin (μU/mL)	22.13 (6.96)	20.90	12.50-32.60	17.54 (5.07)	16.80	2.00-34.80	0.048*
HOMA	4.82 (1.22)	5.02	2.57-6.85	3.84 (1.46)	3.44	0.46-6.38	0.05*
Glycated haemoglobin (%)	5.46 (0.21)	5.50	5.10-5.80	5.40 (0.30)	5.50	4.80-5.90	0.388
Total cholesterol (mg/dL)	161.80 (20.75)	162.00	122.00-197.00	150.40 (19.10)	153.00	111.00-	0.041*
HDL (mg/dL)	47.00 (8.35)	45.00	35.00-58.00	49.26 (10.13)	46.00	187.00	0.094
LDL (mg/dL)	92.26 (15.51)	90.00	59.00-113.00	84.46 (16.68)	82.00	35.00-62.00	0.069
Triglycerides (mg/dL)	97.26 (34.40)	94.00	55.00-182.00	68.20 (17.20)	68.00	65.00-122.00	0.001*
Total cholesterol/HDL	3.50 (0.57)	3.41	2.75-4.84	3.13 (0.55)	3.02	48.00-105.00	0.012*
TG/HDL	2.16 (0.91)	2.21	1.05-3.95	1.46 (0.51)	1.44	2.48-4.07	0.004*
ApoA (mg/dL)	109.06 (16.17)	104.00	93.00-142.00	108.73 (13.58)	102.00	0.79-2.28	0.637
ApoB (mg/dL)	67.13 (7.26)	66.00	53,00-81,00	66.06 (12.87)	62.00	94.00-132.00	0.220
ApoA/ApoB	1.62 (0.16)	1.63	1.35-1.92	1.67 (0.27)	1.68	55.00-108.00	0.078
ALT (U/l)	32.46 (21.76)	27.00	12.00-82.00	26.26 (11.73)	24.00	1.22-2.08	0.123
AST (U/l)	27.80 (9.77)	24.00	20.00-51.00	25.06 (7.48)	23.00	10.00-46.00	0.016*
GGT (U/l)	13.53 (4.03)	12.00	8.00-19.00	15.06 (8.92)	11.00	17.00-38.00	0.937
C-reactive protein (mg/L)	3.47 (4.61)	1.00	0.33-15.0	2.66 (2.61)	1.00	9.00-36.00	0.754
Systolic blood pressure (mmHg)	118.13 (10.61)	118.00	100-135	116.47 (9.27)	120.00	0.33-6.60	0.484
Diastolic blood pressure (mmHg)	67.47 (9.98)	67.00	54-85	67.20 (10.57)	70.00	100-131 50-80	0.944

§ Statistical significance between baseline and end of the study (Student t test for paired data or Wilcoxon test). *Statistically significant

Comparison between groups

Tables 17-21 compare the two groups (DHA vs. placebo) with regards to anthropometric data, blood profile, pressure and ultrasonography, both at baseline and at the end of the study. At analysis of repeated measures with treatment as fixed factor no significant difference was found between groups (minimum p=0.216).

TABLE 17. Anthropometric characteristics of children (DHA vs. placebo), at baseline (T=0) and at the end of the study (T=1). Data are mean (SD).

	DHA (n=15)	**Placebo (n=15)**	*P*§
Height (m)			
Baseline	1,55 (1.41)	1.55 (0,09)	0.806
End of the study	1,57 (0.08)	1.57 (0,08)	0.838
P§§	0.002*	0.003*	
Weight (Kg)			
Baseline	72.28 (48.70)	72.86 (13.53)	0.870
End of the study	72.08 (14.64)	73.54 (15.49)	0.806
P§§	0.910	0.649	
BMI (Kg/m^2)			
Baseline	29.89 (4.02)	29.89 (3.81)	1.000
End of the study	28.95 (4.33)	19.18 (4.47)	0.870
P§§	0.088	0.233	
BMI z-score			
Baseline	2.25 (0.49)	2.27 (0.47)	0.935
End of the study	1.90 (0.95)	1.95 (0.96)	0.902
P§§	0.078	0.198	
Waist circumference (cm)			
Baseline	86.59 (9.07)	86.39 (8.89)	0.902
End of the study	86.57 (9.50)	86.80 (9.76)	0.902
P§§	0.609	0.865	
Triceps skinfold (mm)			
Baseline	40.62 (27.60)	40.54 (27.68)	1.000
End of the study	31.84 (7.07)	31.50 (7.25)	0.935
P§§	0.245	0.140	

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test). §§ Statistical significance of within group longitudinal variation (Student t test for paired data or Wilcoxon test).

* Statistically significant

Table 18. Glycemic profile of children (DHA vs. placebo), at baseline (T=0) and at the end of the study (T=1). Data are mean (SD)

	DHA (n=15)	**Placebo (n=15)**	*P*§
Glucose (mg/dl)			
Baseline	89.86 (18.04)	91.86 (17.14)	0.713
End of the study	84.13 (9.91)	85.86 (9.51)	0.512
P§§	0.054	0.059	
Insulin (μU/mL)			
Baseline	21.52 (6.85)	22.13 (6.96)	0.838
End of the study	14.07 (4.58)	17.54 (5.07)	0.050*
P§§	0.001*	0.048*	
HOMA			
Baseline	4.58 (1.21)	4.82 (1.22)	0.539
End of the study	3.05 (1.06)	3.84 (1.46)	0.101
P§§	0.001*	0.05*	
Glycated haemoglobin (%)			
Baseline	5.42 (0.22)	5.46 (0.21)	0.624
End of the study	5.36 (0.32)	5.40 (0.30)	0.683
P§§	0.422	0.388	

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test). §§ Statistical significance of within group longitudinal variation (Student t test for paired data or Wilcoxon test).

* Statistically significant

TABLE 19. Lipid profile of children (DHA vs. placebo), at baseline (T=0) and at the end of the study (T=1). Data are mean (SD)

	DHA (n=15)	Placebo (n=15)	*P*§
Total cholesterol (mg/dL)			
Baseline	160.46 (20.86)	161.80 (20.75)	0.870
End of the study	145.80 (22.15)	150.40 (19.10)	0.595
P§§	0.031*	0.041*	
LDL cholesterol (mg/dL)			
Baseline	92.93 (15.62)	92.26 (15.51)	0.935
End of the study	84.13 (16.89)	84.46 (16.68)	0.902
P§§	0.061	0.069	
HDL cholesterol (mg/dL)			
Baseline	45.26 (7.25)	47.00 (8.35)	0.624
End of the study	45.73 (9.84)	49.26 (10.13)	0.345
P§§	0.777	0.094	
Triglycerides mg/dL)			
Baseline	97.46 (34.67)	97.20 (34.40)	1.000
End of the study	70.60 (15.73)	68.20 (17.20)	0.653
P§§	0.002*	0.001*	
Total cholesterol /HDL			
Basale	3.59(0.53)	3.50 (0.57)	0.653
End of the study	3.25 (0.50)	3.13 (0.55)	0.539
P§§	0.041*	0.012*	
Triglycerides/HDL			
Baseline	2.22 (0.88)	2.16 (0.91)	0.838
End of the study	1.61 (0.46)	1.46 (0.51)	0.436
P§§	0.020*	0.004*	
ApoA (mg/dL)			
Baseline	106.86 (13.21)	109.06 (16.17)	0.870
End of the study	107.00 (13.03)	108.73 (13.58)	0.775
P§§	0.623	0.637	
ApoB (mg/dL)			
Baseline	66.20 (6.27)	67.13 (7.26)	0.806
End of the study	65.66 (13.10)	66.06 (12.87)	0.838
P§§	0.220	0.220	
ApoA/ApoB			
Baseline	1.61 (0.16)	1.62 (0.16)	0.838
End of the study	1.66 (0.24)	1.67 (0.27)	0.902
P§§	0.078	0.078	

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test). §§ Statistical significance of within group longitudinal variation (Student t test for paired data or Wilcoxon test). * Statistically significant

TABLE 20. Hepatic function indicators of children (DHA vs. placebo), at baseline (T=0) and at the end of the study (T=1). Data are mean (SD)

	DHA (n=15)	**Placebo (n=15)**	*P*§
ALT (U/L)			
Baseline	30.06 (16.53)	32.46 (21.76)	0.935
End of the study	25.73 (9.68)	26.26 (11.73)	0.935
P§§	0.299	0.123	
AST (U/L)			
Baseline	26.60 (8.95)	27.80 (9.77)	0.870
End of the study	24.33 (6.42)	25.06 (7.48)	1.000
P§§	0.081	0.016*	
GGT (U/L)			
Baseline	13.26 (3.53)	13.53 (4.03)	0.935
End of the study	13.73 (6.76)	15.06 (8.92)	1.000
P§§	0.693	0.937	
Steatosis			
Baseline	0.27 (0.467)	0.30 (0.483)	0.918
End of the study	0.27 (0.90)	0.60 (1.26)	0.705
P§§	1.000	0.276	

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test). §§ Statistical significance of within group longitudinal variation (Student t test for paired data or Wilcoxon test).

* Statistically significant

TABLE 21. Blood pressure and C-reactive protein of children (DHA vs. placebo), at baseline (T=0) and at the end of the study (T=1). Data are meaning (SD)

	DHA (n=15)	**Placebo (n=15)**	*P*§
Systolic blood pressure ((mmHg)			
Baseline	115.73 (9.98)	118.13 (10.61)	0.512
End of the study	115.53 (10.48)	116.47 (9.27)	0.838
P§§	0.806	0.484	
Diastolic blood pressure (mmHg)			
Baseline	67.33 (8.07)	67.47 (9.98)	0.935
End of the study	66.73 (11.42)	67.20 (10.57)	0.902
P§§	0.888	0.944	
C-reactive protein (mg/L)			
Baseline	4.39 (5.42)	3.47 (4.61)	0.838
End of the study	3.20 (2.58)	2.66 (2.61)	0.713
P§§	0.649	0.754	

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test). §§ Statistical significance of within group longitudinal variation (Student t test for paired data or Wilcoxon test).

* Statistically significant

No significant difference was found between groups with regards to longitudinal percentage variation [(end of study – baseline)*100/baseline] for any variable except insulin (p=0.046) and HOMA (p=0.045) (Tables 22-26)

TABLE 22. Percentage variation of anthropometric characteristics in DHA and placebo groups. Data are mean (SD)

	DHA (n=15)	**Placebo (n=15)**	*P*§
Height (m)	0.013 (0.011)	0.014 (0.012)	0.775
Weight (Kg)	-0.002 (0.071)	0.007 (0.076)	0.653
BMI (Kg/m²)	-0.031 (0.067)	-0.024 (0.074)	0.838
BMI zscore	-0.172 (0.355)	-0.159 (0.363)	0.806
Waist circumference (cm)	0.001 (0.073)	0.006 (0.073)	0.806
Triceps skinfold (mm)	-0.083 (0.259)	-0.091 (0.251)	1.000

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test).

TABLE 23. Percentage variation of glycemic profile in DHA and placebo groups. Data are mean (SD)

	DHA + (n=15)	**Placebo + (n=15)**	*P*§
Glucose (mg/dl)	-0.047 (0.105)	-0.050 (0.109)	0.838
Insulin (μU/mL)	-0.346 (0.230)	-0.207 (0.117)	0.046*
HOMA	-0.334 (0.213)	-0.203 (0.115)	0.045*
Glycated haemoglobin (%)	-0.010 (0.052)	-0.010 (0.047)	1.000

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test).
* Statistically significant

Table 24. Percentage variation of lipid profile in DHA and placebo groups. Data are mean (SD)

Variable	DHA (n=15)	Placebo (n=15)	*P*§
Total cholesterol (mg/dL)	-0.080 (0.172)	-0.058 (0.159)	0.624
HDL cholesterol (mg/dL)	0.013 (0.158)	0.050 (0.128)	0.624
LDL cholesterol (mg/dL)	-0.076 (0.219)	-0.066 (0.215)	0.870
Triglycerides (mg/dL)	-0.223 (0.217)	-0.258 (0.186)	0.744
Total cholesterol/HDL	-0.085 (0.139)	-0.100 (0.121)	0.838
TG/ HDL cholesterol	-0.189 (0.363)	-0.269 (0.285)	0.567
ApoA (mg/dL)	0.004 (0.083)	0.002 (0.087)	0.902
ApoB (mg/dL)	0.001 (0.220)	-0.003 (0.225)	1.000
ApoA/ApoB	0.028 (0.124)	0.032 (0.128)	0.838

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test).

Table 25. Percentage variation of hepatic function indicators in DHA and placebo groups. Data are mean (SD).

	DHA (n=15)	Placebo (n=15)	*P*§
ALT (U/L)	-0.072 (0.284)	-0.104 (0.260)	0.806
AST (U/L)	-0.062 (0.139)	-0.079 (0.128)	0.595
GGT (U/L)	0.031 (0.318)	0.089 (0.381)	0.806
Steatosis	0.500 (2.121)	1.000 (1.732)	0.700

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test).

Table 26. Percentage variation of blood pressure and C-reactive protein in DHA and placebo groups. Data are mean (SD).

	DHA (n=15)	**Placebo (n=15)**	*P*§
PAS (mmHg)	0.004 (0.119)	-0.008 (0.106)	0.838
PAD (mmHg)	0.002 (0.199)	0.013 (0.208)	0.838
C-reactive protein (mg/L)	1.589 (3.707)	0.957 (2.880)	0.870

§ Statistical significance of difference between groups (Student t test for independent data or Mann-Whitney U test).

Dietary intake of total energy, macronutrients, fibre, overall glycemic index and glycemic load did not differ between groups both at baseline (minimum p = 0.131) and after 6 months (minimum p= 0.215)

10.2 Analysis of Group A (DHA) characteristics at the end of the study

Average anthropometric parameters (Table 10) of Group A (DHA) after 6 months resulted as follows:

- height 1.57 m (SD 0.08)
- weight 72.08 kg (SD 14.64)
- BMI 28.95 kg/m^2 (SD 4.33)
- Waist circumference 86.57 cm (SD 9.50)
- Triceps skinfold 31.84 mm (SD 7.07)

The analysis of data recovered from the breakdown of the FFQ (Table 11) administered to subjects proved an average caloric intake of 2470.85 kcal/ day (SD 418.62), hereby divided in macronutrients classes:

- proteins: 20.23% (SD 5.09), 124.55 g (SD 35.01)
- lipids: 25.34% (SD 4.32), 72.28 g (SD 14.03)
- carbohydrates: 53.07% (SD 6.86), 350.97 g (SD 78.22)

As far as the lipids intake in particular is concerned:

- saturated: 7.61% (SD 1.98), 20.58 g (SD 4.95)
- monounsaturated: 8.38% (SD 1.22), 22.79 g (SD 3.85)
- polyunsaturated: 2.73% (SD 0.77), 7.62 g (SD 3.01).

Average insulnaemia levels resulted equal to 14.07 μU/ml (SD 4.58).

HOMA calculation, as an index of insulin-resistance resulted equal to 3.05 (SD 1.06) (Table 12).
For what concerns the lipids profile (Table 12) average levels resulted as follows:

- Total Cholesterol: 145.80 mg/dL (SD 22.15)
- HDL Cholesterol: 45.73 mg/dL (SD 9.84)
- LDL Cholesterol: 84.13 mg/dL (SD 16.89)
- Triglycerides: 70.60 mg/dl (SD 15.73)
- Total cholesterol/ HDL: 3.25 (SD 0.50)
- Triglycerides/HDL: 1.61 (SD 0.46)
- ApoA: 107.00 mg/dL (SD 13.03)
- ApoB: 65.66 mg/dL (SD 13.10)
- ApoA/ApoB: 1.66 mg/dL (SD 0.24)

Average levels of hepatic function indexes (Table 12) resulted as follows:

- ALT: 25.73 U/L (SD 9.68)
- AST: 24.33 U/L (SD 6.42)
- GGT: 13.73 U/L (SD 6.76)

Arterial pressure detection showed average levels of systolic arterial pressure of 115.53 mmHg (SD 10.48) and of diastolic arterial pressure of 66.73 mmHg (SD 11.43) (Table 12).

10.3 Analysis of Group B (placebo) characteristics at the end of the study

Average anthropometric parameters (Table 14) of Group B (placebo) after 6 months resulted as follows:

- height 1.57 m (SD 0.08)
- weight 73.54 kg (SD 15.49)
- BMI 29.18 kg/m^2 (SD 4.47)
- Waist circumference 86.80 cm (SD 9.76)
- Triceps skinfold 31.54 mm (SD 7.25)

The analysis of the data recovered from the FFQ breakdown (Table 15) administered to subjects showed an average caloric intake of 2416.55 kcal/ day (SD 474.84), hereby divided in macronutrients classes:

- proteins: 20.73% (SD 5.35), 124.54 g (SD 36.95)
- lipids: 25.90% (SD 5.04), 72.20 g (SD 14.81)
- carbohydrates: 52.82% (SD 7.51), 336.38 g (SD 90.60)

As far as the lipids intake in particular is concerned:

- saturated: 7.71% (SD 2.10), 20.30 g (SD 5.24)
- monounsaturated: 8.27% (SD 1.22), 21.97 g (SD 4.12)
- polyunsaturated: 2.78% (SD 0.77), 7.64 g (SD 3.18)

Average insulinaemia levels resulted equal to 7.54 μU/ml (SD 5.07). Mean HOMA was 3.84 (SD 1.46) (Table 16).
For what concerns the lipids profile (Table 16) average levels resulted as follows:

- Total Cholesterol: 150.40 mg/dL (SD 19.10)
- HDL Cholesterol: 49.26 mg/dL (SD 10.13)
- LDL Cholesterol: 84.46 mg/dL (SD 16.68)
- Triglycerides: 68.20 mg/dl (SD 17.20)
- Total Cholesterol/ HDL: 3.13 (SD 0.55)
- Triglycerides/HDL: 1.46 (SD 0.27)
- ApoA: 108.73 mg/dL (SD 13.58)
- ApoB: 66.06 mg/dL (SD 12.87)
- ApoA/ApoB: 1.67 mg/dL (SD 0.27)

Hepatic function average index levels (Table 16) resulted as follows:

- ALT: 26.26 U/L (SD 11.73)
- AST: 25.06 U/L (SD 7.48)
- GGT: 15.06 U/L (SD 8.92)

Arterial pressure detection showed average systolic arterial pressure levels equal to 116.47 mmHg (SD 9.27) and of diastolic arterial pressure equal to 67.20 mmHg (SD 10.57) (Table 16).

10.4 Overall analysis of data concerning the two groups (DHA and placebo) after 6 months

Anthropometric parameters:

- Reduction of BMI and BMI z-score was near to statistical significance in children supplemented with DHA (BMI, p =0.088; BMI z-score, p =0.073) but not in the placebo group (minimum p = 0.198) (Table 20);
- In groups, waist circumference and triceps skinfold measures did not show statistically significant variations (Table 17).

Diet

- Variation of caloric intake was not statistically significant in either groups;
- Protein intake showed a statistically significant increase both for group A (DHA) (p = 0.007) and for Group B (placebo) (p = 0.011), the registered increase specifically concerned proteins of animal origin;
- Lipids intake showed a statistically significant reduction for both groups (Group A p = 0.012; Group B p = 0.050), concerning in particular the monounsaturated fraction (Group A p = 0.028; Group B

p = 0.021)

- Carbohydrates intake did not show a statistically significant variation in either group.
- Dietary intake of total energy, macronutrients, fibre, overall glycemic index and glycemic load were comparable between group both at baseline and after 6 months.

Carbohydrates profile

- Plasma glucose: both groups showed a statistically significant tendency towards a reduction of average fasting plasma glucose (Group A p=0.054, Group B p=0.059)
- Insulin: both groups showed a statistically significant reduction of average fasting insulinaemia levels (Group A p= 0.001, Group B p =0.048) (Table 18). Such improvement is also shown in table 23 where for the said value a negative percentage variation is identified. Insulin average values vary between 21.52 μU/ml (SD 6.85), at the beginning of the study, and 14.07 μU/mL (SD 4.58), on termination of the study in Group A subjects (DHA). Subjects supplemented with placebo showed a variation of insulinaemia values between 22.13 μU/ml (SD 6.96) and 17.54 μU/ml (SD 5.07). Table 18 shows a statistically significant difference for the reduction of insulinaemia values in patients supplemented DHA, in comparison to subjects supplemented the placebo (p=0.050).
- HOMA (insulin-resistance index): both groups showed a statistically significant reduction of average fasting HOMA values (Group A p= 0.001, Group B p= 0.050). Such improvement is also shown in table 23 where a negative percentage variation value for the said index is identified. Moreover a statistically significant tendency towards an improvement of HOMA index (p=0.101) can be highlighted in subjects supplemented with DHA in comparison to that identified in patients supplemented with the placebo. (Table 18).

Lipid profile

- Total cholesterol: both groups showed a statistically significant decrease (Group A p= 0.031, Group B p= 0.041);
- LDL: both groups presented a statistically significant tendency towards a decrease (Group A p= 0.061, Group B p= 0.069);
- HDL: group B showed a statistically significant tendency towards increase (Group B p= 0.094);
- Triglycerides: both groups showed a statistically significant decrease (Group A p= 0.002, Group B p= 0.001);
- Total cholesterol/HDL: both groups showed a statistically significant decrease (Group A p= 0.041, Group B p =0.012);
- Triglycerides/HDL: both groups showed a statistically significant decrease (Group A p= 0.020, Group B p= 0.004);
- Apo A and Apo B: no statistically significant variation;
- ApoA/ApoB: both groups showed a statistically significant tendency towards decrease (Group A p= 0.078, Group B p= 0.078) (Table 19).

Hepatic function indicators

- ALT: no statistically significant variation in either groups;
- AST: statistically significant tendency towards decrease for Group A (p= 0.081), statistically significant decrease for Group B (p= 0.016);
- GGT: no statistically significant variation in either groups (Table 20).

Arterial Pressure

No statistically significant variation in either groups (Table 21).

Ultrasound profile

No statistically significant variation after 6 months (Table 9 and 13).

11. DISCUSSION

The analysis of anthropometric parameters showed a statistically significant increase of height as opposed to a steadiness of weight for both groups. As far as BMI is concerned, a statistically significant tendency towards decrease was observed in the group supplemented with DHA. The latter allows us to state that the suggested nutritional intervention did not have a negative influence on growth.

FFQ breakdown analysis showed, from the very early stages, strongly inappropriate nutritional habits, characterized by a higher caloric intake than what recommended for age and sex. In particular, there were evidences of an excessive intake of proteins and lipids at the expense of the carbohydrates intake. The comparison with the nutritional analysis after 6 months did not show statistically significant variations, especially regarding the caloric intake. A statistically significant tendency was registered concerning lipids (reduction) and proteins (slight increase). The absence of significant data implies the difficulty to modify the nutritional habits of the child and of the family, which was the goal of our educational intervention. A statistically significant reduction of fasting plasma glucose levels was registered for both groups.

Table 18 shows in details how the insulin-resistance profile improvement was greater in those subjects supplemented with DHA in comparison to those who were administered the placebo. The latter improvements, for both insulinaemia and HOMA, insulin-resistance index, are also accentuated by the evaluation of variation percentages values reported in table 23.

The effect of DHA on insulin-sensitivity can be related to its ability to increase the expression of the genes involved in insulin sensitivity and in the intercellular transport of glucose, as literature has already described (Woodman RJ et al., 2002).

As far as the lipid profile, despite at the beginning it appeared normal, a statistically significant reduction was found of total cholesterol and triglycerides values in both groups as well as a statistically significant tendency towards decrease for cholesterol/LDL values. The ratios Total cholesterol/HDL and Triglycerides/HDL resulted statistically significantly reduced for both groups. No variations were reported concerning ApoA and ApoB levels, besides a statistically significant tendency to increase their ratio in both groups. As these effects were registered equally from both groups, they became secondary to the slight reduction of lipid intake observed after 6 months.

Concerning liver steatosis, data did not show any variation in the ultrasound check profile at the end of the study. The latter contrasts with recent studies

reported in literature which following a supplementation of DHA for 6 months proved a slight reduction of hyperecogenicity (Morales AE & Rosenbloom AL, 2004).

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12. CONCLUSIONS

Obesity is a serious public health issue both at adult and paediatric age. The cornerstones for an intervention on the child are modifying nutritional habits and educating to a non-sedentary lifestyle.

Prostaglandins derived from LCPUFA, play an essential role in adipocyte differentiation (Reginato MJ et al., 1998). In adulthood the influence of fatty acids on obesity-related complications has been repeatedly studied (Galgiani JE et al., 2008; ; U.S. Department of Agricolture and U.S. Department of Health and Human Services, 2010), whilst at paediatric age results have always been contradictory (Decsi T et al., 1998; Ailhaud G & Guesnet P, 2004; Okada T et al., 2005).

Through the present study we tried to evaluate whether the dietary supplementation of DHA could have further benefits than a solely nutritional and behavioural therapy. The data collected proved an improvement of glucose and lipid profiles in both groups, in the one supplemented with DHA as well as in the one administered the placebo. However the improvement of the insulin-resistance condition resulted greater in subjects supplemented with DHA than in the ones taking the placebo.

The reduction of insulinaemia levels and of the HOMA index which was also reported for group B (placebo) should be connected to the dietary nutritional intervention and to the encouragement of physical activity. This confirms how the nutritional and behavioural approaches must always be the primary ones when dealing with an obese child. Therefore, despite the supplementation of DHA did improve the insulin-resistance profile, it should anyhow be considered as an auxiliary means when treating the main obesity-related metabolic complications (insulin-resistance and liver steatosis).

The present study did not prove any effect of reduction on the liver steatosis profile. A limit of this study could depend on the duration of the examination period, indeed 6 months may not be sufficient to highlight the long-term efficacy of liver steatosis treatment. Moreover, the limited sample used for the study should be considered a further limitation to it. Furthermore as there were no negative evidences caused by the supplementation of DHA, this would suggest that a new study could be planned increasing the dosage of DHA, to study its long-term effects.

In conclusion, the present randomized double-blinded study proved the effects of DHA on insulin-resistance, but the same were not reported for liver steatosis.

In consideration of such evidences, it is therefore necessary to continue the study increasing the sample number, lengthening the duration of the observation period and assessing the hypothesis for a further study on DHA

supplementation with higher dosages.

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13. GLOSSARY

α-linoleic acid. α-linoleic acid is an organic compound found in many common vegetable oils. In terms of its structure, it is named all-cis-9,12,15-octadecatrienoic acid. In physiological literature, it is given the name 18:3 (n−3). α-Linolenic acid is a carboxylic acid with an 18-carbon chain and three cis double bonds. The first double bond is located at the third carbon from the methyl end of the fatty acid chain, known as the n end. Thus, α-linolenic acid is a polyunsaturated n−3 (omega-3) fatty acid. It is an isomer of gamma-linolenic acid, a polyunsaturated n−6 (omega-6) fatty acid.

Acanthosis nigricans. Acanthosis nigricans is a brown to black, poorly defined, velvety hyperpigmentation of theskin. It is usually found in body folds, such as the posterior and lateral folds of the neck, the axilla, groin, umbilicus, forehead, and other areas. It typically occurs in individuals younger than age 40, may be genetically inherited, and is associated with obesity or endocrinopathies, such as hypothyroidism or hyperthyroidism,acromegaly, polycystic ovary disease, insulin-resistant diabetes, or Cushing's disease

Adiposity rebound. Adiposity rebound is the time at the minimum (nadir) body mass index of children, usually occurring at about 5-6 years of age.

Apolipoproteins. Apolipoproteins are proteins that bind lipids (oil-soluble substances such as fat andcholesterol) to form lipoproteins. They transport the lipids through the lymphatic andcirculatory systems. There are two main classes, Apolipoprotein A, the major protein component of high-density lipoproteins, and Apolipoproteins B form low-density lipoprotein particles.

Arachidonic acid. Arachidonic acid (20:4 n-6, AA) is a polyunsaturated omega-6 fatty acid20:4(ω-6). It is the counterpart to the saturated arachidic acid found in peanut oil, (L. arachis – peanut). In chemical structure, arachidonic acid is a carboxylic acid with a 20-carbon chain and four cis-double bonds. Arachidonic acid is a polyunsaturated fatty acid present in the phospholipids of membranes of the body's cells.

Body Mass Index. Body Mass Index (or Quetelet index, BMI) is a heuristic proxy for estimating human body fat based on an individual's weight and height. Body mass index is defined as the individual's body mass divided by the square of his/her height. The universally used unit of measure in medicine is kg/m^2.

Docosahexaenoic acid. Docosahexaenoic acid (22:6 n-3, DHA) is an omega-3 fatty acidpolyunsaturated that is a primary structural component of the human brain, cerebral cortex, skin, sperm, testicles and retina. It can be synthesized from alpha-linolenic acid or obtained directly from maternal milk or fish oil. DHA's structure is a carboxylic acid(~oic acid) with a 22-carbon chain cis double bonds, the first double bond is located at the third carbon from the omega end.

Eicosapentaenoic acid. Eicosapentaenoic acid (20:5 n-3, EPA) is an omega-3 fatty acidpolyunsaturated. In chemical structure, EPA is a carboxylic acid with a 20-carbon chain and five cis double bonds; the first double bond is located at the third carbon from the omega end. EPA and its metabolites act in the body largely by their interactions with the metabolites of arachidonic acid. EPA acts as a precursor for prostaglandin-3 (which inhibits plateletaggregation), thromboxane-3, and leukotriene-5 groups (alleicosanoids).

Essential fatty acids. Fatty acids that are required by the human body but cannot be made in sufficient quantity from other substrates, and therefore must be obtained from food, are called essential fatty acids. There are two series of essential fatty acids: one has a double bond three carbon atoms removed from the methyl end; the other has a double bond six carbon atoms removed from the methyl end. Humans lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10, as counted from the carboxylic acid side. Two main essential fatty acids are linoleic acid (LA) and alpha-linolenic acid (ALA). They are widely distributed in plant oils.

Fatty Acids. In chemistry, and especially in biochemistry, a fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. Most naturally occurring fatty acids have a chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually derived from triglycerides orphospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP. Many

cell types can use either glucose or fatty acids for this purpose. In particular, heart and skeletal muscle prefer fatty acids. The brain cannot use fatty acids as a source of fuel; it relies on glucose or ketone bodies.
Unsaturated fatty acids have one (mono) or more (poli) double bonds between carbon atoms. Saturated fatty acids are long-chain carboxylic acids that usually have between 12 and 24 carbon atoms and have no double bonds. Thus, saturated fatty acids are saturated with hydrogen.
Long chain polyunsaturated fatty acid is a fatty acid with aliphatic tails 13 to 21 carbons.

Fatty Liver Disease. Fatty liver disease is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis (i.e. abnormal retention of lipids within a cell). Despite having multiple causes, fatty liver can be considered a single disease that occurs worldwide in those with excessive alcohol intake and those who are obese (with or without effects of insulin resistance). The condition is also associated with other diseases that influence fat metabolism. Morphologically, it is difficult to distinguish alcoholic FLD from non-alcoholic FLD, and both show microvesicular and macrovesicular fatty changes at different stages.

Food Frequency Questionnaire . Food frequency questionnaires (FFQ) are designed to easily assess habitual diet by asking about the frequency with which food items or specific food groups are consumed over a reference period (e.g. 6 months or a year). They may be based on an extensive list of food items or a relatively short list of specific foods. They were originally designed to provide descriptive qualitative information about food-consumption patterns.

Glycemic index. Glycemic index provides a measure of how quickly blood sugar levels (i.e. levels of glucose in the blood) rise after eating a particular type of food. The glycemic index estimates how much each gram of available carbohydrate (total carbohydrate minus fibre) in a food raises a person's blood glucose level following consumption of the food, relative to consumption of pure glucose. Glucose has a glycemic index of 100.

Glycemic load. Glycemic load of food estimates how much the food will raise a person's blood glucose level after eating it. One unit of glycemic load approximates the effect of consuming one gram of glucose. Glycemic load accounts for how much carbohydrate is in the food and how much each gram of carbohydrate in the food raises blood glucose levels. Glycemic load is based on the glycemic index. Glycemic load is defined as the grams of available carbohydrate in the food x the food's GI / 100.

Hypertension. Hypertension is a chronic medical condition in which the blood pressure in the arteries is elevated. Normal blood pressure at rest is within the range of 100-140mmHg systolic and 60-90mmHg diastolic.

Homeostasis Model Assessment. Homeostasis Model Assessment (HOMA) is a method used to quantify insulin resistance (HOMA-IR) and beta-cell function (HOMA-β%).

Insulin-resistance. Insulin-resistance is a physiological condition in which cells fail to respond to the normal actions of the hormone insulin. Insulin job is to deliver sugar to cells to provide them with energy. the inverse of insulin resistance if the insulin sensitivity.

Linoleic acid. Linoleic acid (18:2 n-6; LA) is an unsaturated n-6 fatty acid belonging to one of the two families of essential fatty acids. It is a colourless liquid at room temperature. Chemically, linoleic acid is a carboxylic acid with an 18-carbon chain and two cis double bonds; the first double bond is located at the sixth carbon from the methyl end.

Lipoprotein. A **lipoprotein** is a biochemical assembly that contains both proteins and lipids, bound to the proteins, which allow fats to move through the water inside and outside cells. The proteins serve to emulsify the lipid (otherwise called fat) molecules. Many enzymes, transporters, structural proteins, antigens, adhesins, and toxins are lipoproteins. Examples include the high-density (HDL) and low-density (LDL) lipoproteins, which enable fats to be carried in the blood stream, the transmembrane proteins of the mitochondrion and the chloroplast, and bacterial lipoproteins.

Obesity. Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health. Adults are considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight in kilograms by the square of the person's height in metres, exceeds 30 kg/m^2. For children, the International Obesity Task Force recommends using appropriate age- and sex-adjusted BMI curves, and defines obesity over the curve having value 30 kg/m^2 at the age of 18 years.

Peroxisome proliferator-activated receptor. In the field of molecular biology, the peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, protein), and tumorigenesis of higher organisms. Three types of PPARs have been identified: alpha, gamma, and delta (beta). Alpha is expressed in liver, kidney, heart, muscle, adipose tissue, and others; Gamma is expressed in three forms, including heart, muscle, colon, kidney, pancreas, and spleen, in adipose tissue (30 amino acids longer) macrophages, large intestine, white adipose tissue; Delta/Beta is expressed in many tissues but markedly in brain, adipose tissue, and skin

Quantitative Insulin-Sensitivity Check Index. Quantitative Insulin-Sensitivity Check Index (QUICKI) is an indicator of insulin sensitivity that is derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose. This index has the advantage of that it can be obtained from a fasting blood sample, and is the preferred method for certain types of clinical research.

Transaminase. In biochemistry, a transaminase or an aminotransferase is an enzyme that catalyzes a type of reaction between an amino acid and an α-keto acid. Two important transaminase enzymes are serum aspartate transaninase (*AST*) and alanine transaminase (ALT).

RIASSUNTO

INTRODUZIONE: L'obesità infantile è una patologia di sempre maggior interesse, in cui vi è un'elevata prevalenza di insulino-resistenza, quale complicanza metabolica. I bambini obesi rispetto ai normopeso evidenziano un significativo incremento dei valori plasmatici di LCPUFA della serie n-6 e più bassi livelli di DHA associati. Diversi studi condotti su adulti hanno mostrato che bassi livelli di DHA, e un elevato rapporto n-6/n-3 LCPUFA nei fosfolipidi di membrana a livello del muscolo scheletrico sono stati associati all'insulino-resistenza.

SCOPO: primario: verificare se la supplementazione con DHA, in associazione ad un intervento nutrizionale- comportamentale, presenti un'efficacia maggiore nel migliorare l'insulino-resistenza, rispetto al solo intervento nutrizionale, in bambini affetti da obesità essenziale. Secondario: verificare se il miglioramento della steatosi epatica sia associato alla supplementazione con DHA.

SOGGETTI E METODI: si tratta di uno studio longitudinale randomizzato in doppio cieco iniziato il 1 Gennaio 2010. Fino al 30 Settembre 2012 sono stati reclutati 30 bambini, di cui 14 maschi e 16 femmine di età compresa tra 8-13 anni, affetti da obesità essenziale, definita secondo i criteri dell'International Obesity Task Force (IOTF), giunti alla nostra osservazione presso l'Ambulatorio di Nutrizione Clinica della Clinica Pediatrica dell'Ospedale S. Paolo di Milano e a quella della Clinica Pediatrica dell'Ospedale Federico II di Napoli. I bambini sono stati sottoposti ad intervento nutrizionale-comportamentale e sono stati randomizzati in due gruppi:

1) *Gruppo A*: soggetti supplementati con DHA (derivato algale) al dosaggio di 500 mg/die,
2) *Gruppo B*: soggetti supplementati con olio di germe di grano (placebo).

Il dosaggio del DHA è stato stabilito in modo da fornire 2.4 grammi di DHA/ 1000 Kcal, ovvero il 2 % circa del fabbisogno calorico giornaliero.

Prima della supplementazione (basale) e dopo 6 mesi sono state effettuate le seguenti valutazioni: esame clinico e valutazione della crescita (P, H, BMI, circonferenza vita, plica tricipitale, PA), valutazione nutrizionale mediante questionario delle frequenze alimentari (FFQ), esami ematochimici (glicemia, insulinemia, profilo lipidico, funzionalità epatica), ECG, ecografia epatica

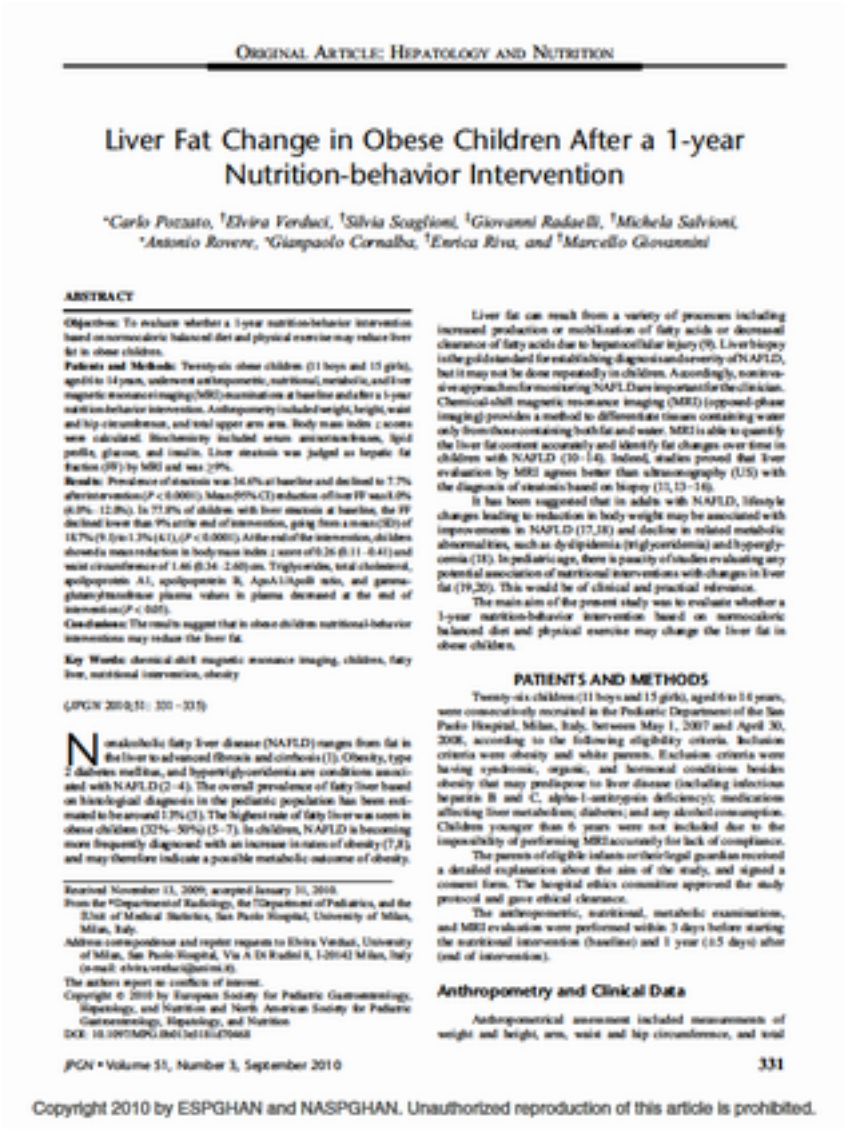
RISULTATI: al basale i due gruppi (A e B) non hanno presentato tra loro differenze statisticamente significative per le variabili considerate nello studio. Al termine dello studio entrambi i gruppi hanno mostrato una riduzione statisticamente significativa dell'insuline e dell'HOMA. Il miglioramento

del quadro di insulino-resistenza risulta essere maggiore nel gruppo supplementato con DHA rispetto al gruppo placebo. In entrambi i gruppi si è verificata una riduzione della % di soggetti affetti da steatosi epatica: nel gruppo A è passata dal 14% al 7% (P=0.655), nel gruppo B dal 20% al 13% (P=0.275). Il miglioramento non è risultato differente dal punto di vista statistico tra i due gruppi (P=0.775).

CONCLUSIONI: i cardini d'intervento nel bambino obeso sono la modificazione delle abitudini alimentari e l'educazione alla non sedentarietà: stili di vita adeguati sono in grado non solo di ridurre l'eccesso ponderale, ma anche di migliorare il profilo glucidico-insulinemico, come osservato nel gruppo placebo. Il nostro studio ha però evidenziato come la supplementazione con DHA possa giocare un ruolo importante sullo stato di insulino-resistenza, riducendo i livelli di insulinemia e dell'indice ad essa correlato (HOMA) maggiormente rispetto al sono intervento nutrizionale. Per quanto riguarda l'effetto sulla steatosi epatica, non è stato evidenziato alcuna differenza tra i due gruppi, placebo e supplementato con DHA. Sono necessari ulteriori studi di adeguata numerosità per valutare gli effettivi vantaggi della supplementazione con DHA in aggiunta all'intervento nutrizionale sulle principali complicanze metaboliche dell'obesità in età pediatrica.

APPENDIX 1. RESULTS OF THE RESEARCH (PUBBLICATONS)

1. Pozzato C, Verduci E, Scaglioni S, Radaelli G, Salvioni M, Rovere A, Cornalba G, Riva E, Giovannini M. Liver fat change in obese children after a 1-year nutrition-behavior intervention. J Pediatr Gastroenterol Nutr 2010;51(3):331-5.



Liver Fat Change in Obese Children After a 1-year Nutrition-behavior Intervention

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ABSTRACT

Objectives: To evaluate whether a 1-year nutrition-behavior intervention based on normocaloric balanced diet and physical exercise may reduce liver fat in obese children.

Patients and Methods: Twenty-six obese children (11 boys and 15 girls), aged 6 to 14 years, underwent anthropometric, nutritional, metabolic, and liver magnetic resonance imaging (MRI) examinations at baseline and after a 1-year nutrition-behavior intervention. Anthropometry included weight, height, waist and hip circumference, and total upper arm area. Body mass index *z* scores were calculated. Biochemistry included serum aminotransferases, lipid profile, glucose, and insulin. Liver steatosis was judged as hepatic fat fraction (FF) by MRI and was ≥9%.

Results: Prevalence of steatosis was 34.6% at baseline and declined to 7.7% after intervention ($P < 0.0001$). Mean (95% CI) reduction of liver FF was 3.0% (0.0%–12.8%). In 77.8% of children with liver steatosis at baseline, the FF declined lower than 9% at the end of intervention, going from a mean (SD) of 18.7% (9.1) to 1.3% (4.1), ($P < 0.0001$). At the end of the intervention, children showed a mean reduction in body mass index *z* score of 0.26 (0.11–0.41) and waist circumference of 1.46 (0.34–2.60) cm. Triglycerides, total cholesterol, apolipoprotein A1, apolipoprotein B, ApoA1/ApoB ratio, and gamma-glutamyltranspeptidase plasma values in plasma decreased at the end of intervention ($P < 0.05$).

Conclusions: The results suggest that in obese children nutritional-behavior interventions may reduce the liver fat.

Key Words: chemical-shift magnetic resonance imaging, children, fatty liver, nutritional intervention, obesity

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Nonalcoholic fatty liver disease (NAFLD) ranges from fat in the liver to advanced fibrosis and cirrhosis (1). Obesity, type 2 diabetes mellitus, and hypertriglyceridemia are conditions associated with NAFLD (2–4). The overall prevalence of fatty liver based on histological diagnosis in the pediatric population has been estimated to be around 1.3% (5). The highest rate of fatty liver was seen in obese children (32%–50%) (5–7). In children, NAFLD is becoming more frequently diagnosed with an increase in rates of obesity (7,8), and may therefore indicate a possible metabolic outcome of obesity.

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Liver fat can result from a variety of processes including increased production or mobilization of fatty acids or decreased clearance of fatty acids due to hepatocellular injury (9). Liver biopsy is the gold standard for establishing diagnosis and severity of NAFLD, but it may not be done repeatedly in children. Accordingly, noninvasive approaches for monitoring NAFLD are important for the clinician. Chemical-shift magnetic resonance imaging (MRI) (opposed-phase imaging) provides a method to differentiate tissues containing water only from those containing both fat and water. MRI is able to quantify the liver fat content accurately and identify fat changes over time in children with NAFLD (10–14). Indeed, studies proved that liver evaluation by MRI agrees better than ultrasonography (US) with the diagnosis of steatosis based on biopsy (13,15–16).

It has been suggested that in adults with NAFLD, lifestyle changes leading to reduction in body weight may be associated with improvements in NAFLD (17,18) and decline in related metabolic abnormalities, such as dyslipidemia (triglyceridemia) and hyperglycemia (18). In pediatric age, there is paucity of studies evaluating any potential association of nutritional interventions with changes in liver fat (19,20). This would be of clinical and practical relevance.

The main aim of the present study was to evaluate whether a 1-year nutrition-behavior intervention based on normocaloric balanced diet and physical exercise may change the liver fat in obese children.

PATIENTS AND METHODS

Twenty-six children (11 boys and 15 girls), aged 6 to 14 years, were consecutively recruited in the Pediatric Department of the San Paolo Hospital, Milan, Italy, between May 1, 2007 and April 30, 2008, according to the following eligibility criteria. Inclusion criteria were obesity and white parents. Exclusion criteria were having syndromic, organic, and hormonal conditions besides obesity that may predispose to liver disease (including infectious hepatitis B and C, alpha-1-antitrypsin deficiency); medications affecting liver metabolism; diabetes; and any alcohol consumption. Children younger than 6 years were not included due to the impossibility of performing MRI accurately for lack of compliance.

The parents of eligible infants or their legal guardian received a detailed explanation about the aim of the study, and signed a consent form. The hospital ethics committee approved the study protocol and gave ethical clearance.

The anthropometric, nutritional, metabolic examinations, and MRI evaluation were performed within 3 days before starting the nutritional intervention (baseline) and 1 year (±5 days) after (end of intervention).

Anthropometry and Clinical Data

Anthropometrical assessment included measurements of weight and height, arm, waist and hip circumference, and total

2. Verduci E, Radaelli G, Salvioni M, Riva E, Giovannini M. Plasma long-chain fatty acids profile and metabolic outcomes in normolipidaemic obese children after one-year nutritional intervention. Acta Paediatr. 2011;100(4):585-9.

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REGULAR ARTICLE

Plasma long-chain fatty acids profile and metabolic outcomes in normolipidaemic obese children after one-year nutritional intervention

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Keywords
Long-chain polyunsaturated fatty acids, Nutritional intervention, Obese children

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ABSTRACT

Aim: To assess the association between changes in plasma long-chain polyunsaturated fatty acids (LCPUFAs) profile and metabolic outcomes after 1-year nutritional intervention in normolipidaemic obese children.

Methods: Fifty-seven normolipidaemic obese children, aged 8–13 years, were recruited in the study. Body mass index (BMI) z-scores were calculated. Fasting blood samples were analysed for insulin, glucose, lipid profile and fatty acid (FA) levels at baseline and after an 1-year nutritional-behaviour intervention. Insulin resistance was estimated by homeostatic model assessment (HOMA).

Results: Fifty-one obese children completed the study. At the end of the intervention, the children showed decreased BMI z-score (mean reduction 0.25; 95% confidence interval [CI], 0.18–0.31), HOMA index (1.6; 0.6–2.5), plasma-saturated FA (1.49; 0.67–2.31 mg/dL), C20:3n-9 (0.05; 0.03–0.07 mg/dL) and increased plasma levels of monounsaturated FA (mean increase 1.35; 0.63–2.07 mg/dL), n-6 PUFA (1.02; 0.08–1.97 mg/dL), n-3 PUFA (0.24; 0.07–0.40 mg/dL), C20:4n-6 (0.37; 0.11–0.63 mg/dL), C18:3n-3 (0.04; 0.01–0.07 mg/dL), C22:6n-3 (0.30; 0.17–0.42 mg/dL) and the C22:6n-3/C20:4n-6 ratio (0.02; 0.01–0.05 mg/dL) ratio.

Conclusions: Nutritional interventions may improve plasma LCPUFA profile and metabolic outcomes of normolipidaemic obese children.

INTRODUCTION

Obesity in childhood is associated with adult obesity and development of the metabolic syndrome, which is a major health problem, involving cardiovascular disease and type II diabetes (1–3). Insulin resistance (IR) is a major component of the metabolic syndrome in obese children (4). While quality of dietary fats might be related to the metabolic syndrome (5), the relationship between the long-chain plasma polyunsaturated fatty acids (LCPUFAs) profile and obesity has been scantily investigated in younger ages, and results are controversial (6–8).

From a biochemical standpoint, prostaglandins, derivatives of LCPUFAs, may play a critical role in adipocyte differentiation (9). It has been suggested that in obese adults, a hypocaloric low-fat dietary intervention aimed to reduce body weight may be associated with increased incorporation of LCPUFA n-3, especially docosahexaenoic acid (DHA, 22:6n-3), and reduced saturated FAs in skeletal muscle membrane phospholipids (10) that may affect insulin action (11).

Despite the potential clinical and practical relevance, there is lack of studies in current literature evaluating any potential association of nutritional interventions with changes in LCPUFA profile in paediatric age.

The main aim of this study was to assess whether an association may exist in normolipidaemic obese children between changes in plasma LCPUFA profile and metabolic outcomes after 1-year intervention based on normocaloric balanced diet and physical activity.

PATIENTS AND METHODS

Fifty-seven obese children (31 boys and 26 girls) were recruited among patients consecutively admitted to the Department of Paediatrics, San Paolo Hospital, Milan, between 1 January and 30 December 2008 for routine examinations according to the following eligibility criteria.

Inclusion criteria were as follows: age 8–13 years, weight at birth ≥2500 g, gestational age 37–42 weeks, singleton birth, no neonatal disease or congenital malformation, children having white parents and residing in Milan or neighbourhood. Exclusion criteria were as follows: having syndromic, organic and hormonal conditions besides obesity, dysmetabolic diseases and fatty liver disease. Children having hyperlipidaemia, according to Italian guidelines (12), were further excluded to prevent possible biases effect on FA status (13).