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EFFECT OF A LOW GLYCEMIC INDEX MEDITERRANEAN DIET ON NON-ALCOHOLIC FATTY LIVER DISEASE. A RANDOMIZED CONTROLLED CLINICI TRIAL

G. MISCIAGNA[1], M. DEL PILAR DÍAZ[6], D.V. CARAMIA[1], C. BONFIGLIO[1], I. FRANCO[1], M.R. NOVIELLO[2], M. CHILOIRO[2], D.I. ABBRESCIA[1], A. MIRIZZI[1], M. TANZI[1], M.G. CARUSO[3,4], M. CORREALE[5], R. REDDAVIDE[4], R. INGUAGGIATO[4], A.M. CISTERNINO[4], A.R. OSELLA[1]

1. Laboratory of Epidemiology and Biostatistics; 2. Department of Radiology; 3. Laboratory of Biochemical Nutrition; 4. Clinical Nutrition Outpatient Clinic; 5. Laboratory of Clinical Chemistry, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Castellana Grotte (BA), Italy; 6. Instituto de Investigaciones en Ciencias de la Salud (INICSA), CONICET y Facultad de Ciencias Médicas. Universidad Nacional de Córdoba, Córdoba, Argentina. Corresponding author: Alberto Rubén Osella, PhD, Laboratory of Epidemiology and Biostatistics, IRCCS Saverio de Bellis, Via Turi 27, 70013 Castellana Grotte (BA), Italy, Tel: +39 0804994655, Fax: +39 0804994650, e-mail: arosella@irccsdebellis.it

Abstract: *Introduction:* Non-Alcoholic Fatty Liver Disease (NAFLD) is currently the most common form of liver disease worldwide affecting all ages and ethnic groups and it has become a consistent threat even in young people. Our aim was to estimate the effect of a Low Glycemic Index Mediterranean Diet (LGIMD) on the NAFLD score as measured by a Liver Ultrasonography (LUS). *Design:* NUTRIzione in EPAtologia (NUTRIEPA) is a population-based Double-Blind RCT. Data were collected in 2011 and analyzed in 2013-14. *Setting/participants:* 98 men and women coming from Putignano (Puglia, Southern Italy) were drawn from a previous randomly sampled population-based study and identified as having moderate or severe NAFLD. *Intervention:* The intervention strategy was the assignment of a LGIMD or a control diet. *Outcome measures:* The main outcome measure was NAFLD score, defined by LUS. *Results:* After randomization, 50 subjects were assigned to a LGIMD and 48 to a control diet. The study lasted six months and all participants were subject to monthly controls/checks. Adherence to the LGIMD as measured by Mediterranean Adequacy Index (MAI) showed a median of 10.1. A negative interaction between time and LGIMD on the NAFLD score (-4.14, 95% CI -6.78,-1.49) was observed, and became more evident at the sixth month (-4.43, 95%CI -7.15, -1.71). A positive effect of the interaction among LGIMD, time and age (Third month: 0.07, 95% CI 0.02, 0.12; Sixth month: 0.08, 95% CI 0.03,0.13) was also observed. *Conclusions:* LGIMD was found to decrease the NAFLD score in a relatively short time. Encouraging those subjects who do not seek medical attention but still have NAFLD to follow a LGIMD and other life-style interventions, may reduce the degree of severity of the disease. Dietary intervention of this kind, could also form the cornerstone of primary prevention of Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease.

Key words: Hepatic steatosis, Mediterranean diet, low glycemic index Mediterranean diet.

Introduction

NAFLD consists of a broad spectrum of liver diseases (1). NAFLD is currently the most common form of liver disease worldwide affecting all ages and ethnic groups. On a global level, prevalence varies between 20% and 33%. In northern Italy, prevalence of NAFLD was found to be approximately 25% in the general population, and is associated with most features of metabolic syndrome (2). NAFLD increases with age, BMI and is common in Type 2 Diabetes Mellitus (T2DM) (3). Rising prevalence of obesity and T2DM (particularly in younger people) makes NAFLD a growing threat at both the clinical and the population level (4).

Excessive hepatic fat deposition is a hallmark of NAFLD. It is linked to insulin resistance (5) and is a risk factor for T2DM and cardiovascular disease.

Although, it is predicted that some forms of NAFLD will be the leading cause of liver transplantation in the US by 2020, the optimal treatment of NAFLD remains uncertain due to the difficulties associated with adopting changes in individual's lifestyle, adverse effect of drug therapies and selection criteria, availability, and the cost of bariatric surgery (6).

Lifestyle modification is the standard treatment for NAFLD, despite the existence of little robust evidence to support this recommendation (7). This approach encompasses dietary, exercise and other behavioral changes, due to the fact that they offer a range of health benefits.

The Mediterranean Diet (MD) is a dietary pattern that has long been associated with favorable health outcomes (8, 9) and greater adherence to MD has been reported with beneficial effect on the severity of NAFLD (10). Most of the studies however, have been conducted at the clinical level where patients are highly selected (8).

A recent trial on subjects with Metabolic Syndrome (11) has shown that an energy-unrestricted MD, supplemented with extra-virgin olive oil or nuts, resulted in a substantial reduction of the risk of cardiovascular events and general mortality. The results of this study are consistent with others about the role of MD on Metabolic Syndrome (12) and diabetes (13).

In order to estimate the prevalence of some liver diseases in this Mediterranean area of Southern Italy a cohort was assembled (14) and a NAFLD prevalence of 24.6% was found.

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Our hypothesis was that a MD intervention would lead to greater improvements in NAFLD in this free-living population, than a diet based on the Italian National Research Institute for Foods and Nutrition (INRAN) guidelines. The standard MD of Crete and Nicotera, reported in the Ancel Keys' Seven Countries Study, used integral flour (flour from stone mills) for bread and other carbohydrate rich food (15) and in this study a Low Glycemic Index Mediterranean Diet (LGIMD), based on that diet, was experimented. The objective of this RCT was to estimate the effect of a LGIMD on the NAFLD score as measured by Liver Ultrasonography (LUS).

Materials and methods

Study design

NutriEpa was a parallel-group randomized controlled clinical trial. The sampled population was taken from the NutriEp survey, conducted at the Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) "Saverio de Bellis" (Castellana Grotte, Italy), from July 2005 to January 2007 and its details have been published elsewhere (14). In brief, in collaboration with 12 General Practitioners (GP) working in Putignano (Puglia, Italy) and, after testing the hypothesis that the sex-age group specific mean was the same among the general population and subjects of the GP clinics (p= 0.15), a random sample was drawn (for those individuals 18 years old, and older) from the GP patients' list. 2550 subjects were invited to participate in the survey; among these subjects, 2301 (90.2%) provided their written consent (according to the Helsinki Declaration).

Participant Selection

The trial was designed and conducted by the authors, and the protocol was approved initially by the Technical-Scientific review board and then by the Ethical Institutional one. Subjects with NAFLD were identified during the NutriEp enrollment process. Eligible participants were those individuals identified as having moderate or severe NAFLD (n=203). The exclusion criteria included: 1) overt cardiovascular disease and revascularization procedures; 2) stroke; 3) clinical peripheral artery disease; 4) T2DM (current treatment with insulin or oral hypoglycemic drugs, fasting glucose >126 mg/dl, or casual glucose >200 mg/dl; 5) more than 20 gr/daily of alcohol intake; 6) severe medical condition that may impair the person to participate in a nutritional intervention study; 7) people following a special diet or involved in a program for weight loss, or who had experienced recent weight loss and 8) inability to follow a MD for religious or other reasons. Subjects were invited to participate in the trial, and after a new assessment of the severity of NAFLD using LUS, those who agreed to participate provided, in written form, their consent for participating in the trial. The trial took place at the Laboratory of Epidemiology and Biostatistics of the IRCCS "Saverio de Bellis", Castellana Grotte (Italy), from February to November 2011 and data were analyzed in 2013-2014. All experimental subjects participated on voluntary basis.

Randomization and Masking

Participants were randomly assigned, according to a computerized random numbers sequence, to 1 of 2 treatment groups and a one-to-one ratio was used to allocate subjects.

Blinding and equipoise were strictly maintained by emphasizing to the intervention staff and participants that each diet adhered to healthy principles. With the exception of the dietitians, investigators and staff were unaware of the subjects' diet assignment. Although each dietitian followed the participant for the duration of the trial, individual assignment was made on daily random basis. Staff members who obtained outcome measurements were not informed about diet assignment. Only one of two radiologists performed outcome measurements each day and this order was also randomly assigned. In the outcome measurements relating to the third and sixth months, radiologists were unaware of the previous measurement.

Sample Size

Sample size was estimated taken into account the repeated measurement of the outcome. From a previous study (16), the mean (SD) score of NAFLD was estimated to be 4.5 (1) and 4.0 (0.5) for the treatment and control group respectively; the type I error was fixed at 0.05 (one sided) level and statistical power to 0.9. The correlation between baseline/follow-up measurements of the outcome was set to 0.4. A sample size of n1=n2=36 was estimated, in order to obtain a 1 point reduction in NAFLD score in the LGIMD group after six months.

Exposure Measurements

On the first visit, subjects were interviewed to complete a pre-coded questionnaire regarding socio-demographic issues, medical history and potential risk factors pertaining to some liver diseases. Alcohol consumption was probed (17) and the European Prospective Investigation on Cancer (EPIC) questionnaire about dietary habits was also completed. Baseline physical activity was measured by asking participants to wear a biaxial accelerometer (SenseWear®PRO3TM) over a period of seven days, including weekends.

On the second visit, a fasting venous blood sample was collected. Liver function tests and others biochemical serum markers were assessed using standard laboratory test techniques.

Body weight and height, as well as blood pressure, were measured in standard conditions at baseline, at the third and sixth month. At this point in time, dietician also reviewed (and completed if necessary) the EPIC questionnaire, taking the opportunity to highlight the advantages of following such diets.

During this second visit, at the beginning of February 2011, participants were randomly assigned to one of two dietary interventions: LGIMD or INRAN diet. The purpose of the study was explained in detail in a face-to-face interview. All participants received training so as to be able to properly fill in the Dietary Record. Personal advice was also provided in both groups, at the baseline visit and monthly thereafter. The dietary records were constructed using a weekly sheet, in which participants indicated all the food they had consumed during the course of the day. Portion size was determined taking into account the answer given in the EPIC questionnaire. The dietary record was controlled weekly during the first month of the trial, and monthly thereafter. Follow-up anthropometric measures were taken and Bioelectrical Impedance Analysis was performed monthly. LUS was performed at the third and sixth month, together with the collection of a fasting venous sample. The monthly follow-up visit also included a face-to-face interview with the dietician in order to assess the diet followed by the subject and to give, if needed, personal recommendations to achieve the "group assigned" goal.

Outcome Measurement

All subjects underwent LUS (Hitachi HI Vision E) testing. To obtain a semi-quantitative evaluation of fat in the liver, a scoring system was adopted (16, 18). NAFLD was then categorized as: absent (0), mild (1-2), moderate (3-4) and severe (5-6). If the subject met the trial inclusion criteria, he was invited to participate in the trial and convened for a second visit one week later. A sub-sample of 30 subjects (ten subjects at enrollment, first and second follow-up respectively randomly chosen) underwent LUS by the two radiologists separately. An overall weighted Kappa of 0.9 was obtained.

Dietary Interventions

A six-month intervention period for both the study and control diets was chosen. No advice was given as to total calories to be consumed or the level of physical activity to be undertaken.

Detailed information, relating to the LGIMD intervention and control (recommended by the World Health Organization and followed by INRAN (19), can be found in the Supplemental on line data.. Foods in LGIMD have all a low Glycemic Index (GI) and no more than 10% of total daily calories coming from saturated fats. The LGIMD was high in monounsatured fatty acids (MUFA) from olive oil and contained also omega-3 polyunsatured fatty acids (ω3PUFA), from both plant and marine sources.

The recommended diets were provided in brochure format, with graphical explanations organized according to a traffic light system: with a list of foods that can be consumed frequently (green foods), sometimes (yellow foods) and never (red foods). The brochure also contained a dietary record, where participants daily indicated the code of each food consumed at breakfast, lunch, dinner and during snack time. A detailed description of both the intervention and control diets (the brochures provided to participants) are available as supplementary on line material. The Mediterranean Adequacy Index (MAI) was chosen as a relevant measure to evaluate the adherence to both the intervention and control diets (20). A median value of 7.5 with an inter-quantile range (IQR) of 5.4 was, as established by the reference Italian Mediterranean Diet, expected (20).

Statistical Analysis

The primary analysis was intention-to-treat and involved all participants who were randomly assigned. In this paper only socio-demographic, biochemical, BMI and Physical Activity (at baseline) data were considered. For descriptive purposes, age at enrollment (<40, 40-59 and ≥60 years old), BMI (Normal, Overweight and Obesity), Glutamic pyruvic and Glutamic oxaloacetic transaminases (altered ≥40 U/l), Triglycerides (altered ≥165 mg/dl), Cholesterol (altered ≥200 mg/dl for men and ≥220 mg/dl for women), γ-glutamyl transpeptidase (altered >25 UI/l for men and >14 UI/l for women), Insulin (altered >29 μUI/ml), Glucose (altered ≥ 127 mg/dl) and physical activity (Low <3 METs, Moderate 3-6 METs and High >6 METs) were categorized. Using age at enrollment, number of households, education, employment position, civil status and whether the individual was retired, Factor Analysis was performed to create a composite indicator of SES (21). The resulting scale (index of socio-economic position) was then standardized, reversed (higher values correspond to higher socio-economic position) and categorized as low (<0.25 quantile), medium (0.25-0.74 quantile) and high ≥ 0.75 quantile.

Cross-tabulations between interventions and socio-demographic, life-style and biological variables were performed and proportion differences were assessed using a χ^2 test.

Dietary Records were analyzed using the MètaDieta® software and the results expressed as a percentage of total calorie intake for each food item consumed. In order to calculate the MAI, the four central months of the trial were considered; the first, second, third, and fourth week of the second, third, fourth, and fifth month respectively were chosen. MAI was calculated according to age-class, gender, and month to clearly describe the adherence during the trial. Furthermore, the Fatty Liver Index (FLI) (22) was estimated and its statistical analysis was performed by using a Wilcoxon matched-pairs signed-ranks test.

A Generalized Estimating Equation (GEE) (23) was performed to estimate the effect of a LGIMD and control diets on the NAFLD score (outcome). GEE models are useful in biomedical studies to estimate how the average of an outcome changes with covariates, allowing correlated response data (repeated measurements on each subject). A gamma distribution (link identity) for the response was assumed and an unstructured correlation matrix was set to the data. Gender (categorical), BMI, Liver Enzymes, Cholesterol, Triglycerides, Insulin, Glucose, physical activity level (MET/min/week-1) and age (continuous variables) were included as covariates. The results obtained are expressed in natural scale as mean ± 95% Confidence Interval (95% CI). Diet Marginal Average effects were estimated in order to illustrate intervention through age.

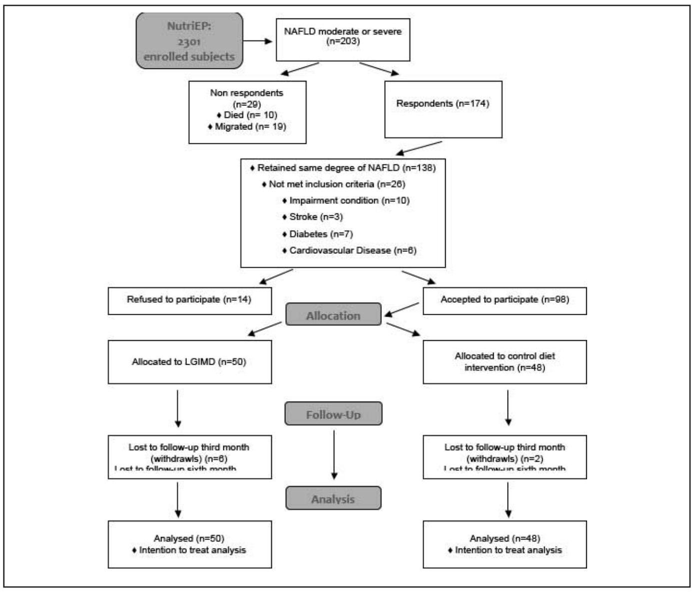
The statistical analysis was carried out using the statistical software, Stata version 12.1.

Results

During the NutriEp study, 203 participants were identified as having moderate or severe NAFLD; 174 of these individuals responded to our letter to take part in the trial. Of these 174, 138 had preserved the grade of severity of NAFLD (Figure 1).

Figure 1

NutriEpa Flow Diagram. Putignano (BA), Italy, 2011



After completion of the second visit, 98 subjects agreed to participate in the trial, and were randomly selected for either the LGIMD group (n=50) or the control diet one (n=48). 6 individuals were subsequently lost in the follow-up in the intervention group and 2 in a control group. The socio-demographic characteristics of the subjects who participated in the trial are reported in Table 1. There were more men than women reflecting the distribution of NAFLD in this population. No other significant differences were found and all subject characteristics were perfectly balanced. The biochemical characteristics, BMI, FLI and NAFLD score of the participants, both at the baseline and after six months, are given in Table 2. FLI median and inter quantile range were similar at enrollment, whereas at six months they were statistically significant lower, especially for the LGIMD group. Lower levels of SGPT, Triglycerides and Glycemia were found in both groups after six months. Lower levels of Gamma-GT and HDL-Cholesterol were also observed, but only in the LGIMD group.

MAI by age-class, gender and month is shown in Table 3. Adherence to the LGIMD resulted in a median MAI of 10.1 (IQR 7.0). Adherence was found to be lower amongst women (8.4), than among men (10.5), to increase with age for men, but was found to be higher in younger women. Adherence to the control diet showed a median MAI of 4.8 (IQR 6.7), was lower amongst males (4.8) than females (8.4). Younger males (<40 years old) and younger and oldest females (<40 years old and >60 years old), showed a MAI of 10.1, 13.0 and 10.1 respectively.

Table 1

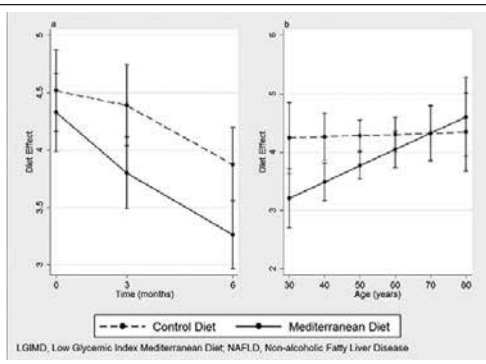
Baseline Characteristics of NutriEpa Study Participants. Putignano (BA), Italy, 2011

	DIET	
	CONTROL	**LGIMD**
Age category (years)*	No (%)	No (%)
< 30	4 (100.0)	0 (0.0)
30-39	6 (42.9)	8 (57.1)
40-49	6 (35.3)	11 (64.7)
50-59	14 (42.4)	19 (57.6)
60-69	15 (65.2)	8 (34.8)
70-79	3 (42.9)	4 (57.1)
Gender*		
Male	38 (52.8)	34 (47.2)
Female	10 (38.5)	16 (61.5)
Education*		
Illiterate	0 (0.0)	3 (100.0)
Elementary School	15 (57.7)	11 (42.3)
Middle School	12 (4404)	15 (55.6)
High School	20 (52.6)	18 (47.4)
College	1 (25.0)	3 (75.0)
Job*		
Farmer	1 (33.3)	2 (66.7)
Worker	5 (55.6)	4 (44.4)
Employee	21 (51.2)	20 (48.8)
Trader	1 (50.0)	1 (50.0)
Freelance	4 (50.0)	4 (50.0)
Housewife	2 (25.0)	6 (75.0)
Craftsman	14 (51.9)	13 (48.1)
SEP*		
Low	2 (50.0)	2 (50.0)
Medium	38 (51.4)	36 (48.6)
High	4 (44.4)	5 (55.6)
Status*		
Single	4 (57.1)	3 (42.9)
Married	42 (47.7)	46 (52.3)
Widowed	2 (66.7)	1 (33.3)
Body Mass Index		
Normal	0 (0.0)	3 (6.0)
Overweight	13 (27.1)	13 (26.0)
Obesity	35 (72.9)	34 (68.0)
NAFLD		
Absent	0 (0.0)	0 (0.0)
Mild	0 (0.0)	0 (0.0)
Moderate	34 (70.8)	35 (70.0)
Severe	14 (29.2)	15 (30.0)
Total	48 (49.0)	50 (51.0)

*Pearson $\chi 2$ p>0.10. LGIMD: Low Glycemic Index Mediterranean Diet; SEP: Socio-Economic Position.

The GEE analysis results are presented in Table 4. Significant interaction between the effect of LGIMD and time on the NAFLD score (Figure 2) was observed at both the third (-4.53, 95% CI --7.28, -1.78) and sixth month (-4.64, 95% CI -7.30, -1.99) of follow up. Also, interaction among LGIMD, time and age was significant. Predictive margins of diet vs Age are showed in Figure 2. Related to control diet all marginal average were lower ($p < 0.05$) up to 55 years old. After this cut point no diet effect was observed.

Figure 2
Effect and Contrast of LIGMD vs Control Diet (with 95% CI) on NAFLD Score. NutriEpa. Putignano (BA), Italy, 2011



No statistically significant effect was found between gender and age, between LGIMD and age or age and time.

Discussion

In this RCT, a LGIMD, which is rich in MUFA and ω3PUFA, with no more than 10% of total daily calories deriving from saturated fats and a low GI, was associated with a more intense reduction of the NAFLD score, as measured by LUS, than a control diet following the INRAN guidelines until 55 years old.

Although NAFLD is globally the most common form of liver disease and is now a significant cause of chronic liver disease, optimal treatment remains uncertain. The high prevalence of NAFLD observed in Western countries is probably due to the concurrent epidemics of overweight/obesity and associated metabolic complications, which are all recognized risk factors for NAFLD (24, 26).

Current therapy focuses on modifying individuals' lifestyle, such as diet and physical activity and potential pharmacologic treatments (26). Usually, NAFLD management includes weight reduction and increased physical activity (27, 28).

In this RCT, an energy unrestricted LGIMD was given as concerns have been raised with regards to the difficulty of reducing and maintaining the weight reduction over the long term (29).

Our intervention diet was rich in MUFAs and ω3PUFA. Olive oil, almost the only oil consumed in Puglia (30), is the major source of MUFAs. High MUFAs diets have found to be associated with an improvement on lipids profile, as well as glycemic control in humans (31) and animals (32). The first one includes decreased fasting plasma triacylglycerol and VLDL-Cholesterol concentrations without accompanying decrease in HDL (31).

PUFAs have also been shown to have an effect in NAFLD, the most commonly found being ω3PUFA. Animal studies, have shown that a ω3PUFA enriched diet increases insulin sensitivity (33), reduces intra-hepatic triglycerides (34) content and ameliorate steatohepatitis in rats (35). Evidence from cross-sectional studies show a lower consumption of ω3PUFA (36) and a higher ω6PUFA/ω3PUFA ratio in NAFLD subjects (37). Evidence from clinical trials also highlights the beneficial effect on NAFLD of a ω3PUFA supplemented diet, even in the absence of weight loss (38,39). To ensure an adequate ω3PUFA intake, our subjects were advised to eat purslane (Portulaca oleracea), which is eaten extensively in soups and salads in this Mediterranean Area (40).

Low-fat, low-saturated, low GI diet, as characterized by the MD of the 1960s, has showed to decrease liver fat in women (41) and middle-aged men (42) and also reduced fatty acids synthesis, after the substitution of more complex carbohydrates for sugar. It has been shown that even small decreases in liver fat maybe clinically relevant in subjects with high liver fat, (43) especially if any diet can be sustained for a longer period of time.

It is interesting that in this RCT the effect of LGIMD is found to be a decreasing trend until 55 years of age for both men and women, as it is showed in Figure 2. The "two hit model" (44) proposed for NAFLD involves excessive hepatocyte triglycerides accumulation, resulting from insulin resistance (first hit). As the first hit occurs at a young age, the MD could influence the natural history of NAFLD, by lowering the likelihood of the progression of the disease. Moreover, it has been hypothesized that diet composition may affect the severity of NAFLD by influencing the second hit. From a public health viewpoint, it is important to detect NAFLD at a relatively young age; treatment of NAFLD may form part of the primary prevention of T2DM and coronary heart disease. A recently published study has revealed for example, that the main cause of mortality amongst diabetics between 30 and 89 years old may be attributable to NAFLD (4). Meticulous assessment methods for adherence to diet must be implemented. In this trial MAI was adopted, as it is useful for individual evaluation and longitudinal studies. The median MAI found in our study was close to that of families in Crete in 1958 (44), although a higher MAI was observed amongst older men. Higher adherence to MD had been already observed in the population of southern Italy and the Greek Islands, although it has been decreasing along the time (45). The LGIMD given in this trial has shown a high adherence, probably due to an accurate follow-up of subjects and a favorable cultural background in terms of dietary habits (46).

Table 2
Biochemical Characteristics of the Participants at Baseline and Sixth Month. NutriEpa. Putignano (BA), Italy, 2011

	Baseline		Sixth Month	
	Diet		Diet	
	Control*	LGIMD*	Control#	LGIMD#
	No. (%)	No. (%)	No. (%)	No. (%)
Body Mass Index				
Normal	0 (0.0)	3 (6.0)	0 (0.0)	5 (10.3)
Overweight	13 (27.1)	13 (26.0)	20 (44.2)	19 (43.6)
Obesity	35 (72.9)	34 (68.0)	26 (55.8)	20 (46.2)
NAFLD				
Absent	0 (0.0)	0 (0.0)	4 (9.3)	4 (10.3)
Mild	0 (0.0)	0 (0.0)	8 (16.3)	14 (30.8)
Moderate	34 (70.8)	35 (70.0)	27 (58.1)	24 (53.8)
Severe	14 (29.2)	15 (30.0)	7 (16.3)	2 (5.1)
FLI‡	82.28(70.31-90.38)	81.19(49.19-90.24)	72.69(49.59-83.94)	57.72(27.33-73.14)
SGOT				
Normal	48 (100.0)	50 (100.0)	43 (100.0)	39 (100.0)
Altered	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SGPT				
Normal	44 (91.7)	44 (88.0)	45 (97.7)	39 (100.0)
Altered	4 (8.3)	6 (12.0)	1 (2.3)	0 (0.0)
Gamma-GT				
Normal	41 (85.4)	37 (74.0)	40 (86.0)	36 (82.1)
Altered	7 (14.6)	13 (26.0)	6 (14.0)	8 (17.9)
Cholesterol				
Normal	25 (52.1)	29 (58.0)	24 (51.2)	25 (56.4)
Altered	23 (47.9)	21 (42.0)	22 (48.8)	19 (43.6)
HDL-Cholesterol				
Normal	47 (97.1)	45 (90.0)	44 (95.3)	43 (97.4)
Altered	1 (2.1)	5 (10.0)	2 (4.7)	1 (2.6)
Triglycerides				
Normal	24 (50.0)	22 (44.0)	33 (72.1)	27 (61.5)
Altered	14 (50.0)	28 (56.0)	13 (27.9)	17 (38.5)
Insulin				
Normal	44 (91.7)	42 (84.0)	37 (81.4)	30 (69.2)
Altered	4 (8.3)	8 (16.0)	9 (18.6)	14 (30.8)
Glycemia				
Normal	40 (83.3)	36 (72.0)	46 (100.0)	44 (100.0)
Altered	8 (16.7)	14 (28.0)	0 (0.0)	0 (0.0)
Total	48 (100.0)	50 (100.0)	46 (100.0)	44 (100.0)

Abbreviations: FLI: Fatty Liver Index; SGOT: Serum Glutamic-Oxalacetic Transaminase; SGPT: Serum Glutamic-PyruvateTransaminase; Gamma-GT: Gamma-Glutamyl Transferase; *,#Pearson $\chi2$ $p<0.05$. Median (Inter Quantile Range)

Table 3
Mediterranean Adequacy Index by Gender, Age and Month of the NutriEpa Trial

		Second Month			Third Month			Fourth Month			Fifth Month		
		Percentiles											
Males Age (years)	Diet	25	Median	75	25	Median	75	25	Median	75	25	Median	75
< 40	LGIMD	10.1	10.1	10.1	10.1	10.1	10.1	3.9	10.1	10.1	3.9	10.1	10.1
	CTR	2.4	8.5	10.4	2.4	4.9	8.5	2.4	4.9	8.5	2.4	4.9	8.5
40-59	LGIMD	7.4	11.8	17.2	7.4	11.8	17.2	6.8	11.0	16.0	6.8	11.0	16.0
	CTR	2.9	8.6	9.9	4.8	6.3	9.9	2.9	7.8	9.9	2.9	6.3	9.9
60 >	LGIMD	12.5	21.1	45.1	12.5	21.1	66.6	12.5	21.1	66.6	12.5	12.5	21.1
	CTR	1.5	3.3	6.4	1.5	3.3	6.4	2.1	3.3	6.4	2.1	3.3	6.4
Females Age (years)													
< 40	LGIMD	7.6	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
	CTR	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
40-59	LGIMD	7.0	8.5	9.5	7.0	8.5	10.1	7.0	8.5	10.1	7.0	8.5	10.1
	CTR	0.9	5.7	5.7	0.2	0.9	5.7	0.2	5.7	5.7	0.9	0.9	5.7
60 >	LGIMD	4.5	5.3	13.8	4.5	5.3	13.8	4.5	5.3	13.8	4.5	5.3	13.8
	CTR	8.9	10.1	15.1	8.9	10.1	15.1	8.9	10.1	15.1	8.9	10.1	15.1

Abbreviations: LGIMD, Low Glycemic Index Mediterranean Diet; CTR, Control.

Table 4
Effect of Mediterranean Diet on Stages of Non-alcoholic Fatty Liver Disease. NutriEpa, Putignano (BA), Italy 2011

	Coefficient	95%CI[1]	
LGIMD[2]	0.94	-1.34	3.21
Time (3rd Month)	1.21	-0.75	3.16
Time (6th Month)	-0.81	-2.85	1.23
LGIMD*Time (3rd Month)	-4.14**	-6.78	-1.49
LGIMD*Time (6th Month)	-4.43**	-7.15	-1.71
Age	0.01	-0.02	0.04
LGIMD*Age	-0.02	-0.06	0.02
Time (3rd Month)* Age	-0.03	-0.06	0.01
Time (6th Month)*Age	0.00	-0.03	0.04
LIGMD*Time (3rd Month)* Age	0.07**	0.02	0.12
LIGMD*Time (6th Month)* Age	0.08**	0.03	0.13
Gender (Female)	0.30	-0.09	0.70

1. 95% Confidence Interval; 2. Low Glycemic Index Mediterranean; Diet; **: p< 0.01; *: p< 0.05

Strengths and limitations

Some methodological issues need, however, to be considered. The strengths of this study include: the characteristics of the study subjects, who are drawn from a survey of a population sample, the statistically adequate sample size and the controlled nature of the diet intervention. Although a few subjects were lost in the follow-up stages, the small numbers involved do not give rise for concern. An intention-to-treat analysis was applied, thus there is no reason to assume that non-adherence to the protocol is related to prognosis; this RCT provides therefore an unbiased assessment of treatment efficacy (47). To control for the possible presence of residual confounding effects several covariates were included in the GEE model in an attempt to obtain more precise and valid estimates. The GEE approach estimates coefficients of the covariates considering the correlated response. Also predictive marginal averages, after adjustment, can be obtained by using this strategy which might be useful to describe mean differences along covariates of interest. Diagnosis of NAFLD was performed by LUS which has generally considerable sensitivity and specificity (48, 49), but may fail to detect hepatic fat content <25-30% (50), thus underestimating the actual liver fat infiltration, the effect of this non-differential misclassification (due to the randomization and blinding of the operator) could, however, only produce a bias toward the null (51).

Conclusions

This study (NutriEpa) compares a LGIMD with the standard national recommended diet and illustrates that a LGIMD is more effective than the standard diet in reducing NAFLD scores in subjects who do not seek medical attention. Other relevant findings include that changing dietary quality composition is a more realistic alternative to energy restricted diets and that dietary intervention at a relative young age may, as recently published, be part of primary prevention of T2DM and coronary heart disease (49).

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