EFFICIENCY OF TWO PHYSICAL ACTIVITY PROGRAMS ON NON-ALCOHOLIC FATTY LIVER DISEASE. A RANDOMIZED CONTROLLED CLINICAL TRIAL.

EFICIENCIA DE DOS PROGRAMAS DE ACTIVIDAD FISICA SOBRE LA ESTEATOSIS HEPATICA NO ALCOHOLICA. UN TRIAL CLINICO RANDOMIZATO.

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Conceptos clave:

A) Que se sabe sobre el tema: Lifestyle interventions are the only effective known measures to manage NAFLD.

B) Que aporta este trabajo: Our objective was to estimate the effectiveness of two physical activity program, based on the results of a Randomized Clinical Trial. We hypothesized that an aerobic program is more efficient in reducing fatty liver content and may be prescribed by the General Practitioner. It is easily implemented in any setting (outdoor and indoor) and it is less expensive than any other form of physical activity.

Abstract:

Introduction: To estimate the efficiency of two physical activity programs on NAFLD. Methods: Participants come from a survey conducted in southern Italy. Subjects with moderate or severe NAFLD were invited to participate. After giving informed consent, they completed a questionnaire, underwent ultrasonography and anthropometric measurements. Then they were randomized an Aerobic or a Combined Exercise program and followed up for six months. The first group followed a program of moderate aerobic activity lasting 30 minutes, 5 days per week. The second group did aerobic training with the addition of muscle training involving the large muscle groups, stimulating them to make more intense efforts, for a duration of 60 minutes, at least, 3 days a week. Compliance with the programs was measured. A mixed linear model was applied to the data. Results: Compliance with Aerobic Exercise was homogeneous and increased over time. Combined Program compliance was equal to 100%. There was no significant difference in the NAFLD mean score by treatment at baseline and after six months. However, there was a significant reduction in the NAFLD mean score for treatments after six months. The NAFLD measured score was reduced by 22% in the Aerobic treatment group when confronted with the other program. In the Combined program, after 6 months, results showed to be less effective than the Aerobic Exercise in reducing the NAFLD score. Conclusions: An aerobic exercise program is a realistic intervention which could be included as a part of primary prevention of several chronic diseases.

*Keywords***:** *liver steatosis; aerobic exercise; resistance training; clinical trial.*

Resumen:

Introducción: Estimar la eficiencia de dos programas de actividad física sobre la NAFLD. Métodos: Sujetos con NAFLD moderada o severa que habían participado a un estudio de pobación en el sur de Italia fueron invitados a participar. Fue completado un cuestionario, se tomaron medidas antropométricas y se realizó una ecografia hepática. Cada participante proveyó el consenso informato. Los participantes fueron aleatorizados a un programa aerobico (actividad aerobica moderada per 30 minutos, 5 veces a la semana) o un programa mixto (aerobico más entrenamiento de la fuerza de los grandes grupos musculares, 60 minutos, tres veces a la semana). Fue medida la adherencia a los programas y se aplicó un modelo linear mixto a los datos. Resultados: El programa aerobico tuvo muy buena adherencia y esta aumentó con el tiempo mientras el programa mixto tuvo 100% de adherencia. No hubo diferencias estadísticamente significativas en el score de NAFLD al enrolamiento, mientras hubo diferencias estadísticamente significativas en el score medio de NAFLD después de 6 meses pero el score en el grupo del programa aerobico tuvo una reducción del 22% más intensa que el programa mixto. Este último programa por lo tanto se ha mostrado menos efectivo que el programa aerobico. Conclusión: Un programa de actividad física aerobica es un tratamiento realístico que podría ser efectuado no solo para la NAFLD sino también como prevención primaria de otras enfermedades crónicas.

*Palabras clave***:** *esteatosis hepática; ejercicio aeróbico; entrenamiento de resistencia; ensayo clínico.*

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the Western World[1,2] It is considered the hepatic manifestation of metabolic syndrome and is commonly associated with obesity and diabetes[3]. Moreover, NAFLD is an independent risk factor for cardiometabolic disease[4]. Worldwide prevalence ranges between 20% and 33%. In northern Italy, the prevalence of NAFLD was found to be approximately 25% in the general population and associated with most features of metabolic syndrome[5]. Since there are few pharmacological agents available to reduce ectopic fat, lifestyle interventions have become a focus of research. Weight loss is the recommended management strategy. Weight loss via diet and physical activity reduces liver fat[6]. Nevertheless, a reduction of more than 3% of the body weight is needed to gain hepatic benefit, and greater weight loss leads to superior benefits[7]. Furthermore, several studies have shown that lifestyle modifications based on a reduced energy intake and/or increased physical activity induce improvements in biochemical and metabolic parameters, improve intrahepatic triacylglycerol concentrations and reduce steatosis and necro-inflammation, as detected in paired histologic analyses[8-10]. Both aerobic and resistance training-based exercise programs improve liver fat contents[11]. Several studies have shown that aerobic exercise programs of different intensity and duration, with or without weight loss, improve liver fat content[12]. On the other hand, also resistance training leads to a reduction of liver fat[13] and both approaches have recently been reported to be equally effective[14]. However, most studies have been hospital-based, conducted on highly selected patients. To estimate the prevalence of some liver diseases in this Mediterranean area of Southern Italy, a cohort was assembled[15] and a NAFLD prevalence of 24.6% was found. In addition, a sex-age specific NAFLD prevalence was observed. As most NAFLD patients are seen at the primary care setting the objective of this Randomized Clinical Trial (RCT) was to estimate the efficiency of two different Physical Activity Programs on the NAFLD score as measured by Liver Ultrasonography (LUS) in this free-living population resident in the Mediterranean Area.

Methods

Study design

This study is registered at www.clinicaltrials.gov NCTO1798719. NutriEpatt was a parallel-group randomized controlled clinical trial. The sampled population was drawn from the NutriEp survey, conducted at the National Institute of Gastroenterology, “Saverio de Bellis” Research Hospital (Castellana Grotte, Italy), from July 2005 to January 2007. Details have been published elsewhere[15]. 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 attending the GP clinics, a random sample was drawn from the GP patients’ lists: 2550 subjects were invited to participate in the survey. Of these subjects, 2301 (90.2%) gave written consent to take part.

Participant Selection

Subjects with NAFLD were identified during the NutriEp enrolment 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) Type 2 Diabetes Mellitus (T2DM) (current treatment with insulin or oral hypoglycaemic drugs, fasting glucose >126 mg/dl, or chance glucose values >200 mg/dl); 5) good physical condition and normal exercise tolerance test; 6) no other serious medical condition; 7) no pregnancy. Subjects were invited to participate in the trial, and after a new assessment of the severity of NAFLD using LUS, those who agreed to take part provided written informed consent. The trial was designed and conducted by the authors at the Laboratory of Epidemiology and Biostatistics of the National Institute of Gastroenterology, “Saverio de Bellis”, Research Hospital, Castellana Grotte (Italy), from March to December 2013 and data were analysed in 2016.

Randomization and Masking

Participants were randomly assigned, according to a computerized random numbers sequence, to one of the two treatment groups using a one-to-one ratio for allocation. Randomized group assignment was

issued on completion of the baseline assessments. Participants and exercise supervision personnel could not practicably be blinded to group assignments but the radiologists measuring the main study outcome were blinded. Each physical activity group was followed by an exercise supervisor, who was initially assigned on a random basis.

Sample Size

Sample size was estimated taking into account repeated measurements 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. Probabilistic errors type I and II were fixed at 5% and 10%, respectively. Then the power of the study reached 90%. The correlation between baseline/follow-up (FU) measurements of the outcome was set to 0.4. A minimum sample size of n1=n2=40 was estimated, to obtain a 1-point reduction in the NAFLD score after six months.

Intervention

Aerobic Exercise

Four 45-minute non-consecutive day sessions of moderate intensity aerobic activity (measured heart rate to ensure 65-75% VO2). Aerobic exercise intensity was monitored every 5 minutes using an automated heart rate monitor. Total weekly exercise duration: 180 minutes. The intervention was done outdoors or indoors. The outdoor structure was a communal circuit 3 km long located in Putignano, where it is possible to do physical activity.

Combined Exercise

Three non-consecutive day sessions including: a) 30 minutes of moderate intensity aerobic activity (measured heart rate to ensure 65-75% VO2). Aerobic exercise intensity was monitored every 5 minutes using an automated heart rate monitor; b) two sets of 11 exercises, each to volitional fatigue: leg press, adductor/abductor machine, gluteus machine, biceps curl, leg curl, triceps extension, three different abdominal exercises, lat machine, low row, shoulder flexion. Approximate duration of each session was about 40 minutes. The weight lifting was increased when 10 repetitions were completed in good form. Total weekly exercise duration: 210 minutes. The intervention was done at 4 gyms located in Putignano: Nadir, Inda Club, New Sporting House and GymEnjoy.

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[17] and dietary habits (European Prospective Investigation on Cancer and Nutrition, EPIC) were also probed. Subjects were invited to complete a four-day (including one week-end day) dietary sheet to estimate the actual dietary behavior and the energy intake. Participants also completed the International Physical Activity questionnaire-IPAQ[18]. On the second visit, a fasting venous blood sample was collected. Liver function tests and other biochemical serum markers were assessed using standard laboratory tests. At this point in time, the staff also reviewed (and completed if necessary) the EPIC questionnaire, taking the opportunity to highlight the advantages of following the physical activity programs.

Body weight, height, and blood pressure were measured in standard conditions. During this second visit, participants were randomly assigned to one of two Physical Activity Interventions. The purpose of the study was explained in detail in a face-to-face interview. Personal advice was also provided in both groups, at the baseline visit and monthly thereafter. At the second visit, participants were instructed by a nutritionist to maintain the calorie intake determined at baseline. The monthly FU visit also included a face-to-face interview with the exercise supervisor to assess the compliance with the Physical Activity Program assigned and to give, if needed, personal recommendations to achieve the “group assigned goal". During the baseline period, participants were instructed by the nutritionist to continue their diet in order to maintain the calorie intake, and to record the daily consumption of selected foods. All exposure, anthropometric, biochemical and outcome measures were repeated at the third and sixth month of follow-up. All intervention providers were highly expert and were specifically trained over two months before the trial. The Exercise Supervisors were specifically trained to reach the research goals as programmed. Nutritionists held meetings to tell participants not to change their dietary habits.

Fitness Evaluation

To evaluate the initial physical condition, the right training program and to compare the initial with the FU assessment of physical condition, three field tests were carried out: cardiorespiratory, strength and flexibility fitness. Cardio-respiratory fitness was assessed by means of the 2-km Walking test, suitable for adults[19] whereas strength and flexibility fitness were evaluated by means of the push-up test (also called press up test) [20] and the Sit and Reach test, respectively.[21] All tests were repeated at the third and sixth month of FU.

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[22]. NAFLD was then categorized as: absent (0), mild (1-2), moderate (3-4) and severe (5-6). A sub-sample of 30 subjects (ten subjects at enrolment, first and second FU, respectively, randomly chosen) underwent LUS by the two radiologists separately. An overall weighted Kappa of 0.9 was obtained. Only one of the 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.

Statistical Analysis

Statistical analysis was performed with Stata, version 14.1. The primary analysis was intention-to-treat. In this paper only socio-demographic, biochemical, BMI and Fitness Index (at baseline) data were considered. For descriptive purposes, age at enrolment (<40, 40-59 and ≥60 years old), BMI (Normal, Overweight, Obese), 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 UI/ml), Glucose (altered ≥ 127 mg/dl), physical activity (Low <3 METs, Moderate 3-6 METs and High >6 METs), Systolic Blood Pressure (high >130 mmHg) and Diastolic Blood Pressure (high >90 mmHg) were categorized. A composite indicator of Socio-Economic Position (SEP) was built. Cross-tabulations between interventions and socio-demographic, life-style and biological variables were performed and proportion differences test was applied. For the compliance to the physical activity programs (APAP), the four central months were assessed. The first, second, third, and fourth week of the second, third, fourth, and fifth month, respectively, were chosen to evaluate compliance. APAP was estimated as the proportion of effective gym or field sessions frequency/time/intensity in relation to the expected weekly frequency, time per session and intensity for the Aerobic Exercise group plus expected load for the Combined Exercise group. APAP was estimated by age-group, gender, and month, and expressed as percentage. A Mixed Linear Model was built for the NAFLD score (outcome). Gender (categorical), BMI, Fitness Index, Cholesterol, Triglycerides, HOMA-Test Insulin, Glucose, and Age (continuous) were included as covariates as well as an interaction between Treatment and Time. The results are expressed as estimated coefficient and 95% Confidence Interval (95% CI).

Patient involvement

After the assessment of NAFLD persistence at the first visit, subjects were invited to participate in small groups (6 subjects). In these groups they were informed by the exercise supervisor and nutritionist about the research question and outcomes measures. All included subjects had the opportunity to choose where to do their aerobic program (indoor/outdoor), how to do it (walking, cycling, treadmill walking) and on which week-days. They could choose the gym closest to their home. Participant subjects had the opportunity to monitor their performance each time.

During the trial, subjects otherwise participated at working groups each month to discuss about feelings, difficulties and all other aspects of their participation in the trial.

At the end of the trial, a workshop (subjects and operators) was organized and the results were communicated.

All experimental subjects participated on a voluntary basis, and the protocol was approved initially by the Technical-Scientific Review Board and then by the Institutional Ethics Committee of the National Institute of

Gastroenterology, "Saverio de Bellis" Research Hospital (DDG n° 207, 07/04/2010). All participants provided written consent according to the Helsinki Declaration.

Results

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

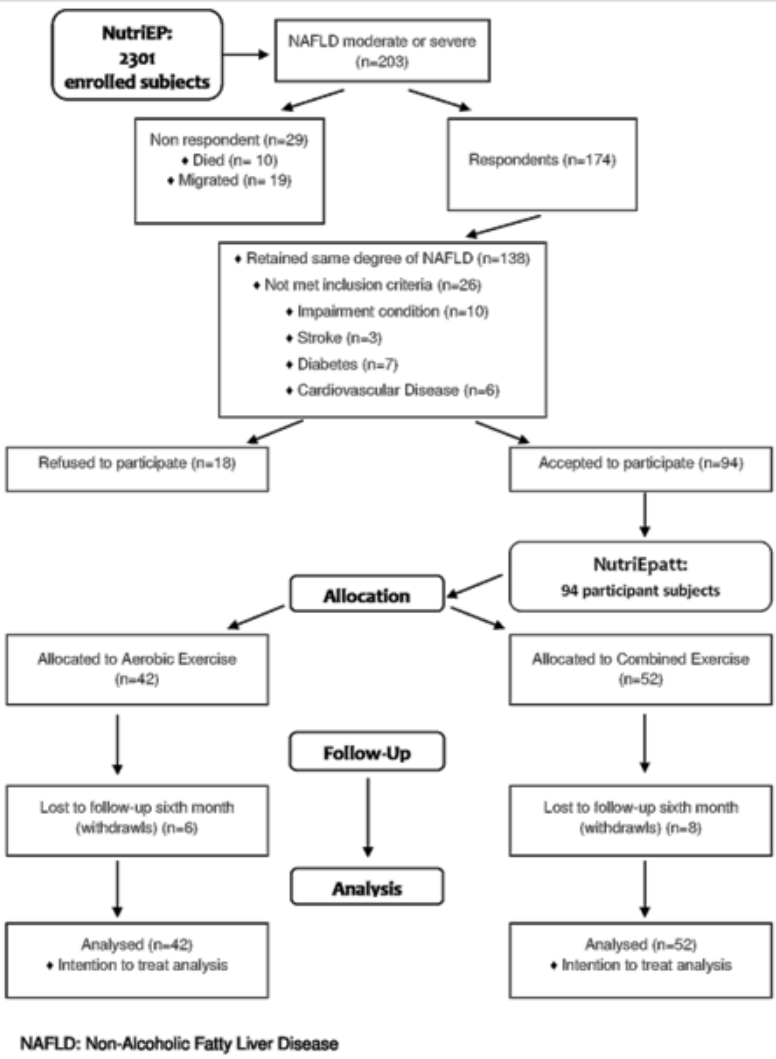

Figure N° 1. NutriEpatt Flowchart. Castellana Grotte (BA), Italy

Ninety-four individuals agreed to participate and were randomly assigned to the Aerobic Exercise (n=42, six lost to FU) or Combined Exercise (n=52, 8 lost to FU) group. ***Table 1*** shows socio-demographic and some phenotypic characteristics of subjects by treatment group.

Table N°1: Biological and socio-demographic characteristics, NAFLD and Fitness scores (at baseline) of subjects by treatment groups. NutriEpatt project, Putignano (BA), Italy, 2013.

	Treatment			
	Aerobic		Combined	
	No.	%	No.	%
Gender*				
Male	36	46.2	42	53.8
Female	6	37.5	10	62.5
Age(years)*				
<50	10	45.5	12	54.5
50-64	26	48.1	28	51.9
65 or >	6	33.3	12	66.7
Education*				

Illiterate	2	100.0	0	0.0
Elementary School	4	18.2	18	81.8
Middle School	12	46.2	14	53.8
High School	20	52.6	18	47.4
College	4	66.7	2	33.3
Job*				
Farmer	2	33.3	4	66.7
Worker	4	33.3	8	66.7
Employee	10	41.7	14	58.3
Trader	4	50.0	4	50.0
Freelance	6	75.0	2	25.0
Pensioneer	8	66.7	4	33.3
Housewife	4	40.0	6	60.0
Craftsman	4	28.6	10	71.4
Status*				
Single	2	100.0	0	0.0
Married	38	42.2	52	57.8
Widowed	2	100.0	0	0.0
SEP*				
Low	4	20.0	16	80.0
Medium	24	60.0	16	40.0
High	10	50.0	10	50.0
Body Mass Index*				
Overweight	14	58.3	10	41.7
Obesity I	18	37.5	30	62.5
Obesity II	10	45.5	12	54.5
NAFLD*				
Moderate	18	42.9	24	57.1
Severe	24	46.2	28	53.8
Fitness Score*				
Clearly below average	34	44.7	42	55.3
Slightly bellow average	4	33.3	8	66.7
On average	4	66.7	2	33.3
Total	42	44.7	52	55.3

*Proportion differences were not significant (α=0.05)

There were more men than women and most subjects were between 50 to 64 years old. An elevated percentage of people had finished high school education. Almost every participant was married, presented obesity and had a fitness score clearly below average. Men were mainly employees, freelance or pensioners, whereas women were mainly employees and artisans. Most participants belonged to the medium or low SEP. Approximately half the subjects had a severe NAFLD score. The Aerobic Exercise NAFLD mean score was 4.9, SE 0.17 at baseline and 2.8, SE 0.4 after six months. The Combined Exercise NAFLD mean score was 4.6, SE 0.12 at baseline and 3.13, SE 0.4 after intervention. There was no significant difference in the NAFLD mean score by treatment at baseline (p=0.22) and after six months (p=0.65). However, there was a significant reduction in the NAFLD mean score for treatments after six months (p< 0.01). About half the participants showed an altered HOMA-Test, SGPT, γ-glutamyl transpeptidase, Total Cholesterol and Systolic Blood Pressure. (Table 2).

Table N° 2: Biochemical characteristics (at baseline) of subjects by treatment groups. NutriEpatt project, Putignano (BA), Italy, 2013

	Treatment			
	Aerobic Exercise		**Mix Exercise**	
	No.	**%**	**No.**	**%**
HOMA Test (Baseline)*				
Normal	22	42.3	30	57.7
Altered	20	47.6	22	52.4
SGOT				
Normal	42	44.7	52	55.3
SGPT*				
Normal	34	43.6	44	56.4
Altered	8	50.0	8	50.0
Gamma-GT*				

Normal	36	46.2	42	53.8
Altered	6	37.5	10	62.5
Cholesterol*				
Normal	22	44.0	28	56.0
Altered	20	45.5	24	54.5
HDL-Cholesterol*				
Normal	38	47.5	42	52.5
Altered	4	28.6	10	71.4
Triglycerides*				
Normal	30	53.6	26	46.4
Altered	12	31.6	26	68.4
Systolic Blood Pressure (Baseline)*				
Normal	30	40.5	44	59.5
High	12	60.0	8	40.0
Diastolic Blood Pressure (Baseline)*				
Normal	42	44.7	52	55.3
Total	42	44.7	52	55.3

For males, compliance to Aerobic Exercise was homogeneous in terms of weekly frequency, expected time per session (increasing over time) and expected intensity. On the contrary, women presented a lower compliance mainly as regards the second item. Regarding the Combined Program, compliance was equal to 100% each month for all subjects and there were no trouble. (Table 3)

Table N° 3: Compliance with the physical activity program in the Aerobic Exercise group: percentage of the expected weekly frequency, time per session and intensity by sex and age. NutriEpatt project, Putignano (BA), Italy, 2013

		Frequency (%)		Expected Time (%)		Expected Intensity (%)	
		Male	Female	Male	Female	Male	Female
Age (yrs)	Month						
<55	2nd	100		76.7		99.4	
	3rd	91.7		77.5		97.4	
	4th	100		92.1		94.4	
	5th	100		96.1		97.9	
55-64	2nd	100	100	71.4	65.0	97.8	100
	3rd	100	100	88.7	90.0	96.8	98.6
	4th	100	66.7	77.8	66.7	85.4	64.5
	5th	100	83.3	90.0	83.3	90.2	81.7
>64	2nd	100		60.0		100	
	3rd	100		80.0		100	
	4th	90.2		78.2		88.8	
	5th	100		87.3		96.5	

A complete factorial structure for treatments and time (main and interaction effects) was included in the model, adjusting for gender, age, BMI and biochemical covariates. The Aerobic Exercise program was chosen as baseline. Even though the joint effect between treatment and time was significant, the strong main effect of the latter was noteworthy. After 6 months of physical activity, the measured NAFLD score was reduced, on average, by 22%. The Combined program after 6 months was less effective than Aerobic Exercise in reducing the NAFLD score (the interaction term estimate was positive and equal to 0.5749, p=0.033). These results are displayed in ***Figure 2.***

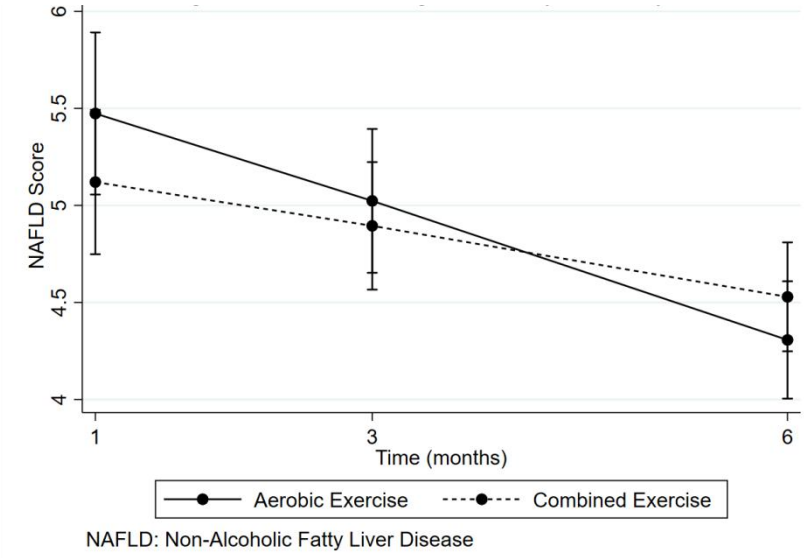

Figure N° 2. Effect of Two Programms of Physical Activity on NAFLD

The mixed model presented an improvement of the estimation process at the linear regression model (p<0.001 for the likelihood ratio test, ***Table 4***).

Table N° 4: Results from the mixed linear regression model of the NAFLD score (outcome): estimates of physical activity programs and time effects. NutriEpatt project, Putignano (BA), Italy, 2013.

	Coefficients	**Standard Error**	**p value**	**95%CI**
Combined Program (Aerobic)	-0.353	0.281	0.211	-0.905 0.199
Time				
3 months	-0.450	0.185	0.015	-0.813 -0.086
6 months	-1.166	0.216	0.001	-1.591 -0.741
Treatment*Time				
Combined-3 months	0.224	0.238	0.347	-0.243 0.692
Combined-6 months	0.574	0.270	0.033	0.045 1.104
Gender				
Female (Male)	-0.781	0.228	0.001	-1.229 -0.332
Age (continuous**)**	0.012	0.009	0.177	-0.005 0.030
BMI (continuous**)**	0.168	0.021	0.001	0.126 0.209
Random effects Parameters	Estimate	Standard Error		95%CI
Subject cluster				
Var(Time)	0.017	0.007		0.008 0.040

LR test vs. linear model: chi2(3) = 69.24; Prob > chi2 = 0.0000. Adjusted for HOMA Test, Gamma GT, Tryglicerides and Cholesterol; 95%CI: 95% Confidence Interval

A high proportion of the outcome variability was captured by this model (conditional intra-class coefficient).

Discussion

In this RCT, the Aerobic Exercise program was associated with a more intense reduction of the NAFLD score than the Combined Exercise program, measured by LUS. It indicates that 45 min, four times a week, of moderate-intensity walking (75% VO2 max obtained from HRM) results in a significant decrease (22%) of the NAFLD mean score.

Regular physical activity is now a therapy to prevent the onset and progression of several chronic diseases including NAFLD[23]. As the efficacy of exercise is now acknowledged, research has focused on the optimal type, duration, frequency and intensity of physical activity training and most studies have conformed to doses recommended by the American College of Sport Medicine[12]. Exercise influences hepatic metabolism and the adoption of either aerobic or strength exercise regimes results in a reduction of hepatic fat accumulation, increased insulin sensitivity and fat oxidation, even without weight loss[24]. Aerobic exercise seems to increase the intracellular synthesis of triglycerides at muscle level, decrease fatty acids metabolites accumulation and suppress the inflammatory state associated with insulin resistance[25]. Although the risk of developing NAFLD or more advanced grades of liver diseases seems to be exercise dose-related[26], recently no differences among different intensity aerobic exercise regimens were found: all of them reduced liver fat[14]. Resistance exercise seems to have a beneficial effect on NAFLD by enhancing circulating fatty acids and glucose uptake. Thus, it reduces the impact of hepatic insulin-stimulated de novo lipogenesis[24]. The efficacy of resistance training is not as clear as after aerobic exercise but still reduces liver fat[27]. Recently, a report has been published stating that, at the community level, a combined exercise program is effective in reducing liver fat in NAFLD patients, with an effect that is proportional to the degree of weight loss but can occur even without it. Moreover, several effect modifiers such as age, sex and ethnicity have been suggested as high-risk factors in the general population[27]. This work aimed to estimate the effectiveness of two different programs of physical activity in this Mediterranean area, at population level. The Aerobic Exercise program was more effective in reducing liver fat as measured by LUS. It is noteworthy that both programs may be associated with considerable health benefits because they do not only reduce liver fat content but also contribute to improve many health-related life domains[28].

This study has several strengths, namely the characteristics of the study subjects, drawn from a population sample survey; the statistically adequate sample size and the controlled nature of the physical activity intervention. A few subjects were lost over the FU stages. An intention-to-treat analysis was applied, so there is no reason to assume that deviation from the protocol is related to prognosis[29]. To control for residual confounding, several covariates were included in the linear mixed model in order to obtain more precise and reliable estimates. The diagnosis of NAFLD was made with LUS, which generally features considerable sensitivity and specificity[30] but may fail to detect hepatic fat content <25-30%, thus underestimating the actual liver fat. The effect of this non-differential misclassification could, however, only produce a bias toward the null. Moreover, other diagnostic methods such as liver biopsy, proton magnetic resonance spectroscopy or computed tomography are impossible in this setting for ethical or economic reasons. The NutriEpatt study compares the efficiency of an Aerobic Exercise with a Combined Exercise program and shows that the Aerobic Exercise program is more effective in reducing NAFLD scores in subjects who do not seek medical attention. This finding is relevant, since Aerobic exercise programs are simple to implement and the cost-efficiency is higher. In addition, an Aerobic exercise program is a realistic intervention which could be included as a part of primary prevention of metabolic and cardiovascular diseases.

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