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A “healthy diet-optimal sleep” lifestyle pattern is inversely associated with liver stiffness and insulin resistance in patients with non-alcoholic fatty liver disease

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Abstract

Objectives: Several lifestyle habits have been described as risk factors for non-alcoholic fatty liver disease (NAFLD). Given that both healthy and unhealthy habits tend to cluster, the aim of this study was to identify lifestyle patterns and explore their potential associations with clinical characteristics of individuals with NAFLD.

Methods: One hundred-thirty-six consecutive patients with ultrasound-proven NAFLD were included. Diet and physical activity level were assessed through appropriate questionnaires. Habitual night sleep hours and duration of midday naps (siesta) were recorded. Optimal sleep duration was defined as sleep hours ≥ 7 and ≤ 9 hours/day. Lifestyle patterns were identified using principal component analysis.

Results: Eight components were derived explaining 67% of total variation of lifestyle characteristics. Lifestyle pattern 3, namely high consumption of low-fat dairy products, vegetables, fish and optimal sleep duration was negatively associated with insulin resistance ($\beta = -1.66$, $P = 0.008$) and liver stiffness ($\beta = -1.62$, $P = 0.05$), after controlling for age, sex, BMI, energy intake, smoking habits, adiponectin and tumor necrosis factor- α . Lifestyle pattern 1, namely high consumption of full-fat dairy products, refined cereals, potatoes, red meat and high television viewing time was positively associated with insulin resistance ($\beta = 1.66$, $P = 0.005$), although this association was weakened after adjusting for adiponectin and tumor necrosis factor- α .

Conclusion: A “healthy diet-optimal sleep” lifestyle pattern was beneficially associated with insulin resistance and liver stiffness, in NAFLD patients, independently of body weight status and energy intake.

Keywords: NAFLD; insulin resistance; lifestyle pattern; sleep; diet.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered as the hepatic manifestation of the metabolic syndrome (Marchesini et al. 2001) with insulin resistance representing the basic underlying pathophysiological mechanism. Caused by fat accumulation in the liver (liver fat > 5% of liver weight), which is not due to excess alcohol consumption or other causes of steatosis, NAFLD is nowadays the most common cause of chronic liver diseases in Western countries (Ratzliff et al. 2010), a consequence of the rising prevalence of obesity (Masarone et al. 2014).

Several lifestyle factors can affect the risk for developing NAFLD, such as positive energy balance, unhealthy food choices and physical inactivity (EASL–EASD–EASO 2016). Moreover, epidemiological studies suggest that cigarette smoking is associated with NAFLD development (Liu et al. 2013; Zein et al. 2011), although results are still conflicting, especially in regard to its role in NAFLD progression (Yilmaz et al. 2010; Zein et al. 2011). Regarding dietary habits, mounting evidence supports associations between individual food groups and NAFLD risk or presence (Marchesini et al. 2015), but data from dietary patterns analyses, consisting a more holistic approach of dietary intake estimation, are rather limited. Results from *a posteriori* analysis suggest that high adherence to high-carbohydrate sugary load dietary patterns is associated with increased likelihood of having NAFLD (Jia et al. 2015), whereas higher adherence to a healthy pattern, characterized by fruits, vegetables and other high-fiber foods, is inversely associated with NAFLD presence (Adriano et al. 2016; Yang et al. 2015). Similarly, data from *a priori* analyses have revealed potential benefits of the adherence to the Mediterranean diet both for the prevention and the treatment of NAFLD (Abenavoli et al. 2014). In specific, higher adherence to the Mediterranean dietary pattern has been associated with less severe

NAFLD (Kontogianni et al. 2014), lower likelihood of having metabolic syndrome in individuals with NAFLD (Georgoulis et al. 2015), whereas according to a randomized cross-over clinical trial, adherence to the Mediterranean diet improved liver fat content, insulin levels and insulin sensitivity (Ryan et al. 2013).

Furthermore, other behaviors such as physical activity and sleep habits have been also suggested to affect NAFLD risk or severity. NAFLD patients seem to spend more time on sedentary activities (Hallsworth et al. 2015; Ryu et al. 2015) and to have a lower level of physical activity compared to healthy controls (Ryu et al. 2015). Regarding sleep disturbances, they have been linked to increased risk for obesity (Nielsen et al. 2011), insulin resistance (Mesarwi et al. 2013) and cardiometabolic risk (Badran et al. 2015), whereas studies exploring the effect of sleep habits on NAFLD risk are limited, relating both sleep duration and quality with increased risk of the disease (Bernsmeier et al. 2015; Kim et al. 2013).

Principal component analysis (PCA) is a multivariate statistical method used to identify potential patterns by reducing the dimensionality and variation of the analyzed variables (Holand 2008). By clustering variables that co-vary, instead of analyzing independent associations of individual lifestyle items, they form a component that can be associated with several characteristics of the study's sample (Holand 2008). Thus, the aim of this cross-sectional study was to identify clustering of several lifestyle behaviors and to explore their potential associations with clinical and laboratory characteristics of NAFLD patients.

Materials and methods

Study sample consisted of 136 consecutive adult (19–72 years old) patients with newly diagnosed NAFLD (within 6 months). All patients visited one of two outpatient liver clinics (Academic Department of Gastroenterology, Laiko General Hospital of Athens or 2nd Academic Department of Internal Medicine, Hippokration General Hospital of Athens, Greece) between May 2013 and December 2015. Definition of NAFLD was based on the following criteria: elevated alanine aminotransferase (ALT) and/or gamma-glutamyltranspeptidase (GGT) levels, evidence of hepatic steatosis on ultrasound and/or compatible liver histology and no other causes of liver injury or steatosis. Patients with positive serological markers for hepatitis B (HBsAg), hepatitis C (anti-HCV) and human immunodeficiency virus (anti-HIV), weekly alcohol consumption more than 210 g for men or 140 g for women, use of potentially hepatotoxic or steatogenic agents (i.e., amiodarone, valproate, glucocorticosteroids, tamoxifen, methotrexate and other chemotherapeutic agents), evidence of metabolic or autoimmune liver disease, diabetes or presence of known systemic disease with potential liver involvement were excluded. Participants who had changed their eating habits since NAFLD diagnosis or were on a weight loss diet were also excluded from the study. Data for HBsAg, anti-HBc, anti-HBs, anti-HCV, anti-HIV and liver autoantibodies (anti-nuclear, anti-smooth muscles, anti-microsomal, anti-mitochondrial) were retrieved from medical records. History of alcohol intake was taken from the patients and was confirmed by the patients' relatives or friends. Patients, after their initial evaluation, were encouraged to participate in a randomized controlled clinical trial, aiming at evaluating the potential benefit of the Mediterranean diet or the Mediterranean lifestyle compared to usual care ([ClinicalTrials.gov ID: NCT01894438](https://clinicaltrials.gov/ct2/show/study/NCT01894438)).

The study was approved by the Ethics Committees of “Laiko General Hospital of Athens” and of “Harokopio University” and was also carried out in accordance with the Declaration of Helsinki (Helsinki 1997).

Lifestyle assessment

Participants’ dietary habits over the last six months were assessed through a semi-quantitative 69-item food frequency questionnaire (FFQ) validated for the Greek population (Bountziouka et al. 2012). Based on this FFQ, consumption of main food groups, namely total, low fat and full fat dairy products; total, refined and non-refined cereals; red meat, poultry, fish, legumes, vegetables, fruits, sweets, soft drinks and nuts were calculated in servings per day. Servings were determined based on the dietary guidelines for Greek adults published in 1999 (Greece 1999). Dietary intake was also assessed through two non-consecutive 24-h dietary recalls, which were analyzed in terms of energy, macronutrient and micronutrient intakes using Nutritionist Pro version 2.2 (Axxya Systems, USA).

Assessment of physical activity (PA) level was based on a validated for the Greek population questionnaire (Athens Physical Activity Questionnaire in Greek adult- APAQ) (Kavouras et al. 2016). Total volume of activity was determined by calculating total metabolic equivalent of task (MET) min per day (MET-min/day) (Kavouras et al. 2016). Mean television viewing hours (TV hours/day) was also extracted and was used as an estimate of sedentary behaviors.

Habitual total night sleep hours, as well as duration of midday naps (siesta), were self reported and recorded both for weekdays and weekend days. Short duration siesta was defined as midday sleep or resting (≤ 1 hour/day) and optimal sleep duration was defined as sleep hours ≥ 7 and ≤ 9 hours/day (Mukherjee et al. 2015).

Information regarding smoking habits and routine medication use was also recorded. Participants were also asked to report their marital status.

Anthropometric assessment

Body weight was measured with a digital scale (Seca Robusta 813, Hamburg, Germany) to the nearest 100 g, and height with stadiometer to the nearest 0.5 cm. Body mass index was calculated as weight (kg) divided by height squared (m^2). Waist circumference was tape measured to the nearest 0.1 cm. Increased WC was defined as >102 cm for men and >88 cm for women.

Biochemical and inflammatory markers

Blood samples of patients were collected after an overnight fast (12-hours in total) on the same day that dietary, physical activity and sleep assessment was performed. Serum and plasma were isolated and immediately frozen at -80°C . Glucose, total cholesterol and high density lipoprotein cholesterol (HDL-C) were measured using enzymatic colorimetric method (Cobas® 8000 analyzer, Roche), and insulin levels with chemiluminescence method (E170 modular analyzer, Roche). The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the formula by Matthews et al. (Matthews et al. 1985). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (Friedewald et al. 1972). Triglycerides, ALT, aspartate aminotransferase (AST), and GGT were measured with chromatometric enzymatic method (Cobas® 8000 analyzer, Roche). Tumor necrosis factor- α (TNF- α) and adiponectin were measured by sensitivity enzyme-linked immunosorbent assay (ELISA, Quantikine/immunoassay kit, R&D Systems, Minneapolis, MN, USA).

Shear wave elastography

Reliable liver stiffness measurements (LSM) (in kPa) were performed in 110 of the 136 total patients by 2-dimension shear wave elastography of the liver. LSM were operated using the Aixplorer[®] MultiWave[™] ultrasound system (Supersonic Imagine S.A., Aix-en-Provence, France) with the single crystal curved probe at frequencies 1-6 MHz. The examination was considered to be reliable if at least 5 valid measurements were obtained based on good sonographic signal, combined with <25% ratio of SD to the mean LSM (mean value of all valid measurements per examination). Patients were categorized based on the LSM results, as reviewed by Abevanoli et al. (Abevanoli and Beaugrand 2012) into those with $LSM \geq 7$ and $LSM < 7$ kPa.

Statistical analysis

The normality of the data was assessed using Shapiro-Wilk test and graphically through histograms. Qualitative variables are presented as mean values $\pm$ standard deviation or median (interquartile range, 25th-75th percentile) depending on their normality, whereas categorical variables are presented as absolute frequencies. Comparisons between food groups intake were made using the student t test.

Lifestyle patterns were identified using principal components analysis (PCA) (Cureton E 1983). The Kaiser-Meyer-Olkin (KMO) criterion was applied to ensure intercorrelations between the entered variables and pattern analysis. Furthermore, the orthogonal rotation (varimax option) was used to derive optimal non correlated components (lifestyle patterns). The information used was rotated to increase the representation of each food group/lifestyle pattern to a component. The selection of the retained components was based on the proportion of the variance explained by the lifestyle components used. Particularly, eight components were retained explaining 67% of the total variation in lifestyle characteristics examined. The lifestyle

components (patterns) were characterized according to the scores of dietary/lifestyle variables correlated most with the component (scores >0.40).

Predominant lifestyle components were thereafter divided into tertiles according to principal component scores (i.e. low, medium and high adherence to each tertile), reflecting the degree of adherence to the lifestyle pattern. To further assess all associations across tertiles of lifestyle patterns and biochemical characteristics of NAFLD patients, the Jonckheere-Terpstra test was used (Ali et al. 2015).

Multiple linear regression analysis was used to estimate all relevant associations between insulin resistance, liver outcomes (i.e. ALT, AST, GGT and liver stiffness) and lifestyle patterns derived from the PCA, after adjustment for several confounders, including age (continuous), sex (male vs. female), BMI (continuous), energy intake (continuous), smoking (yes vs. no), adiponectin (continuous) and TNF- α (continuous). Log-transformation of dependent variables, i.e. HOMA-IR and LSM, was made for achieving normality distribution. The results from the regression models are presented as standardized beta coefficients which were antilogarithm-transformed.

All reported P values were based on two-sided tests and compared to a significance level of 5%. Statistical Package for Social Sciences software (version 21.0, SPSS 2012, Chicago, Illinois, USA) was used for all the statistical analyses.

Results

Demographic, dietary, lifestyle and biochemical characteristics of NAFLD patients are shown in **Table 1**. The majority of the participants were men, obese and with

increased WC. Only 63.2% of patients reported optimal sleep duration (i.e. ≥ 7 and ≤ 9 hours/day). Regarding food groups, patients were consuming more servings of refined than non-refined cereals per day ($P < 0.001$) and more red meat compared to poultry or fish (all $P < 0.001$) per day. Concerning biochemical characteristics, patients, as expected, had increased liver enzymes. The median of LSM in the sample was 6.8 (5.3-8.3) kPa and 42.7% had $\text{LSM} \geq 7$ kPa.

Principal component analysis extracted eight components explaining 67% of the total variation in lifestyle characteristics examined [**Table 2**; in bold are the coefficients with the highest correlation (> 0.40) within the component]. The KMO criterion was 0.571 suggesting a strong intercorrelation between the variables used in the analysis. The corresponding components, characterising each pattern, are described below: 1 = Full fat dairy products, refined cereals, potatoes, red meat, TV hours/day; 2 = Fruit, vegetables, coffee, nuts; 3 = Low fat dairy products, vegetables, fish, optimal sleep duration; 4 = Legumes, poultry, less optimal sleep hours; 5 = Total MET-min/day, short duration siesta; 6 = Non refined cereals, coffee; 7 = Sweets; 8 = Smoking.

From the lifestyle patterns derived, only adherence to two of them (i.e. lifestyle component 1 characterised as “unhealthy diet-sedentary behavior lifestyle pattern” and lifestyle component 3 characterised as “healthy diet-optimal sleep lifestyle pattern”) was associated with several patients’ characteristics as shown in **Table 3**. In specific, a negative significant trend was found for HDL-C and adiponectin levels and a positive one for ALT levels, while moving to greater adherence to lifestyle pattern 1. Unexpectedly, higher adherence to this pattern was also associated with a negative trend in GGT levels ($P = 0.019$). On the contrary,

higher scores of lifestyle pattern 3 were associated with inverse trends in insulin levels and LSM (all $P \leq 0.05$).

Potential associations between lifestyle patterns and insulin, HOMA-IR, liver enzymes (ALT and GGT), HDL and adiponectin, as well as liver stiffness, were further explored using multivariate regression analysis, after controlling for potential confounders, namely age, sex and BMI, energy intake and smoking, as well as adiponectin and TNF- α (**Table 4**). Lifestyle component 3 (i.e., healthy diet-optimal sleep lifestyle pattern), was negatively associated with HOMA-IR (standardized $\beta = -1.66$, $P = 0.008$) and LSM (standardized $\beta = -1.62$, $P = 0.05$), after controlling for the aforementioned confounding factors. Lifestyle component 1 (i.e. unhealthy diet-sedentary behavior lifestyle pattern), was positively associated with HOMA-IR (standardized $\beta = 1.66$, $P = 0.005$), however this association was weakened after adjusting for inflammation indices, i.e., adiponectin and TNF- α levels ($p = 0.14$) (**Table 4**, Model 3), while adiponectin levels were inversely associated with insulin resistance in this model (standardized $\beta = -2.39$, $P < 0.001$). Using binary logistic regression analysis, lifestyle components were not found to be significantly associated with more severe disease, as depicted by liver stiffness ≥ 7 kPa (data not shown). Moreover, when patients were categorized as having or not insulin resistance (defined as $\text{HOMA-IR} \geq 3$), adherence to lifestyle component 3 tended to be negatively associated ($\text{OR} = 0.67$, $P = 0.13$), whereas lifestyle component 1 was not associated ($P = 0.61$) with the likelihood of having insulin resistance, after controlling for several confounders (i.e. age, sex, BMI, energy intake, smoking, adiponectin and TNF- α).

A negative association was also found between HDL-C (standardized $\beta = -2.29$, $P < 0.001$) and component 1, after controlling for age, sex and BMI, energy intake and smoking, as well as adiponectin and TNF- α . The negative trend between

component 1 and adiponectin was lost after controlling for age and sex, whereas for GGT after controlling for age only. No other associations were found between these two or the rest of the PCA components and any other assessed characteristics (data not shown).

Discussion

In this cross-sectional study, lifestyle patterns and their potential associations with clinical and laboratory characteristics of NAFLD patients were examined. Using PCA analysis, the derived lifestyle pattern 3, characterized as “healthy diet-optimal sleep lifestyle pattern” (i.e. low fat dairy products, vegetables, fish and optimal sleep duration), was negatively associated with liver stiffness and insulin resistance, independently of BMI and energy intake and after adjusting for specific confounding factors.

Evidence exploring associations between *a posteriori* driven patterns and NAFLD characteristics, namely insulin resistance or liver stiffness, as estimated by transient elastography, is sparse. Adriano et al. showed an inverse association between a healthy pattern, rich in vegetables/legumes, white meat, olive oil, margarine and bread/toast and NAFLD presence in the elderly (Adriano et al. 2016). Additionally, a study from a Chinese population (Yang et al. 2015) concluded that adherence to a pattern high in coarse grains, tubers, vegetables, mushroom and kelp/seaweed, cooked meat and beans is associated with lower risk of NAFLD. Other studies using *a priori* analysis have associated greater adherence to the Mediterranean dietary pattern with lower insulin resistance and severity of steatosis (Kontogianni et al. 2014), as well as with lower odds of having metabolic syndrome (Georgoulis et al. 2015). Furthermore, adherence to healthy dietary patterns has been linked to better insulin sensitivity in

samples of middle-aged women, young adults or older individuals (Anderson et al. 2012; Deshmukh-Taskar et al. 2009; Esmailzadeh et al. 2007).

Moreover, in the healthy dietary pattern derived in this study, all the foods included (i.e. low fat dairies, vegetables and fish) are in general foods with anti-inflammatory and antioxidants properties (Sears 2015) that have been previously linked to lower insulin resistance (Rideout et al. 2013; Torris et al. 2016) and lower likelihood of NAFLD (Finelli and Tarantino 2012; Zelber-Sagi et al. 2007). Bearing in mind that lifestyle modifications remain the cornerstone therapy for treating NAFLD, healthy dietary patterns like the one emerged in the current study or the Mediterranean diet could also be integral parts of NAFLD treatment (Abenavoli et al. 2014).

The present study also advocates that a healthy dietary pattern combined with optimal sleep duration (i.e. ≥ 7 and ≤ 9 hours/day) (component 3) is associated with favorable characteristics of NAFLD patients, independently of BMI and energy intake. Evidence regarding the association between sleep habits and the development of NAFLD is limited, let alone its synergistic effect with diet. A recent study (Kim et al. 2013) concluded that short sleep duration (≤ 5 hours vs. > 7 hours) has been associated with increased risk of NAFLD, but according to authors this association remained only in men, after BMI was taken into account. Additionally, in the study of Imaizumi et al. short sleep duration (≤ 6 hours vs. 6 to ≤ 7 hours) was linked to increased risk of NAFLD only in Japanese women and this association was lost after BMI adjustment (Imaizumi et al. 2015). Daytime sleepiness has also been associated with insulin resistance and histologic features of NAFLD (Bernsmeier et al. 2015). Reduced sleep time can lead to increased food intake through leptin and ghrelin regulation (Copinschi et al. 2014), and through increasing preference for high-

energy foods with rich carbohydrate content, including sweets, salty snacks and starchy foods (Dashti et al. 2015). Sleep deprivation seems to share common metabolic pathways with the stress system by activating hypothalamic-pituitary-adrenal (HPA) axis (Hirotsu et al. 2015). Finally, excessive daytime sleepiness reduces people's engagement in physical activity and increases engagement in sedentary behaviors (Zimberg et al. 2012).

In this study, lifestyle pattern 1, characterized as “unhealthy diet-sedentary behavior” lifestyle pattern (i.e. full fat dairy products, refined cereals, potatoes, red meat consumption and TV hours/day), was also positively associated with insulin resistance, but this association became marginally insignificant after controlling for adiponectin and TNF- α levels, while adiponectin levels were inversely associated with insulin resistance in this model. Therefore, one could speculate, that the inverse association initially found between lifestyle pattern 1 and insulin sensitivity was reflecting higher inflammatory load, given that participants with high adherence in this lifestyle pattern had significantly lower adiponectin levels and tended to have higher TNF- α levels (Table 3). Indeed, full fat dairy products, refined cereals, potatoes and red meats have been constantly linked to higher inflammation indices (Azadbakht and Esmailzadeh 2009; Buyken et al. 2010; Kontogianni et al. 2006; Ley et al. 2014), whereas it has been shown that high sedentary behavior is associated with a worse inflammatory profile (Henson et al. 2013; Leon-Latre et al. 2014). Moreover, the inverse association between adiponectin levels and insulin resistance that was revealed in regression model 3 was anticipated given the pathophysiology of NAFLD. According to current knowledge, insulin resistance and adipocyte dysfunction are the initial hits of NAFLD onset (Buzzetti et al. 2016), whereas imbalance of adipocytokines (e.g. low adiponectin, high TNF- α) may also result in the development

of insulin resistance and NAFLD (Polyzos et al. 2010b), by playing a crucial role in the regulation of glucose and lipid metabolism and in the control of inflammation in insulin-sensitive tissues (Polyzos et al. 2010a).

Regarding smoking habits these were not associated with variables related to NAFLD in this study. Data from experimental studies suggest that smoking prompts oxidative stress-mediated hepatocellular apoptosis, implicating liver injury (Azzalini et al. 2010). Nevertheless, the effects of active or passive smoking on NAFLD development and progression are still controversial and more studies are needed to better clarify this association (Liu et al. 2013; Yilmaz et al. 2010; Zein et al. 2011).

The present study has some limitations. In specific, this is a cross-sectional study and thus it is not suitable for cause-effect relationships. Moreover, PCA analysis method depends on several subjective decisions that should be made by investigators during the extraction of the components. Furthermore, the population examined in the present study has a specific genetic and cultural profile and thus results should be confirmed to other populations as well. However, by clustering lifestyle variables that co-vary using PCA approach, instead of analyzing independent associations of individual lifestyle items, we identified interpretable lifestyle patterns, associated with NAFLD characteristics which could be used for the prevention of the disease.

The present study supports that higher adherence to a healthy lifestyle pattern, characterized by high consumption of low fat dairy products, vegetables and fish, as well as by optimal sleep duration, is associated with lower insulin resistance and liver stiffness in a sample of NAFLD patients, independently of body weight status and energy intake.

Conflict of interest

Authors have no conflicts of interest to declare. Christina N. Katsagoni has received a Fellowship of Excellence for Post-graduate Research in Greece - Siemens Program, awarded by the State Scholarships Foundation (I.K.Y.).

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Table 1: Descriptive characteristics of patients with non-alcoholic fatty liver disease (NAFLD).

Variables	Total sample (n=136)
Age (years)	47.0 ± 11.6
Sex (men), n (%)	91, (66.9)
Body Mass Index (kg/m ²)	30.6 (27.18-32.83)
Increased waist circumference ^a , n (%)	92 (67.6)
Marital status Married, n (%)	 95, (69.9)
Smoking (yes), n (%)	34, (25)
<i>Physical activity</i>	
Total MET-min/day	1981 (1801-2205)
Television viewing hours/day (hours)	3.0 (2.0-4.2)
<i>Sleep habits</i>	
Short duration siesta (yes) ^b , n (%)	105, (77.2)
Optimal Sleep Duration ^c , n (%)	86, (63.2)
<i>Dietary habits</i>	

Energy intake (Kcal/day)	1797 (1486-2225)
Full fat dairy (serv./day)	1.12 (0.28-3.06)
Low fat dairy (serv./day)	0.92 (0.15-1.9)
Refined cereals (serv./day)	4.48 (2.55-7.65)
Non refined cereals (serv./day)	0.21 (0.0-1.14)
Red meat (serv./day)	1.47 (0.91-2.26)
Poultry (serv./day)	0.52 (0.24-0.67)
Fish (serv./day)	1.64 (0.85-2.69)
Legumes (serv./day)	0.52 (0.28-0.52)
Vegetables (serv./day)	2.42 (1.78-3.71)
Fruits (serv./day)	1.64 (0.85-2.69)
Potatoes (serv./day)	0.43 (0.22-0.86)
Sweets (serv./day)	1.28 (0.57-2.21)
Soft drinks (serv./day)	0.0 (0.0-0.12)
Nuts (serv./day)	0.18 (0.0-0.36)
Coffee consumption (times/day)	1.50 (1.0-2.5)
<i>Biochemical characteristics</i>	
HOMA-IR	3.0 (1.8-4.3)

Total Cholesterol (mg/dL)	204±40.8
Triglycerides (mg/dL)	119 (90-171)
HDL-C (mg/dL)	35.0 (23.7-47.2)
LDL-C (mg/dl)	140±40.8
ALT (IU/L)	58 (44-86)
AST (IU/L)	34.0 (26-44)
GGT (IU/L)	57.0 (35-124)
TNF-a (pg/mL)	4.1 (2.1-6.0)
Adiponectin (µg/mL)	4.7 (3.2-8.2)

Data are presented as mean ± SD or median (interquartile range) or otherwise mentioned.

^a >102 cm for males & > 88 cm for females, ^b Midday sleep or resting ≤1 hour/day, ^c ≥7 and ≤9hours/day

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase; HDL-C: high-density lipoprotein-cholesterol; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; LDL-C: low-density lipoprotein-cholesterol, METs: Metabolic equivalent of task(s), NAFLD: non-alcoholic fatty liver disease, Serv./day: servings per day according to dietary guidelines for Greek adults, TNF-a: tumor necrosis factor-a.

Table 2: Score coefficients derived from principal components analysis (PCA) regarding dietary/lifestyle patterns of NAFLD patients^a.

	Component							
	1	2	3	4	5	6	7	8
Full fat dairy products	0.819	0.113	0.001	-0.040	0.045	-0.041	-0.170	0.036
Low fat dairy products	-0.199	0.231	0.771	-0.040	-0.068	-0.042	0.150	-0.148
Non refined cereals	-0.096	0.081	0.181	0.179	0.029	0.818	-0.178	-0.059
Refined cereals	0.887	0.118	-0.032	-0.054	0.035	0.019	-0.108	-0.022
Fruit	-0.031	0.791	0.173	0.177	-0.009	-0.013	-0.077	-0.211
Vegetables	0.325	0.428	0.513	0.230	-0.163	-0.066	-0.246	-0.075
Potatoes	0.781	-0.040	-0.008	0.120	-0.100	0.004	0.046	0.026
Legumes	0.036	0.126	-0.010	0.681	-0.023	0.061	-0.078	-0.102
Fish	0.077	-0.168	0.530	0.173	-0.117	0.316	0.112	0.044
Red meat	0.644	-0.104	0.099	0.315	0.124	0.078	0.194	0.014
Poultry	0.159	-0.044	0.101	0.732	0.086	0.060	0.068	0.211
Coffee	0.242	0.463	-0.151	-0.255	-0.055	0.553	0.265	0.093
Sweets	-0.060	0.065	0.068	-0.002	0.035	-0.097	0.896	-0.036
Nuts	0.064	0.731	-0.034	-0.021	0.035	0.113	0.104	0.141
Total MET-min/day	0.059	0.116	0.015	0.054	0.827	-0.210	-0.025	0.185
Optimal Sleep duration ^b	0.147	-0.119	0.483	-0.421	0.273	0.183	-0.175	0.241
Short duration siesta ^c	-0.012	-0.111	-0.228	-0.032	0.666	0.218	0.101	-0.234
Television viewing hours per day	0.410	0.055	-0.112	-0.039	-0.320	-0.115	-0.324	0.068
Current smoking	0.017	-0.012	-0.058	0.034	-0.010	-0.021	-0.041	0.902

Explained Variance (%)	16.5	11.0	7.9	7.6	6.9	5.9	5.7	5.4
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^a Score coefficients are similar to the correlation coefficients. Numbers in bold indicate foods/habits with the highest correlation (>0.40) within the component.

^b ≥ 7 and ≤ 9 hours/day

^c Midday sleep or resting ≤ 1 hour/day

METs: Metabolic equivalent of task(s), NAFLD: non-alcoholic fatty liver disease.

Description of components: 1 = Full fat dairy, refined cereals, potatoes, red meat, TV-hours/day; 2 = Fruit, vegetables, Coffee, nuts; 3 = Low fat dairy, vegetables, fish, optimal sleep duration; 4 = Legumes, poultry, less optimal sleep hours; 5 = Total MET-min/day, short sleep siesta; 6 = Non refined, coffee; 7 = Sweets; 8 = Smoking.

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Table 3: Trends associations across tertiles of lifestyle patterns 1 and 3 and NAFLD patients characteristics.

	Lifestyle pattern 1: Full fat dairy products, refined cereals, potatoes, red meat, TV-hours/day			P value for trend ^a	Lifestyle pattern 3: Low fat dairy products, vegetables, fish, optimal sleep duration			P value for trend ^a
	Low adherence	Medium adherence	High adherence		Low adherence	Medium adherence	High adherence	
Age (years)	49.2±11.2	49.1±11.6	42.8±11.2	0.01	45.3±11.7	47.6±10.9	48.1±12.2	0.20
Sex, mens (n,%)	27, (58.7)	26, (57.8)	38, (84.4)	0.01	31, (68.9)	30, (66.7)	30, (65.2)	0.93
BMI (kg/m ²)	29.8 (27.7-33.5)	30.0 (26.4-35.0)	30.1 (26.8-32.1)	0.40	29.1 (26.6-33.5)	39.8 (27.0-34.6)	30.2 (27.7-31.9)	0.73
WC (cm)	104.5 (95-109)	102 (96-112)	102 (96-111)	0.99	102 (96-111)	103 (95-116)	103 (96-109)	0.51
Glucose (mg/dl)	88.6±9.6	92.8±11.1	91.7±13.5	0.08	91.2±12.9	90.4±11.7	91.5±10.1	0.28
Insulin (μIU/ml)	11.0 (9-18.5)	12.0 (8-18)	14.0 (11-20)	0.08	16.0 (11-19)	13.0 (9-23)	11.0 (8.1-16.5)	0.05
HOMA-IR	2.3 (1.7-4.0)	2.8 (1.7-4.4)	3.2 (2.7-4.8)	0.06	3.3 (2.2-4.8)	3.0 (1.9-5.2)	2.6 (1.7-3.3)	0.11
Total cholesterol (mg/dl)	205±41.8	208±36.5	201± 44.5	0.63	201±47.9	212±36.6	201±36.7	0.95
LDL cholesterol	136±41.5	139±39.7	145± 41.6	0.33	137±46.2	146±36.6	138±39.2	0.94

(mg/dl)								
HDL cholesterol (mg/dl)	39.0 (28.7- 53.2)	37.0 (27- 51)	24.0 (19- 31)	<0.001	31.0 (23.5- 42.5)	36.0 (27.2- 47.5)	38.0 (22.0- 48.5)	0.33
Triglycerides (mg/dl)	120.0 (76-156)	118 (84- 171)	130 (96- 178)	0.24	126 (95.5- 173)	124 (72.2- 194.5)	118 (88.5- 150.5)	0.31
ALT (IU/L)	55.0 (42- 79)	57.0 (33- 75)	71.0 (51- 98)	0.03	59.0 (48- 95)	57 (42-89)	56 (37-83)	0.17
AST (IU/L)	36.0 (26- 41)	33.0 (23- 41)	39.0 (28- 49)	0.21	36.0 (26- 47)	34 (26-46)	34 (25-41)	0.29
GGT (IU/L)	82.3 (37- 156)	73.0 (36- 128)	39.0 (30- 85)	0.02	47 (34- 126)	71 (37- 150)	51 (33-107)	0.86
Adiponectin (µg/ml)	5.5 (3.5- 9.5)	5.2 (3.8- 8.9)	4.2 (2.9- 6.0)	0.05	4.9 (3.6- 8.2)	4.5 (3.0- 9.8)	5.0 (3.2- 8.1)	0.88
TNF-a (pg/ml)	3.2 (1.8- 5.7)	4.1 (2.8- 6.0)	4.9 (1.7- 6.5)	0.18	4.9 (2.9- 6.7)	4.1 (1.7- 6.0)	3.9 (1.8- 6.0)	0.36
Liver Stiffness (kPa)	7.0 (5.6- 8.3)	6.4 (5.2- 9.1)	6.8 (5.2- 8.3)	0.59	7.3 (5.8- 10.2)	6.7 (5.7- 8.7)	6.6 (5.0- 7.4)	0.04

Data are presented as mean ± SD or median (interquartile range).

^a P-value showing trends of NAFLD patients characteristics across the tertiles of lifestyle patterns 1 and 3 using Jonckheere-Terpstra trend test.

ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMI: Body mass index, GGT: gamma-glutamyltransferase, HDL: high-density lipoprotein, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, NAFLD: non-alcoholic fatty liver disease, TNF-a: Tumor necrosis factor-a, WC: waist circumference.

Table 4: Linear regression analysis models, exploring the association between lifestyle patterns 1 and 3 and several characteristics of NAFLD patients.

Lifestyle patterns	Dependent variable HOMA-IR [‡]		Dependent variable LSM [‡]	
	standardized β [†]	P*	standardized β [†]	P*
Model 1^a				
Lifestyle pattern 1	1.62	0.005	1.23	0.27
Lifestyle pattern 3	-1.17	0.016	-1.54	0.022
Model 2^b				
Lifestyle pattern 1	1.66	0.005	1.23	0.30
Lifestyle pattern 3	-1.48	0.02	-1.48	0.05
Model 3^c				
Lifestyle pattern 1	1.31	0.14	1.28	0.34
Lifestyle pattern 3	-1.66	0.008	-1.62	0.05

^a Model 1: adjusted for age, sex, BMI

^b Model 2: adjusted for age, sex, BMI, energy intake, smoking habits

^c Model 3: adjusted for age, sex, BMI, energy intake, smoking habits, adiponectin, tumor necrosis factor- α

*P-value as derived by multiple linear regression analysis showing associations between lifestyle patterns and HOMA-IR or LSM.

†standardized beta coefficients after their conversion to natural numbers.

‡Log-transformation of dependent variables was made for achieving normality distribution.

Lifestyle pattern 1: Full fat dairy products, refined cereals, potatoes, red meat, television-hours/day.

Lifestyle pattern 3: Low fat dairy products, vegetables, fish, optimal sleep duration.

BMI: body mass index; HOMA-IR: homeostatic model assessment of insulin resistance; LSM: Reliable liver stiffness measurements; NAFLD: non alcoholic fatty liver disease.