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The Effect of the Spanish Ketogenic Mediterranean Diet on Nonalcoholic Fatty Liver Disease: A Pilot Study

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ABSTRACT The "Spanish Ketogenic Mediterranean Diet" (SKMD) has been shown to be an effective and safe way to cure patients suffering from metabolic syndrome (MS). Keeping in mind that nonalcoholic fatty liver disease (NAFLD) is closely associated with MS, the purpose of this study was to evaluate the potential therapeutic properties under free living conditions of the SKMD in patients with MS (following the International Diabetes Federation [IDF] consensus guidelines) and NAFLD (suspected by using a cutoff value of alanine aminotransferase [ALT] levels of >40 U/L and confirmed by abdominal ultrasonography) over a 12-week period. A prospective study was carried out in 14 obese men meeting the inclusion criteria and whose body mass index (BMI) and age were 36.58 ± 0.54 kg/m^2 and 41.18 ± 2.28 years, respectively. Statistical differences between the parameters studied before and after administration of the SKMD (week 0 and 12) were analyzed by paired Student's *t* test (continuous variables) and the χ^2 test (discontinuous variables). $P < .05$ was considered statistically significant. There was an extremely significant ($P < .001$) improvement in body weight (from 109.79 kg to 95.86 kg), low-density lipoprotein-cholesterol (from 123.43 mg/dL to 100.35 mg/dL), ALT (from 71.92 U/L to 37.07 U/L), aspartate aminotransferase (from 47.71 U/L to 29.57 U/L), steatosis degree (complete fatty liver regression was observed in 21.4% of the patients, and an overall reduction was found in 92.86% of the patients), and all the parameters studied associated with the MS: BMI (from 36.99 kg/m^2 to 32.42 kg/m^2), waist circumference (from 114.01 cm to 98.59 cm), fasting plasma glucose (from 118.57 mg/dL to 90.14 mg/dL), triacylglycerols (from 232.64 mg/dL to 111.21 mg/dL), high-density lipoprotein-cholesterol (HDLc) (from 42.81 mg/dL to 58.71 mg/dL), systolic blood pressure (from 142.86 mm Hg to 125.36 mm Hg), and diastolic blood pressure (from 89.64 mm Hg to 77.86 mm Hg). After the diet all the subjects were free of MS according to the IDF definition, and 100% of them had normal triacylglycerols and HDLc levels, in spite of the fact that 100% of them still had a BMI of >30 kg/m^2. We conclude that the SKMD could be an effective and safe way to treat patients suffering from MS and the associated NAFLD.

KEY WORDS: • ***ketogenic diet*** • ***low carbohydrate diet*** • ***metabolic syndrome*** • ***nonalcoholic fatty liver disease*** • ***protein diet*** • ***"Spanish Ketogenic Mediterranean Diet"***

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is closely associated with the metabolic syndrome (MS).[1] Thus, insulin resistance with dyslipidemia, especially hypertriglyceridemia,[2] obesity, and type 2 diabetes mellitus are typical in patients with NAFLD.[3] The connection between obesity and the development of NAFLD has also been well proved with numerous studies showing that 70–80% of people with a body mass index (BMI) of >30 kg/m^2 have hepatic steatosis.[4,5] These associations of obesity, dyslipidemia, and type 2 diabetes with NAFLD are important because it is well known that they represent the typical constellation of insulin resistance, and for that reason nowadays insulin resistance is considered as an essential pathophysiologic key responsible for the genesis of NAFLD.[3]

Most patients with NAFLD have no symptoms or signs of liver disease at the time of diagnosis, although many patients report fatigue or malaise and a sensation of fullness or discomfort on the right side of the upper abdomen.[6]

The diagnosis of NAFLD is based on two criteria—presence of a fatty liver or steatohepatitis[7]—and determining the nonalcoholic nature of the disease process[8] requires the exclusion of alcohol abuse as the cause of liver disease. Some patients' livers are very vulnerable to alcohol, so, for example, the daily consumption should be less than 30 g of alcohol for men (360 mL of wine) and 20 g for women (240 mL of wine).[6]

Although is well known that the liver biopsy is the gold standard for the diagnosis of NAFLD, it is invasive and associated with discomfort and some risks; for that reason it has a low acceptance rate, and it is not frequently performed in NAFLD patients.[9] Unlike liver biopsy, sonography is not invasive and is the most widely available and the cheapest of the modalities for the diagnosis of NAFLD. Besides, it can achieve for MS a diagnostic sensitivity of 91.7% and specificity of 100% in NAFLD detection.[1] The determination

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of levels of serum alanine aminotransferase (ALT) (also known as glutamic pyruvic transaminase) has been used most frequently as a screening test to diagnose presumed NAFLD in a large population.[10,11] The sensitivity and specificity of ALT values in distinguishing hepatic steatosis and steatohepatitis have been studied in morbidly obese individuals undergoing bariatric surgery. It was seen that by using a cutoff value of $>40\,U/L$, ALT values diagnosed steatosis with a sensitivity of 45% and specificity of 100%.[12]

Moreover, patients with NAFLD usually have, in addition to high levels of ALT, a ratio of aspartate aminotransferase (AST) (also known as glutamic oxaloacetic transaminase) to ALT of <1, but this ratio increases as fibrosis advances, leading to a loss of its diagnostic accuracy in patients with cirrhotic NAFLD.[13] However, it is important to keep in mind that this relationship with liver enzymes could be moderated when individuals are close to the Mediterranean dietary pattern.[14]

In connection with natural NAFDL treatments, seal oils (*n*-3 polyunsaturated fatty acids) and ketogenic diets have been shown to be useful. The *n*-3 polyunsaturated fatty acids from seal oils ($6\,g/day$) have been shown to be safe and efficacious for patients with NAFLD associated with hyperlipidemia and can improve their total symptom scores and ALT and serum lipid levels and bring about normalization of ultrasonographic evidence.[15]

On the other hand, carbohydrate restriction has been proved to improve all of the features of MS in normal subjects, patients with MS, and patients with diabetes, and these beneficial effects do not require weight loss.[16,17] This is not strange if we consider that the ketogenic diets promote, in addition to weight loss,[18,19] a non-atherogenic lipid profile, lower blood pressure, and decrease resistance to insulin with an improvement in blood levels of glucose and insulin.[18,20] Keeping in mind all these therapeutic effects, it is not strange that low carbohydrate ketogenic diets have also shown to promote significant weight loss and histological improvement of fatty liver disease.[21] In addition to these findings, recent clinical studies have shown that the ''Spanish Ketogenic Mediterranean Diet'' (SKMD) is an effective therapy for the treatment of obesity, dyslipidemia, hypertension,[22,23] and the MS.[23]

We therefore hypothesized that SKMD might also lead to improvements in NAFLD. The purpose of this pilot study was to assess the effects of the SKMD on both ultrasonographic parameters and serologic measures of liver function in obese patients with MS and NAFLD.

SUBJECTS AND METHODS

Subjects

A prospective study was carried out at a general medicine consultation (Córdoba, Spain) in 14 overweight male subjects with MS and NAFLD. The BMI and age were $36.58 \pm 0.54\,kg/m^2$ and 41.18 ± 2.28 years, respectively.

Inclusion criteria were a diet based on carbohydrate foods ($>50\%$ of daily energy intake), desire to lose weight, hepatic transaminase levels three times or less normal values (AST and ALT $\leq 120\,U/L$), normal renal function (plasma creatinine $\leq 1.3\,mg/dL$ and plasma urea $\leq 40\,mg/dL$), not to have antecedents of gout or high uric acid, not to have exercise, alcoholic, and smoking habits, not to be pregnant or lactating, BMI $\geq 30\,kg/m^2$, age ≥ 18 years and ≤ 65 years, and not to be taking medication. Exclusion criteria included antecedents of alcohol overuse, viral hepatitis, hemochromatosis, autoimmune hepatitis, primary sclerosing cholangitis or primary biliary cirrhosis, Wilson's disease, α-1 antitrypsin deficiency, and history of any other hepatic, gastrointestinal, renal, cardiovascular, or hematological disorders.

Patients measured their body's ketosis state every morning by ketone urine-testing strips, and the status was confirmed every week by the physician with ketone blood-testing strips.

The Ethics and Clinical Investigation Committee of the ''Spanish Medical Association of the Proteinic Diet'' approved the study protocol, informed consent form, and subject informational materials. Patient anonymity was preserved.

Procedures

The MS was diagnosed following the International Diabetes Federation (IDF) consensus guidelines (Table 1).[24]

NAFLD was suspected by using a cutoff value for ALT levels of $>40\,U/L$ and confirmed by abdominal ultrasonography. Ultrasound scans were performed by a trained operator who was blinded to the treatment of participants. The severity of steatosis or steatosis degree was also scored using a 4-point validated scale as follows: grade 0 = normal echogenicity, grade 1 = slight, grade 2 = moderate, and grade 3 = severe.[25]

The description of the diet, the supplementation, and the techniques or procedures for all the measurements we used were the same as those previously reported, respectively, in the Diet, Supplements, and Measurements sections of our prior publication[23] with one exception: a lower red wine

Table 1. The New International Diabetes Federation Definition for the Metabolic Syndrome

Factor	*Criteria*
Raised triglycerides	$\geq 150\,mg/dL$ or specific treatment for this abnormality
Reduced high-density lipoprotein-cholesterol	$<40\,mg/dL$ (1.03 mmol/L) in men, $<50\,mg/dL$ (1.29 mmol/L) in women, or specific treatment for this abnormality
Raised BP	Systolic BP ≥ 130 mm Hg or diastolic BP ≥ 85 mm Hg or specific treatment for this abnormality
Raised fasting plasma glucose	$\geq 100\,mg/dL$ or specific treatment for this abnormality

According to the new International Diabetes Federation definition, for a person to be defined as having the metabolic syndrome they must have central obesity (body mass index of $>30\,kg/m^2$ or waist circumference in Europeans for men of $\geq 94\,cm$ and women of $\geq 80\,cm$) plus any two of the previous factors.

BP, blood pressure.

consumption (200–300 mL daily instead of 200–400 mL). As we said, some patients livers are very vulnerable to alcohol, so we decided to reduce the maximum daily red wine consumption from 400 mL to 300 mL. It was distributed as 100–150 mL each at lunch and dinner.

Data analysis

Statistical differences between the continuous variables before and after administration of the SKMD (week 0 and 12) were analyzed by paired Student's *t* test with SPSS version 12.0 software (SPSS Inc., Chicago, IL, USA), and data are expressed as mean ± SEM values. The parameters studied were weight, waist circumference, BMI, systolic blood pressure, diastolic blood pressure, high-density lipoprotein-cholesterol (HDLc), triacylglycerols, glucose, ALT, AST, and ALT/AST ratio. Before the Student's *t* test, Kolmogorov–Smirnov and Shapiro–Wilk tests were used for testing normality, and the assumption of homoscedasticity was determined with the *F*-Snedecor test. The steatosis degree parameter (discontinuous variable) was performed using a χ^2 test. $P < .05$ was considered statistically significant.

RESULTS AND DISCUSSION

Normal distribution and the assumption of homoscedasticity were verified. The changes in all the parameters studied are shown in Table 2.

There was an extremely significant ($P < .001$) improvement in body weight (from 109.79 kg to 95.86 kg), low-density lipoprotein-cholesterol (from 123.43 mg/dL to 100.35 mg/dL), ALT (from 71.92 U/L to 37.07 U/L), AST (from 47.71 U/L to 29.57 U/L), steatosis degree (complete fatty liver regression was observed in 21.4% of the patients, and an overall reduction was found in 92.86% of the patients), and all the parameters studied associated with MS: BMI (from 36.99 kg/m^2 to 32.42 kg/m^2), waist circumference (from 114.01 cm to 98.59 cm), fasting plasma glucose (from 118.57 mg/dL to 90.14 mg/dL), triacylglycerols (from 232.64 mg/dL to 111.21 mg/dL), HDLc (from 42.81 mg/dL to 58.71 mg/dL), systolic blood pressure (from 142.86 mm Hg to 125.36 mm Hg), and diastolic blood pressure (from 89.64 mm Hg to 77.86 mm Hg). After the diet all the subjects were free of MS according to the IDF definition, and 100% of them had normal triacylglycerols and HDLc levels, in spite of the fact that 100% of them still had a BMI of > 30 kg/m^2 due to the short term of the study.

If we consider the results taken in conjunction with the fact that the scientific literature demonstrates that the basic components of the SKMD are effective ways to improve or even treat the MS, we can state that the components of the SKMD may exert a synergic effect indeed.

We can see that our results are similar to those previously reported with the SKMD[22,23] for the improvement in cardiovascular parameters, weight loss, and the MS, and we agree this diet could be an effective and safe way to cure patients suffering from MS.[23] Moreover, we found an improvement in NAFLD proved through normalization of transaminase levels and regression of steatosis degree. Our results have been shown to be faster than those obtained independently by a ketogenic diet[21] or a diet with ω-3 fish oil supplementation[15] for the improvement of the fatty liver disease. Maybe because of the ketogenic nature of the SKMD, its richness in ω-3 fish oil and virgin olive oil exerts a synergic effect indeed. We also have to point out that

Table 2. Changes in Levels of Parameters Associated and not Associated with the Metabolic Syndrome Definition Before and After the Spanish Ketogenic Mediterranean Diet

	Week 0	*Week 12*
Parameter associated with MS		
BMI (kg/m^2)	36.99 ± 0.66 (100%)	32.42 ± 0.63 (100%)
Waist circumference (cm)	114.01 ± 3.17 (100%)	98.59 ± 3.06 (64.29%)
Glycemia (mg/dL)	118.57 ± 2.05 (100%)	90.14 ± 1.59 (0%)
Triacylglycerols (mg/dL)	232.64 ± 10.34 (100%)	111.21 ± 2.78 (0%)
HDLc (mg/dL)	42.81 ± 1.34 (42.86%)	58.71 ± 1.32 (0%)
BP (mm Hg)		
Systolic	142.86 ± 2.39 (100%)	125.36 ± 1.84 (28.57%)
Diastolic	89.64 ± 1.35 (93%)	77.86 ± 1.27 (14.29%)
Patients with MS [*n* (%)]	14 (100%)	0 (0%)
Parameter not associated with MS		
Weight (kg)	109.79 ± 3.07	95.86 ± 2.76
LDLc (mg/dL)	123.43 ± 4.26	100.36 ± 1.84
ALT (U/L)	71.92 ± 3.57	37.07 ± 1.62
AST (U/L)	47.71 ± 2.81	29.57 ± 1.13
AST/ALT	0.62 ± 0.02	0.80 ± 0.02
Steatosis degree of 0/1/2/3 (%)	0/18.2/36.4/9.1	21.4/64.3/14.3/0

Data are mean ± SEM values (percentage of patients with pathological values of metabolic syndrome [MS]) according to the new International Diabetes Federation definition. The *P* value was extremely significant ($P < 0.001$) in all the parameters studied.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDLc, high-density lipoprotein-cholesterol; LDLc, low-density lipoprotein-cholesterol.

many components of olive oil exert, through different mechanisms of action, a favorable effect on fatty liver.[26]

Moreover, if we have in mind that NAFLD is closely associated with the MS,[1] we are not surprised with our results because the cure of MS should be associated with at least an improvement in NAFLD. Although after the diet all the subjects were free from MS, 100% of them still have a BMI of $>30\,kg/m^2$, and an overall reduction in fatty liver regression was found in 92.86% of the patients, not just the 21.4% of the patients who had a complete fatty liver regression. With these findings, our hypothesis is that with the SKMD, the regression of the MS is faster than the weight loss and the regression of NAFLD, and maybe a longer time consuming this diet is necessary to achieve a complete fatty liver regression besides the weight loss.

In conclusion, treatment of NAFLD associated with MS with SKMD seems to be safe and efficacious, improving levels of transaminases, especially ALT, the severity of steatosis, and all the parameters associated with MS. Further study is needed to confirm these results.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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