Low physical activity and energy dense Malaysian foods are associated with non-alcoholic fatty liver disease in centrally obese but not in non-centrally obese patients with diabetes mellitus

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Running Title: Diet, physical activity and NAFLD in diabetics

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Data analysis and interpretation: WKC, PCT
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ABSTRACT

Objective: To study the dietary intake and level of physical activity (PA) of patients with diabetes mellitus and the association with non-alcoholic fatty liver disease (NAFLD).

Methods: Consecutive adult patients with type 2 diabetes mellitus seen in our hospital diabetes clinic were enrolled. The Global Physical Activity Questionnaire and a semi-quantitative food-frequency questionnaire were used to assess PA and dietary intake, respectively. Diagnosis of NAFLD was ultrasound-based and following exclusion of significant alcohol intake and other causes of chronic liver disease. Results: Data for 299 patients were analyzed (mean age 63.3±10.5 years old, 41.1% male). Prevalence of NAFLD was 49.2%. Patients with low PA were more likely to have NAFLD (OR=1.75, 95% CI=1.03-2.99, p=0.029). There was no significant difference in energy intake, intake of macronutrients and percentage energy intake from each macronutrient, high sugar food, high cholesterol food and high SFA food between patients with and without NAFLD. Among centrally obese patients, patients with low PA and in the highest quartile of percentage energy intake from fat (OR=4.03, 95% CI=1.12-15.0, p=0.015), high cholesterol food (OR=3.61, 95% CI=1.37-9.72, p=0.004) and high SFA food (OR=2.67, 95% CI=1.08-6.67, p=0.019) were most likely to have NAFLD. Among those who were not centrally obese, PA and percentage energy intake from fat, high cholesterol food and high SFA food was not associated with NAFLD. Conclusion: Low PA and high percentage energy intake from fat, high cholesterol food and high SFA food is associated with NAFLD in centrally obese but not in non-centrally obese patients with diabetes mellitus.

Key Words: diet, physical activity, non-alcoholic fatty liver disease, diabetes mellitus, obesity

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver conditions, ranging from benign steatosis to non-alcoholic steatohepatitis (NASH) to fibrosis and cirrhosis. It is closely related to the metabolic syndrome and has become a common cause of chronic liver disease parallel to the increase in metabolic syndrome in populations worldwide. In the Asian-Pacific region, the prevalence of NAFLD has increased remarkably over the years, affecting up to 30% of the general population. In Malaysia, the prevalence of NAFLD was reported to be 22.7% in a study on individuals attending health-check in a suburban medical facility. The prevalence of NAFLD is even higher among patients with diabetes
mellitus (DM) and has been reported to be up to 70%.\textsuperscript{3} In a recent study on patients attending the diabetes clinic in our hospital, we found that close to half of the patients had NAFLD and independent predictors for NAFLD were central obesity and elevated serum alanine aminotransferase level.\textsuperscript{4}

Diet and physical activity are important factors in NAFLD. A case-control study identified increased fructose consumption as a risk factor for NAFLD.\textsuperscript{5} This was supported by a population-based cross-sectional study that found increased intake of soft drink as an independent predictor for NAFLD.\textsuperscript{5} Higher percentage energy from fat has also been found to be an independent factor for NAFLD in a case-control study\textsuperscript{7} and in a randomized study.\textsuperscript{8} Higher carbohydrate intake,\textsuperscript{9} particularly simple carbohydrates,\textsuperscript{10} and higher fat intake, particularly excessive amount of n-6 polyunsaturated fatty acids (PUFAs)\textsuperscript{11} have been implicated in NASH, the more severe form of NAFLD. The importance of diet and physical activity in NAFLD is also reflected by the many interventional trials that have looked at energy restrictions, with and without increased physical activity to reduce liver fat.\textsuperscript{12} To the best of our knowledge, there is to date no published study that looked specifically at the association of diet and physical activity with NAFLD in patients with diabetes mellitus. Hence, we embarked on this study to look for any association of diet and physical activity with NAFLD in patients with diabetes mellitus.

**METHODS AND MATERIALS**

The study was a cross-sectional study on consecutive patients seen at the Diabetes Clinic of University of Malaya Medical Centre (UMMC) between November 2011 and April 2012. Patients who have been included but returned for follow-up during the study period were identified and not included a second time. Patients with known chronic liver disease other than fatty liver and patients who did not agree to participate were excluded. The study was approved by the University of Malaya Medical Center Medical Ethics Committee and informed consent was obtained from all included patients.

Demographic and anthropometric data and relevant clinical and laboratory data were obtained using a standard protocol. Alcohol intake was estimated using the quantity-frequency method.\textsuperscript{13} Alcohol intake was estimated based on patient’s self-reported frequency and quantity of intake of each of the 3 main types of alcoholic beverages i.e. beer, wine and spirit. Frequency of intake was divided into 7 categories i.e. almost every day, 5 or 6 days a week, 3 or 4 days a week, once or twice a week, once or twice a month, once every couple of months and once or twice a year. Each of these categories provided a multiplying factor for
calculation of alcohol intake per week. Information on average intake during each drinking session was captured using common serving measurements and this was translated into units of alcohol based on the volume consumed and the alcohol by volume for each of the types of alcoholic beverages. Units of alcohol consumed in a week in the form of beer, wine and spirit was calculated separately and summed up to give an estimate of alcohol intake per week for each patient. Significant alcohol intake was defined as more than 21 units per week for men and more than 14 units per week for women.\textsuperscript{14}

The Global Physical Activity Questionnaire (GPAQ) was used to measure physical activity. The GPAQ categorizes level of physical activity into low, moderate and high according to reported frequency and duration of different intensities of physical activity in three domains: work, travel and leisure.\textsuperscript{15} A semi-quantitative food-frequency questionnaire (FFQ) comprising 200 common Malaysian food items was used to assess dietary intake. This FFQ had similar estimates of dietary intakes when compared with three days 24-hour dietary recall and is an adequate tool for estimation of dietary intakes for epidemiological studies in Malaysia.\textsuperscript{16} Patients who did not do the FFQ and patients whose FFQ was incomplete were excluded from analysis. The dietary composition of each food item was based on a standard reference.\textsuperscript{17} Food items were further categorized as high sugar, high cholesterol and/or high saturated fatty acid (SFA) where applicable (Appendix 1). The daily energy intake, intake of carbohydrate, protein and fat, the percentage energy from carbohydrate, protein and fat, and the percentage energy from high sugar, high cholesterol and high SFA food were estimated for each patient.

Weight and height were measured using standardized equipment. Body mass index (BMI) was calculated by dividing weight in kilogram by the square of height in meters. Patients were categorized as underweight (BMI<18.5 kg/m\textsuperscript{2}), normal (18.5 kg/m\textsuperscript{2} ≤ BMI < 23.0 kg/m\textsuperscript{2}), overweight (23.0 kg/m\textsuperscript{2} ≤ BMI < 25.0 kg/m\textsuperscript{2}) or obese (BMI≥25.0 kg/m\textsuperscript{2}).\textsuperscript{18} Waist circumference (WC) was measured at the mid-point between the lowest margin of the least palpable rib and the top of the iliac crest in the standing position. Central obesity was defined as WC > 90 cm for men and > 80 cm for women [19]. Blood pressure was measured in the sitting position using standardized equipment. A patient was considered hypertensive if there was a self-reported history of hypertension, if the patient was on anti-hypertensive medication(s), if the systolic blood pressure was ≥ 130 mmHg, or if the diastolic blood pressure was ≥ 85 mmHg.\textsuperscript{19}

All patients had venous blood drawn after an overnight fast for blood sugar, glycated hemoglobin (HbA1c), lipid profile and liver function test as routine before their appointment.
at the Diabetes Clinic. Biochemical measurements were performed using standard laboratory procedures. A patient was considered to have dyslipidemia if there was a self-reported history of dyslipidemia, if the patient was on lipid-lowering medication(s), if the serum total cholesterol was $\geq 5.2$ mmol/L, if the serum triglyceride was $\geq 1.7$ mmol/L, if the serum HDL was $< 1.0$ mmol/L for men or $< 1.3$ mmol/L for women, or if the serum LDL was $\geq 3.4$ mmol/L. A patient was considered to have metabolic syndrome if two or more of the following were present: central obesity, hypertension, hypertriglyceridemia and low serum HDL (according to the aforementioned cut-offs). Additional venous blood was drawn on the day of study for viral hepatitis B and C serology. The Elecsys HBsAg II assay (Roche, Mannheim, Germany) and the Elecsys Anti-HCV II assay (Roche, Mannheim, Germany) were used to test for viral hepatitis B and C infection, respectively.

Diagnosis of NAFLD was by trans-abdominal ultrasonography following exclusion of significant alcohol intake, use of medications known to cause fatty liver and other causes of chronic liver disease. The following criteria were used for ultrasound diagnosis of fatty liver: increased echogenicity, posterior attenuation and loss of intra-hepatic architectural details. Investigators involved in other parts of the study were blinded to the ultrasonography findings, vice versa.

Data were analyzed using a standard statistical software program (SPSS 15.0). Continuous variables were expressed as mean $\pm$ standard deviation or median with inter-quartile range, and analyzed using student’s t-test or Mann-Whitney U test where appropriate. Categorical variables were expressed as percentage and analyzed using chi-square test or Fisher exact test where appropriate. Percentage energy from each macronutrient and from high sugar food, high cholesterol food and high SFA food were stratified into quartiles and the prevalence of NAFLD was compared across quartiles for each of the variables. The prevalence of NAFLD was also compared between the highest quartile and lower quartiles for each of the variables. Percentage energy from each macronutrient and from high sugar food, high cholesterol food and high SFA food was analyzed individually with level of physical activity to look for any association with prevalence of NAFLD. Further analysis was performed for patients who were and were not centrally obese.

RESULTS
Data for 299 patients were analyzed (Figure 1). The characteristics of patients included in the analysis were similar to that of the entire cohort that has been reported elsewhere. Mean age of the study population was $63.3 \pm 10.5$ years old with 41.1% male. The majority (81.9%)
completed at least lower secondary education with median income of RM 1000 (RM 667 - RM 2000) per household person per month. Mean duration since diagnosis of diabetes mellitus was 16.6±9.7 years. Most patients had hypertension (90.3%) and dyslipidemia (97.7%). Central obesity was seen in 71.9% and the majority (95.7%) had metabolic syndrome. The prevalence of NAFLD was 49.2%. On multivariate analysis, independent factors associated with NAFLD were central obesity and raised serum ALT level. Central obesity was associated with NAFLD on multivariate analysis while obesity was not (data not shown). Hence, central obesity instead of obesity was used for further analysis of dietary intake and physical activity. More than half (53.8%) of patients had low level of physical activity, while the percentage of patients with moderate and high level of physical activity was 34.1% and 12.0%, respectively. Patients with low level of physical activity were more likely to have NAFLD compared with patients with moderate level of physical activity (OR=1.75, 95 % CI =1.03-2.99, p=0.029).

The daily energy intake according to food groups for patients with and without NAFLD is shown in Table 1. There was no significant difference in the daily energy intake from the different food groups between patients with and without NAFLD. There was also no significant difference in the daily energy intake from animal foods (i.e. fish, seafood and products; meat and meat products; eggs; milk and milk products) and plant foods (i.e. soy products; legumes and nuts; coconut milk and grated coconut; rice and cereals; vegetables; fruits) between patients with and without NAFLD (data not shown). The findings were similar when analysis was performed separately for patients who were and were not centrally obese (data not shown).

There was no significant difference in energy intake, intake of macronutrients, percentage energy from each macronutrient and from high sugar, high cholesterol and high SFA foods between patients with and without NAFLD (Table 2). When percentage energy from each macronutrient and from high sugar, high cholesterol and high SFA foods was stratified according to quartiles, no significant difference in distribution of patients with and without NAFLD was seen across quartiles (data not shown) and between the highest and lower quartiles (Table 3). The findings were similar when analysis was performed separately for patients who were and were not centrally obese (data not shown).

Among centrally obese patients, patients with low level of physical activity and in the highest quartile of percentage energy from fat were the most likely to have NAFLD (OR=4.03, 95% CI=1.12-15.0, p=0.015). Among patients who were not centrally obese, level of physical activity and percentage energy from fat was not associated with NAFLD.
(Table 4). Among centrally obese patients, patients with low level of physical activity and in the highest quartile of percentage energy from high cholesterol food (OR=3.61, 95% CI=1.37-9.72, \( p = 0.004 \)) and high SFA food (OR=2.67, 95% CI=1.08-6.67, \( p = 0.019 \)) were most likely to have NAFLD. Among patients who were not centrally obese, the level of physical activity and percentage energy from high cholesterol and high SFA foods was again not associated with NAFLD (Table 5). These findings were not affected when adjusted for age and gender, and other components of metabolic syndrome (data not shown).

The energy contribution of the different polyunsaturated fatty acids is shown in Table 6. There was no significant difference in the energy contribution of the different polyunsaturated fatty acids and the n-3/n-6 fatty acid ratio between patients with and without NAFLD. There was no significant difference in these parameters when analysis was performed separately for patients who were and were not centrally obese (data not shown).

**DISCUSSION**

Obesity is the result of energy intake in excess of expenditure over time. Given sufficient time, even a relatively small imbalance between energy intake and expenditure can lead to obesity. Moreover, such imbalance and the resultant weight change may occur at some times but not others.\(^{22}\) Consequently, detecting and linking differences in dietary intake and physical activity to the development of obesity and its associated conditions such as NAFLD is difficult even under the best circumstances. Hence, it came as no surprise that we did not detect any significant differences in the energy intake, the intake of macronutrients, and the percentage of energy from each macronutrient and from high sugar, high cholesterol and high SFA foods between patients with and without NAFLD in our cross-sectional study of patients with diabetes mellitus.

In a retrospective analysis of data from a large population-based cross-sectional study that used physical activity monitors, Gerber and colleagues showed that average physical activity and moderate/vigorous physical activity was significantly lower in patients with NAFLD or DM compared to those without either conditions. Average physical activity and moderate/vigorous physical activity were lowest among patients with coexisting NAFLD and DM.\(^{23}\) This is consistent with our findings, that diabetic patients with NAFLD were more likely to have low level of physical activity compared to diabetic patients without NAFLD. Whether low level of physical activity is the cause or effect of NAFLD is unclear. Reduced energy expenditure due to low level of physical activity can theoretically contribute to energy excess, weight gain, obesity and NAFLD. On the other hand, obese patients have lower
cardio-respiratory fitness and lower level of physical activity. These associations can potentially form a self-perpetuating vicious cycle that promotes NAFLD.

We also found that a low level of physical activity and high percentage of energy from fat, high cholesterol food and high SFA food was associated with NAFLD in centrally obese but not in non-centrally obese diabetic patients. Obese patients have increased insulin resistance with impaired insulin-mediated glucose uptake in adipose tissue and skeletal muscle. The resultant increase in blood glucose concentration leads to increased glucose uptake in the liver, a process which is insulin-independent. This leads to increase de novo lipogenesis (DNL). FFAs from DNL are a significant source of accumulated fat in the liver after serum non-esterified fatty acids (NEFAs). The latter is also increased with increased insulin resistance due to impaired insulin-mediated suppression of triglyceride hydrolysis in adipose tissue. Low levels of physical activity aggravates insulin resistance. This, along with increased dietary fat makes obese patients with diabetes mellitus more susceptible to NAFLD compared to their non-obese counterparts. Dietary cholesterol has been shown to exacerbate hepatic steatosis and inflammation in animal model. In a study comparing non-obese and obese NAFLD patients, dietary cholesterol was superabundant in non-obese NAFLD patients suggesting an important role in development of NAFLD. Accumulation of SFA in the liver also plays an important role in the pathogenesis of NAFLD and has been shown to exacerbate liver inflammation and injury in various studies at the cellular and molecular level.

The findings from our study are novel and complement that from other previous studies. However, as our study population consisted of patients with diabetes mellitus only, it should not be generalized to patients without diabetes mellitus. The findings from this study may not be generalized to non-Malaysian populations as this was a study conducted in Malaysia. However, we feel that it does provide some useful insights on patients with diabetes mellitus in general. Moreover, the findings are biologically plausible. Further studies should be performed to see if similar findings are observed in other populations. Despite our effort, there were several other limitations in our study. Dietary intake and physical activity were self-reported and could be subjected to bias. However, strict and continuous measurement of dietary intake and physical activity over time would not be practical in cross-sectional studies involving fairly large number of patients like this and has its own inherent way of causing bias too. We did use previously validated questionnaires to capture information on dietary intake and physical activity. Dietary composition for many Malaysian food items is not complete so we were not able to look at the sugar, cholesterol and SFA content of each individual food items. Instead, we categorized food items into high sugar food, high
cholesterol food and high SFA food for analysis. Lastly, the diagnosis of fatty liver was based on ultrasound and not histopathological examination of liver biopsy specimen. While the latter is more accurate to diagnose fatty liver, it is invasive and not feasible in our study. Ultrasonography is by far the most commonly used method to diagnose fatty liver in clinical practice and in epidemiological studies with good sensitivity (89%) and specificity (93%) in moderate and severe fatty liver.

Based on findings from our study, we conclude that a low level of physical activity and energy dense Malaysian foods are associated with NAFLD in centrally obese but not in non-centrally obese patients with diabetes mellitus. We are not implying that there should be different lifestyle recommendations for the two groups, but are simply putting forward direct evidence from a cross-sectional study that low level of physical activity and poor dietary habits have different impact on centrally obese and non-centrally obese patients with diabetes mellitus in regards to NAFLD.

ACKNOWLEDGEMENTS
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CONFLICT OF INTEREST AND FUNDING DISCLOSURE
The authors have no conflict of interest to disclose. This study was funded in full by University of Malaya Research Grant (UMRG), Project No.: RG375-11HTM.

REFERENCES


Appendix 1

High sugar food
Sweetened condensed milk, sugar, kaya (coconut jam), fruit jam, chocolate cereal, syrup drink, bandung (syrup-flavored milk), lemonade, iced lemon tea, carbonated drink, doughnut, various types of sweet kuih (bite-sized snack), various types of cake, muffin, chocolate, ice-cream, chocolate biscuits, sweets.

High cholesterol food
Full cream milk powder, cheese, sambal (chili-based sauce), deep-fried beef lung, deep-fried chicken liver, other offal, sambal ikan bilis (chili-based sauce with anchovies), squid, prawn, crab, fried egg, boiled egg, salted egg, full cream yogurt, ice-cream.

High SFA food
Roti canai (flatbread), roti telur (flatbread with egg), sambal, nasi lemak (rice cooked in coconut milk), fried rice, nasi minyak (ghee rice), biryani rice, chicken rice, fried chicken, curry with coconut milk, kuah masak lemak (creamy coconut sauce), kurma sauce, kuah rendang (a spicy sauce made from coconut milk and mixture of ground spices), deep-fried fish, deep-fried meat, deep-fried beef lung, deep-fried anchovies, sambal ikan bilis, chicken burger, beef burger, sausage/frankfurter, nuggets, French fries, cekodok pisang (bite-sized snack made from banana), banana fritter, shrimp fritter, curry puff, fried spring roll, mung bean fritter, vadai kacang dhal (fritter-type snack made from pulses), various types of cakes, chocolate, ice-cream, chocolate biscuits.

Table 1. Daily energy intake (kcal/day) by food groups for patients with and without NAFLD

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Patients with NAFLD</th>
<th>Patients without NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish, seafood and products</td>
<td>60.2 (26.9-117)</td>
<td>56.5 (25.5-102)</td>
</tr>
<tr>
<td>Meats and meat products</td>
<td>88.3 (39.2-179)</td>
<td>91.6 (37.6-167)</td>
</tr>
<tr>
<td>Eggs</td>
<td>6.8 (2.3-16.5)</td>
<td>6.4 (2.3-15.9)</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td>42.2 (0-123)</td>
<td>28.2 (0-92.9)</td>
</tr>
<tr>
<td>Soy products</td>
<td>11.2 (3.2-27.3)</td>
<td>11.2 (5.6-24.6)</td>
</tr>
<tr>
<td>Legumes and nuts</td>
<td>11.9 (0-78.4)</td>
<td>26.9 (0-83.5)</td>
</tr>
<tr>
<td>Coconut milk and grated coconut</td>
<td>0 (0-6.9)</td>
<td>0 (0-6.1)</td>
</tr>
<tr>
<td>Rice and cereals</td>
<td>683 (481-910)</td>
<td>702 (499-930)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>7.0 (3.1-13.7)</td>
<td>7.1 (4.1-13.9)</td>
</tr>
<tr>
<td>Fruits</td>
<td>67.4 (29.0-119)</td>
<td>65.6 (39.5-126)</td>
</tr>
<tr>
<td>Non-alcoholic beverages</td>
<td>12.2 (6.0-28.0)</td>
<td>10.1 (4.9-20.7)</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.8 (0-22.8)</td>
<td>5.0 (0-29.9)</td>
</tr>
<tr>
<td>Palm oil</td>
<td>22.5 (3.7-51.2)</td>
<td>22.2 (6.3-54.0)</td>
</tr>
</tbody>
</table>

There was no significant difference in the daily energy intake from the different food groups between patients with and without NAFLD.
Table 2. Daily energy intake, intake of macronutrients, percentage energy from each macronutrient, and percentage energy from high sugar food, high cholesterol food and high SFA food in patients with and without NAFLD

<table>
<thead>
<tr>
<th></th>
<th>Patients with NAFLD</th>
<th>Patients without NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (kcal/day)</td>
<td>1272 (946-1600)</td>
<td>1242 (1001-1631)</td>
</tr>
<tr>
<td>Carbohydrate (g/day) % total energy</td>
<td>171 (127-224)</td>
<td>165 (136-226)</td>
</tr>
<tr>
<td>Protein (g/day) % total energy</td>
<td>52.0 (39.7-70.5)</td>
<td>53.1 (39.9-70.5)</td>
</tr>
<tr>
<td>Fat (g/day) % total energy</td>
<td>17.1±3.7</td>
<td>16.8±3.0</td>
</tr>
<tr>
<td>% total energy from high sugar food</td>
<td>37.4 (27.0-51.7)</td>
<td>37.4 (27.6-54.4)</td>
</tr>
<tr>
<td>% total energy from high cholesterol food</td>
<td>2.5 (0.3-7.0)</td>
<td>2.8 (0.5-5.6)</td>
</tr>
<tr>
<td>% total energy from high SFA food</td>
<td>3.0 (0.9-7.9)</td>
<td>2.4 (0.8-5.9)</td>
</tr>
<tr>
<td>% total energy from high SFA food</td>
<td>12.7 (5.7-23.5)</td>
<td>11.7 (4.9-18.4)</td>
</tr>
</tbody>
</table>

There was no significant difference in daily energy intake, intake of macronutrients, percentage energy from each macronutrient, and percentage energy from high sugar food, high cholesterol food and high SFA food between patients with and without NAFLD.

Table 3. Distribution of patients with and without NAFLD in the highest and lower quartiles of percentage energy from each macronutrient and in the highest and lower quartiles of percentage energy from high sugar food, high cholesterol food and high SFA food.

<table>
<thead>
<tr>
<th></th>
<th>Patients with NAFLD</th>
<th>Patients without NAFLD</th>
<th>OR</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>% total energy from carbohydrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest quartile (≥61.0%)</td>
<td>38 (25.9)</td>
<td>36 (23.7)</td>
<td>1.12</td>
<td>0.64-1.96</td>
</tr>
<tr>
<td>Lower quartiles (&lt;61.0%)</td>
<td>109 (74.1)</td>
<td>116 (76.3)</td>
<td></td>
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<tr>
<td>% total energy from protein</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Highest quartile (≥19.1%)</td>
<td>37 (25.2)</td>
<td>36 (23.7)</td>
<td>1.08</td>
<td>0.62-1.90</td>
</tr>
<tr>
<td>Lower quartiles (&lt;19.1%)</td>
<td>110 (74.8)</td>
<td>116 (76.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% total energy from fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest quartile (≥31.5%)</td>
<td>37 (25.2)</td>
<td>38 (25.0)</td>
<td>1.01</td>
<td>0.58-1.76</td>
</tr>
<tr>
<td>Lower quartiles (&lt;31.5%)</td>
<td>110 (74.8)</td>
<td>114 (75.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% total energy from high sugar food</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest quartile (≥6.22%)</td>
<td>38 (25.9)</td>
<td>28 (18.4)</td>
<td>1.54</td>
<td>0.86-2.78</td>
</tr>
<tr>
<td>Lower quartiles (&lt;6.22%)</td>
<td>109 (74.1)</td>
<td>124 (81.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% total energy from high cholesterol food</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest quartile (≥6.74%)</td>
<td>44 (29.9)</td>
<td>31 (20.4)</td>
<td>1.67</td>
<td>0.95-2.93</td>
</tr>
<tr>
<td>Lower quartiles (&lt;6.74%)</td>
<td>103 (70.1)</td>
<td>121 (79.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% total energy from high SFA food</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest quartile (≥20.2%)</td>
<td>43 (29.3)</td>
<td>32 (21.1)</td>
<td>1.55</td>
<td>0.89-2.72</td>
</tr>
<tr>
<td>Lower quartiles (&lt;20.2%)</td>
<td>104 (70.7)</td>
<td>120 (78.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no significant difference in the distribution of patients with and without NAFLD in the highest and lower quartiles of percentage energy from each macronutrient and in the highest and lower quartiles of percentage energy from high sugar food, high cholesterol food and high SFA food.
Table 4. Distribution of patients with and without NAFLD according to level of physical activity and highest and lower quartiles of percentage energy from each macronutrient

<table>
<thead>
<tr>
<th></th>
<th>Patients with NAFLD</th>
<th>Patients without NAFLD</th>
<th>OR</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a) Carbohydrate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>19 (12.9 %)</td>
<td>19 (12.5 %)</td>
<td>0.78</td>
<td>0.35-1.72</td>
</tr>
<tr>
<td>A2</td>
<td>69 (46.9 %)</td>
<td>54 (35.5 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B1</td>
<td>19 (12.9 %)</td>
<td>17 (11.2 %)</td>
<td>0.87</td>
<td>0.39-1.97</td>
</tr>
<tr>
<td>B2*</td>
<td>40 (27.2 %)</td>
<td>62 (40.8 %)</td>
<td>0.50</td>
<td>0.29-0.89</td>
</tr>
<tr>
<td>Patients with central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>17 (14.3 %)</td>
<td>14 (14.6 %)</td>
<td>0.62</td>
<td>0.25-1.54</td>
</tr>
<tr>
<td>A2</td>
<td>59 (49.6 %)</td>
<td>30 (31.2 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B1</td>
<td>16 (13.4 %)</td>
<td>12 (12.5 %)</td>
<td>0.68</td>
<td>0.26-1.76</td>
</tr>
<tr>
<td>B2**</td>
<td>27 (22.7 %)</td>
<td>40 (41.7 %)</td>
<td>0.34</td>
<td>0.17-0.70</td>
</tr>
<tr>
<td>Patients without central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>2 (7.1 %)</td>
<td>5 (8.9 %)</td>
<td>0.96</td>
<td>0.11-7.28</td>
</tr>
<tr>
<td>A2</td>
<td>10 (35.7 %)</td>
<td>24 (42.9 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B1</td>
<td>3 (10.7 %)</td>
<td>5 (8.9 %)</td>
<td>1.44</td>
<td>0.22-9.13</td>
</tr>
<tr>
<td>B2</td>
<td>13 (46.4 %)</td>
<td>22 (39.3 %)</td>
<td>1.42</td>
<td>0.46-4.39</td>
</tr>
<tr>
<td><strong>b) Protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>23 (15.6 %)</td>
<td>16 (10.5 %)</td>
<td>1.88</td>
<td>0.84-4.26</td>
</tr>
<tr>
<td>A2</td>
<td>65 (44.2 %)</td>
<td>57 (37.5 %)</td>
<td>1.50</td>
<td>0.85-2.62</td>
</tr>
<tr>
<td>B1</td>
<td>14 (9.5 %)</td>
<td>20 (13.2 %)</td>
<td>0.92</td>
<td>0.39-2.16</td>
</tr>
<tr>
<td>B2</td>
<td>45 (30.6 %)</td>
<td>59 (38.8 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients with central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1*</td>
<td>22 (18.5 %)</td>
<td>11 (11.5 %)</td>
<td>2.44</td>
<td>0.95-6.33</td>
</tr>
<tr>
<td>A2*</td>
<td>54 (45.4 %)</td>
<td>33 (34.4 %)</td>
<td>1.99</td>
<td>1.00-3.97</td>
</tr>
<tr>
<td>B1</td>
<td>11 (9.2 %)</td>
<td>13 (13.5 %)</td>
<td>1.03</td>
<td>0.37-2.88</td>
</tr>
<tr>
<td>B2</td>
<td>32 (26.9 %)</td>
<td>39 (40.6 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients without central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>1 (3.6 %)</td>
<td>5 (8.9 %)</td>
<td>0.31</td>
<td>0.01-3.37</td>
</tr>
<tr>
<td>A2</td>
<td>11 (39.3 %)</td>
<td>24 (42.9 %)</td>
<td>0.71</td>
<td>0.23-2.14</td>
</tr>
<tr>
<td>B1</td>
<td>3 (10.7 %)</td>
<td>7 (12.5 %)</td>
<td>0.66</td>
<td>0.11-3.66</td>
</tr>
<tr>
<td>B2</td>
<td>13 (46.4 %)</td>
<td>20 (35.7 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td><strong>c) Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1*</td>
<td>27 (18.4 %)</td>
<td>18 (11.8 %)</td>
<td>3.00</td>
<td>1.03-8.88</td>
</tr>
<tr>
<td>A2</td>
<td>61 (41.5 %)</td>
<td>55 (36.2 %)</td>
<td>2.22</td>
<td>0.89-5.61</td>
</tr>
<tr>
<td>B1</td>
<td>10 (6.8 %)</td>
<td>20 (13.2 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B2</td>
<td>49 (33.3 %)</td>
<td>59 (38.8 %)</td>
<td>1.66</td>
<td>0.66-4.23</td>
</tr>
<tr>
<td>Patients with central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1*</td>
<td>23 (19.3 %)</td>
<td>10 (10.4 %)</td>
<td>4.03</td>
<td>1.12-15.0</td>
</tr>
<tr>
<td>A2*</td>
<td>53 (44.5 %)</td>
<td>34 (35.4 %)</td>
<td>2.73</td>
<td>0.94-8.05</td>
</tr>
<tr>
<td>B1</td>
<td>8 (6.7 %)</td>
<td>14 (14.6 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B2</td>
<td>35 (29.4 %)</td>
<td>38 (39.6 %)</td>
<td>1.61</td>
<td>0.55-4.83</td>
</tr>
<tr>
<td>Patients without central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>4 (14.3 %)</td>
<td>8 (14.3 %)</td>
<td>1.50</td>
<td>0.14-17.5</td>
</tr>
<tr>
<td>A2</td>
<td>8 (28.6 %)</td>
<td>21 (37.5 %)</td>
<td>1.14</td>
<td>0.15-10.3</td>
</tr>
<tr>
<td>B1</td>
<td>2 (7.1 %)</td>
<td>6 (10.7 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B2</td>
<td>14 (50.0 %)</td>
<td>21 (37.5 %)</td>
<td>2.00</td>
<td>0.29-16.9</td>
</tr>
</tbody>
</table>

Significantly different from the comparative group: *, p < 0.05; **, p < 0.01.
A1 = low level of physical activity and highest quartile of percentage energy from corresponding macronutrient.
A2 = low level of physical activity and lower quartiles of percentage energy from corresponding macronutrient.
B1 = moderate or high level of physical activity and highest quartile of percentage energy from corresponding macronutrient.
B2 = moderate or high level of physical activity and lower quartiles of percentage energy from corresponding macronutrient.
Table 5. Distribution of patients with and without NAFLD according to level of physical activity and highest and lower quartiles of percentage energy from high sugar food, high cholesterol food and high SFA food

<table>
<thead>
<tr>
<th></th>
<th>Patients with NAFLD</th>
<th>Patients without NAFLD</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High sugar food</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>22 (15.0 %)</td>
<td>17 (11.2 %)</td>
<td>2.05</td>
<td>0.92-4.58</td>
</tr>
<tr>
<td>A2*</td>
<td>66 (44.9 %)</td>
<td>56 (36.8 %)</td>
<td>1.86</td>
<td>1.07-3.25</td>
</tr>
<tr>
<td>B1</td>
<td>16 (10.9 %)</td>
<td>11 (7.2 %)</td>
<td>2.30</td>
<td>0.90-5.91</td>
</tr>
<tr>
<td>B2</td>
<td>43 (29.3 %)</td>
<td>68 (44.7 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients with central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1*</td>
<td>20 (16.8 %)</td>
<td>9 (9.4 %)</td>
<td>3.23</td>
<td>1.19-8.89</td>
</tr>
<tr>
<td>A2**</td>
<td>56 (47.1 %)</td>
<td>35 (36.5 %)</td>
<td>2.32</td>
<td>1.19-4.55</td>
</tr>
<tr>
<td>B1</td>
<td>12 (10.1 %)</td>
<td>7 (7.3 %)</td>
<td>2.49</td>
<td>0.79-7.99</td>
</tr>
<tr>
<td>B2</td>
<td>31 (26.1 %)</td>
<td>45 (46.9 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients without central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>2 (7.1 %)</td>
<td>8 (14.3 %)</td>
<td>0.48</td>
<td>0.06-3.12</td>
</tr>
<tr>
<td>A2</td>
<td>10 (35.7 %)</td>
<td>21 (37.5 %)</td>
<td>0.91</td>
<td>0.29-2.87</td>
</tr>
<tr>
<td>B1</td>
<td>4 (14.3 %)</td>
<td>4 (7.1 %)</td>
<td>1.92</td>
<td>0.32-11.6</td>
</tr>
<tr>
<td>B2</td>
<td>12 (42.9 %)</td>
<td>23 (41.1 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td><strong>High cholesterol food</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1**</td>
<td>31 (21.1 %)</td>
<td>15 (9.9 %)</td>
<td>2.83</td>
<td>1.30-6.24</td>
</tr>
<tr>
<td>A2</td>
<td>57 (38.8 %)</td>
<td>58 (38.2 %)</td>
<td>1.35</td>
<td>0.77-2.36</td>
</tr>
<tr>
<td>B1</td>
<td>13 (8.8 %)</td>
<td>16 (8.8 %)</td>
<td>1.11</td>
<td>0.45-2.74</td>
</tr>
<tr>
<td>B2</td>
<td>46 (31.3 %)</td>
<td>63 (31.3 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients with central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1**</td>
<td>26 (21.8 %)</td>
<td>9 (9.4 %)</td>
<td>3.61</td>
<td>1.37-9.72</td>
</tr>
<tr>
<td>A2</td>
<td>50 (42.0 %)</td>
<td>35 (36.5 %)</td>
<td>1.79</td>
<td>0.90-5.34</td>
</tr>
<tr>
<td>B1</td>
<td>11 (9.2 %)</td>
<td>12 (12.5 %)</td>
<td>1.15</td>
<td>0.40-3.24</td>
</tr>
<tr>
<td>B2</td>
<td>32 (26.9 %)</td>
<td>40 (41.6 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients without central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>5 (17.9 %)</td>
<td>6 (10.7 %)</td>
<td>1.37</td>
<td>0.29-6.48</td>
</tr>
<tr>
<td>A2</td>
<td>7 (25.0 %)</td>
<td>23 (41.1 %)</td>
<td>0.50</td>
<td>0.15-1.65</td>
</tr>
<tr>
<td>B1</td>
<td>2 (7.1 %)</td>
<td>4 (7.1 %)</td>
<td>0.82</td>
<td>0.09-6.39</td>
</tr>
<tr>
<td>B2</td>
<td>14 (50.0 %)</td>
<td>23 (41.1 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td><strong>High SFA food</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1*</td>
<td>29 (19.7 %)</td>
<td>19 (12.5 %)</td>
<td>2.24</td>
<td>1.06-4.74</td>
</tr>
<tr>
<td>A2</td>
<td>59 (40.1 %)</td>
<td>54 (35.5 %)</td>
<td>1.60</td>
<td>0.91-2.82</td>
</tr>
<tr>
<td>B1</td>
<td>14 (9.5 %)</td>
<td>13 (8.6 %)</td>
<td>1.58</td>
<td>0.63-3.99</td>
</tr>
<tr>
<td>B2</td>
<td>45 (30.6 %)</td>
<td>66 (43.4 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients with central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1*</td>
<td>25 (21.0 %)</td>
<td>12 (12.5 %)</td>
<td>2.67</td>
<td>1.08-6.67</td>
</tr>
<tr>
<td>A2*</td>
<td>51 (42.9 %)</td>
<td>32 (33.3 %)</td>
<td>2.04</td>
<td>1.03-4.08</td>
</tr>
<tr>
<td>B1</td>
<td>11 (9.2 %)</td>
<td>11 (11.3 %)</td>
<td>1.28</td>
<td>0.45-3.69</td>
</tr>
<tr>
<td>B2*</td>
<td>32 (26.9 %)</td>
<td>41 (42.7 %)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Patients without central obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>4 (14.3 %)</td>
<td>7 (12.5 %)</td>
<td>1.10</td>
<td>0.22-5.37</td>
</tr>
<tr>
<td>A2</td>
<td>8 (28.6 %)</td>
<td>22 (39.3 %)</td>
<td>0.70</td>
<td>0.21-2.25</td>
</tr>
<tr>
<td>B1</td>
<td>3 (10.7 %)</td>
<td>2 (3.6 %)</td>
<td>2.88</td>
<td>0.33-29.1</td>
</tr>
<tr>
<td>B2</td>
<td>13 (46.4 %)</td>
<td>25 (44.6 %)</td>
<td>1</td>
<td>–</td>
</tr>
</tbody>
</table>

Significantly different from the comparative group: *, p < 0.05; **, p < 0.01.
A1 = low level of physical activity and highest quartile of percentage energy from corresponding food type.
A2 = low level of physical activity and lower quartiles of percentage energy from corresponding food type.
B1 = moderate or high level of physical activity and highest quartile of percentage energy from corresponding food type.
B2 = moderate or high level of physical activity and lower quartiles of percentage energy from corresponding food type.
Table 6. Energy contribution of the different polyunsaturated fatty acids in patients with and without NALFD

<table>
<thead>
<tr>
<th></th>
<th>Patient with NAFLD</th>
<th>Patient without NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>6.37 (3.69-9.79)</td>
<td>6.56 (3.96-10.4)</td>
</tr>
<tr>
<td>n-6 fatty acids</td>
<td>5.99 (3.42-9.32)</td>
<td>6.22 (3.61-9.78)</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>5.86 (3.42-8.75)</td>
<td>6.01 (3.33-9.30)</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>0.0017 (0.0006-0.602)</td>
<td>0.0011 (0.0007-0.323)</td>
</tr>
<tr>
<td>n-3 fatty acids</td>
<td>0.42 (0.30-0.55)</td>
<td>0.45 (0.30-0.61)</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>0.31 (0.22-0.41)</td>
<td>0.33 (0.20-0.48)</td>
</tr>
<tr>
<td>Eicosapentenoic acid</td>
<td>0.03 (0.02-0.06)</td>
<td>0.03 (0.02-0.05)</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>0.06 (0.04-0.10)</td>
<td>0.06 (0.04-0.10)</td>
</tr>
<tr>
<td>n-3/n-6 fatty acid ratio</td>
<td>0.07 (0.05-0.09)</td>
<td>0.07 (0.05-0.11)</td>
</tr>
</tbody>
</table>

There was no significant difference in the energy contribution of the different polyunsaturated fatty acids and the n-3/n-6 fatty acid ratio between patients with and without NAFLD.

Figure 1 Flow diagram showing patients who were included/excluded in the study