Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): A population based study☆

Shira Zelber-Sagi1,3,†, Dorit Nitzan-Kaluski2,3, Rebecca Goldsmith2, Muriel Webb1, Laurie Blendis1, Zamir Halpern1,3, Ran Oren1,3,*

1The Liver Unit, Department of Gastroenterology, Tel Aviv Sourasky Medical Center, Tel Aviv 64239, Israel
2The Food and Nutrition Administration, Ministry of Health, 2 Ben Tabai St. Jerusalem, Israel
3The Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv 69978, Israel

Background/Aims: Weight loss is considered therapeutic for patients with NAFLD. However, there is no epidemiological evidence that dietary habits are associated with NAFLD. Dietary patterns associated with primary NAFLD were investigated.

Methods: A cross-sectional study of a sub-sample (n = 375) of the Israeli National Health and Nutrition Survey. Exclusion criteria were any known etiology for secondary NAFLD. Participants underwent an abdominal ultrasound, biochemical tests, dietary and anthropometric evaluations. A semi-quantitative food-frequency questionnaire was administered.

Results: After exclusion, 349 volunteers (52.7% male, mean age 50.7 ± 10.4, 30.9% primary NAFLD) were included. The NAFLD group consumed almost twice the amount of soft drinks (P = 0.03) and 27% more meat (P < 0.001). In contrast, the NAFLD group consumed somewhat less fish rich in omega-3 (P = 0.056). Adjusting for age, gender, BMI and total calories, intake of soft drinks and meat was significantly associated with an increased risk for NAFLD (OR = 1.45, 1.13–1.85 95% CI and OR = 1.37, 1.04–1.83 95% CI, respectively).

Conclusions: NAFLD patients have a higher intake of soft drinks and meat and a tendency towards a lower intake of fish rich in omega-3. Moreover, a higher intake of soft drinks and meat is associated with an increased risk of NAFLD, independently of age, gender, BMI and total calories.

© 2007 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Keywords: NAFLD; Nutrition; Soft drinks; Meat; Omega-3

1. Introduction

Obesity is strongly associated with hepatic steatosis in humans [1]. Therefore, the usual management of NAFLD includes gradual weight reduction and increase in physical activity [2–7]. It is possible that altering dietary macronutrient composition could modulate NAFLD even without weight loss [8]. However, it remains uncertain whether excessive food consumption per se causes fatty liver. It is also unclear whether or not diets that are enriched in certain types of food are more likely to cause fatty liver than others [1].

A few recent studies on selected NAFLD/NASH patients were designed to address these questions [9–12]. The results of these studies are not uniform. Some highlight the positive association between carbohydrate intake and histological inflammation [12], or the presence of the metabolic syndrome [10]. Others report an association between a higher intake of saturated fat...
and cholesterol and a lower intake of poly-unsaturated Fat [11], or a higher intake of total fat with a higher n - 6/n - 3 fatty acid ratio [9].

Although the association between dietary intake and the components of the metabolic syndrome has been analyzed, the studies did not focus on NAFLD and hence there is no epidemiological evidence that dietary habits are associated with fatty liver [13]. The present study is an epidemiological study designed to identify specific long term dietary patterns that might be associated with NAFLD.

2. Patients and methods

2.1. Study design

Data were collected prospectively from January 2003 to March 2004. The study population consisted of 799 participants, a sub-group of 3280 Israeli adults (24–70 years of age) who were randomly sampled from the national population registry and interviewed in the First Israeli National Health and Nutrition Survey (the MABAT Survey) [14]. Individuals with any of the following were excluded from the study: alcohol consumption ≥ 30 g/day in men or ≥ 20 g/day in women [15–17], presence of HBsAg or anti-HCV antibodies, fatty liver suspected to be secondary to hepatotoxic drugs, inflammatory bowel disease, prior surgery that could cause fatty liver, or celiac disease. Pregnant women were also excluded. As described elsewhere [18,19], five hundred and thirty-nine of the 799 individuals in the sub-sample were located. Of these 239 (44.3%) agreed to participate in the study, as did 136 of their spouses or acquaintances. Thus, the study population consisted of 375 subjects prior to the application of exclusion criteria.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institution’s Human Research Committee. All study participants signed informed consent.

2.2. Study measurements

The one-time evaluation included an interview, anthropometric measurements, biochemical tests and ultrasound (US), all performed on the same day.

2.3. Interview

A face-to-face interview was carried out in all cases by the same interviewer. To avoid information bias, both the subject and the interviewer were unaware of the results of the examination.

The first questionnaire included demographic data, health status and current alcohol intake. The second questionnaire was a detailed semi-quantitative food-frequency questionnaire (FFQ) adapted to the Israeli population. The FFQ has the advantage of providing information on long term nutritional habits, which is conceptually more important than intake on a few specific days and is the most commonly used method in epidemiological studies [20]. The FFQ was devised by the Food and Nutrition Administration, Ministry of Health, based on data from the 24-h recall questionnaires used in the MABAT Survey. The FFQ included 111 food items with specified serving sizes that are described by using natural portions (e.g., one banana and one slice of pizza) or standard weight and volume measures of the servings commonly consumed in this study population. For each food item, participants indicated their average frequency of consumption over the past year in terms of the number of specified serving sizes consumed per day/week/month, less than once a month, or never. The selected frequency category for each food item was converted to a daily intake. The nutrient components of each food item were taken from the Israeli National Nutrient Database (BINAT), Food and Nutrition Administration, Ministry of Health.

2.4. Anthropometric measurements

All patients underwent measurements of weight, height and waist and hip circumference. Measurements were taken by the interviewer at the end of the interview, following a uniform protocol that included repeat measurements for each participant. If the two measurements differed by more than 0.5 cm, a third measurement was taken. When the two measurements were similar their mean was calculated. When a third measurement was required the mean of the third measurement and the closest to it of the first two measurements was calculated.

2.5. Biochemical tests

Each participant underwent biochemical testing, following a 12 h fast, for liver enzymes, serum lipid profile and fasting serum glucose and serum insulin levels. All biochemical assessments were performed in the same laboratory by standard laboratory methods.

2.6. Radiological examination

Fatty liver was diagnosed by abdominal US using standardized criteria [21]. US was performed in all subjects with the same equipment (Hitachi EUB 6500 with a 3.5 MHz convex probe). The same operator, who was unaware of the clinical and laboratory results, performed all US procedures.

2.7. Statistical analysis

Statistical analyses were performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA) software.

Continuous variables are presented as means ± SD. To test differences in continuous variables between the two groups the independent samples t-test (for normally distributed variables) or the Mann–Whitney U test (if non-parametric tests were required) was performed. Associations between nominal variables were performed with the Pearson’s χ² test. The suspected association between NAFLD and nutrition was tested on several levels: macro- and micro-nutrients and different food groups, using both the absolute amount (crude intake) as well as calorie adjusted.

A multivariate logistic regression analysis was performed to test the adjusted association between nutritional variables and NAFLD. The nutritional model was performed in two blocks: the first included age, gender, BMI and total calorie intake that were forced into the model and the second added a stepwise regression that included major nutrients or groups of food items: total carbohydrates, total fat, saturated fat, mono-unsaturated fat, poly-unsaturated fat, fiber, fruits, vegetables, carbohydrates from different sweet food items and soft drinks, protein from high- and low-fat meat, protein from high- and low-fat dairy products, protein from all types of meat or fish or dairy together and separately, and protein from fish rich in omega-3.

A value of P < 0.05 was considered statistically significant for all analyses.

3. Results

3.1. Characteristics of the study population

Three hundred and forty-nine volunteers (52.7% male, mean age 50.7 ± 10.4 SD [24–42]) who met the inclusion and exclusion criteria were included in the analysis. The mean BMI was 27.2 ± 4.5. Fasting serum levels of glucose (90.8 ± 19.5 mg/dl), insulin (22.3 ± 11.8 μU/ml), triglycerides (117.0 ± 61 mg/dl) and liver enzymes (22.0 ± 10.0 U/L for ALT and 23.0 ± 6.4 for AST) were within the normal range. The prevalence of primary NAFLD, diag-
nosed by ultrasound, in the study population was 30.9% \((n = 108)\) (95% CI 26–36%). Detailed information on the study population has been described elsewhere [18,19].

3.2. Comparison of macro-nutrient intake between subjects diagnosed as NAFLD versus normal liver

Among men and women together no significant difference was found in macro-nutrient intake between the groups (Table 1). However, women with NAFLD had a significantly higher consumption of calories (2702.5 ± 1114.0 kcal/day vs. 2277.8 ± 902.5, \(P = 0.016\)) and other macro-nutrients as fat (118.5 ± 54.3 g/day vs. 97.8 ± 44.4, \(P = 0.017\)), carbohydrates (312.2 ± 149.3 g/day vs. 266.6 ± 114.1, \(P = 0.045\)) and protein (118.0 ± 51.1 g/day vs. 98.9 ± 44.9, \(P = 0.026\)). No significant difference was found among men.

Further comparisons of major macro-nutrient intake were performed by adjusting for total caloric intake. No significant differences were found between the groups in the percent of fat or carbohydrates intake, for men and women together and separately (Table 2). In men and women together the NAFLD group tended to consume a larger percent of protein but this difference did not reach statistical significance (\(P = 0.08\)). Among men this higher intake of protein was statistically significant (\(P = 0.04\)), whereas among women it was not.

3.3. Comparison of the composition of fat intake, adjusted for total fat intake, between subjects diagnosed as NAFLD versus normal liver

The intake of different types of fat (saturated, mono-unsaturated, poly-unsaturated and cholesterol) was compared between the two groups after adjusting for total fat intake. In men and women together and separately no significant difference was found between the groups (data not shown).

3.4. Comparison of average daily carbohydrate intake from sweet foods

Carbohydrate consumption from sweet food items was classified according to the following categories:

1. Soft drinks (sweetened with sugar).
2. Sugar, honey, candy, soft drinks, cakes, cookies, chocolate and chocolate snacks, ice-cream, mousse, sweetened morning cereals.

The NAFLD group consumed almost twice the amount of carbohydrates from soft drinks (23.3 ± 42.0 vs. 12.3 ± 24.1 g/day, \(P = 0.03\)) (Fig. 1).

3.5. Comparison of average daily protein intake from different sources between subjects diagnosed as NAFLD versus normal liver

Protein consumption was classified as fat meat (beef, liver, sausage, hot dog, lamb) and/or low-fat meat (chicken, turkey) and/or fish (pond fish, sea fish, North Sea fish), fish rich in omega-3 fatty acids (North Sea fish, i.e., salmon, herring, mackerel, tuna, hake), high-fat dairy products (yellow cheese, cream cheese, cream) and/or low-fat dairy products (milk, yoghurt, low-fat cheese with up to 5% fat). The NAFLD group consumed 27% more meat protein from all types of meat (high-fat plus low-fat) with a mean of 33.3 ± 22.8 versus 26.2 ± 17.9 g/day (\(P < 0.001\)) (Fig. 1). In contrast, the NAFLD group consumed somewhat a little less protein.

Table 1

<table>
<thead>
<tr>
<th>Parameter (U)</th>
<th>NAFLD ((n = 108))</th>
<th>Normal liver ((n = 241))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>51.4 ± 9.8</td>
<td>50.4 ± 10.7</td>
<td>0.41</td>
</tr>
<tr>
<td>Gender (% males)</td>
<td>63.9%</td>
<td>47.7%</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 ± 4.6</td>
<td>25.8 ± 3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>2493.2 ± 1013.4</td>
<td>2381.6 ± 1009.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>103.8 ± 48.3</td>
<td>100.3 ± 49.1</td>
<td>0.54</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>295.5 ± 139.2</td>
<td>282.1 ± 125.7</td>
<td>0.37</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>110.2 ± 45.6</td>
<td>102.0 ± 45.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>27.0 ± 12.1</td>
<td>26.4 ± 12.7</td>
<td>0.69</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>34.0 ± 18.7</td>
<td>33.2 ± 19.0</td>
<td>0.72</td>
</tr>
<tr>
<td>MUFAs (g)</td>
<td>37.4 ± 18.0</td>
<td>39.1 ± 19.7</td>
<td>0.43</td>
</tr>
<tr>
<td>PUFAs (g)</td>
<td>21.4 ± 9.9</td>
<td>20.7 ± 11.0</td>
<td>0.56</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>314.4 ± 154.4</td>
<td>306.0 ± 167.6</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; MUFAs, mono-unsaturated fat; PUFAs, poly-unsaturated fat.

Table 2

<table>
<thead>
<tr>
<th>Gender Nutrient (%)</th>
<th>NAFLD ((n = 69))</th>
<th>Normal liver ((n = 115))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men and women together</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>37.4 ± 6.7</td>
<td>37.7 ± 6.4</td>
<td>0.76</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>46.9 ± 8.2</td>
<td>47.4 ± 8.0</td>
<td>0.63</td>
</tr>
<tr>
<td>Protein</td>
<td>18.2 ± 4.1</td>
<td>17.4 ± 3.4</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Further comparisons of major macro-nutrient intake were performed by adjusting for total caloric intake. No significant differences were found between the groups in the percent of fat or carbohydrates intake, for men and women together and separately (Table 2). In men and women together the NAFLD group tended to consume a larger percent of protein but this difference did not reach statistical significance (\(P = 0.08\)). Among men this higher intake of protein was statistically significant (\(P = 0.04\)), whereas among women it was not.
from fish rich in omega-3 (3.7 ± 4.3 vs. 4.9 ± 7.2 g/day, \( P = 0.056 \)). This difference did not reach statistical significance.

No significant difference was found in other categories of protein intake.

### 3.6. Multivariate analysis of the association between nutrition and NAFLD

Adjusting for age, gender, BMI and total calories, intake of carbohydrates from soft drinks and protein from all types of meat was significantly associated with an increased risk for NAFLD (OR = 1.45, 1.13–1.85 95% CI per 31.1 g/day increment [1 SD] and OR = 1.37, 1.04–1.83 95% CI per 19.8 g/day increment [1 SD], respectively). In contrast, intake of protein from fish rich in omega-3 tended to reduce the risk for NAFLD (OR = 0.73, 0.52–1.04 95% CI per 6.4 g/day increment [1 SD]) (Table 3).

When intake of protein from all types of meat was stratified to quartiles (first < 16.7 g/day, 16.7 ≤ second < 25.0, 25.0 ≤ third < 36.0, fourth ≥ 36.0) the age, gender, BMI and total calorie intake adjusted OR for NAFLD significantly increased with the increase in quartiles, \( P = 0.01 \) (Fig. 2). The percent of subjects diagnosed as NAFLD in the fourth quartile of meat protein intake was 46% compared to 17% in the first quartile \( P = 0.001 \) (Fig. 3).

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR (95% CI)</th>
<th>( P )-Wald</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.004 (0.98–1.03)</td>
<td>0.76</td>
</tr>
<tr>
<td>Male gender</td>
<td>1.64 (0.99–2.70)</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>1.07 (1.03–1.11)</td>
<td>0.001</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1.00 (1.00–1.00)</td>
<td>0.99</td>
</tr>
<tr>
<td>Carbohydrates from soft drinks (31.1 g [1 SD])(^a)</td>
<td>1.45 (1.13–1.85)</td>
<td>0.005</td>
</tr>
<tr>
<td>Protein from all types</td>
<td>1.37 (1.04–1.83)</td>
<td>0.03</td>
</tr>
<tr>
<td>of meat (19.8 g [1 SD])(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein from fish rich in</td>
<td>0.73 (0.52–1.04)</td>
<td>0.08</td>
</tr>
<tr>
<td>omega-3 (6.4 g [1 SD])(^c)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. The Wald test was used to assess the significance of each logit. \( P\)-LR for the full model <0.001.

\(^a\) Soft drinks sweetened with sugar.

\(^b\) Protein from all kinds of meat: beef, liver, sausage, hot dog, lamb, chicken, turkey.

\(^c\) Fish rich in omega-3 fatty acids: North Sea fish, i.e., salmon, herring, mackerel, tuna, hake.
4. Discussion

The results of this study show that a higher intake of soft drinks and of meat was significantly associated with an increased risk for NAFLD, while a higher intake of fish rich in omega-3 tended to reduce the risk for NAFLD.

Unlike Musso et al. [11] who reported that the dietary intake of NASH patients was richer in saturated fat and in cholesterol and was poorer in PUFA, fiber, ascorbic acid and tocopherol compared to matched controls, we found no significant difference in the intake of different types of fats or antioxidants and fiber. However, our findings of higher meat intake and a tendency to a lower intake of fish rich in omega-3 in NAFLD patients are consistent with a recent paper by Cortez-Pinto et al. who reported a higher intake of n − 6 fatty acids (mostly found in meat) and n − 6/n − 3 ratio in NASH patients [9].

The only statistically significant difference in a nutritional source for simple carbohydrates was the consumption of soft drinks sweetened with sugar. An increase of 31 g/day in sugar intake from soft drinks (equal to about one can of an average soft drink) raises the OR for NAFLD by 1.45 (1.13–1.85, 95% CI) (Table 3).

Based on the association of the increased high-fructose corn syrup consumption with obesity, as well as the hepatic effects of high-fructose diets in animal models, it is reasonable to suggest that NAFLD patients should limit their high-fructose corn syrup consumption, although definitive data are lacking [8].

A higher consumption of sugar-sweetened beverages was found to be associated with a greater magnitude of weight gain and an increased risk for development of type 2 diabetes, possibly by providing excess calories and large amounts of rapidly absorbable sugars [22].

Various NADPH-inducing lipogenic hepatic enzymes are generated following a sucrose-rich diet. Thus, these diets increase the hepatic synthesis of triglycerides. Rats and humans that are fed either sucrose- or fructose-enriched diets develop fatty livers [23,24].

To our knowledge, a direct association between NAFLD and protein intake has never been investigated, but there are reports of an association between protein intake and insulin resistance or diabetes.

In the present study, men with NAFLD consumed a significantly larger percent of protein in their diet (Table 2), but with further adjustment for age and BMI (in the multivariate analysis) this association was not statistically significant (P = 0.27). However, we did find a significant and independent association with meat protein intake. A consumption of 36 g/day (about 110 g of cooked red meat), or more, results in an OR of 3.6 (1.7–7.8, 95% CI) for NAFLD (Fig. 2).

High protein intake is associated with insulin resistance and glucose intolerance [25,26] and might even increase the incidence of type-2 diabetes [27–30]. In particular, high consumption of red meat, especially processed meat, shows a positive association with the risk of type-2 diabetes [27,28]. We did not find an association with the specific consumption of fat or processed meat but rather with the consumption of all types of meat.

There are several potential explanations for the association between meat intake and type-2 diabetes. First, higher amounts of saturated fat and cholesterol in meat could increase the risk of diabetes [28]. Second, preservatives and additives contained in processed meats may contribute to the increased risk [27]. Third, the increased risk with higher meat intake might be due to dietary factors associated with meat intake, in particular adherence to a “Western” dietary pattern [28]. Another possible explanation is a higher consumption of iron and especially heme-iron that may play a role in the pathogenesis of NAFLD by increasing oxidative stress [31]. However, we did not find any significant difference in dietary iron consumption [19].

In the present study, higher consumption of fish rich in omega-3 appeared to reduce the risk for NAFLD, but this association did not reach statistical significance. The lack of statistical significance may be due to insufficient statistical power, especially since the consumption of fish rich in omega-3 is not very common among Israelis.

Support for the possible protective role of omega-3 fatty acids can be found in the results of a pilot study which showed that PUFA supplementation yielded a beneficial effect on liver enzymes and steatosis [32]. Insulin resistance may be accompanied by a deficiency of omega-3 PUFAs in serum and tissues [33,34]. Furthermore, low levels of circulating n − 3 PUFAs are associated with higher lipogenesis and hepatic uptake of circulating free fatty acids along with decreased fatty acid oxidation and VLDL synthesis [35–37].

Women with NAFLD had a significantly higher consumption of calories compared to women with normal livers. Surprisingly, no such difference was found among men. Moreover, calorie intake among men diagnosed as NAFLD was even lower than the calorie intake of women diagnosed as NAFLD. This gender difference may be explained by the lower reporting of energy intake in dietary self-reports in men [38–40] perhaps since women are typically more conscious of serving sizes than men [38].

In applying dietary assessment the possibility of recall bias or reporting bias should be considered. Recall bias could be introduced by subjects (e.g., obese individuals) who are more knowledgeable about healthy diets. In this study most people were unaware of whether they had NAFLD or not. The participants were informed of their US results only after they completed their dietary questionnaires. Thus, there is no reason to believe that NAFLD was the source of a recall bias. To prevent interviewer bias the interviewer was also unaware of...
the US results. Therefore, we believe that in our study this misclassification bias is non-differential so a true association might only be underestimated [41].

Since the magnitude of underreporting depends on gender, age and BMI [38,39] these potential confounders were adjusted for in the nutritional multivariate analysis. Energy intake was also considered as a possible confounding factor in the univariate and multivariate analysis. It has been shown that when the nutrient values are energy-adjusted or are expressed as percentages of energy intake, the effect of underreporting is minimized [39].

There are methodological limitations to this study. The suboptimal sample does not enable us to represent the entire adult population of Israel. However, it is unlikely that there is a differential response bias in this study since most people are unaware that they have NAFLD. The diagnosis of NAFLD was not based on liver biopsy. Still, US is a reasonably established accurate tool for diagnosing NAFLD [42] and a non-differential misclassification bias that might occur could only reduce the strength of the associations.

In conclusion, NAFLD patients have a higher intake of soft drinks and meat and a tendency to a lower intake of fish rich in omega-3. The higher intake of soft drinks and meat is associated with an increased risk of NAFLD, independent of age, gender, BMI and total calories, but could also represent an unhealthy dietary pattern that might lead to NAFLD. These associations need to be confirmed in larger, ideally prospective, studies designed to further clarify the pathogenesis of NAFLD and to establish evidence-based nutritional recommendations for its prevention and treatment.

Acknowledgements

The authors thank the Eduarda and Dr. Moshe Ishay Institute for the study of the Effect of Natural Food on the Quality of Life and Human Health, Tel-Aviv University for providing a grant to this study.

References


