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Journal of the American College of Nutrition

ISSN: 0731-5724 (Print) 1541-1087 (Online) Journal homepage: http://www.tandfonline.com/loi/uacn20

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To cite this article: Monica Dinu, Anne Whittaker, Giuditta Pagliai, Ilaria Giangrandi, Barbara Colombini, Anna Maria Gori, Claudia Fiorillo, Matteo Becatti, Alessandro Casini, Stefano Benedettelli & Francesco Sofi (2018) A Khorasan Wheat-Based Replacement Diet Improves Risk Profile of Patients With Nonalcoholic Fatty Liver Disease (NAFLD): A Randomized Clinical Trial, Journal of the American College of Nutrition, 37:6, 508-514, DOI: 10.1080/07315724.2018.1445047

To link to this article: https://doi.org/10.1080/07315724.2018.1445047

Published online: 13 Apr 2018.

JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION
2018, VOL. 37, NO. 6, 508–514
https://doi.org/10.1080/07315724.2018.1445047

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A Khorasan Wheat-Based Replacement Diet Improves Risk Profile of Patients With Nonalcoholic Fatty Liver Disease (NAFLD): A Randomized Clinical Trial

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ABSTRACT

Objective: KAMUT khorasan is an ancient grain with widely acclaimed health benefits. The aim of this study was to investigate the effects of a replacement diet with ancient khorasan wheat products in patients with NAFLD, in comparison to a similar replacement diet with control products made from organic semi-whole-grain modern wheat.

Methods: Forty NAFLD patients (12 M/28 F; age 55.2 ± 10.4 years) with mild to moderate liver steatosis were included. The experimental design was a randomized, double-blind, parallel-arm study with 20 participants assigned to consume either KAMUT khorasan or control wheat products (pasta, bread, crackers, biscuits) over a 3-month period. Anthropometric measurements, blood analyses, and ultrasonography examination were performed at both the beginning and end of each dietary intervention.

Results: After the implementation of a general linear model for repeated measurements adjusted for baseline demographic details, risk factors, and medication, alanine aminotransferase (ALT) was significantly reduced by 12%, aspartate aminotransferase (AST) by 14%, alkaline phosphatase (ALP) by 8%, and cholesterol by 6% only in the khorasan group ($p < 0.05$ for all). Similarly, significant reductions in circulating proinflammatory tumor necrosis factor-alpha by 50%, interleukin I-receptor antagonist-alpha by 37%, interleukin-8 by 24%, and interferon gamma by 24% were evident only in participants who consumed the khorasan products ($p < 0.05$ for all). Finally, significant improvements in the liver steatosis grading, Doppler perfusion index values, and reactive oxygen species (ROS) production were evident after consumption of both the khorasan and control products.

Conclusions: This study suggests that a short-term replacement diet with ancient KAMUT khorasan products is most effective in reducing metabolic risk factors and ameliorating the liver profile in patients with NAFLD.

ARTICLE HISTORY
Received 29 November 2017
Accepted 21 February 2018

KEYWORDS
NAFLD; khorasan; ancient grain; risk factors; steatosis

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the accumulation of fats in the liver of patients who do not consume excessive alcohol. NAFLD encompasses a wide spectrum of conditions, ranging from the asymptomatic accumulation of triglyceride deposits (hepatic steatosis) to the potentially progressive nonalcoholic steatohepatitis (NASH), also characterized by inflammation, ballooning, and moderate fibrosis, to more advanced forms including cirrhosis, liver failure, and hepatocellular carcinoma (1,2). The estimated prevalence of NAFLD in the adult population ranges from 20 to 30% (15–20% in women and 30–40% in men), increasing up to 70% and 90% in type 2 diabetes mellitus (T2DM) and obese populations, respectively (1−3). Multiple parallel factors, including insulin resistance, oxidative stress, inflammation, and altered gut microflora, acting synergistically (multiple-hit hypothesis) in genetically predisposed individuals, are implicated in the development and progression of the disease (1,4). Moreover, there is growing evidence that NAFLD is a multisystem disease affecting extrahepatic organs and regulatory pathways, as in T2DM and cardiovascular disease (CVD), with the latter being responsible for the majority of NAFLD deaths (3).

NAFLD in the hepatic steatosis phase can be reversed by lifestyle modifications, with the "gold standard" treatment including weight loss, dietary therapy, and physical activity (2). Although a relatively smaller proportion of patients with

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NAFLD have NASH, the latter is more difficult to treat, and preventing the progression of hepatic steatosis to NASH is of primary importance in the treatment of NAFLD (2,5). In general, dietetic recommendations for NAFLD are the same as those for obesity, T2DM, and dyslipidemia; however, clinical dietary trials on patients with NAFLD are scarce (2). In a single study, the Mediterranean diet was shown to reduce hepatic steatosis and reduce insulin resistance in patients with nondiabetic NAFLD (6). Given the well-established benefits of the Mediterranean diet, we were particularly interested in focusing on cereal products, which form the basis of the Mediterranean dietary pyramid (with carbohydrates comprising 50–60% of the daily dietary energy). Whole-grain intake was proposed as part of a both prevention and treatment strategy in the treatment of NAFLD (5), but to the best of our knowledge no trials have yet been performed assessing the efficacy of grains on NAFLD patients. Of particular relevance are the positive benefits reported on human health by ancient *Triticum turgidum* subsp. *turanicum (*Khorasan) (7–10). Previous studies demonstrated that ancient khorasan wheat, as the principle cereal grain source in a Mediterranean diet, provides additive protective effects in reducing glucose and insulin, as well as lipid, oxidative, and inflammatory risk factors, in populations with acute coronary syndrome and T2DM (9,10).

Given the shared risk factors (visceral fat, dyslipidemia, insulin resistance, inflammation, oxidative stress), particularly between NAFLD and T2DM (3,11), the aim of the present study was to investigate the effects of a replacement diet with ancient khorasan wheat products in NAFLD participants.

Methods and materials

Participants

All patients were recruited from the Unit of Clinical Nutrition of the Department of Experimental and Clinical Medicine, University of Florence, Careggi University Hospital. The study population was comprised of 40 volunteers (12 males; 28 females), aged 55.2 ± 10.4 years, with a body mass index (BMI) of 28.8 ± 4.1 kg m^{-2}. Inclusion criteria were the presence of bright liver echotexture based on ultrasonography, and at least 18 years of age. Exclusion criteria were as follows: wheat allergies including celiac disease and gluten intolerance; excessive alcohol consumption (>30 g daily), T2DM, viral hepatitis, NASH, and other chronic liver diseases (including autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hereditary haemochromatosis, Wilson's disease, and alpha-1 antitrypsin deficiency). Patients were instructed not to alter their dietary or lifestyle habits, and written informed consent was then obtained from each participant before the start of the trial. The institutional review board at the University of Florence approved the study protocol.

Experimental design

The study was a randomized, double-blinded trial with two parallel arms, aimed at testing whether a replacement diet with khorasan wheat products and/or control wheat products was effective in ameliorating NAFLD parameters. The participants were divided into two groups, which were respectively assigned to consume either khorasan products or control products for the duration of the study. Participants were instructed to exclude from their respective diets other cereal grains, which were then "replaced" by either the ancient khorasan or control products during the experimental intervention phase. The food products were packaged with no labels attached to the packages, and patients were informed that all products to be administered were organic and prepared by artisan methods. A single intervention phase was initiated in early May 2016 and was completed at the end of July 2016. Participants in both groups received 500 g per week of pasta, 150 g per day of bread, 250 g per week of crackers, and 250 g per week of biscuits over the 3-month period. Patients were advised to eat the products according to their normal cereal consumption habits, which were documented at baseline. On average, a patient's daily intake of both ancient khorasan and control semolina was 62 g dry weight, whereas daily intake of khorasan or control flour (from all the products consumed) amounted to 140 g dry weight. The caloric intake was estimated to be equal between the khorasan and modern wheat, representing approximately 722 kcal (50–55% of daily energy intake).

Experimental and control wheat

The experimental wheat utilized in the present study was organic khorasan wheat (*Tritucum turgidum* subsp. *turanicum*), KAMUT brand, provided by the Kamut Enterprises of Europe (KEE), Belgium. KAMUT is a registered trademark of Kamut International, Ltd., and Kamut Enterprises of Europe, bvba, guarantees the wheat is pure ancient khorasan wheat and is organically grown and processed. As the control, modern organically cultivated varieties of *Triticum durum* and *Titicum aestivum*, respectively, obtained in a mix were used. Both the experimental and control seeds were milled at Molino Angelini (*Lucca area, Italy*) to produce semi-whole-grain semolina and flour. The classification characteristics in terms of required ash contents representative of semi-whole-grain semolina and flour are presented in our previous articles (9,10). Pastificio Artigiano FABBRI s.a.s. (Strada in Chianti, Firenze, Italy) prepared the pasta (with no additives) from both the khorasan and control semolina, according to artisan manufacturing procedures. The bakery selected was Forno Garbo (Firenze, Italy). Similarly, naturally leavened Tuscan-style sourdough bread was prepared by the bakery. Crackers were prepared with olive oil and had no additives.

Data collection and measurements

Participants were both interviewed and examined at Careggi Hospital (Florence) through the use of standardized methods. The information obtained served as descriptive supplementary information pertaining to the current study population. All subjects were examined between 7:00 a.m. and 9:30 a.m. after an overnight fasting period. Moreover, participants were requested not to engage in strenuous physical activity during the day before the examination. During the interview, details regarding demographics, personal medical history (weight, hypertension, and dyslipidemia), medication, and lifestyle

habits (related to smoking habit, diet, and physical exercise) were obtained. BMI was calculated as weight (kg) per height squared (m^{-2}). Patients were classified as overweight if their BMI was more than 25 kg m^{-2} but less than 30 kg m^{-2}, and obese if their BMI was 30 kg m^{-2} or more. Blood pressure was measured on the patient's right upper arm in a sitting position. Hypertension (raised blood pressure) was defined as systolic blood pressure 140 mm Hg or more and/or diastolic blood pressure 90 mm Hg, in accordance with the guidelines of the European Society of Cardiology. Dyslipidemia was identified according to the Third Report of the National Cholesterol Education Program (NCEP-III), or if patients reported taking antidyslipidemic drugs, as verified by the physician. Regarding lifestyle habits, a sedentary lifestyle was reported if physical activity was absent over the preceding 6 months. Current smokers at the time of the physical examination were noted. Participant adherence to a Mediterranean diet was evaluated from a questionnaire that permitted the assignment of an overall score (minimum 0 points, maximum 18 points), ranking the degree of adherence (12). The questionnaire was designed to include 3 score categories (minimum 0 points, maximum 2 points) relating the consumption of each food group (cereals, fruit, vegetables, legumes, olive oil, meat products, dairy products, fish, and alcohol) comprising the Mediterranean diet.

B-mode and duplex Doppler ultrasound

As part of the examination, both B-mode ultrasound and duplex Doppler ultrasound were performed on each participant (13). The same expert operator was employed to perform the ultrasonography (high-quality ultrasound device equipped with a multifrequency convex array transducer, Voluson 530 DMT, Kretz Technik AG, Zipf, Austria) both at baseline and at the conclusion of the experimental intervention. Either 3.5 or 7.5 MHz ultrasound frequency was used for the liver scan and detection of vascular parameters. In B-mode imaging, liver echotexture was scored on a 4-grade scale, through comparison to right kidney cortical echogenicity. The following criteria were used: grade 0, steatosis absent; grade 1, mild steatosis (homogeneous and light liver echotexture with patent intrahepatic vascular pattern and no posterior attenuation); grade 2, moderate steatosis (moderate increase in liver echotexture with partial dimming of the intrahepatic vessels and early posterior attenuation), and grade 3, severe steatosis (diffuse increase in liver echotexture with no visible presence of the intrahepatic vessels and heavy posterior attenuation).

Duplex Doppler ultrasound was used to provide a quantitative measurement of fatty storage based on the Doppler perfusion index (DPI). The DPI is defined as the ratio between hepatic artery blood flow and total liver blood flow, with DPI values being inversely correlated to increasing severity in the histological grading of fatty liver (14).

Measurement of biochemical, oxidative stress, and inflammatory parameters

During the examination, venous blood samples were collected into evacuated plastic tubes (Vacutainer). Samples were centrifuged at 3000 × g for 15 min (4°C), and stored in aliquots at −80°C until further analysis. Cholesterol subtypes, triglycerides, glucose, insulin, serum electrolytes, and standard liver panel enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (gGT), and alkaline phosphatase (ALP), were measured according to standard conventional laboratory methods. Pro- and anti-inflammatory cytokines were determined with the Bio-Plex cytokine assay (Bio-Rad Laboratories, Inc., Hercules, CA), according to the manufacturer's instructions. Leukocyte (lymphocyte, monocyte, and granulocyte) reactive oxygen species (ROS) generation was measured as reported previously (15). Similarly, fatty acid peroxidation was determined by measuring malondialdehyde using the thiobarbituric acid-reactive substance assay kit (Oxitek-ZeptoMetrix Corporation Buffalo, NY). Total antioxidant capacity, accounting for total hydrophilic ROS scavengers, was measured using the ORAC (oxygen radical absorbance capacity) assay.

Statistical analysis

The statistical package PASW 20.0 for Macintosh (SPSS, Inc., Chicago, IL) was utilized. Results were expressed either as mean ± SD or as median and range, as appropriate. The analyses were simplified by calculating the absolute change for each variable tested (mean value at baseline subtracted from the mean value after intervention for each subject) with independent-sample *t*-tests. Data were analyzed using paired t-tests for significant differences between changes observed during experimental and control intervention periods. Moreover, in order to compare the effect of khorasan products versus baseline and versus the control products, a general linear model for repeated measurements, after adjustment for age, gender, BMI and fat mass (%) changes, hypertension, smoking, and medication, was performed. For this model, data were logarithmically transformed, and back-computed as the geometric mean and standard deviation, for data presentation; $p < 0.05$ was considered to indicate statistical significance.

Results

Study population characteristics

Baseline characteristics of the study population, including the grade of steatosis, traditional risk factors, lifestyle habits, and diet quality, are presented in Table 1. The majority of the participants (70%) were graded 1 (mild steatosis), while the remaining participants were graded 2 (moderate steatosis). Half of the population was sedentary, and all participants were within the overweight to obese BMI range (28.8 ± 4.1 kg m^{-2}). No statistically significant differences between the two groups with regard to baseline demographic, clinical, and nutritional characteristics were observed (data not reported). Behavioral risk factors related to smoking habit and sedentary lifestyle were unmodified during the course of the study, whereas the remaining modifiable risk factors were maintained under control by

Table 1. Demographic, body composition, risk factors, grade of steatosis, and adherence to mediterranean diet of the study population at baseline, according to randomization.

Variable	Total ($n = 40$)	Khorasan ($n = 20$)	Control ($n = 20$)	p Value
Age, mean ± SD, years	55.2 ± 10.4	56.4 ± 7.7	53.6 ± 12.2	0.371
BMI, mean ± SD, kg m^{-2}	28.8 ± 4.1	29.1 ± 4.2	28.4 ± 4.1	0.655
Fat mass, %	36.0 ± 9.3	36.4 ± 8.5	35.2 ± 10.2	0.779
Sex (M/F)	12/28	6/14	6/14	0.999
Hypercholesterolemia, *n* (%)	24 (60)	12 (60)	12 (60)	0.999
Current smokers, *n* (%)	8 (20)	5 (25)	3 (15)	0.492
Sedentary lifestyle, *n* (%)	20 (50)	14 (70)	6 (30)	0.011
Doppler perfusion index	0.21 ± 0.06	0.21 ± 0.06	0.21 ± 0.05	0.870
Steatosis grade				
Mild, *n* (%)	28 (70)	14 (70)	14 (70)	0.999
Moderate, *n* (%)	12 (30)	6 (30)	6 (30)	0.999
MDS, mean ± SD	12.0 ± 2.4	12.4 ± 2.6	11.5 ± 2.1	0.192

The p value was calculated with t-test for independent variables.
BMI = body mass index; MDS = Mediterranean diet score; SD= standard deviation.

medicinal therapies. There was a significant decrease in the fat mass percentage after the 3-month dietary intervention for both the ancient khorasan and control groups (mean reduction: −1.4 ± 1.8% and −0.8 ± 1.3% respectively), but the change (from pre- to postintervention) between the two groups was not significantly different ($p > 0.05$).

Modifications in the biochemical profile

In order to evaluate the differences in change from pre- to post-intervention between ancient khorasan and control groups, we applied a general linear model adjusted for age, gender, BMI and fat mass (%) changes, hypertension, smoking, and medication. As reported in Table 2, a significant decrease by 6% in total cholesterol was evident only for the ancient khorasan group ($p < 0.05$). We also observed a trend for a significant decrease of glycemia by 7% after the period of intervention with ancient khorasan wheat, but the result did not reach statistical significance when the fully adjusted model was applied. The panel of liver function enzymes significantly improved only in the khorasan group, as seen by a reduction of AST by 12%, ALT by 14%, and ALP by 8% ($p < 0.05$ for all), whereas a significant worsening effect was observed for AST and ALT in the control group. Finally, potassium and magnesium were slightly but significantly increased only after consumption of the khorasan products. There were significant differences between study arms regarding the absolute changes (mean value at baseline subtracted from the mean value after intervention for each subject) in glucose, AST, ALT, ALP, and potassium.

Modifications in the oxidative stress profile

As showed in Table 3, after the 3-month dietary intervention period a significant decrease by 25% in ROS produced by the monocytes was evident in only the khorasan group. On the other hand, significant decreases in ROS derived from red blood cells (RBC), lymphocytes (L), and granulocytes (G) were observed in both the ancient khorasan and control groups, with greater reductions in the ancient khorasan group (RBC derived ROS reduced by 20% vs. 12%; L-derived ROS by 21% vs. 15%; G-derived ROS by 16% vs. 15%) ($p < 0.05$ for all). No significant changes were evident for total antioxidant capacity (ORAC method) or in lipid peroxidation (thiobarbituric acid-reactive substances [TBARS] method). Absolute changes in all parameters of the antioxidant profile in the ancient khorasan group were not significantly different with respect to those in the control group.

Modifications in the inflammatory profile

Significant decreases in the levels of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) by 50%, interleukin (IL)-1Rα by 37%, IL-8 by 24%, and interferon-γ by 24%, were only evident in the ancient khorasan group after the 3-month dietary invention ($p < 0.05$ for all) (Table 4). A significant increase in IL-12 by 28% was also reported for only the ancient khorasan group. Significant differences in comparative change between the khorasan and control groups, respectively, were evident for IL-4, IL-6, IL-8, interferon-γ–inducible protein-10, and TNF-α,

Table 2. Modification of Lipid Variables, Glycemic Profile Variables, Liver Enzymes, and Minerals in the Two Different Interventions.

Variable	Khorasan pre	Khorasan post	p Value[b]	Control pre	Control post	p Value[b]	Change khorasan	Change control	p Value[c]
Cholesterol, mg/dL	222.7 ± 38.6	208.4 ± 36.2[a]	0.013	212.9 ± 25	215.1 ± 28.2	0.701	−14.3 ± 23.2	2.20 ± 23.4	0.096
LDL-cholesterol, mg/dL	145.3 ± 36.5	133.8 ± 32.8[a]	0.119	130.1 ± 42.5	130.5 ± 35.7	0.994	−11.5 ± 25.4	0.05 ± 25.9	0.117
HDL-cholesterol, mg/dL	55.3 ± 12.7	53.6 ± 13.4	0.203	55.9 ± 16.9	53.1 ± 13.6	0.328	−1.75 ± 6.0	−2.80 ± 11.4	0.989
Triglycerides, mg/dL	139.2 ± 85.9	120.3 ± 45.6	0.120	124.7 ± 49.2	133.3 ± 50.3	0.354	−18.9 ± 48.2	8.60 ± 34.0	0.163
Glucose, g/L	1.01 ± 0.49	0.94 ± 0.33[a]	0.072	0.97 ± 0.18	0.96 ± 0.17	0.569	−0.08 ± 0.18	−0.01 ± 0.06	0.045
Insulin, mU/L	10.2 ± 3.27	10.6 ± 3.68	0.611	12.0 ± 11.0	12.1 ± 11.6	0.977	0.32 ± 2.66	0.02 ± 3.32	0.685
AST, U/L	25.8 ± 20.1	22.1 ± 16.7[a]	0.011	19.5 ± 7.1	23.8 ± 10.7[a]	0.001	−3.70 ± 4.86	4.35 ± 5.27	0.001
ALT, U/L	40.0 ± 32.3	35.4 ± 25.9[a]	0.017	34.8 ± 13.6	37.8 ± 14.2[a]	0.019	−4.55 ± 8.37	2.95 ± 5.42	0.002
gGT, U/L	31.4 ± 15.3	29.3 ± 9.44	0.211	29.6 ± 13.9	32.2 ± 17.6	0.067	−2.10 ± 8.57	2.55 ± 6.87	0.071
ALP, U/L	82.1 ± 24.3	75.9 ± 22.2[a]	0.001	78.7 ± 20.3	81.2 ± 21.2	0.304	−6.15 ± 6.88	2.50 ± 9.78	0.001
Potassium, mEq/L	4.33 ± 0.23	4.42 ± 0.21[a]	0.048	4.31 ± 0.31	4.07 ± 0.24[a]	0.009	0.09 ± 0.17	−0.35 ± 0.37	0.001
Magnesium, mg/dL	2.12 ± 0.15	2.20 ± 0.13[a]	0.020	2.04 ± 0.21	2.10 ± 0.22	0.092	0.09 ± 0.16	0.07 ± 0.18	0.966
Iron, μg/dL	82.0 ± 28.8	87.1 ± 29.6	0.504	88.0 ± 22.1	95.4 ± 39.3	0.311	5.10 ± 30.6	7.45 ± 37.9	0.968

AST = aspartate aminotransferase; ALT = alanine aminotransferase; gGT = gamma-glutamyltransferase; ALP = alkaline phosphatase.
Data are reported as mean and standard deviation.
[a]Significant at $p < 0.05$ for paired t-test (pre vs. post).
[b]General linear model for repeated measurements adjusted for age, gender, BMI and fat mass (%) change, hypertension, smoking, and medication.
[c]Significant at $p < 0.05$ for independent t-test (for changes between the khorasan and control groups).

Table 3. Modification of Oxidative Stress Parameters in the Two Different Interventions.

Variable	Khorasan pre	Khorasan post	*p* Value[b]	Control pre	Control post	*p* Value[b]	Change khorasan	Change control	*p* Value[c]
RBC-derived ROS, RFU	491.8 ± 83.7	395.6 ± 75.3[a]	0.001	524.1 ± 97.7	459.1 ± 121.5[a]	0.015	−96.3 (−239.0; 32.3)	−65.1 (−349.1; 173.0)	0.234
L-derived ROS, RFU	938.2 ± 246.0	739.4 ± 173.1[a]	0.003	822.6 ± 201.9	696.7 ± 224.8[a]	0.005	−198.9 (−979.5; 174.0)	−125.9 (−471.0; 113.5)	0.387
M-derived ROS, RFU	1764.9 ± 529.7	1321.8 ± 240.1[a]	0.001	1542 ± 391.8	1470 ± 476.6	0.627	−443.1 (−2138; 193.6)	−72.0 (−903.0; 1811)	0.088
G-derived ROS, RFU	2365.9 ± 451.1	1999.9 ± 367.2[a]	0.002	2407 ± 500.6	2043 ± 651.6[a]	0.014	−366.0 (−2025; 246.0)	−364.2 (1299; 825.0)	0.735
ORAC, μmol/mL	16.5 ± 3.54	18.0 ± 3.56	0.145	17.3 ± 2.90	17.4 ± 3.33	0.692	1.50 (−2.51; 13.4)	0.14 (−4.18; 3.03)	0.482
TBARS, nmol/mL	8.19 ± 4.27	8.37 ± 3.71	0.809	8.35 ± 6.01	8.60 ± 5.53	0.765	0.18 (−7.17; 6.71)	0.25 (−8.20; 5.66)	0.829

RBC = red blood cells; ROS = reactive oxygen species; RFU = relative fluorescence unit; L = lymphocytes; M = monocytes; G = granulocytes; ORAC = oxygen radical absorbance capacity; TBARS = thiobarbituric acid-reactive substance.
Data are reported as mean and standard deviation.
[a]Significant at $p < 0.05$ for paired *t*-test (pre vs. post).
[b] General linear model for repeated measurements adjusted for age, gender, BMI and fat mass (%) change, hypertension, smoking, and medication.
[c]Significant at $p < 0.05$ for independent *t*-test (for changes between the khorasan and control groups).

primarily attributable to significant improvements effects in the ancient khorasan group.

Liver steatosis grading and Doppler ultrasound examination

Following the 3-month dietary intervention, there was a significant improvement in steatosis grading for both the ancient khorasan and control groups, with no significant differences reported between the latter. Six and 7 participants in the ancient khorasan and control groups, respectively, displayed a regression from liver steatosis to normal liver (grade 0). In addition, in the ancient khorasan and control groups, 4 and 2 of the original 6 individuals, respectively, regressed from a moderate to a mild degree of steatosis after the dietary intervention period.

With the improvements in steatosis grading, the DPI improved significantly in both the ancient khorasan (pre: 0.21 ± 0.06; post: 0.23 ± 0.05; $p = 0.01$) and control (pre: 0.21 ± 0.05; post: 0.22 ± 0.06; $p = 0.006$) groups. Overall, a 10% improvement was evident in the khorasan group and a 5% improvement in the control group, with no significant differences between groups.

Discussion

NAFLD, considered the hepatic manifestation of the metabolic syndrome, is an emerging liver disease warranting extensive research (16). Clinical dietary trials on patients with NAFLD are scarce (2), and to the best of our knowledge, no trials have yet been performed assessing the efficacy of cereals on NAFLD patients. The present study shows, for the first time, that khorasan wheat is more effective than modern wheat in improving the risk profile of patients with NAFLD, confirming the original research hypothesis. Particularly noteworthy is the significant improvement in the circulating levels of cholesterol, magnesium, potassium, liver function enzymes, and proinflammatory cytokines evident only in subjects who consumed the khorasan products. On the other hand, improvements in steatosis grading (regression to either normal liver or lower steatosis grading), related DPI, fat mass, and ROS production in circulating red blood cells and leukocytes were observed, suggesting that the products, which were prepared using organic semi-wholegrain semolina or flour according to artisan procedures, represented an improved quality in comparison to cereal products normally consumed by the participants.

In our previous studies (8,9,17−18), khorasan wheat was found to be richer in macro- and microelements, particularly in comparison to the control modern soft wheat. Nutritional analysis found higher selenium and carotenoid content, and higher levels of magnesium, phosphorus, potassium, and zinc. Slight but significantly higher levels of polyphenol secondary metabolites were found as well. Through the use of liquid chromatography coupled with time-of-flight mass spectroscopy

Table 4. Modification of Inflammatory Parameters in the Two Study Interventions.

Variable	Khorasan pre	Khorasan post	*p* Value[b]	Control pre	Control post	*p* Value[b]	Change khorasan	Change control	*p* Value[c]
Interleukin-1rα, pg/mL	53.8 ± 54.8	33.9 ± 39.6[a]	0.015	55.1 ± 53.9	45.2 ± 45.2	0.098	−20.0 (−144.3; 12.6)	−9.89 (−86.3; 21.8)	0.117
Interleukin-4, pg/mL	0.42 ± 0.29	0.40 ± 0.27	0.876	0.66 ± 0.35	0.87 ± 0.47[a]	0.012	−0.01 (−0.67; 0.64)	0.21 (−0.70; 0.94)	0.048
Interleukin-6, pg/mL	2.86 ± 1.66	2.73 ± 1.48	0.625	1.69 ±1.19	2.48 ±1.71[a]	0.010	−0.13 (−2.76; 1.86)	0.78 (−1.15; 3.95)	0.048
Interleukin-8, pg/mL	9.52 ± 5.09	7.18 ± 3.29[a]	0.043	6.18 ± 2.78	7.60 ± 2.30[a]	0.001	−2.34 (−17.1; 1.75)	1.42 (−1.05; 4.38)	0.001
Interleukin-12, pg/mL	15.7 ± 11.9	20.1 ± 14.7[a]	0.013	29.8 ± 22.8	27.3 ± 18.6	0.245	4.37 (−4.21; 25.1)	−2.46 (−19.2; 8.85)	0.018
Interleukin-17, pg/mL	4.12 ± 2.92	5.31 ± 3.46	0.114	4.32 ± 2.48	5.54 ± 5.52	0.296	1.19 (−3.27; 9.87)	1.22 (−9.02; 15.3)	0.968
INF-gamma, pg/mL	30.5 ± 23.0	23.3 ± 17.2[a]	0.007	18.6 ± 11.7	17.8 ± 13.3	0.835	−7.18 (−38.3; 10.7)	−0.78 (−38.3; 43.7)	0.273
IP-10, pg/mL	984.7 ± 774.5	866.4 ± 669	0.144	901.7 ± 514.3	1229 ± 1084	0.051	−118.4 (−824.4; 636.2)	327.4 (−328.8; 3372)	0.008
MCP-1, pg/mL	41.8 ± 25.1	44.3 ± 35.6	0.694	44.7 ±44.4	36.9 ± 26.3	0.502	2.51 (−35.8; 90.9)	−7.80 (−150.3; 39.4)	0.978
MIP-1beta, pg/mL	71.9 ± 40.5	66.6 ± 30.4	0.359	90.2 ± 44.8	92.4 ± 33.5	0.799	−5.29 (−47.6; 46.4)	2.20 (−44.7; 128.6)	0.766
TNF-alpha, pg/mL	10.1 ± 8.95	5.06 ± 3.83[a]	0.026	6.45 ± 3.41	7.28 ± 3.59[a]	0.042	−5.02 (−30.6; 0.92)	0.83 (−1.31; 4.43)	0.001
VEGF, pg/mL	80.1 ± 106.2	65.8 ± 81.8[a]	0.131	91.7 ± 77.4	92.2 ± 100	0.940	−14.4 (−101.8; 68.4)	0.54 (−63.9; 114.5)	0.490

INF-gamma = interferon-gamma; IP-10= interferon-γ–inducible protein-10; MCP-1 = monocyte chemotactic protein-1; MIP-1beta = macrophage inflammatory protein-1 beta; TNF-alpha = tumour necrosis factor-alpha; VEGF = vascular endothelial growth factor.
Data are reported as mean and standard deviation.
[a]Significant at $p < 0.05$ for paired *t*-test (pre vs. post).
[b]General linear model for repeated measurements adjusted for age, gender, BMI and fat mass (%) change, hypertension, smoking, and medication.
[c]Significant at $p < 0.05$ for independent *t*-test (for changes between the khorasan and control groups).

analysis, moreover, khorasan wheat was shown to contain two unique compounds, namely, coumarin and ferulic acid isomer (19). To date, no single functional constituent, responsible for improvements in the evaluated parameters, has yet been identified. It is possible that the beneficial effects are attributable to synergistic effects within the spectrum of various functional constituents present in khorasan wheat.

Relevant to NAFLD pathology, the most studied proinflammatory cytokines are TNF-α, IL-6, the IL-1 family (including IL-1Rα), and IL-18 (4). Results from human studies (4) have implicated the involvement of TNF-α in every setting relating to NAFLD pathogenesis (steatosis, necrosis, apoptosis, and fibrosis). The reduction in TNF-α levels corroborated previous findings, showing that there appears to be a threshold baseline level of TNF-α in human subjects, above which ancient khorasan wheat products induce a significant decrease in TNF-α (8,9) and below which there is no effect (10). Given that higher basal levels of TNF-α represent a risk factor in the development and progression of NAFLD (4), the significant decline of TNF-α after consumption of ancient khorasan is promising. The significant decrease in IL-1Rα following the consumption of ancient khorasan products is potentially relevant in secondary prevention, given that higher levels of IL-1Ra are reported to be positively related to obesity and the progression of the disease in human subjects (4). Of great interest is that the significant decrease in proinflammatory cytokines, evident in the ancient khorasan group, occurred independently of the significant decreases in percentage fat mass, steatosis grading, and ROS production, evident for both the ancient khorasan and control groups. There is extensive literature coverage indicating that stimulation of proinflammatory gene expression occurs as result of redox-sensitive transcription factors such as nuclear factor-kappa B, as well as the stress induced by the deposition of fat (1). The present result suggests that the decreases in proinflammatory cytokines were not an indirect consequence related to the decrease in liver fat or oxidative stress, but rather a possible direct effect related to components in the ancient khorasan products acting on proinflammatory expression. As hypothesized previously (9,10), these functional effects on inflammatory cytokines may reflect the existence of novel secondary metabolite molecules or specific polyphenol isoforms, not evident in the conventional varieties.

As regards the antioxidant capacity, the present results suggest an improved antioxidant profile of the ancient khorasan wheat used in this study. Interestingly, total polyphenol content and antiradical activity in ancient khorasan were not quantitatively different from those of the control modern wheat, even if previous studies reported only slight but significantly higher levels of total polyphenol secondary metabolites, only when compared to control modern soft wheat. Nonetheless, given that the generation of ROS can induce lipid peroxidation leading to inflammation, as well as hepatic insulin resistance, and is involved in all stages of atherosclerotic plaque formation (16), the significant decrease in ROS-derived blood cells is similarly a repeatable characteristic of ancient khorasan wheat. The liver function enzymes ALT, AST, and ALP, an indirect measure of inflammation, decreased significantly only after consumption of the ancient khorasan products. High selenium levels are a widely reported characteristic of ancient khorasan wheat. Similarly, higher levels of both magnesium and potassium, in comparison to the modern wheat, have also been reported and are likely attributable to the increases in circulatory magnesium and potassium in both the present and previous studies (8,9).

The present study was subject to limitations. In comparison to crossover trials (in which participants consume both experimental and control products), in parallel-arm studies the participants consume either the experimental or control products and differences obtained due to individual participant characteristics could not be excluded, notwithstanding the similar baseline demographic, clinical, and nutritional characteristics between the groups prior to the initiation of the study. Moreover, it was not possible to take into consideration any lifestyle changes that may have impacted on the study, although participants were under strict instruction to maintain their usual lifestyle patterns during the consumption of both the khorasan and control products.

In conclusion, a short-term replacement trial with the ancient khorasan wheat improved biochemical and inflammatory parameters in mild-to-moderate NAFLD participants. These beneficial effects were not evident after the consumption of modern control wheat products. The cultivation/transformation techniques employed in the production of both the ancient khorasan and control products lend support to the improvements in steatosis grading, DPI, and fat mass over those provided by cereal products normally consumed in the diet.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported in part by a grant from Kamut Enterprises of Europe (KEE), Oudenaarde, Belgium.

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