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Original Research Article

Effect of an Asian-adapted Mediterranean diet and pentadecanoic acid on fatty liver disease: the TANGO randomized controlled trial

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A B S T R A C T

Background: Weight loss is the most effective treatment for nonalcoholic fatty liver disease (NAFLD). There is evidence that the Mediterranean diets rich in unsaturated fatty acids and fiber have beneficial effects on weight homeostasis and metabolic risk factors in individuals with NAFLD. Studies have also shown that higher circulating concentrations of pentadecanoic acid (C15:0) are associated with a lower risk for NAFLD.

Objectives: To examine the effects of a Mediterranean-like, culturally contextualized Asian diet rich in fiber and unsaturated fatty acids, with or without C15:0 supplementation, in Chinese females with NAFLD.

Methods: In a double-blinded, parallel-design, randomized controlled trial, 88 Chinese females with NAFLD were randomly assigned to 1 of the 3 groups for 12 wk: diet with C15:0 supplementation ($n = 31$), diet without C15:0 supplementation ($n = 28$), or control (habitual diet and no C15:0 supplementation, $n = 29$). At baseline and after the intervention, body fat percentage, intrahepatic lipid content, muscle and abdominal fat, liver enzymes, cardiometabolic risk factors, and gut microbiome were assessed.

Results: In the intention-to-treat analysis, weight reductions of 4.0 ± 0.5 kg (5.3%), 3.4 ± 0.5 kg (4.5%), and 1.5 ± 0.5 kg (2.1%) were achieved in the diet-with-C15:0, diet without-C15:0, and the control groups, respectively. The proton density fat fraction (PDFF) of the liver decreased by 33%, 30%, and 10%, respectively. Both diet groups achieved significantly greater reductions in body weight, liver PDFF, total cholesterol, gamma-glutamyl transferase, and triglyceride concentrations compared with the control group. C15:0 supplementation reduced LDL-cholesterol further, and increased the abundance of *Bifidobacterium adolescentis*. Fat mass, visceral adipose tissue, subcutaneous abdominal adipose tissue (deep and superficial), insulin, glycated hemoglobin, and blood pressure decreased significantly in all groups, in parallel with weight loss.

Conclusion: Mild weight loss induced by a Mediterranean-like diet adapted for Asians has multiple beneficial health effects in females with NAFLD. C15:0 supplementation lowers LDL-cholesterol and may cause beneficial shifts in the gut microbiome.

Trial registration number: This trial was registered at the clinicaltrials.gov as NCT05259475.

Keywords: NAFLD, Weight loss, Pentadecanoic acid, C15:0, Asian-adapted Mediterranean diet, Chinese female

Abbreviations: BP, blood pressure; C15:0, pentadecanoic acid; CAP, controlled attenuation parameter; Diet + C15, Diet with C15:0 supplementation; Diet – C15, Diet without C15:0 supplementation; HbA_{1C}, glycated hemoglobin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PDFF, proton density fat fraction; RMR, resting metabolic rate; ROIs, regions of interest; SAT, subcutaneous abdominal adipose tissue; STAI, State-Trait Anxiety Inventory; VAT, visceral adipose tissue.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and affects ~25% of all adults worldwide [1,2]. In Asia, the estimated prevalence of NAFLD is ~30% and is projected to increase further over the next 10 year [3]. There is limited epidemiological data on the prevalence of NAFLD in Singapore; however, a small observational study reported a prevalence of ~40% [4].

NAFLD is defined as the accumulation of triglycerides in the liver exceeding 5% of the liver's overall weight, in the absence of significant alcohol consumption [1]. About 1 in 5 people with NAFLD progresses to nonalcoholic steatohepatitis (NASH), which is characterized by steatosis, infiltration by inflammatory cells, and different stages of fibrosis (from no fibrosis to advanced tissue scarring) [5]. NASH may progress to cirrhosis or even hepatocellular carcinoma [6], with liver cancer being the second most common cause of years of life lost among all cancers [7]. NAFLD is strongly associated with obesity, type 2 diabetes, hypertension, and dyslipidemia [1] and is expected to pose an important public health challenge in tandem with the pandemics of obesity and diabetes [8]. A number of lifestyle factors have been associated with the development and progression of NAFLD, including diets rich in fructose and sugar-sweetened beverages, high saturated fat, diets low in omega-3 and omega-6 fatty acids, and inadequate physical activity [9,10].

Currently, there is no approved pharmacotherapy for the treatment of NAFLD or NASH. Diet and other lifestyle modifications leading to weight loss comprise the only clinical guideline [11,12]. Moderate weight loss of 3%–5% has been consistently shown to improve body composition, hepatic fat and liver histology, and these benefits increase linearly with more weight loss [13–19]. Furthermore, there is evidence that Mediterranean-style diets that do not explicitly aim at weight loss have multiple benefits in the management of NALFD [20,21]. Substituting saturated fatty acids with MUFA or PUFA decreases liver fat and improves the lipid profile and other metabolic risk factors, whereas high glycemic index carbohydrates and simple sugars (for example, fructose) have adverse effects [20,21]

Recently, the health effects of odd-chain fatty acids, such as pentadecanoic acid (C15:0), have been highlighted, despite that they comprise <1% of total plasma fatty acid concentration [22]. C15:0 is present in trace levels in dairy fat and ruminant meat [23,24]. Epidemiological studies find that higher circulating levels of C15:0 are associated with lower risk for metabolic syndrome-related disorders and lower NAFLD-activity scores [25]. Moreover, a study in children reported that plasma C15:0 concentrations are inversely correlated with the degree of hepatic steatosis [26].

To date, several randomized controlled trials (RCT), reviews, and meta-analyses have assessed the effect of weight loss in individuals with NAFLD, with most studies undertaken in Europe and North America [27]. Few RCTs have explored the role of macronutrient composition, particularly in Asian populations, and little is known about the effect of C15:0 supplementation. Therefore, the aim of this study was to investigate the effects of an Asian-adapted Mediterranean-like diet rich in fiber, MUFA and PUFA, with or without a C15:0 supplementation on body weight and composition, liver fat content, and metabolic function in females with NAFLD.

Methods

Study design

The TANGO (Ectopic Fat in Singaporean Women – the Culprit Leading to Gestational Diabetes, Metabolic Syndrome, and Type 2 Diabetes) study was a 12-wk double-blinded, parallel-design RCT that examined the effects of a calorie-restricted diet with C15:0 supplementation, "Diet + C15" ($n = 31$) or a calorie-restricted diet without C15:0 supplementation, "Diet – C15" ($n = 28$), against a standard hypocaloric control diet, "Control" ($n = 29$). Ethics approval was obtained from the Domain Specific Review Board of the National Healthcare Group in Singapore, and all participants provided their signed informed consent before enrolment.

Participants

Chinese females aged 21–45 y who had a BMI between 23 and 35 kg/m^2 were recruited from the community between October 2021 and March 2022 (Figure 1). Participants had to confirm their Chinese ethnicity by answering a binary question (yes/no) at screening. The presence of NAFLD was assessed by liver ultrasound imaging (FibroScan)—which is a noninvasive, simple, and fast diagnostic method [28]. Participants with liver controlled attenuation parameter (CAP) scores ≥268 dB/m were included if they also fulfilled the other inclusion criteria. Participants had no prior history of diabetes mellitus, other than gestational diabetes, and all had nondiabetic fasting plasma glucose (<7.0 mmol/L) and glycated hemoglobin (HbA_{1C} <6.5%) concentrations during screening. Those with evidence of significant organ system dysfunction or disease, those who were pregnant, lactating, consuming alcohol regularly (on ≥4 d per week, or ≥6 drinks per week), those using medications known to affect metabolism or gut microbiota (for example, antibiotics and oral contraceptives), and those suffering from severe diarrhea and recent weight loss (≥5% over the past 3 months) were excluded from the study. Participants were allowed to continue taking any dietary supplements they habitually consumed, but they were instructed not to make any changes during the study period. Only 3 of them consumed probiotics.

Randomization

The randomization codes were generated using the "block Rand" (version 1.5) R package [29] and allocated based on the recruitment sequence. Random block sizes of 3, 6, and 9 were used to ensure balance in sample size across the 3 arms of the trial by a blinded statistician who was not involved in the intervention. Participants were randomly assigned into 3 color-coded groups (blue, yellow, and green). Blue and yellow were the diet intervention groups with or without C15:0 supplementation, respectively, and green was the control group. The C15:0 supplement was included in soymilk, and soymilk cartons were labeled in blue or yellow and delivered to participants assigned to the corresponding groups. Both the study team and the participants were blinded until the intervention and data analyses were completed. By mistake, 1 participant randomly assigned to the diet without C15:0 received soymilk with C15:0 on the first delivery, and was therefore switched to that group for the remainder of the intervention, whereas another participant randomly assigned to the diet with C15:0 was provided with 28 packets of yellow soymilk without C15:0 during weeks 4–8, but her data were included in the originally assigned diet group.

Diet intervention

All participants received counseling from a registered dietitian focusing on making healthier food choices and reducing total energy intake to facilitate weight loss. They were recommended to consume moderately low-calorie diets (1,000–1,500 kcal/d) during the 12-wk intervention, estimated to induce an energy deficit of 500–1,000 kcal/d relative to energy needs for weight maintenance. Daily energy

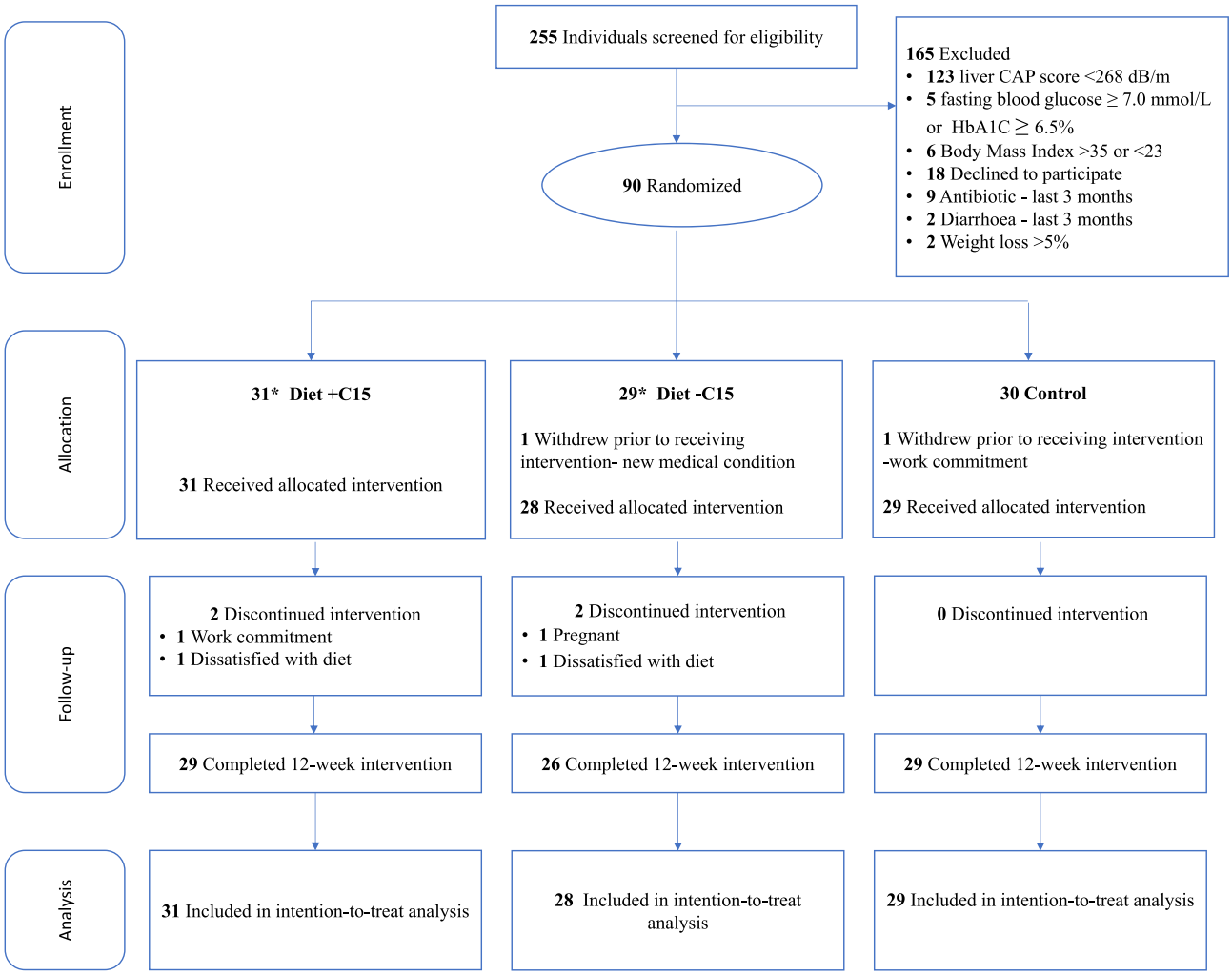


FIGURE 1. Flowchart of participants through the study. *Instead of 30 participants because of 1 participant who was allocated to Diet – C15 but was wrongly delivered the soya milk with C15 supplementation on the first delivery and thereafter her intervention was continued with C15 supplementation. CAP, controlled attenuation parameter; Diet + C15, diet with C15:0 supplementation; Diet – C15, diet without C15:0 supplementation; HbA1C, glycated hemoglobin.

requirements were calculated by using the measured resting metabolic rate (RMR) and multiplying by a physical activity level of 1.3, as all participants were sedentary. Standard measurement cups were provided to facilitate better management of portion size intake. Dietary counseling also aimed at promoting healthy eating habits based on the My Healthy Plate from the Singapore Health Promotion Board [30], focusing on consuming adequate fruits and vegetables, fish (≥2 portions weekly), choosing whole-grain products instead of refined ones, choosing low-fat options for dairy products (milk, yogurt, and cheese) and lean meat products, using healthier oils (for example, olive oil) instead of butter and oils rich in saturated fat, limiting added sugar intake, and minimizing intake of ruminant meat (beef and lamb) and butter.

Participants assigned to the Mediterranean-like diet groups received, in addition to the general dietetic advice, nutrition education on the Mediterranean diet and food components and were required to consume 12 frozen study meals/wk, and soymilk once daily (with or without 300 mg of C15:0) throughout the 12-wk intervention. The 12 frozen meals (providing an average of 350 kcal each, with 36% of energy from carbohydrates, 21% from protein, 33% from MUFA and PUFA; and 7 g of fiber) were prepared in line with the Asian cuisine. The diet was high in fiber, MUFA and PUFA, whole-grain products, legumes, vegetables, salmon, plant-based protein, nuts, fruits, and high-polyphenol extra virgin olive oil. The calorie content of the soymilk supplement, both with or without C15:0, was 108 kcal (38% of energy from carbohydrates, 22% from protein, 31% from MUFA and PUFA; and 4 g of fiber). The frozen meals and soymilk were sourced and produced in a single batch and provided by Wilmar International Ltd (Singapore). Almonds, frozen vegetables, frozen soy-based protein, oat bran, millet, and olive oil were provided to the 2 diet groups. All these food items, except the frozen meals as such, are widely available in grocery stores in Singapore.

To encourage compliance, participants were contacted by phone after the first 2 wk and met every 4 wk with a registered dietitian. Adherence to the dietary intervention was evaluated by meal checklists completed daily by the participants in the 2 diet groups. No frozen meals or soymilk were provided to control participants, but they had access to the dietitian consultations focusing on healthier food choices and weight loss during their monthly visits. Almost 80% of the participants in the control group opted to meet with dietitians during their monthly visits.

Energy and macronutrient intakes in all groups were evaluated by 3-day food diaries completed at baseline. At the end of the intervention (week 12), dietary intakes were calculated from 3 days selected randomly from the meal checklist in the 2 diet groups and from the 3-day food diary in the control group. Dietary intake was assessed by

using Foodworks 8 diet analysis software (Xyrus Software Australia Pty. Ltd.), which utilizes databases for Australian foods (AusFoods 2019 and AusBrands 2019) and local Singaporean foods (Energy & Nutrient Composition of Food, Singapore Health Promotion Board).

Clinical visits and outcome assessment

The participants completed 4 clinical visits for study-related measurements at weeks 0 (baseline), 4, 8, and 12 (end of intervention). At each visit, they arrived at the Human Development Research Centre at the National University of Singapore (NUS) campus in the morning, after having fasted overnight. Body weight was measured, and fasting blood samples were collected on all 4 visits. RMR, liver fat content, body composition, and fat distribution were measured at baseline and at the end of the intervention.

Participants wore an OURA ring (Oura Ring Heritage, Oura Health Oy) on the index, middle, or ring finger of either hand for 7–10 days before week 0 and during weeks 10–11 for sleep and physical activity monitoring. They were encouraged to stay physically active, but no formal exercise prescription was given. Daily step count data were downloaded from the cloud-based OURA Teams platform at each visit. Data for the first and last days of each wear period (7–10 days in total), and for days with >180 min/d of nonwear time were removed before analysis. We obtained data for 84 participants at baseline and 81 participants at week 12 (missing data were imputed by carrying forward the last observation).

Mental well-being was assessed at baseline and week 12 by using the self-reported State-Trait Anxiety Inventory (STAI) and Beck's Depression Inventory-II (BDI-II). The STAI consists of 2 subscales (State and Trait) to assess temporary anxiety levels over a recent period (State), and long-standing anxiety over a longer period (Trait) [31]. The BDI-II is widely used to assess the existence and severity of symptoms of depression and predict the severity of clinical depressive symptoms [32].

Anthropometric parameters (weight, height, hip, and waist circumferences) and systolic and diastolic blood pressures (BPs) were measured according to routine standardized procedures. Blood was collected through venipuncture after 10–12 h of fasting. Fasting glucose, insulin, HbA_{1C}, liver enzymes, total plasma triglyceride, total cholesterol, and LDL and HDL-cholesterol concentrations were determined by standard methods at the National University Hospital Referral Laboratory (accredited by the College of American Pathologists). C15:0 in plasma phospholipids, reflecting chronic dietary intake, was measured by the dried plasma spot method in conjunction with gas chromatography [33]. The HOMA-IR score was calculated as an index of whole-body insulin sensitivity by multiplying fasting insulin concentration with fasting glucose concentration divided by 22.5 [34].

Selfcollection kits for fecal samples consisting of the DNA/RNA shield fecal collection tube (Zymo Research) and the OMNImet.GUT all-in-one system (DNA Genotek) were given to the study participants. They were instructed to collect fecal samples within 24 h of a clinical visit at week 0 (baseline) and weeks 2 (transport by courier), 4, 8, and 12. We analyzed metagenomic data from fecal samples. The stool sample examination was performed using a DNA isolation kit (Maxwell® 16 FFS Nucleic Acid Extraction Kit; Promega). The DNA isolation and sequencing workflow underwent validation with the ZymoBIOMICS Gut Microbiome Standard (Zymo Research) and revealed close congruence with the standard. Metagenomics data was preprocessed to remove host contaminants using KneadData and compositional analysis was performed using MetaPhlAn v3.0 [35,36].

Participants had their O_2 consumption and CO_2 production measured continuously for 20 min, while breathing under a ventilated hood, and RMR was determined by using indirect calorimetry (Q-NRG Portable Metabolic Monitor, COSMED). Fat mass and fat-free mass were determined by bioelectrical impedance analysis (Impedimed, SFB7).

Liver morphology was assessed by ultrasound imaging (FibroScan 502, Echosens). Intra-abdominal fat [visceral adipose tissue (VAT)] and subcutaneous abdominal adipose tissue (SAT) volumes were determined by MRI using Siemens Prisma 3T MR scanner (Siemens Healthcare). A deep learning-based automatic segmentation algorithm followed by manual editing was used to delineate and quantify the VAT, deep SAT, and superficial SAT compartments [37]. Liver and pancreatic fat contents were determined using a multiecho Dixon fat-water imaging sequence and a body matrix coil. Multiple regions of interest (ROIs) were selected within the liver and pancreas (head-body and tail), carefully excluding blood vessels and boundaries, and liver and pancreatic fat were quantified as the mean proton density fat fraction (PDFF) within the selected ROIs [38,39].

Skeletal muscle fat content in the soleus was determined using magnetic resonance spectroscopy. The spectrum was quantified using LCModel [40] and the amount of intramyocellular lipids was calculated and expressed as a ratio with respect to water and corrected for transverse relaxation time (T2) losses [41].

Statistical analysis

The primary outcome of the study was liver PDFF. Power calculations for sample size determination were based on the anticipated means of the diet and control groups [42]. Based on the results of a previous diet intervention reporting a statistically significant decrease in liver fat content after a multicomponent fiber-rich diet compared with a control diet [43], we assumed a liver fat content (mean and standard deviation) of $9.5 \pm 8.9\%$ in the control group and $5.2 \pm 4.8\%$ in the diet group (irrespective of C15:0 supplementation). A sample size of $n = 27$ per arm was required to detect this difference at the 5% level of statistical significance and with 90% power. Assuming an overall study drop-out rate of 10%, we planned to recruit a total of 90 participants for the 3-arm study ($n = 30$ per group).

The data analysis was done based on the intention-to-treat principle with last observation carried forward for imputing missing data for 5 participants (4 discontinued interventions during the study and 1 declined blood sampling after week 0). Data measured at 2 timepoints (weeks 0 and 12) were analyzed with SPSS version 26 (IBM SPSS) by using repeated measures analysis of variance, with 1 within-subjects factor (time, with 2 levels: baseline compared with 12 wk) and 1 between-subjects factor (diet group, with 3 levels: diet with C15:0 compared with diet without C15:0 compared with control). Significant main effects and time-by-diet interactions were followed by Sidak's post hoc tests to adjust for multiple comparisons and evaluate differences before and after the intervention and among diet groups.

Data measured at 4 timepoints (weeks 0, 4, 8, and 12) were modeled using linear mixed modeling in R 4.2.2 (R Core Team, 2022) using the "lme4" package [44] to assess changes from baseline to post-treatment. For each model, we included the metabolic parameter of interest as the dependent variable and added fixed effects for treatment, time, as well as the interaction between treatment and time, including a random intercept for participants: Metabolic Parameter ~ Treatment + Time + Treatment*Time + (1|Participant). Normality was assessed visually using QQ plots and further tested with the Kolmogorov–Smirnov test. Log transformation of data was undertaken to achieve normality when req

uired. A significant interaction between treatment and time was followed with Benjamini–Hochberg correction for multiple comparisons.

Results are reported as means ± SE or medians and quartiles (quartile 1 and quartile 3), or as mean differences with 95% confidence interval. Statistical significance was assessed at $P < 0.05$. The results for gut microbiota were modeled using a mixed effect Bayesian framework using the formula: Gut microbiome ~ Treatment + Time + Treatment*Time + (1|Participant), model priors were estimated empirically from data and model estimates represented by posterior distributions, allowing the understanding of the uncertainty these estimates. The posterior distributions were used to compute the posterior probabilities of observing this deviation ($P_{beta\_coefficient} < 0$ or $P_{beta\_coefficient} > 0$).

Results

Participants

In total, 255 Chinese females living in Singapore were assessed for eligibility and 90 of them were enrolled and randomly assigned. However, only 88 participants (diet with C15:0, $n = 31$; diet without C15:0, $n = 28$; and control, $n = 29$) attended the baseline visit (week 0) and 84 completed the study (Figure 1). At baseline, participants had a mean age of 35.7 y, a mean BMI of 28.4 kg/m^2, and a mean liver CAP score of 310.5 dB/m. Baseline characteristics are shown in Table 1.

Changes in weight, body composition and liver fat

Most anthropometric, body composition, and whole body and liver adiposity parameters improved significantly at the end of the intervention in all 3 groups (all $P < 0.05$, Table 2). The 2 diet groups had significantly greater reductions in body weight, BMI, and liver PDFF compared with the control group (relative weight change: 5.3 ± 0.7%, 4.5 ± 0.6%, and 2.1 ± 0.6% in diet with C15:0, diet without C15:0, and control, respectively and relative liver PDFF change: 32.6 ± 4.5%, 30.1 ± 4.1%, and 10.3 ± 5.9%, respectively). Changes in liver PDFF distribution were more marked among participants in the 2 diet groups (with or without C15:0) than among those in the control group (Figure 2). The reductions in total fat mass ($P = 0.004$), VAT ($P = 0.039$), SAT ($P = 0.006$) and superficial SAT ($P = 0.002$) were significantly greater in the diet with C15:0 group compared with the control group, but there were no significant differences in any of these parameters between the 2 Mediterranean-like diet groups with or without C15:0 supplementation (Table 2).

Changes in adiposity measures (BMI, fat mass, waist circumference, VAT, and SAT volumes) correlated directly with improvements in liver PDFF and CAP scores when groups were collapsed, and all subjects were analyzed together (Table 3).

Changes in metabolic parameters, physical activity, mental well-being and sleep

The cardiometabolic risk factor profile improved during the study in all groups (Table 2 and Figure 3). Systolic and diastolic BPs, insulin, HOMA-IR, and HbA_{1C} decreased with weight loss and to the same extent in all 3 groups. Total and LDL-cholesterol, triglyceride, and gamma-glutamyl transferase concentrations decreased significantly only in the 2 diet groups. The reduction in LDL-cholesterol was significantly greater with than without C15:0 supplementation, but no other differences were found between the 2 Mediterranean-like diet groups (Figure 3). The concentration of C15:0, as expected, was significantly higher at the end of the study in the diet group with C15:0 supplementation compared with the other 2 groups (Table 2).

No significant changes occurred in RMR, physical activity and sleep duration, in any group (Table 2). The mental well-being scores (STAI and BDI-II) improved statistically significantly and without any

TABLE 1
Baseline characteristics of study participants[1]

	Diet + C15 ($N = 31$)	Diet – C15 ($N = 28$)	Control ($N = 29$)
Age, y	36.7 ± 1.0	35.7 ± 1.3	34.6 ± 1.5
Weight (kg)	74.2 ± 1.6	76.2 ± 1.7	71.6 ± 1.6
BMI (kg/m^2)	28.6 ± 0.5	29.3 ± 0.5	27.4 ± 0.5
Body fat (%)	35.5 ± 0.8	35.8 ± 0.8	34.7 ± 0.8
CAP Score (dB/m)	314.5 ± 5.5	315.0 ± 5.8	301.8 ± 5.7
Liver PDFF (%)	12.4 ± 1.4	15.7 ± 1.5	8.8 ± 1.5
Systolic BP (mmHg)	120 ± 2	126 ± 2	121 ± 2
Diastolic BP (mmHg)	77 ± 2	81 ± 2	78 ± 2
Fasting glucose (mmol/L)	5.0 (4.7, 5.3)	5.0 (4.8, 5.1)	4.8 (4.5, 5.2)
Insulin (mU/L)	11.7 (8.8, 13.7)	12.3 (8.2, 14.1)	9.6 (7.7, 12.4)
HOMA-IR	2.6 (1.9, 3.0)	2.5 (1.9, 3.3)	2.1 (1.7, 2.9)
HbA_{1C} (%)	5.5 (5.2, 5.9)	5.5 (5.3, 6.3)	5.3 (5.2, 6.1)
Total cholesterol (mmol/L)	5.7 ± 0.2	5.1 ± 0.2	5.3 ± 0.2
HDL-cholesterol (mmol/L)	1.4 (1.3, 1.6)	1.4 (1.2, 1.5)	1.4 (1.3, 1.6)
LDL-cholesterol (mmol/L)	3.6 ± 0.1	3.2 ± 0.2	3.4 ± 0.1
Triglycerides (mmol/L)	1.2 (1.0, 1.4)	1.1 (0.8, 1.4)	1.1 (0.8, 1.2)
RMR (kcal/d)	1381 ± 44	1471 ± 46	1354 ± 45
Physical activity (steps/d), $N = 84$	9,087 (6508, 11,512)	9,017 (7136, 9660)	8,846 (7186, 12,010)
Sleep duration (h/d), $N = 85$	6.7 ± 0.1	6.2 ± 0.1	6.4 ± 0.1
STAI: State	30.0 (26.0, 38.0)	35.0 (25.5, 39.2)	35.0 (31.0, 41.0)
STAI: Traits	37.0 (32.0, 42.5)	39.5 (35.0, 48.2)	39.0 (32.0, 47.0)
BDI-II	6.0 (2.0, 10.0)	6.0 (3.0, 11.0)	7.0 (2.0, 13.0)

Abbreviations: BDI-II, Beck's Depression Inventory-II; BP, blood pressure; CAP, controlled attenuation parameter; Diet + C15, diet with C15:0 supplementation; Diet – C15, diet without C15:0 supplementation; HbA_{1C}, glycated hemoglobin; ITT, intention to treat; PDFF, Proton Density Fat Fraction; RMR, Resting Metabolic Rate; STAI, State-Trait Anxiety Inventory.

[1] Values are means ± SEs or medians (quartile 1 and quartile 3) for 88 participants (per ITT).

TABLE 2
Changes in body composition and metabolic parameters in study participants after the 12-wk interventions[1]

	Diet + C15 ($N = 31$)	Diet – C15 ($N = 28$)	Control ($N = 29$)	Time	Diet	Interaction
Anthropometry and fat deposition						
Body weight (kg)	−4.0 (−5.0, −3.1)[2]	−3.4 (−4.3, −2.3)[2]	−1.5 (−2.5, −0.5)[2,3]	<0.001	0.108	<0.001
BMI (kg/m^2)	−1.5 (−1.8, −1.2)[2]	−1.3 (−1.7, −0.9)[2]	−0.6 (−1.0, −0.2)[2,3]	<0.001	0.015	<0.001
Fat mass (kg)	−2.9 (−3.7, −2.2)[2,4]	−2.6 (−3.4, −1.8)[2]	−1.1 (−1.9, −0.4)[2]	<0.001	0.385	0.004
Fat-free mass (kg)	−1.1 (−1.9, −0.4)[2]	−0.7 (−1.4, 0.1)[2]	−0.4 (−1.2, 0.3)[2]	0.001	0.440	0.412
Waist circumference (cm)	−2.1 (−3.7, −0.5)[2]	−3.5(−5.2, −1.8)[2]	−1.8 (−3.5, −0.2)[2]	<0.001	0.037	0.342
VAT (cc)	−226 (−294, −158)[2,4]	−201 (−273, −130)[2]	−105 (−174, −34)[2]	<0.001	0.252	0.039
SAT (cc)	−477 (−587, −366)[2,4]	−376 (−492, −259)[2]	−213 (−327, −99)[2]	<0.001	0.151	0.006
Superficial SAT (cc)	−327 (−397, −257)[2,4]	−258 (−331, −184)[2]	−143 (−215, −71)[2]	<0.001	0.150	0.002
Deep SAT (cc)	−150 (−197, −102)[2]	−118 (−167, −68)[2]	−70 (−119, −21)[2]	<0.001	0.242	0.071
IMCL/water (%)	−0.1 ± 0.2	−0.2 ± 0.2	−0.0 ± 0.2	0.296	0.086	0.657
Pancreatic head-body PDFF (%)	−0.0 (−0.6, 0.5)	0.1 (−0.5, 0.7)	0.3 (−0.3, 0.8)	0.539	0.466	0.745
Pancreatic tail PDFF (%)	0.1 (−0.7, 0.8)	−0.2 (−1.0, 0.6)	−0.0 (−0.8, 0.8)	0.868	0.782	0.867
Liver markers						
Liver PDFF (%)	−4.4 (−5.9, −2.9)[2]	−5.5 (−7.1, −3.9)[2]	−1.5 (−3.0, 0.1)[3]	<0.001	0.027	0.001
CAP Score (dB/m)	−39.2 (−55.1, −23.4)[2]	−44.9 (−61.5, −28.2)[2]	−39.3 (−55.6, −22.9)[2]	<0.001	0.325	0.859
Metabolic and lifestyle markers						
Systolic BP (mmHg)	−6 (−9, −2)[2,6]	−4 (−7, 0)[2]	−4 (−8, −1)[2]	<0.001	0.031	0.758
Diastolic BP (mmHg)	−5 (−7, −2)[2]	−3 (−5, 0)[2]	−2 (−5, 1)[2]	<0.001	0.077	0.313
HbA_{1C} (%)	−0.1 (−0.2, −0.1)[2]	−0.1 (−0.1, 0.0)[2]	−0.1 (−0.2, −0.0)[2]	<0.001	0.449	0.357
C15:0 (ng/mL)	24 (−27, 74)[5,7,8]	−74 (−127, −21)	−8 (−59, 42)	0.193	0.005	0.031
RMR (kcal/d)	−27 (−117, 63)	−78 (−173, 17)	−26 (−119, 67)	0.109	0.192	0.675
Physical activity (steps/d), $N = 84$	513 (−582, 1609)	218 (−937, 1373)	199 (−956, 1354)	0.349	0.668	0.906
Sleep duration (h/d), $N = 85$	−0.0 (−0.2, 0.2)	0.1 (−0.1, 0.3)	0.1 (−0.1, 0.3)	0.213	0.05	0.485
STAI: State	−4.7 (−7.6, −1.8)[2]	−3.3 (−6.4, −0.2)[2]	−3.0 (−6.0, 0.0)	<0.001	0.625	0.696
STAI: Traits	−4.2 (−6.9, −1.6)[2]	−5.1 (−7.9, −2.3)[2]	−3.5 (−6.3, −0.8)[2]	<0.001	0.626	0.712
BDI-II	−2.7 (−4.8, −0.7)[2]	−2.6 (−4.7, −0.5)[2]	−3.3 (−5.4, −1.2)[2]	<0.001	0.897	0.880

Abbreviations: BDI-II, Beck's Depression Inventory-II; BP, blood pressure; CAP, controlled attenuation parameter; Diet + C15, diet with C15:0 supplementation; Diet – C15, diet without C15:0 supplementation; HbA_{1C}, glycated hemoglobin A1C; IMCL, Intramyocellular Lipid; ITT, intention to treat; PDFF, Proton Density Fat Fraction; RMR, Resting Metabolic Rate; SAT, Subcutaneous Abdominal Adipose Tissue; STAI, State-Trait Anxiety Inventory; VAT, visceral adipose tissue.

[1] Values are means difference (95% confidence interval) for 88 participants (per ITT). Data were analyzed by repeated measures ANOVA (time-by-diet) for parameters measured at 2 timepoints and linear mixed models for parameters measured at 4 timepoints.

[2] $P < 0.05$ vs. baseline in the same group, from Sidak post hoc test.

[3] $P < 0.05$ vs. changes in both diet groups, from Sidak post hoc test.

[4] $P < 0.05$ vs. changes in the control group, from Sidak post hoc test.

[5] $P < 0.05$ vs. changes in diet – C15, from Sidak post hoc test.

[6] $P < 0.05$ vs. diet − C15 at week 12, from Sidak post hoc test.

[7] $P < 0.05$ vs. diet – C15 at week 12, from Sidak post hoc test.

[8] $P < 0.05$ vs. control at week 12, from Sidak post hoc test.

differences between groups; however, the magnitude of improvement was likely not clinically meaningful. Both before and after the intervention, in all groups, these scores were within the low-moderate range for anxiety and the minimum range for depression.

Dietary intake

Self-reported compliance to provided frozen meals was 84 ± 4% and 82 ± 5% for the diet groups with and without C15:0, respectively. Overall, meal acceptance among participants in the 2 diet groups ranged from 74% to 96%, and no adverse effects were reported. All participants reduced their total energy intake by decreasing consumption of all macronutrients that is, carbohydrate, protein, and fat (Table 4). The decreases in energy (kcal) and protein (g) intakes were significantly greater in the 2 diet groups compared with the control group. The decrease in total fat intake in all groups was exclusively because of a reduction in saturated fatty acids (SFA); absolute MUFA and PUFA intakes did not change in any group, but both were lower in the control group than the 2 diet groups before and after the intervention (Table 4). In relative terms, however (as % of total energy intake), MUFA and PUFA intakes increased in the 2 diet groups and did not change in the control group. Fiber intake increased in the 2 diet groups and decreased in the control group. Changes in nutrient intake were consistent with dietary advice and provided meals.

Changes in gut microbiome

PERMANOVA analysis revealed significant longitudinal trends for the overall microbiome (time-by-diet interaction, $P < 0.05$, Figure 4). Bayesian mixed-effects modeling was used to study the impact of the interventions on the gut microbiome. *Bacteroides ovatus* [diet without C15:0 supplementation (Diet – C15): β = 0.04; and diet with C15:0 supplementation (Diet + C15): β = 0.08] and *Fusobacterium mortiferum* (Diet – C15: β = −0.041; and Diet + C15: β = −0.042) showed consistent longitudinal trends in the 2 diet groups compared with the control group (Figure 4). The abundance of *Bifidobacterium adolescentis* was increased by C15:0 supplementation (β = 0.22 and β = 0.35 against Control and Diet – C15, respectively) whereas the abundance of *Bacteroides dorei* (β = −0.16) and *Bacteroides stercoris* (β = −0.19) was reduced by C15:0 supplementation (Figure 5).

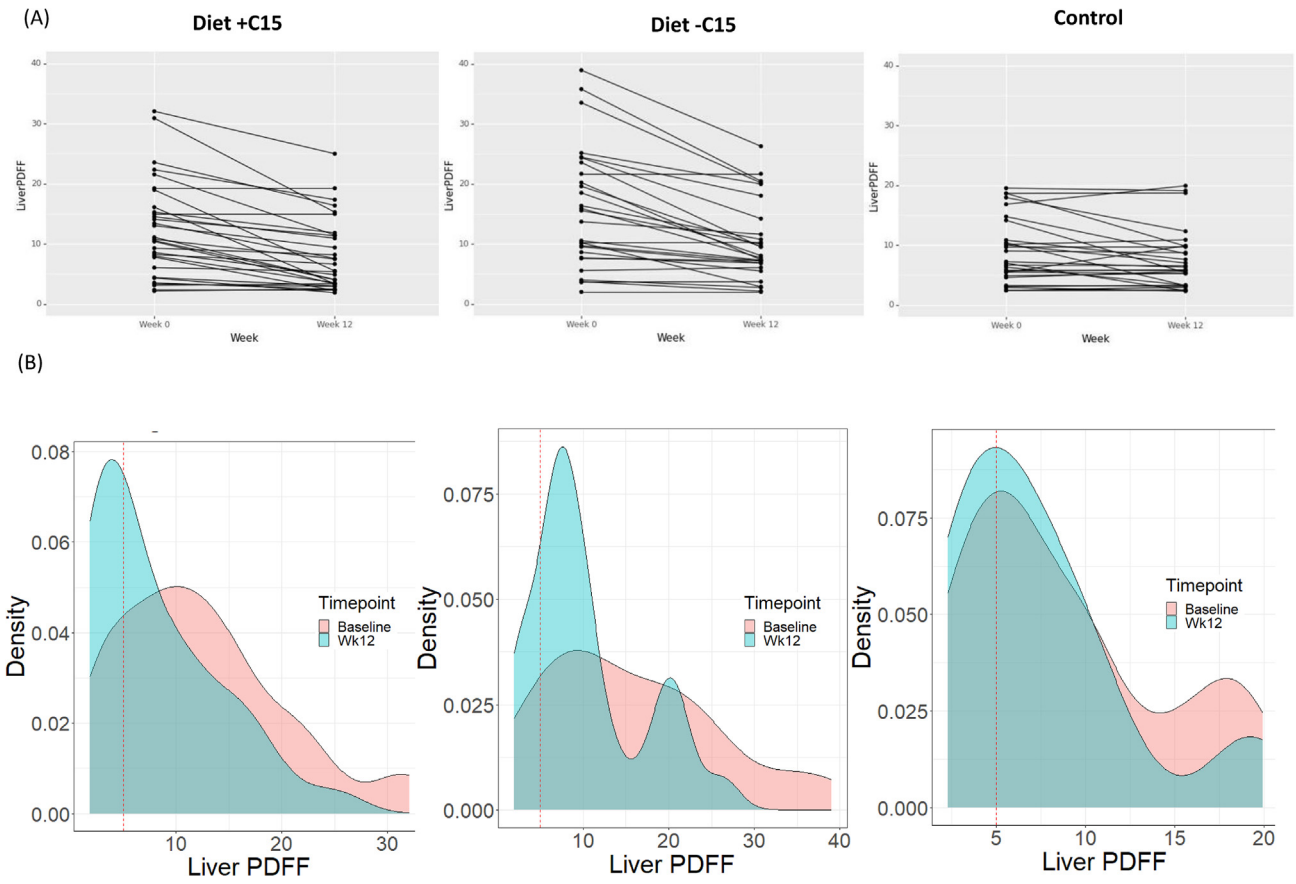


FIGURE 2. Changes in liver PDFF (%). (A) Absolute individual changes in liver PDFF after the 12-wk intervention. (B) Changes in liver PDFF distribution were more marked among participants in the 2 diet groups (with or without C15:0) than among those in the control group. Diet + C15, diet with C15:0 supplementation; Diet – C15, diet without C15:0 supplementation; PDFF, proton density fat fraction.

TABLE 3
Correlations between changes in body composition and metabolic parameters[1]

	BMI		CAP score		Liver PDFF	
	Coeff.	*P*	Coeff.	*P*	Coeff.	*P*
BMI (kg/m^2)	—	—	0.39	<0.001	0.57	<0.001
Fat mass (kg)	0.71	<0.001	0.43	<0.001	0.46	<0.001
Fat-free mass (kg)	0.63	<0.001	0.08	0.46	0.31	0.003
Waist circumference (cm)	0.53	<0.001	0.27	<0.001	0.37	<0.001
VAT (cc)	0.81	<0.001	0.48	<0.001	0.57	<0.001
SAT (cc)	0.84	<0.001	0.42	<0.001	0.58	<0.001
Liver PDFF (%)	0.67	<0.001	0.19	0.07	—	—
IMCL/water (%)	0.00	0.98	0.05	0.62	0.00	0.90
Pancreatic head-body PDFF (%)	0.13	0.24	0.21	0.06	0.18	0.10
Pancreatic tail PDFF (%)	0.09	0.44	0.06	0.61	0.14	0.21

Abbreviations: CAP, controlled attenuation parameter; IMCL, Intramyocellular Lipid; PDFF, Proton Density Fat Fraction; SAT, Subcutaneous Abdominal Adipose Tissue; VAT, visceral adipose tissue.

[1] Values are Pearson or Spearman correlation coefficients (Coeff.) and corresponding *P* values for 88 participants.

Discussion

In this study, we evaluated the effects of a calorie-restricted Mediterranean-like, Asian-adapted diet rich in fiber, MUFA and PUFA on body weight and composition, body fat distribution, ectopic fat, and metabolic function markers in Chinese females with NAFLD. We further evaluated the effects of additional supplementation with C15:0. All groups, including the control group (provided with nutrition counseling but no meals), achieved reductions in body weight and liver fat, although the magnitude of improvement in the 2 Mediterranean-like diet intervention groups was greater than in the control group, together with greater reductions in some metabolic risk factors (total cholesterol and triglyceride concentrations). C15:0 supplementation caused a further reduction in LDL-cholesterol compared with no

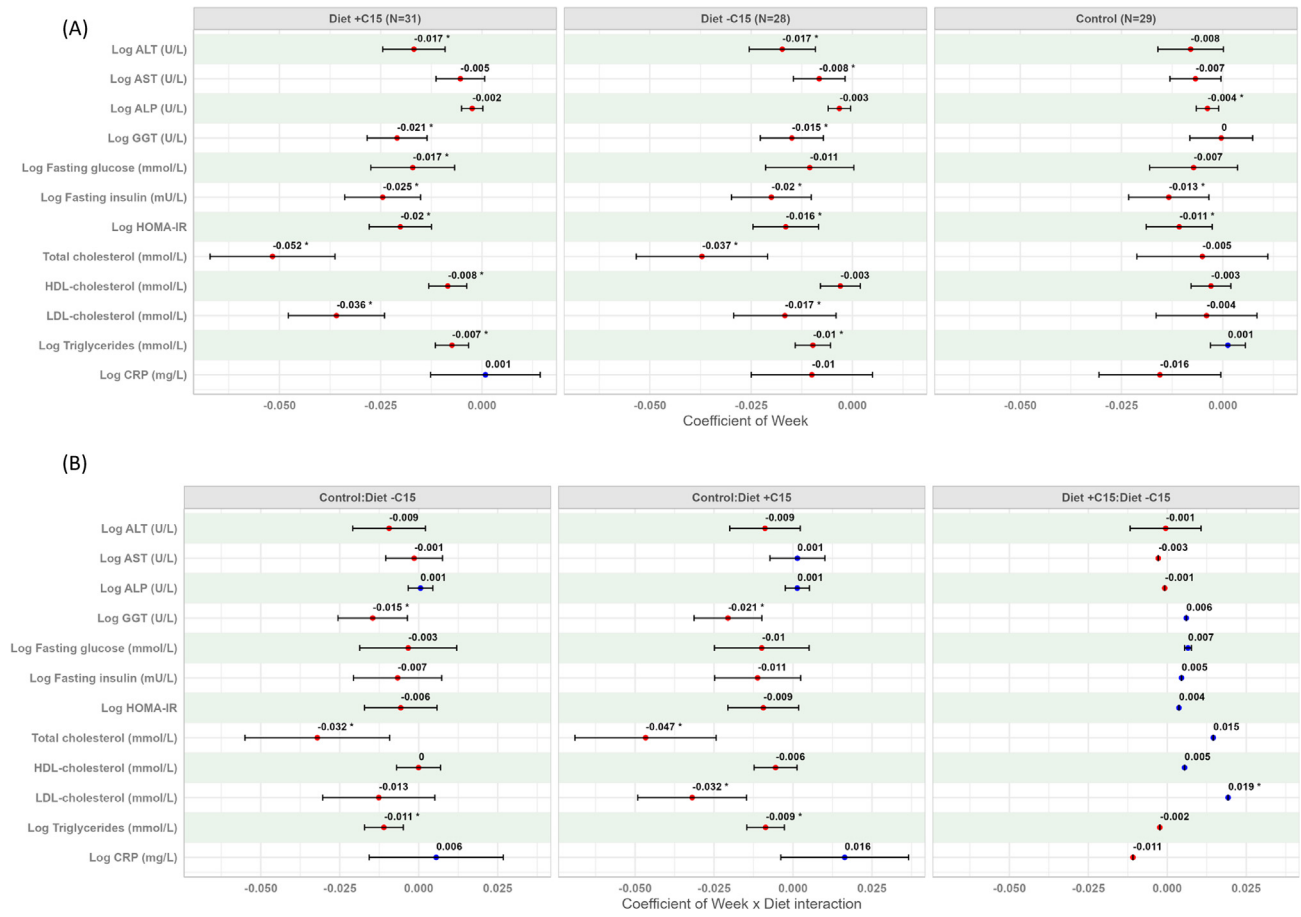


FIGURE 3. Metabolic parameters change in intervention. Values are of coefficient of Week × Diet interaction from Linear Mixed Model. *represent *P* value <0.05 with Benjamini–Hochberg correction. (A) Showing individual changes of parameters in the respective groups. (B) Showing pairwise Control vs. Diet-C15, Control vs. Diet+C15 and Diet+C15 vs. Diet-C15. In the pairwise comparisons, the former is the reference compared with the latter with positive effects (blue) and negative effects (red). ALT, alanine transaminase, ALP, alkaline phosphatase, AST, aspartate transaminase, CRP, C-reactive protein, Diet + C15, diet with C15:0 supplementation, Diet-C15, diet without C15:0 supplementation, GGT, gamma-glutamyl transferase.

supplementation. These observations suggest that several characteristics of the Mediterranean diet can be adapted and incorporated in an Asian context and provide beneficial effects on body weight homeostasis, fat deposition in various adipose tissue depots, liver fat, and metabolic risk factor profiles in individuals with NAFLD. Furthermore, the magnitude of weight loss emerged as the primary determinant of the extent of improvement in NAFLD parameters.

We have demonstrated that weight loss can be achieved in Chinese females with NAFLD after 12 wk of reduced calorie intake, with no concomitant changes in physical activity. Meal planning is often the most challenging aspect of a weight loss diet, and we demonstrate that providing 2 frozen meals/day with almonds, frozen vegetables, frozen soy-based protein, oat bran, millet, and olive oil—that is, shifting the quality of the diet to a more Mediterranean-like pattern—promotes greater weight loss and metabolic benefits. A wider range of healthy food choices, based on regional foods and adapting to cultural diversity, is therefore important and could facilitate adherence to such a diet among Asian populations.

During the 12-wk intervention, participants in the 2 diet groups (with and without C15:0 supplementation) lost 5.4% and 4.5% of their baseline body weight. Concurrently, the liver PDFF decreased by 33% and 30%, respectively (relative changes from baseline). Instead, body weight and liver fat decreased by 2.1% and 10%, respectively, in the control group. These findings confirm observations from previous studies indicating that even small decreases in body weight (3%–5%) result in considerable reductions in liver fat (30%–50%) [13,14], and that liver fat decreases dose-dependently with the amount of weight loss [15,16]. Although more participants in the diet group with C15:0 than without C15:0 achieved liver PDFF values <5% after the intervention (45% and 18%, respectively), that is, values which are no longer diagnostic of NAFLD, baseline differences in PDFF between groups (Table 1) preclude drawing any conclusions regarding C15:0 supplementation. The changes in liver PDFF distribution were very similar in these 2 diet groups (Figure 2), so the same reduction in PDFF would naturally result in more individuals in the C15:0 groups (who started from lower values at baseline) reaching absolute values below 5% at the end of the intervention.

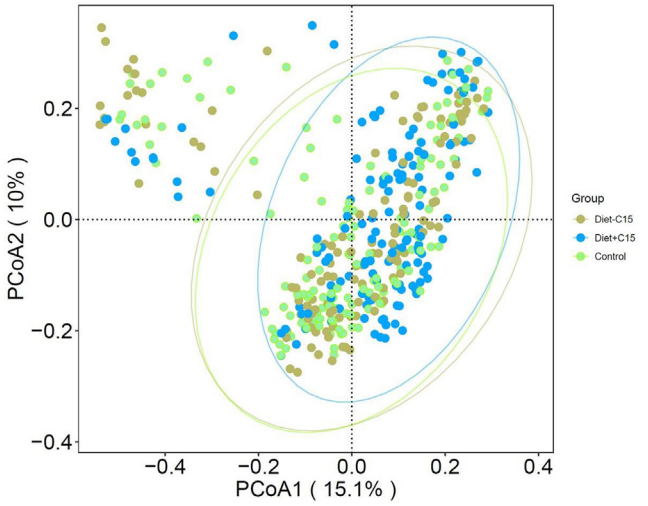
Our results support the notion that dietary energy deficit and weight loss are the cornerstones for the management of NAFLD, although there is some evidence to suggest that macronutrient composition or diet quality also affects liver fat content and metabolic profile. In 2 short-term RCTs (6–8 wk), isocaloric Mediterranean-like diets reduced liver fat compared with low-fat high-carbohydrate control diets, despite maintaining a stable body weight [18,45]. The traditional Mediterranean diet is characterized by high intakes of olive oil (rich in MUFA), nuts, fruits, vegetables, and fish and low intakes of red meat, dairy products and added sugars; and wine in moderation together with meals [46]. Our experimental meals were designed to have similar

TABLE 4
Daily nutrient intakes of study participants before and after the 12-wk interventions[1]

	Diet + C15 (N = 31)		Diet – C15 (N = 28)		Control (N = 29)		Time	Diet	Interaction
	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12			
Dietary intake									
Energy (kcal/d)	1915 ± 71	1263 ± 66[2,6]	1960 ± 75	1290 ± 69[2,6]	1856 ± 73	1530 ± 68[2,3]	<0.001	0.457	0.002
Carbohydrate (g/d)	197.6 ± 9.5	120.0 ± 8.1[2,6,2]	214.2 ± 10.0	124.1 ± 8.6[2,6,5]	210.8 ± 9.8	163.1 ± 8.4[2]	<0.001	0.033	0.010
Carbohydrate (% E)	41.2 ± 1.1	37.8 ± 1.3[2,6,7]	43.4 ± 1.2	37.8 ± 1.3[2]	45.5 ± 1.2	42.5 ± 1.3[2]	<0.001	0.007	0.381
Protein (g/d)	96.3 ± 3.7	67.0 ± 2.6[2,6]	91.9 ± 3.9	66.6 ± 2.8[2,6]	83.9 ± 3.8	80.7 ± 2.7[2,3]	<0.001	0.704	<0.001
Protein (% E)	20.2 ± 0.6	21.5 ± 0.6[2]	19.0 ± 0.6	21.3 ± 0.7[2]	18.2 ± 0.6	21.7 ± 0.6[2]	<0.001	0.374	0.100
Total fat (g/d)	80.1 ± 3.7	56.0 ± 3.6[2]	80.4 ± 3.9	57.3 ± 3.8[2]	73.8 ± 3.8	60.4 ± 3.7[2]	<0.001	0.927	0.150
Total fat (% E)	37.6 ± 1.0	39.7 ± 1.0[2,6,7]	36.9 ± 1.0	39.8 ± 1.0[2,6,7]	35.6 ± 1.0	34.9 ± 1.0[2]	0.050	0.006	0.116
Saturated (g/d)	27.4 ± 1.5	14.7 ± 1.6[2,4,6]	26.6 ± 1.6	15.3 ± 1.7[2]	26.7 ± 1.6	20.6 ± 1.6[2]	<0.001	0.254	0.025
Saturated (% E)	12.9 ± 0.5	10.0 ± 0.5[2]	12.2 ± 0.5	10.3 ± 0.5[2]	12.9 ± 0.5	11.8 ± 0.5[2]	<0.001	0.166	0.143
Monounsaturated (g/d)	23.8 ± 1.6	23.6 ± 1.2[6,7]	24.6 ± 1.6	23.4 ± 1.3[6,7]	22.1 ± 1.6	17.5 ± 1.3	0.075	0.011	0.258
Monounsaturated (% E)	11.2 ± 0.6	17.2 ± 0.7[2,6]	11.5 ± 0.7	16.7 ± 0.7[2,6]	10.7 ± 0.7	10.3 ± 0.7[2,3]	<0.001	<0.001	<0.001
Polyunsaturated (g/d)	9.6 ± 0.8	10.4 ± 0.5[6,7]	10.2 ± 0.8	10.3 ± 0.5[6,7]	9.2 ± 0.8	7.0 ± 0.5	0.383	0.004	0.055
Polyunsaturated (% E)	4.6 ± 0.3	7.6±0.3[2,6]	4.7 ± 0.3	7.5±0.3[2,6]	4.5 ± 0.3	4.1 ± 0.3[3]	<0.001	<0.001	<0.001
Fiber (g/d)	17.8 ± 1.1	20.9±0.9[2,6]	19.2 ± 1.2	22.3 ± 1.0[2,6]	19.0 ± 1.2	15.0 ± 0.9[2,3]	0.285	0.014	<0.001

Abbreviations: Diet + C15, diet with C15:0 supplementation; Diet – C15, diet without C15:0 supplementation; % E, percent of total energy intake; ITT, intention to treat.

[1] Values are means + SEs for 88 participants (per ITT). Data were analyzed by repeated measures ANOVA (time-by-diet).
[2] $P < 0.05$ vs. baseline in the same group, from Sidak post hoc test.
[3] $P < 0.05$ vs. changes in both diet groups, from Sidak post hoc test.
[4] $P < 0.05$ vs. changes in diet + C15, from Sidak post hoc test.
[5] $P < 0.05$ vs. changes in diet – C15, from Sidak post hoc test.
[6] $P < 0.05$ vs. control at week 12, from Sidak post hoc test.
[7] $P < 0.05$ vs. control at week 0, from Sidak post hoc test.



PERMANOVA TEST:

	R^2	*P*-value
Intervention:	0.017	0.001
Time:	0.0046	0.001
Intervention * Time:	0.0058	0.047

BETA DISPERSION TEST:

	F	*P*-value
Intervention:	0.0877	0.916
Time:	0.1595	0.957

FIGURE 4. Change in overall microbiome structure. Principal coordinates analysis (PCoA) plot showing the variation of the gut microbiome in the groups: Diet – C15, Diet + C15, and Control, proportion of variation denoted on the axes. Eclipses represent the 95% confidence level estimated by a multivariate *t*-distribution for the respective groups. PERMANOVA test indicates significant centroid separation across intervention, time as well as interaction effects. Beta dispersion is not significant for both intervention and time. Diet + C15, diet with C15:0 supplementation; Diet – C15, diet without C15:0 supplementation.

characteristics, that is, rich in MUFA, PUFA, and fiber, and were adapted culturally to the local (Asian) cuisine. However, our results cannot dissect the relative contribution of the Mediterranean-like dietary pattern from the reduction in energy intake, as both diet groups lost more weight than the control group, concomitant to greater reductions in total energy intake.

C15:0 is an odd-chain SFA present in whole-fat milk but represents only 1% of all fatty acids in milk [23]. High intakes of SFA from the diet have been associated with an increased risk of cardiovascular disease and type 2 diabetes [47,48], but higher circulating levels of odd-chain SFA have been associated with a lower risk of metabolic syndrome, type 2 diabetes and NASH [25]. One study recently reported that plasma C15:0 methyl ester concentration was inversely correlated with liver fat in children [26], but we could not replicate this observation in our study. We found that C15:0 supplementation induced changes in several bacteria species in the gut microbiome, and these shifts are consistent with purported beneficial effects. The abundance of *Bifidobacterium adolescentis* was increased whereas the abundances of *Bacteroides dorei* and *Bacteroides stercoris* were reduced by C15:0 supplementation.

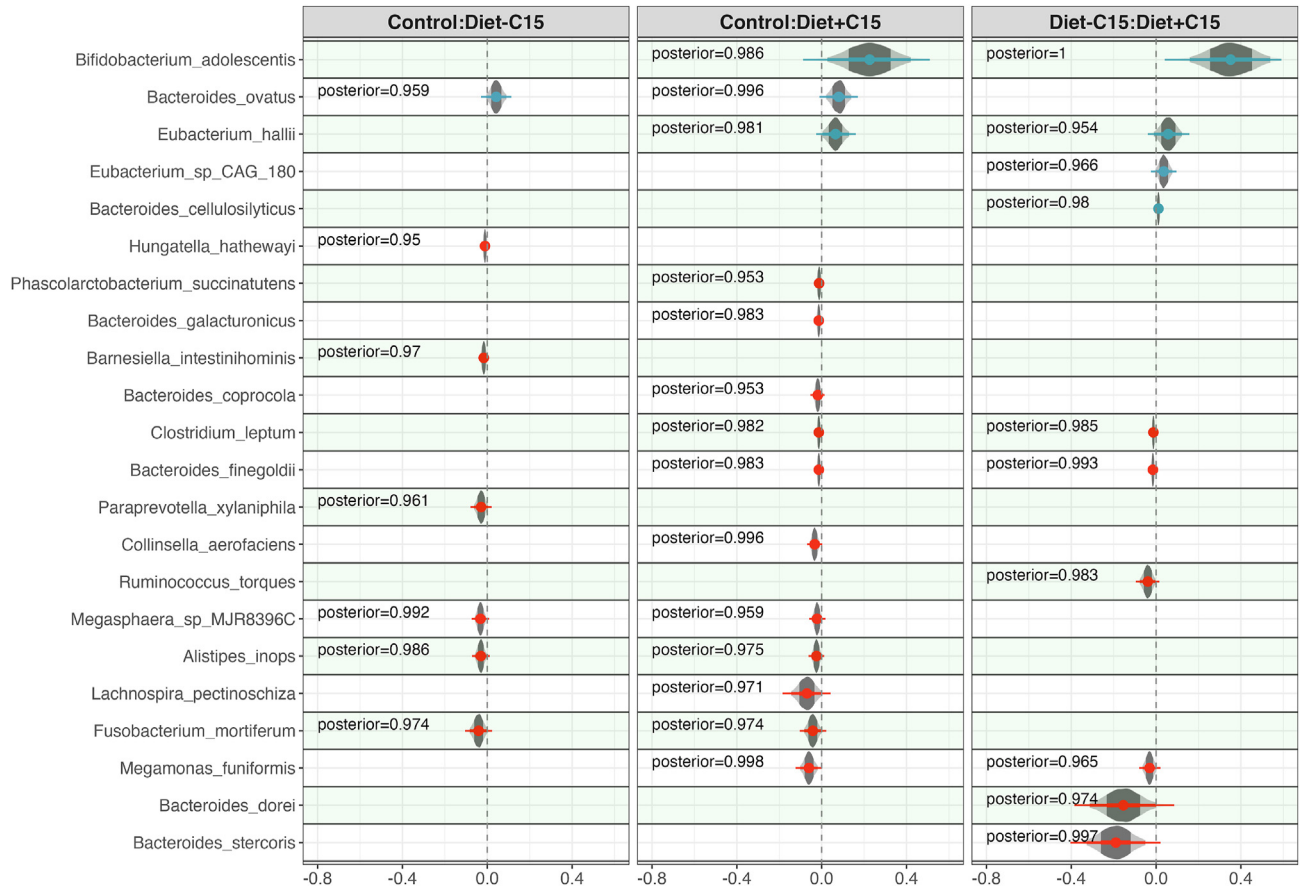


FIGURE 5. Microbiome species changes in intervention. Model posterior distributions from a Bayesian mixed model showing pairwise Control vs. Diet – C15, Control vs. Diet + C15 and Diet – C15 vs. Diet + C15 (darker gray represents 66% of the posterior and lighter gray represents 95% of the posterior). In the pairwise comparisons, the former is the reference compared with the latter with positive effects (blue) and negative effects (red). Diet + C15, diet with C15:0 supplementation; Diet – C15, diet without C15:0 supplementation.

Bifidobacteria abundance is lower in older people and NASH patients, and lower in infants delivered by Cesarean section than those delivered naturally (whereas it increases with lactation) [49,50]. These findings suggest potential gut health benefits from C15:0 supplementation. However, *Bacteroides dorei* and *stercoris* decreased over time in the diet group supplemented with C15:0. The roles of *Bacteroides* are controversial and previous reports have described both beneficial and adverse effects [51]. *Bacteroides ovatus*, which suppresses inflammation in the gastrointestinal tract [52,53], was up-regulated, whereas *Fusobacterium mortiferum*, which is associated with diarrhea [54], was down-regulated in both diet groups compared with the control group.

Our study has several strengths but also limitations. We recruited a relatively large number of Chinese females with NAFLD, most of whom completed the study (drop-out rate <5%). Furthermore, supplementation with C15:0 was double-blinded to minimize bias. Interpretation of our findings, however, is limited by the relatively short duration of the study (12 wk) but this is comparable to most previous studies with similar aims and outcomes [19,27]. Furthermore, we did not consider age, BMI, or liver CAP scores in our randomization process and this may have led to somewhat less well-balanced groups at baseline. Lastly, we assessed metabolic function only on the basis of static plasma concentrations in the fasted state rather than by using dynamic metabolic tests (for example, oral glucose or mixed meal challenges, or intravenous challenges).

In conclusion, mild weight loss can be induced by a calorie-restricted Mediterranean-like diet, which has been adapted for the Asian cuisine, and has multiple beneficial health effects in females with NAFLD. These results suggest that healthy dietary choices including more fiber, MUFA, and PUFA are feasible within the Asian context and can help patients with NAFLD lose weight and improve their liver health and metabolic profile. Dietary supplementation with C15:0 independently lowered LDL-cholesterol and may contribute to an overall healthier gut microbiome. Future studies should focus not only on which diet is more effective in managing NAFLD in clinical practice but also on how to sufficiently motivate people to make healthier food choices and maintain healthy dietary habits in the long term.

Author contributions

The authors' responsibilities were as follows – JGE, YSC, LHW, MDM: designed the study; YCC, KLML, EWMC, LL, MAG, MSFK, AZ, VHKT: were involved in data collection; YCC, AZ, FM, MN, KJL, NM, SAS, JY, SSV: analyzed the data except the gut microbiome data by XW, VSBV, KK, XL; YCC, FM, AZ: wrote the manuscript; and all authors: were involved in the manuscript review and approved the version submitted for publication. JGE: is the principal investigator and had primary responsibility for final content. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not reflect the views of the A*STAR.

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Data availability

Data described in the manuscript will be made available upon request pending approval by the corresponding authors.

Conflict of interest

The authors have no conflicts of interest relevant to the content of this article. FM is a member of AJCN's editorial board but played no role in the editorial handling of the manuscript.

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