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Pentadecanoic Acid Supplementation in Young Adults with Overweight and Obesity: A Randomized Controlled Trial

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A B S T R A C T

Background: Obesity and its associated comorbidities are major public health concerns for which nutrition is central to disease prevention and management. Pentadecanoic acid (C15:0) has the potential for beneficial effects on obesity, but supplementation has not been studied in humans.

Objectives: The primary objective was to investigate changes in plasma C15:0 levels after daily supplementation for 12 wk. Additionally, the study aimed to assess safety and tolerability as well as measure potential markers of physiologic response.

Methods: This was a single-center, double-blind, randomized, controlled, 2-arm trial of 200 mg C15:0 or placebo daily for 12 wk in young adults with overweight or obesity.

Results: A total of 30 participants with a mean age of 20.0 ± 2.1 y and a mean body mass index of 33.4 ± 5.3 kg/m^2 were included. In total, 20 participants received C15:0 supplement and 10 received placebo. The mean increase in circulating C15:0 for the treatment group was 1.88 μg/mL greater than that of the placebo group (P = 0.003). No significant adverse events occurred. Half of the participants in the treatment group had a posttreatment C15:0 level >5 μg/mL. In these individuals, there were significantly greater decreases in alanine aminotransferase (−29 U/L, P = 0.001) and aspartate aminotransferase (−6 U/L, P = 0.014), as well as a greater increase in hemoglobin (0.60 g/dL, P = 0.010), as compared with participants that did not reach a posttreatment level >5 μg/mL.

Conclusions: Daily C15:0 supplementation increased circulating C15:0 levels in young adults with overweight or obesity. End-of-treatment C15:0 >5 μg/mL was associated with potentially relevant improvements in clinical indices, warranting further study.

This trial was registered at clinicaltrials.gov as NCT04947176.

Keywords: obesity, pentadecanoic acid, aminotransferases, anemia, fatty liver

Introduction

Obesity is a highly prevalent chronic condition often associated with systemic inflammation and insulin resistance [1–3]. Affecting >40% of United States adults, obesity markedly increases risk of type 2 diabetes mellitus, cardiovascular disease, various cancers, and steatotic liver disease [2–4]. Identifying practical and accessible interventions to mitigate obesity and its related cardiometabolic diseases is essential to reduce morbidity and mortality.

Nutrition plays a vital role in the development and management of obesity, serving as a primary therapeutic approach. However, controversies persist regarding the specific dietary changes that yield optimal outcomes. Recent research has highlighted pentadecanoic acid (C15:0), an odd chain fatty acid (OCFA) found in foods such as whole-fat dairy, ruminant meat, and fish, as a promising essential fatty acid with potential metabolic benefits [5]. Although traditional approaches focus on reducing saturated fat intake, the overlooked bioactive components of whole foods, such as C15:0, may provide additional advantages. Epidemiological studies have associated higher plasma C15:0 concentrations with reduced cardiovascular disease and diabetes risk [6–10]. Preclinical and cell-based studies support the potential of C15:0 supplementation in addressing

Abbreviations: ALT, alanine aminotransferase; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; C15:0, pentadecanoic acid; GGT, gamma-glutamyl transferase; NASH, nonalcoholic steatohepatitis; OCFA, odd chain fatty acid.

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physiologic changes related to inflammation, obesity, and cardiometabolic disease [5,11,12]. Nevertheless, the absence of human trials necessitates randomized, controlled studies to understand the implication of C15:0 supplementation.

The primary objective of this study was to investigate the changes in plasma C15:0 levels after daily supplementation over a 12-wk period. Additionally, the study aimed to assess the safety and tolerability of C15:0 supplementation and measure various clinical indices associated with physiologic responses, including participant-reported symptoms, changes in weight and BMI, and the occurrence of adverse events. By examining these outcomes, this randomized, controlled study of C15:0 supplementation will provide initial insights into its potential role for young adults with overweight and obesity.

Methods

Study population

This study was a single-center, double-blind, randomized, controlled 2-arm trial aimed at determining changes in plasma C15:0 levels in response to daily supplementation of C15:0 over a period of 12 wk. Participants were enrolled at the University of California, San Diego between July 2021 and September 2022. Adults between the ages of 18 and 25 y, who had a BMI $\geq$25 kg/m^2 were eligible to participate. Exclusionary factors included a habitual dietary intake of C15:0 that consistently exceeded 250 mg/d (as assessed by Food Frequency Questionnaire), the presence of type 1 or type 2 diabetes mellitus, liver cirrhosis, and significant alcohol consumption.

Intervention

Participants were assigned via simple randomization to take 200 mg C15:0 or matching placebo daily for 12 wk. Randomization assignments were generated using computerized methods by the study statistician before the commencement of the study. The PROC SURVEYSELECT function with 12,345 seeds was employed to randomize patients in a ratio of 2 treatments to 1 placebo using the group option. The sample size of 30 participants was determined to ensure feasibility in a pilot study and to provide 90% power to detect that a change in plasma C15:0 level is attributed to treatment group assignment, with an effect size of 0.56 or greater, at a significance level of $\alpha = 0.05$. The C15:0 supplement was provided as a pure free fatty acid, whereas the placebo supplements consisted of rice flour. Participants were instructed to ingest two 100-mg capsules each morning over the course of the 12-wk intervention period. The selected dosage was based on findings from single-dose pharmacokinetic studies, which suggested that an oral intake of 200 mg C15:0 is necessary to achieve optimal circulating concentrations of $\geq$5 μg/mL (20 μM, equivalent to 0.2% of fatty acids). [13]. This optimal value was derived from the following preclinical and epidemiological evidence: *1*) primary human cell-based phenotypic profiling demonstrated optimal dose-dependent anti-inflammatory and antifibrotic activities at C15:0 concentrations of 20 μM [5,14], *2*) C15:0 exhibited optimal mitochondrial repair (reactive oxygen species reduction) activity at 20 μM [5], *3*) C15:0 erythrocyte membrane concentrations $\geq$5 μg/mL were associated with attenuated anemia in vivo [15,16], and *4*) plasma C15:0 concentrations $\geq$0.2% are associated with a linear reduced risk of cardiovascular disease in humans [6].

Medical history, medication history, physical examination, and anthropometric assessments [body weight (kg), body height (m), BMI (kg/m^2), and waist/hip circumference (cm)] were obtained at a screening visit before administration of the study agent and at a 12-wk follow-up visit. Participants also completed AUDIT (Alcohol Use Disorders Identification Test), Food Frequency, and self-reported symptom questionnaires at these times. Plasma C15:0 concentration (including that contained in triglycerides, phospholipids, and cholesterol esters) was assessed at screening and 12-wk follow-up via GC/MS, as previously described [17–19]. In brief, total fatty acids were extracted from plasma using hexane and subsequently derivatized to their pentafluorobenzyl esters. These derivatives were then subjected to separation and detection using GC/MS with negative ion chemical ionization. Other laboratory tests collected at screening and 12-wk follow-up included: fasting serum glucose, insulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), high sensitivity C-reactive protein, total cholesterol, HDL cholesterol, LDL cholesterol, and hemoglobin. Adherence was estimated by the percentage of supplement taken based on capsule counts in bottles returned at the 12-wk visit.

Data analysis

Continuous variables were expressed as mean $\pm$ SD, whereas categorical variables were presented as count (percentage). Demographic variables were compared between the placebo and treatment groups using 2-sample *t*-test for continuous variables and the Fisher's test for categorical variables. To assess the impact of C15:0 treatment on the change in plasma C15:0 levels over 12 wk compared with placebo, we utilized the analysis of covariance (ANCOVA)-Change method proposed by N.S. O'Connell [20]. This involved fitting a linear regression model with the change score in the outcome as the dependent variable and treatment type, baseline score for the outcome, and body weight change as predictors. Body weight change from baseline to 12 wk was included as a predictor (except for BMI and body weight analyses, which already account for this change) due to the variability observed in this parameter across participants. Mean adherence, defined as the percentage of capsules taken, was compared across groups using the Wilcoxon test, and the relationship between adherence and the change in circulating C15:0 levels was examined through simple linear regression. For secondary outcome measures, the change in each measure from baseline to 12 wk was compared between placebo and treatment conditions using the ANCOVA-Change method described above. In instances where 12-wk follow-up data were absent for a participant, the last observation carried forward method was employed to impute their 12-wk value in ANCOVA-Change analyses. The correlation of the change in plasma C15:0 levels was assessed against baseline weight and the percentage of capsules consumed using a Spearman correlation test.

An exploratory analysis categorized participants in the treatment group based on their end-of-treatment plasma C15:0 levels relative to the proposed optimal C15:0 concentration of $\geq$5 μg/mL. Concentrations below 5 μg/mL were designated "sub-threshold," and those above 5 μg/mL were designated "threshold," with the threshold value informed by epidemiological and preclinical literature [5,13,21–23]. Because of the sample size and potential outlier influence, the Kruskal–Wallis

test, a nonparametric alternative to 1-way analysis of variance, was employed to compare distributions of change in plasma C15:0 concentration and all secondary indices between the placebo, threshold, and subthreshold treatment groups. This analysis was summarized as median (IQR), excluding 1 participant with missing 12-wk values.

All statistical analyses were performed using R Studio, version 4.1.2 (R Foundation for Statistical Computing). All tests in these analyses were 2-sided and statistical significance was defined as $P < 0.05$.

Results

Study population

In total, 30 participants were randomized, with 20 receiving C15:0 supplement and 10 receiving placebo (Figure 1). All participants in the treatment group and 9 of 10 participants in the placebo group completed the study. Table 1 presents a summary of baseline characteristics for the study population ($n = 30$) separated by treatment assignment. The C15:0 group had a slightly higher mean age compared with the placebo group, with mean ages of 20.5 ± 2.3 and 18.9 ± 0.9 y, respectively ($P = 0.010$). The distribution of race among participants was 36.7% White, 6.7% Asian, 3.3% multiracial, and 53.3% other. Baseline demographics for sex, race, and ethnicity did not vary significantly across the treatment and placebo groups.

All participants had a BMI ≥ 25 kg/m^2 in accordance with study inclusion criteria, and the mean baseline BMI was 33.4 ± 5.3 kg/m^2 (range: 25.9–45.8 kg/m^2), with no significant difference between the treatment and placebo groups. Baseline mean total cholesterol, HDL, LDL, and glucose were normal in both groups, with no significant difference between baseline values in the placebo and treatment groups. Of 30 participants, 26 met ≥1

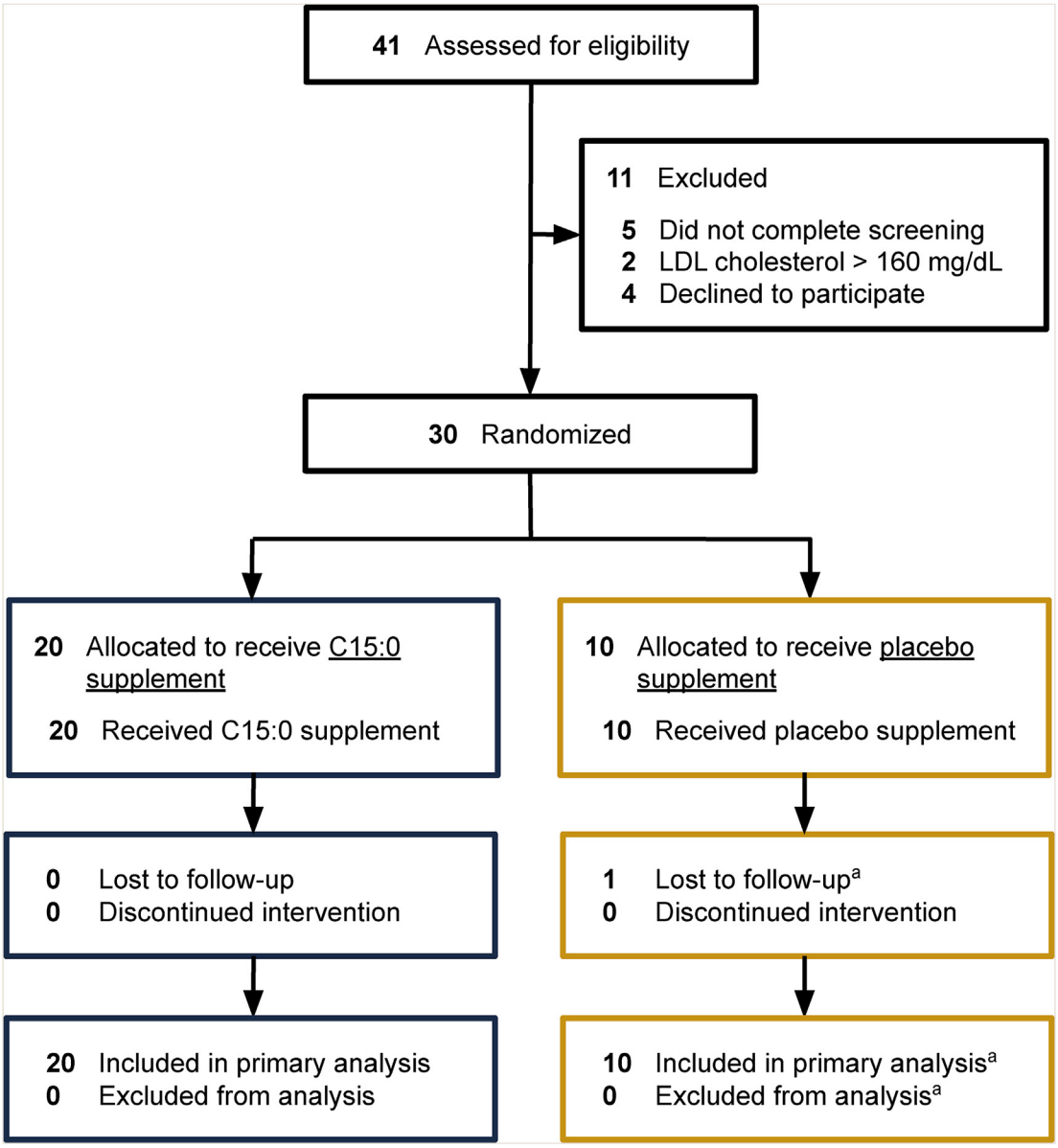

FIGURE 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram of study participants. [1]Included in primary analysis via last observation carried forward (LOCF) assumption, removed from exploratory analysis.

TABLE 1
Baseline characteristics of study participants

Index	Group assignment		Overall $n = 30$	P value
	Treatment $n = 20$	Placebo $n = 10$		
Age (y)	20.5 ± 2.3	18.9 ± 0.9	20.0 ± 2.1	0.010
Sex				
Female	11 (55.0%)	5 (50.0%)	16 (53.3%)	1.000
Male	9 (45.0%)	5 (50.0%)	14 (46.7%)	
Race				
Asian	2 (10.0%)	0 (0.0%)	2 (6.7%)	0.661
Multiracial	1 (5.0%)	0 (0.0%)	1 (3.3%)	
Other	9 (45.0%)	7 (70.0%)	16 (53.3%)	
White	8 (40.0%)	3 (30.0%)	11 (36.7%)	
Hispanic				
No	2 (10.0%)	0 (0.0%)	2 (6.7%)	0.540
Yes	18 (90.0%)	10 (100.0%)	28 (93.3%)	

Data are reported either as mean ± SD or n (%).

criterion for metabolic syndrome, with elevated waist circumference and low HDL cholesterol being the most common. Within this group, 5 participants met 1 criterion, 11 participants met 2 criteria, 7 participants met 3, 2 participants met 4, and 1 participant met all 5. The distribution of the number and type of criteria met by participants was similar across the control and treatment groups.

Primary outcome

Baseline plasma C15:0 concentrations were similar in the placebo and treatment groups (4.33 ± 1.34 μg/mL and 4.23 ± 1.28 μg/mL, respectively). C15:0 supplementation was associated with a significant increase in plasma C15:0 levels at 12 wk, with a mean adjusted increase of 2.07 μg/mL greater than that of the placebo group, after adjusting for baseline C15:0 and change

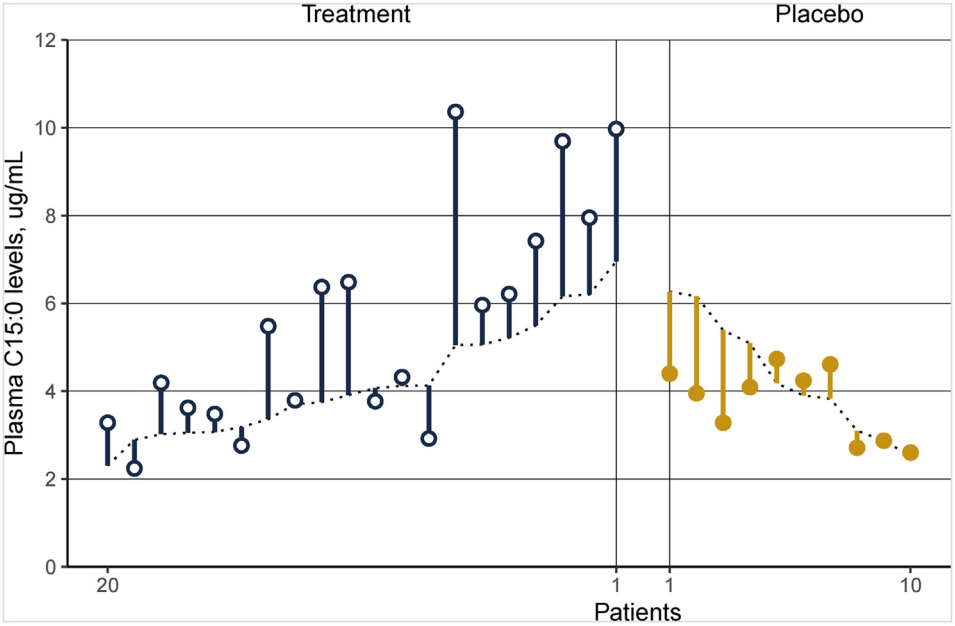

FIGURE 2. Change in plasma C15:0 levels from baseline (dotted line) to 12 wk (circles), by participant, after 12 wk of daily 200 mg C15:0 supplementation (treatment) or placebo. C15:0, pentadecanoic acid. One participant in the placebo group with missing 12-wk data is included here via the last observation carried forward (LOCF) assumption.

TABLE 2
Effect of C15:0 supplementation on secondary outcome measures

Clinical index	Treatment group		Placebo group		Change *P* value
	Baseline	12 wk	Baseline	12 wk	
C15:0 (μg/mL)	4.23 ± 1.28	5.51 ± 2.51	4.33 ± 1.34	3.85 ± 0.80	0.002
Waist circumference (cm)	105.2 ± 13.2	104.1 ± 14.3	107.4 ± 11.4	108.0 ±13.3	0.383
Systolic BP (mmHg)	119 ± 10	119 ± 12	118 ± 10	118 ± 10	0.987
Diastolic BP (mmHg)	71 ± 8	70 ± 9	69 ± 10	68 ± 11	0.886
Body weight (kg)	90.7 ± 18.0	92.4 ± 18.5	96.0 ± 18.2	98.9 ± 16.8	0.139
BMI (kg/m^2)	33.4 ± 5.8	34.0 ± 5.9	33.5 ± 4.6	34.4 ± 4.0	0.142
ALT (U/L)	72 ± 80	67 ± 76	78 ± 61	100 ± 99	0.120
AST (U/L)	45 ± 44	44 ± 42	42 ± 27	49 ± 42	0.303
GGT (U/L)	33 ± 26	32 ± 20	37 ± 21	47 ± 28	0.007
Total cholesterol (mg/dL)	170 ± 31	170 ± 30	166 ± 43	163 ± 41	0.564
HDL (mg/dL)	45 ± 10	47 ± 12	41 ± 8	40 ± 8	0.342
LDL (mg/dL)	99 ± 24	96 ± 23	99 ± 36	100 ± 35	0562
Glucose (mg/dL)	93 ± 9	96 ± 9	96 ± 9	99 ± 13	0.384
Insulin (μIU/mL)	25 ± 16	25 ± 16	26 ± 11	27 ± 12	0.853
CRP (mg/L)	3.5 ± 3.2	3.2 ± 3.9	3.2 ± 2.7	4.4 ± 3.3	0.079
Hemoglobin (g/dL)	14.0 ± 1.8	14.0 ± 1.9	14.3 ± 1.0	14.2 ± 1.0	0.328

Abbreviations: ALT, alanine aminotransferase; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; BP, blood pressure; C15:0, pentadecanoic acid; CRP, high sensitivity C-reactive protein; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low density lipoprotein; LOCF, last observation carried forward.
Data are reported as mean ± SD.
P values are derived from the ANCOVA analysis comparing change in clinical indices (from baseline to 12 wk) between placebo and treatment groups, adjusting for the baseline value of the variable being assessed and change in body weight (except for BMI and body weight, which already account for this change). One participant in the placebo group with missing 12-wk data are included here via the LOCF assumption. Reference ranges: ALT, <30 U/L; AST, <40; GGT, <36 U/L; cholesterol, <200 mg/dL; HDL, >40 mg/dL; LDL, <100 mg/dL; glucose, 70–99 mg/dL; insulin, 2.6–24.9 μIU/mL; CRP, <3.0 mg/L; and hemoglobin, 13.7–15.7 g/dL.

in body weight ($P = 0.003$). It is noteworthy, however, that the extent of this response exhibited substantial variability among individuals within the treatment group, with changes in circulating C15:0 spanning a range of −1.21 to 5.31 μg/mL (Figure 2). Changes in plasma C15:0 levels did not exhibit a significant correlation with baseline weight ($r = -0.110$, $P = 0.644$). Mean adherence (percent of pills taken) among participants was 89.3 ± 10.8% and did not differ significantly between placebo and treatment groups ($P = 0.819$). Within the treatment group, the percentage of prescribed C15:0 capsules consumed was positively correlated with the change in circulating C15:0 levels ($r = 0.650$).

Secondary outcomes

C15:0 supplementation exhibited a favorable safety profile, with no notable occurrence of significant adverse events throughout the duration of the trial. Baseline and end-of-treatment values for secondary endpoints are detailed in Table 2. Supplementation with C15:0 was associated with a significant decrease in GGT with a mean difference of −11 U/L relative to the placebo, adjusted for baseline GGT and change in body weight. No other significant changes were seen with C15:0 treatment of any of the other secondary outcomes (Table 2).

Given the inter-individual variance in treatment response, a nuanced appraisal relative to a proposed threshold of 5 μg/mL [5,13,21–23] was pursued. Changes in C15:0 with participants stratified by end-of-treatment levels relative to this threshold value are presented in Figure 3. Changes in secondary indices for these stratified groups are presented in Supplemental Figure 1. Within the treatment condition, 10 participants had end-of-treatment C15:0 levels above this threshold value (threshold group), and 10 participants had end-of-treatment values below it (subthreshold group). All participants in the placebo group had end-of-treatment C15:0 levels below this

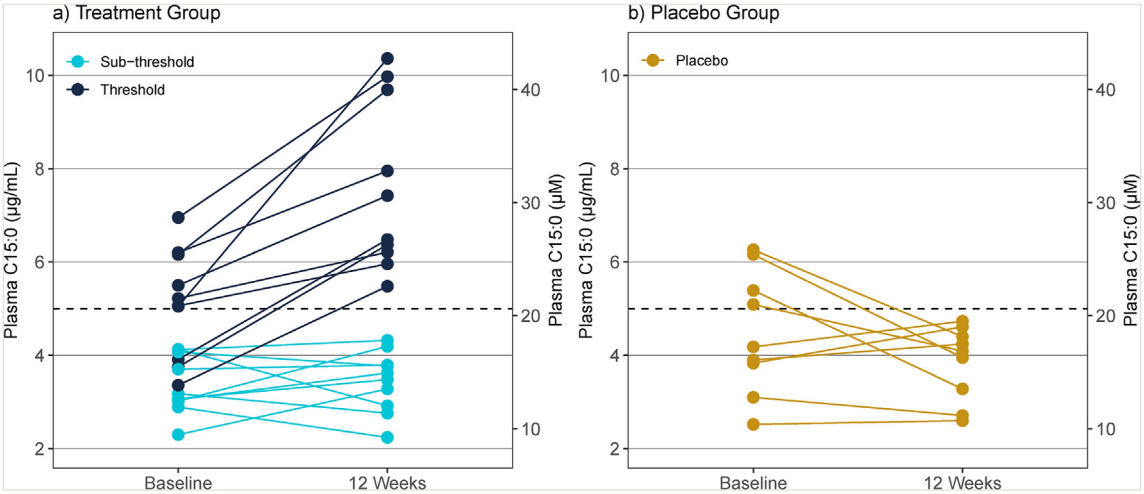

FIGURE 3. Change in plasma C15:0 levels from baseline to 12 wk, by participant, relative to the proposed threshold of 5 µg/mL (20 µM) (dotted line). (A) Participants receiving placebo for 12 wk and (B) Participants receiving 200 mg C15:0 supplement daily, stratified according to end-of-treatment plasma C15:0. C15:0, pentadecanoic acid. One participant in the placebo group with missing 12-wk data are excluded here.

TABLE 3
Threshold analysis

Clinical index	Change in clinical index at week 12 vs. baseline			Kruskal–Wallis *P* value
	Subthreshold *n* = 10	Threshold *n* = 10	Placebo *n* = 9	
C15:0 (µg/mL)	0.15 (−0.39, 0.53)	2.34 (1.79, 2.92)	−0.39 (−1.86, 0.34)	<0.001
Waist circumference (cm)	0 (−7, 2)	0 (−2, 4)	1 (−3, 4)	0.690
Body weight (kg)	3.0 (−0.7, 4.2)	0.7 (0.3, 2.5)	1.0 (−2.4, 2.9)	0.270
BMI (kg/m^2)	1.2 (−0.2, 1.3)	0.3 (0.1, 0.9)	0.3 (−1.0, 0.9)	0.177
Systolic BP (mmHg)	−5 (−9, −3)	4 (−4, 13)	−2 (−5, 4)	0.121
Diastolic BP (mmHg)	−4 (−9, −1)	3 (−2, 11)	3 (−9, 6)	0.137
ALT (U/L)	6 (2, 14)	−29 (−54, −15)	0 (−16, 12)	0.001
AST (U/L)	3 (−1, 6)	−6 (−27, −5)	0 (−6, 9)	0.014
GGT (U/L)	2 (0, 3)	−3 (−5, 1)	5 (−1, 8)	0.056
Triglyceride (mg/dL)	−1 (−24, 9)	−6 (−37, 48)	−3 (−66, 11)	0.869
Total cholesterol (mg/dL)	1 (−4, 6)	5 (−6, 9)	−4 (−19, 12)	0.752
HDL (mg/dL)	2 (−1, 4)	3 (−4, 6)	−2 (−3, 2)	0.680
LDL (mg/dL)	−2 (−8, 4)	−4 (−15, 2)	8 (−17, 13)	0.757
Glucose (mg/dL)	2 (−3, 10)	1 (−2, 5)	3 (0, 11)	0.870
Insulin (µIU/mL)	−1 (−5, 4)	−1 (−3, 12)	3 (−1, 9)	0.458
CRP (mg/L)	−0.2 (−1.3, 1.6)	−0.9 (−1.5, −0.1)	0.3 (−0.5, 2.1)	0.211
Hemoglobin (g/dL)	−0.4 (−0.9, 0.2)	0.6 (0.2, 0.9)	−0.5 (−0.7, −0.4)	0.010

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; C15:0, pentadecanoic acid; CRP, high sensitivity C-reactive protein; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low density lipoprotein; LOCF, last observation carried forward.

Data are reported as median (IQR). One participant in the placebo group with missing 12-wk data are excluded from this analysis. Reference ranges: ALT, <30 U/L; AST, <40; GGT, <36 U/L; cholesterol, <200 mg/dL; HDL, >40 mg/dL; LDL, <100 mg/dL; triglycerides, 10–140 mg/dL; glucose, 70–99 mg/dL; insulin, 2.6–24.9 µIU/mL; CRP, <3.0 mg/L; and hemoglobin, 13.7–15.7 g/dL.

threshold value. When comparing the change in secondary indices between baseline and 12 wk across these 3 groups, participants in the threshold treatment group experienced significantly greater decreases in both serum ALT and AST, as well as a greater increase in hemoglobin (Table 3). Specifically, the median change in ALT from baseline to 12 wk was −29 U/L in the threshold treatment group, 6 U/L in the subthreshold treatment group, and 0 U/L in the placebo group ($P = 0.001$); median change in AST was −6 U/L in the threshold treatment group, 3 U/L in the subthreshold treatment group, and 0 U/L in the placebo group ($P = 0.014$); and median change in hemoglobin was 0.6 g/dL in the threshold treatment group, −0.4 g/dL in the subthreshold treatment group, and −0.5 g/dL in the placebo group ($P = 0.010$). Of note, mean adherence did differ significantly between the threshold and subthreshold treatment groups (95.0 ± 5.8% and 84.3 ± 9.4%, respectively; $P = 0.008$).

Discussion

We performed a double-blind, randomized, controlled, 2-arm trial of daily, oral C15:0 supplementation to evaluate the effect on circulating plasma C15:0 levels in a population of young adults with overweight and obesity. Notably, the mean plasma C15:0 increased significantly for the active treatment group relative to the placebo group. Moreover, C15:0 supplementation was well tolerated without any significant adverse events. Supplementation with C15:0 was associated with a decrease in GGT. Approximately half of the participants taking the C15:0 supplement had a posttreatment plasma level that was >5 μg/mL. Importantly, among these individuals, elevations in circulating C15:0 were associated with improvements in ALT, AST, and hemoglobin.

This study demonstrated that daily, oral C15:0 supplementation increases plasma C15:0 levels in humans. This finding is consistent with preclinical studies conducted in rats and single-dose feeding studies in healthy human participants [5,13]. Interestingly, the extent of this increase exhibited substantial heterogeneity among participants in the treatment group. This variability may be attributable in part to differences in adherence to the supplement regimen, as indicated by the positive relationship between the percent of supplements taken and the change in C15:0 for the treatment group. However, this relationship was not strong enough to explain the response heterogeneity alone, implying differences in C15:0 absorption or metabolism also play a role. Although paired, individual-level data are not available for the studies conducted in rats, the treated rodents exhibited a similarly broad spectrum of plasma C15:0 concentrations after 14-d supplementation, in comparison with the control group. Of note, some treated rats had post-supplementation values that overlapped with those of the control group [5]. Additionally, during human feeding trials with guaranteed adherence, substantial variations in acute alterations of plasma C15:0 levels were documented [13]. These observations collectively imply that the observed variability in the changes in C15:0 levels cannot be solely attributed to adherence considerations. This study was not designed or powered to expound further on this observation, and future studies will need to address these discrepancies.

The decrease in GGT observed with C15:0 supplementation should be interpreted with caution, as it was a secondary outcome in this study. However, the mean relative decrease in GGT of 11 U/L may have clinical relevance. Numerous studies have shown that small differences in GGT, even within the normal laboratory range, are associated with differences in mortality [24–26]. The exploratory analysis of secondary outcomes presented in Table 3 suggests that the plasma level of C15:0 achieved may also have clinical relevance. Participants who reached the threshold of 5 μg/mL by the end-of-treatment were more likely to experience improvements in ALT, AST, and hemoglobin levels. This association between increased plasma C15:0 and improved liver enzyme activity aligns well with previous research investigating C15:0's effects on liver health. Earlier human studies have indicated that higher circulating C15:0 levels are associated with lower indicators of liver disease, including measures such as liver magnetic resonance imaging-proton density fat fraction [27], nonalcoholic fatty liver disease activity scores [11], and hepatocyte ballooning scores [11]. In parallel, epidemiological studies have hinted at the potential benefits of embracing whole-fat dairy consumption for metabolic health. Notably, plasma C15:0 is considered a reliable marker of dairy fat intake [23,28]. In a mouse model of nonalcoholic steatohepatitis (NASH), dietary C15:0 supplementation resulted in reduced AST [11]. Additionally, diets rich in C15:0 were positively associated with liver health in bottlenose dolphins [12]. Furthermore, a recent study in mice demonstrated that dietary inulin intervention led to decreases in both ALT and AST, with researchers proposing that microbial production of C15:0 in the intestine might mediate this effect [29].

The role of C15:0 in anemia is intriguing although less explored. In a rabbit model of NASH accompanied by anemia, C15:0 supplementation led to a robust increase in hemoglobin and red blood cell count [5]. Similarly, a study exploring the effects of a high-OCFA fish diet in bottlenose dolphins found that increased levels of circulating C15:0 positively correlated with hemoglobin levels and that the experimental diet increased erythrocyte membrane OCFAs, including C15:0. Of note, many rats in this study had either anemia or low–normal hemoglobin (12.2–13.2 g/dL) at baseline [15]. The relationship between plasma C15:0 or C15:0 intake and hemoglobin in humans has not been previously addressed. However, a 2016 study did find that premenopausal women with iron deficiency anemia had lower levels of erythrocyte membrane C15:0 [30], which may reflect circulating concentrations [31]. Erythrocyte membrane lipid profile is also a determining factor in rates of lipid peroxidation, a form of oxidative stress in which free radical species attack the double bonds found in unsaturated, but not saturated, fatty acid carbon chains [32]. This process produces lipid peroxide radical species, which, in high quantities, can overwhelm endogenous antioxidant repair mechanisms, resulting in cell damage [32]. Consequently, there exists a plausible role for C15:0 in erythrocyte stability wherein C15:0 deficiency might unfavorably alter the membrane lipid profile (increased PUFA:SFA), thereby increasing lipid peroxidation, cell destruction, and consequent inflammation [33]. This hypothesis will need to be explored in mechanistic studies.

These findings fit with a growing body of evidence suggesting C15:0 may be beneficial for metabolic health [5–9,11,15,27,

34–36]. For example, a recent clinical trial reported reduced LDL when 300 mg C15:0 was added to a Mediterranean diet in women with hepatic steatosis [37]. Because our study focused on changes in plasma C15:0 as the primary outcome, the secondary findings of possible metabolic benefits are preliminary and necessitate further exploration in a larger setting with the expressed goal of addressing these questions.

The strengths of this study lie in its randomized, controlled, double-blind trial design, providing necessary information on C15:0 supplementation in humans. This preliminary step provides a basis for further study of C15:0 and its metabolites in human health and disease. However, it is imperative to acknowledge the limitations inherent in this preliminary study. The relatively small sample size, the 12-wk intervention duration, and the examination of a single dosage regimen underscore the preliminary nature of our findings. Additionally, the selected age range of 18–25 y may exhibit distinct characteristics compared with younger or older age groups. Furthermore, factors such as dietary habits, physical exercise, and menstruation, though not assessed in this pilot study, may influence the outcomes, and necessitate evaluation in subsequent trials. Although the presence of overweight or obesity in our participants aligns with the clinical populations most relevant for C15:0 supplementation, it is noteworthy that only a third of our participants met the full diagnostic criteria for metabolic syndrome. The lack of individuals uniformly presenting with abnormalities in metabolic parameters complicates our ability to draw definitive conclusions about the potential of C15:0 supplementation in addressing the secondary outcomes presented herein. To assess the effect on a specific clinical parameter, subsequent clinical trials will need to require that all participants have clinically relevant abnormalities in that parameter.

In conclusion, our 12-wk clinical trial of oral C15:0 supplementation demonstrated an increase in circulating C15:0 concentrations among young adults with overweight and obesity. However, it's important to note that this increase displayed considerable variability. Notably, for individuals reaching circulating levels $\geq$5 μg/mL, elevations in plasma C15:0 were linked to improvements in laboratory values that hold potential clinical relevance. These preliminary associations warrant formal testing in subsequent clinical trials dedicated to these inquiries.

Author contributions

The authors' contributions were as follows – PAU-N, JBS: designed research; PAU-N, JWS, LFC, KPN, JBS: conducted research; MKR, EL: conducted data analyses; MKR, JBS: drafted the manuscript; all authors: revised the manuscript for intellectual content; MKR, JBS: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Conflict of interest

JBS reports grants to UCSD from Intercept and Genfit. All other authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2024.07.030.

References

[1] C.M. Hales, M.D. Carroll, C.D. Fryar, C.L. Ogden, Prevalence of obesity and severe obesity among adults: United States, 2017–2018, NCHS Data Brief, National Center for Health Statistics, Hyattsville, MD, 2020, p. 360.
[2] P. González-Muniesa, M.-A. Mártinez-González, F.B. Hu, J.-P. Després, Y. Matsuzawa, R.J.F. Loos, et al., Obesity, Nat. Rev. Dis. Primers. 3 (2017) 17034, https://doi.org/10.1038/nrdp.2017.34.
[3] T. Kawai, M.V. Autieri, R. Scalia, Adipose tissue inflammation and metabolic dysfunction in obesity, Am. J. Physiol. Cell Physiol. 320 (3) (2021) C375–C391, https://doi.org/10.1152/ajpcell.00379.2020.
[4] T.M. Powell-Wiley, P. Poirier, L.E. Burke, J.-P. Després, P. Gordon-Larsen, C.J. Lavie, et al., Obesity and cardiovascular disease: a scientific statement from the American Heart Association, Circulation 143 (21) (2021) e984–e1010, https://doi.org/10.1161/CIR.0000000000000973.
[5] S. Venn-Watson, R. Lumpkin, EA. Dennis, Efficacy of dietary odd-chain saturated fatty acid pentadecanoic acid parallels broad associated health benefits in humans: could it be essential? Sci. Rep. 10 (1) (2020) 8161, https://doi.org/10.1038/s41598-020-64960-y.
[6] K. Trieu, S. Bhat, Z. Dai, K. Leander, B. Gigante, F. Qian, et al., Biomarkers of dairy fat intake, incident cardiovascular disease, and all-cause mortality: a cohort study, systematic review, and meta-analysis, PLOS Med 18 (9) (2021) e1003763, https://doi.org/10.1371/journal.pmed.1003763.
[7] F. Imamura, A. Fretts, M. Marklund, A.V. Ardisson Korat, W.-S. Yang, M. Lankinen, et al., Fatty acid biomarkers of dairy fat consumption and incidence of type 2 diabetes: a pooled analysis of prospective cohort studies, PLOS Med 15 (10) (2018) e1002670, https://doi.org/10.1371/journal.pmed.1002670.
[8] N.G. Forouhi, A. Koulman, S.J. Sharp, F. Imamura, J. Kröger, M.B. Schulze, et al., Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study, Lancet. Diabetes Endocrinol 2 (10) (2014) 810–818, https://doi.org/10.1016/S2213-8587(14)70146-9.
[9] L. Huang, J.S. Lin, I.M. Aris, G. Yang, W.-Q. Chen, L.-J. Li, Circulating saturated fatty acids and incident type 2 diabetes: a systematic review and meta-analysis, Nutrients 11 (5) (2019) 998, https://doi.org/10.3390/nu11050998.
[10] M.Y. Yakoob, P. Shi, W.C. Willett, K.M. Rexrode, H. Campos, E.J. Orav, et al., Circulating biomarkers of dairy fat and risk of incident diabetes mellitus among men and women in the United States in two large prospective cohorts, Circulation 133 (17) (2016) 1645–1654, https://doi.org/10.1161/CIRCULATIONAHA.115.018410.
[11] W. Yoo, D. Gjuka, H.L. Stevenson, X. Song, H. Shen, S.Y. Yoo, et al., Fatty acids in non-alcoholic steatohepatitis: focus on pentadecanoic acid, PLOS ONE 12 (12) (2017) e0189965, https://doi.org/10.1371/journal.pone.0189965.
[12] S.K. Venn-Watson, C. Parry, M. Baird, S. Stevenson, K. Carlin, R. Daniels, et al., Increased dietary intake of saturated fatty acid heptadecanoic acid (C17:0) associated with decreasing ferritin and alleviated metabolic syndrome in dolphins, PLOS ONE 10 (7) (2015) e0132117, https://doi.org/10.1371/journal.pone.0132117.
[13] V.A. Stallings, J.T. Mondick, J.I. Schall, J.S. Barrett, M. Wilson, MR. Mascarenhas, Diagnosing malabsorption with systemic lipid profiling: pharmacokinetics of pentadecanoic acid and triheptadecanoic acid following oral administration in healthy subjects and subjects with cystic fibrosis, Int. J. Clin. Pharmacol. Ther. 51 (4) (2013) 263–273, https://doi.org/10.5414/CP201793.
[14] S. Venn-Watson, N.J. Schork, Pentadecanoic acid (C15:0), an essential fatty acid, shares clinically-relevant cell-based activities with leading longevity-enhancing compounds, Nutrients 15 (21) (2023) 4607, https://doi.org/10.3390/nu15214607.
[15] S. Venn-Watson, M. Baird, B. Novick, C. Parry, E.D. Jensen, Modified fish diet shifted serum metabolome and alleviated chronic anemia in

bottlenose dolphins (*Tursiops truncatus*): potential role of odd-chain saturated fatty acids, PLOS ONE 15 (4) (2020) e0230769, https://doi.org/10.1371/journal.pone.0230769.
[16] M.K. Soboleva, V.I. Sharapov, O.R. Grek, Fatty acids of the lipid fraction of erythrocyte membranes and intensity of lipid peroxidation in iron deficiency, Bull. Exp. Biol. Med. 117 (6) (1994) 601–603.
[17] S.A. Lagerstedt, D.R. Hinrichs, S.M. Batt, M.J. Magera, P. Rinaldo, JP. McConnell, Quantitative determination of plasma C8–C26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders, Mol. Genet. Metab. 73 (1) (2001) 38–45, https://doi.org/10.1006/mgme.2001.3170.
[18] F. Stellaard, H.J. ten Brink, R.M. Kok, L. Van Den Heuvel, C. Jakobs, Stable isotope dilution analysis of very long chain fatty acids in plasma, urine and amniotic fluid by electron capture negative ion mass fragmentography, Clin. Chim. Acta. 192 (2) (1990) 133–144, https://doi.org/10.1016/0009-8981(90)90077-6.
[19] H.J. ten Brink, F. Stellaard, C.M. van den Heuvel, R.M. Kok, D.S. Schor, R.J. Wanders, et al., Pristanic acid and phytanic acid in plasma from patients with peroxisomal disorders: stable isotope dilution analysis with electron capture negative ion mass fragmentography, J. Lipid. Res. 33 (1) (1992) 41–47.
[20] N.S. O'Connell, L. Dai, Y. Jiang, J.L. Speiser, R. Ward, W. Wei, et al., Methods for analysis of pre-post data in clinical research: a comparison of five common methods, J. Biom. Biostat. 8 (1) (2017) 1–8, https://doi.org/10.4172/2155-6180.1000334.
[21] S.K. Venn-Watson, C.N. Butterworth, Broader and safer clinically relevant activities of pentadecanoic acid compared to omega-3: evaluation of an emerging essential fatty acid across twelve primary human cell-based disease systems, PLOS ONE 17 (5) (2022) e0268778, https://doi.org/10.1371/journal.pone.0268778.
[22] M.R. Mascarenhas, J. Mondick, J.S. Barrett, M. Wilson, V.A. Stallings, J.I. Schall, Malabsorption blood test: assessing fat absorption in patients with cystic fibrosis and pancreatic insufficiency, J. Clin. Pharmacol. 55 (8) (2015) 854–865, https://doi.org/10.1002/jcph.484.
[23] A. Wolk, B. Vessby, H. Ljung, P. Barrefors, Evaluation of a biological marker of dairy fat intake, Am. J. Clin. Nutr. 68 (2) (1998) 291–295, https://doi.org/10.1093/ajcn/68.2.291.
[24] J. Wang, D. Zhang, R. Huang, X. Li, W. Huang, Gamma-glutamyltransferase and risk of cardiovascular mortality: a dose-response meta-analysis of prospective cohort studies, PLOS ONE 12 (2) (2017) e0172631, https://doi.org/10.1371/journal.pone.0172631.
[25] Y. Long, F. Zeng, J. Shi, H. Tian, T. Chen, Gamma-glutamyltransferase predicts increased risk of mortality: a systematic review and meta-analysis of prospective observational studies, Free Radic. Res. 48 (6) (2014) 716–728, https://doi.org/10.3109/10715762.2014.902055.
[26] L. Kazemi-Shirazi, G. Endler, S. Winkler, T. Schickbauer, O. Wagner, C. Marsik, Gamma glutamyltransferase and long-term survival: is it just the liver? Clin. Chem. 53 (5) (2007) 940–946, https://doi.org/10.1373/clinchem.2006.081620.
[27] M.C. Sawh, M. Wallace, E. Shapiro, N.P. Goyal, K.P. Newton, E.L. Yu, et al., Dairy fat intake, plasma pentadecanoic acid, and plasma iso-heptadecanoic acid are inversely associated with liver fat in children, J. Pediatr. Gastroenterol. Nutr. 72 (4) (2021) e90–e96, https://doi.org/10.1097/MPG.0000000000003040.
[28] A.E. Smedman, I.B. Gustafsson, L.G. Berglund, B.O. Vessby, Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors, Am. J. Clin. Nutr. 69 (1) (1999) 22–29, https://doi.org/10.1093/ajcn/69.1.22.
[29] W. Wei, C.C. Wong, Z. Jia, W. Liu, C. Liu, F. Ji, et al., *Parabacteroides distasonis* uses dietary inulin to suppress NASH via its metabolite pentadecanoic acid, Nat. Microbiol. 8 (8) (2023) 1534–1548, https://doi.org/10.1038/s41564-023-01418-7.
[30] M. Aktas, M. Elmastas, F. Ozcicek, N. Yilmaz, Erythrocyte membrane fatty acid composition in premenopausal patients with iron deficiency anemia, J. Oleo. Sci. 65 (3) (2016) 225–231, https://doi.org/10.5650/jos.ess15211.
[31] C.M. Skeaff, L. Hodson, J.E. McKenzie, Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course, J. Nutr. 136 (3) (2006) 565–569, https://doi.org/10.1093/jn/136.3.565.
[32] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longev. 2014 (2014) 360438, https://doi.org/10.1155/2014/360438.
[33] U.C.S. Yadav, Oxidative stress-induced lipid peroxidation: role in inflammation, in: V. Rani, U.C.S. Yadav (Eds.), Free Radicals in Human Health and Disease, Springer, New Delhi, 2015, pp. 119–129, https://doi.org/10.1007/978-81-322-2035-0_9.
[34] B. Krachler, M. Norberg, J.W. Eriksson, G. Hallmans, I. Johansson, B. Vessby, et al., Fatty acid profile of the erythrocyte membrane preceding development of type 2 diabetes mellitus, Nutr. Metab. Cardiovasc. Dis. 18 (7) (2008) 503–510, https://doi.org/10.1016/j.numecd.2007.04.005.
[35] M. Kratz, T. Baars, S. Guyenet, The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease, Eur. J. Nutr. 52 (1) (2013) 1–24, https://doi.org/10.1007/s00394-012-0418-1.
[36] S. Venn-Watson, J. Reiner, E.D. Jensen, Pentadecanoylcarnitine is a newly discovered endocannabinoid with pleiotropic activities relevant to supporting physical and mental health, Sci. Rep. 12 (1) (2022) 13717, https://doi.org/10.1038/s41598-022-18266-w.
[37] Y.C. Chooi, Q.A. Zhang, F. Magkos, M. Ng, N. Michael, X. Wu, et al., Effect of an Asian-adapted Mediterranean diet and pentadecanoic acid on fatty liver disease: the TANGO randomized controlled trial, Am. J. Clin. Nutr. 119 (3) (2024) 788–799, https://doi.org/10.1016/j.ajcnut.2023.11.013.