Oxidation of dietary linoleate occurs to a greater extent than dietary palmitate *in vivo* in humans

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Abbreviations

PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; FA, fatty acids; CO_2, carbon dioxide; IHTAG, intrahepatic triacylglycerol; TE, total energy; CRU, clinical research unit; RQ, respiratory quotient; REE, resting energy expenditure; NEFA, non-esterified fatty acids; TAG, triacylglycerol; 3OHB, β-hydroxybutyrate; PL, phospholipids; TTR, tracer to tracee ratio; VLDL, very-low density lipoprotein; LPL, lipoprotein lipase; Acyl-CoA, acyl-coenzyme As; CPT-1, carnitine palmitoyl transferase I

Short running heading: Dietary fatty acid oxidation

Abstract

Background: It has been suggested that dietary polyunsaturated fatty acids (PUFA) are partitioned into oxidation pathways to a greater extent than dietary saturated fatty acids (SFA). Whilst this has been demonstrated in animal models, evidence in humans is lacking. The potential divergence in the metabolic fate of these dietary fatty acids (FA) may explain some of the reported differences in ectopic fat deposition with SFA and PUFA enriched diets.

Aims: To compare whole-body oxidation of dietary palmitate and linoleate after consumption of a single test meal.

Methods: In a randomized, crossover design 24 healthy volunteers (12 males and 12 females, matched for age and BMI) underwent two study days separated by 2-week washout period. During each study day participants consumed a standardized test meal which contained [U^{13}C]palmitate or [U^{13}C]linoleate. Blood and breath samples were collected over the 6 hour postprandial period and the ^{13}C enrichment in breath CO_2 samples and plasma lipid fractions was determined.

Results: Appearance of ^{13}C in expired CO_2 was significantly ($p<0.05$) increased after consumption of the meal containing [U^{13}C]linoleate compared to the meal containing [U^{13}C]palmitate. The recovery of tracer was 8.9±1.2% [U^{13}C]linoleate *vs*. 5.6±0.4% [U^{13}C]palmitate ($p<0.05$). The incorporation of ^{13}C from [U^{13}C]palmitate was greater in plasma triacylglycerol and non-esterified fatty acids than [U^{13}C]linoleate, whereas the incorporation of ^{13}C from [U^{13}C]linoleate was greater than [U^{13}C]palmitate in plasma

67 phospholipids. Although $^{13}CO_2$ was significantly ($p<0.05$) higher in females compared to
68 males after consumption of [U^{13}C]palmitate, there was no difference in $^{13}CO_2$ between sexes
69 after consumption of [U^{13}C]linoleate.

70

71 **Conclusions:** We demonstrate that whole-body oxidation of dietary linoleate is
72 comparatively higher than that of dietary palmitate in humans following consumption of a
73 single mixed-test meal. We found indications of sexual dimorphism for dietary palmitate but
74 not dietary linoleate.

75

76 **Keywords: Fat oxidation, linoleate, palmitate, saturated fatty acid, polyunsaturated**
77 **fatty acid, dietary fat**

78

Introduction

Increased intrahepatic triacylglycerol (IHTAG) content is associated with type 2 diabetes and cardiovascular disease [1]. There is increasing evidence that dietary fatty acid (FA) composition may be a significant mediator of IHTAG content. For example, Rosqvist *et al*., [2] demonstrated greater IHTAG accumulation during overfeeding with saturated fat (SFA) compared with n-6 polyunsaturated fat (PUFA) despite similar weight gain, a finding that has also been reported by others following both isocaloric and hypercaloric diets [3-5]. On the basis of these observations, it has been hypothesized that dietary PUFA are partitioned into oxidation, rather than esterification pathways to a greater extent than SFA. Although animal studies support this notion [6-14], evidence in humans is limited.

The influence of FA saturation on whole-body FA oxidation was highlighted by Jones *et al*., [15] who fed six young healthy males a mixed meal containing either ^{13}C-labelled stearate, oleate or linoleate, in random order. They demonstrated that the postprandial appearance of ^{13}C in expired CO_2 (a marker of whole-body fat oxidation) was significantly greater following consumption of the meals containing ^{13}C-oleate and linoleate, than ^{13}C-stearate [15]. Similarly Schmidt *et al*., [16] found whole-body oxidation of oleate was 21% greater than palmitate when feeding ten healthy adults frequent (every 20 min) small mixed composition meals over a seven hour period. Together these studies suggest the postprandial oxidation of dietary unsaturated fat is greater than dietary saturated fat. In contrast, the findings from a small (n=4) study by DeLany *et al*., [17] found no significant differences between the major dietary saturated and unsaturated FA, with the cumulative recoveries of ^{13}C in breath from palmitate, oleate and linoleate being comparable. To date, the majority of studies have only included males, but as we have previously observed sexual dimorphism in dietary FA oxidation [18], it would be of interest to compare the oxidation of dietary SFA and PUFA between males and females. Therefore, the aim of the present study was to

104 compare the appearance of ^{13}C in expired CO_2 in the 6-hour postprandial period following
105 consumption of a standardized mixed test meal containing either [U^{13}C]palmitate or
106 [U^{13}C]linoleate as a marker of whole-body fat oxidation of SFA and PUFA respectively, in
107 healthy males and females.

108

109

Materials and Methods

Participants

Males and females of a similar age and body mass index (BMI) were recruited by word of mouth from the Oxfordshire area and from the Oxford BioBank (www.oxfordbiobank.org.uk [19]. All volunteers were aged 18-65 years, free from any known metabolic disease, had a BMI between 18-30 kg/m^2, were not taking medication known to affect lipid or glucose metabolism, did not smoke, and did not consume alcohol above recommended UK limits (www.gov.uk) assessed at screening. The study was approved by the Portsmouth Research Ethics Committee (REC 12/SC/0267) and all participants gave written informed consent.

Experimental design

This study was a randomized, crossover design. Each participant completed two postprandial study days, which were separated by a 2-week washout period. The experimental study days involved feeding a standardized test meal which was identical except for the addition of either [U^{13}C]palmitate or [U^{13}C]linoleate, which were used to trace whole-body FA oxidation. Randomization was performed by a statistician who was not involved in the day-to-day running of the study, and the authors were blinded as to the study order until the end of the data collection period. All participants were advised to consume a eucaloric control diet (based on the UK EatWell plate) for 3 days before each experimental study day to standardize the run-in diet between participants and between study days. A 3-day diet diary was completed during the initial run-in period before the first study day and participants were asked to repeat this diet during the second pre-study day run-in period. Participants were instructed to avoid food naturally enriched in ^{13}C (e.g. cornflakes, foods rich in corn-starch etc.) during run-in periods. All participants were asked to refrain from alcohol and strenuous

134 exercise for 24-hours prior to the study day. This study was registered at clinicaltrials.gov
135 (NCT03587753).

136

137 *Postprandial study days*

138 The evening before the study day participants consumed a standardized low-fat test meal
139 (commercially available frozen ready-meal: 292kcal, 64.4% total energy (TE) carbohydrate,
140 18.2% TE fat, and 17.4% TE protein). On the morning of the study day, participants came to
141 the Clinical Research Unit (CRU) at the Oxford Centre for Diabetes Endocrinology and
142 Metabolism after an overnight fast of approximately 10-hours. A Teflon catheter was inserted
143 into an antecubital vein to allow for repeated blood sampling, after which a baseline, fasting
144 venous blood sample (0) was collected in heparinized tubes (Sarstedt, Leicester, UK) and
145 immediately spun at 3000*g* in a refrigerated centrifuge (4˚C) for 10 minutes to obtain plasma,
146 which was then stored at -20˚C until analysis. Participants were then fed a mixed test meal
147 containing 44g carbohydrate, 42g of fat and 9.7g of protein to which either 200mg of
148 [U^{13}C]palmitate or [U^{13}C]linoleate was added. The FA composition of the test meal was
149 ~32% palmitate, 35% oleate, 27% linoleate, 5% stearate and 1% trace FA. Blood samples
150 were taken 30, 60, 90, 120, 180, 240, 300 and 360 minutes after consumption of the test meal.

151 Breath samples were collected in EXETAINER® tubes (Labco Ltd, High Wycombe, UK)
152 before consumption of the test meal (0) and then at 30, 60, 90, 120, 180, 240, 300 and 360
153 minutes after consumption of the mixed test meal to determine $^{13}CO_2$ enrichment. Indirect
154 calorimetry was performed in the fasting state and 120- and 360-minutes after meal
155 consumption using a GEM calorimeter (GEMNutrition Ltd., Cheshire, UK) to determine
156 whole-body CO_2 production, whole-body respiratory quotient (RQ), and resting energy

157 expenditure (REE). Body composition was assessed using Lunar iDXA machine (GE
158 Healthcare, USA) during the first study day.

159

160 *Plasma Biochemistry*

161 Plasma glucose, non-esterified fatty acids (NEFA), total and high-density lipoprotein (HDL)
162 cholesterol, TAG, and β-hydroxybutyrate (3OHB), were measured on a semi-automatic
163 analyser (ILab 600/650 clinical chemistry. Warrington, UK). Plasma insulin levels were
164 determined by radioimmunoassay as described [20].

165

166 *FA composition and isotopic enrichment*

167 Total lipids were extracted from plasma fractions and FA methyl esters were prepared as
168 previously described [21]. FA compositions (μmol/100 μmol total FAs) were determined by
169 gas chromatography (GC) [21]. Internal standards of known concentration were added to
170 samples prior to lipid extraction to allow for determination of FA concentration. Enrichment
171 of [U^{13}C]palmitate and [U^{13}C]linoleate in plasma TAG, NEFA and phospholipid (PL)
172 fractions were determined by gas chromatography-combustion-isotope ratio mass
173 spectrometry (GC-C-IRMS). The ^{13}C enrichments expressed as $\delta^{13}C^{0}/_{00}$, were converted to a
174 tracer to tracee ratio (TTR): TTR ($^{13}C/^{12}C$) = $((\delta^{13}C^{0}/_{00}/1000)+1) \times 0.0112372$ x (17/16)). The
175 concentration of ^{13}C in specific lipid fractions was determined by multiplying the TTR by the
176 corresponding palmitate or linoleate concentration in each lipid fraction. The TTR of a
177 fasting sample (0), obtained before participants consumed the test meal, was subtracted from
178 each sample to account for natural abundance of ^{13}C in all lipid fractions measured.

179

Analysis of ^{13}C enrichment in breath CO_2 samples was determined via IRMS, and whole-body meal-derived FA oxidation was calculated by multiplying the CO_2 production ($\dot{V}CO_2$, mmol/min) by the TTR of $^{13}CO_2/^{12}CO_2$ as described [22] and corrected for lean body mass.

Statistical analysis

Despite calculating the TTR of the meals to be similar, we measured a sample of each participants meal and found that the test meals containing [U^{13}C]linoleate was notably lower (by ~37%) than the TTR of the meals containing [U^{13}C]palmitate. Therefore, a correction factor was applied to the TTR of [U^{13}C]linoleate data on a participant to participant basis that was representative of the difference in the TTR of test meals. All data were analyzed using SPSS (V22.0) for windows (SPSS Inc., Chicago, IL, USA). Areas under the curve (AUC) were calculated by the trapezoidal method and were divided by the relevant time period to provide time-averaged values. Fasting biochemical characteristics were compared between visits using a Students paired t-test. Whole-body fat oxidation (primary outcome measure) and postprandial biochemistry during the two postprandial study days ([U^{13}C]palmitate *vs.* [U^{13}C]linoleate) was compared using a two-way repeated measures ANOVA and Bonferroni post hoc analysis where appropriate. Sex-specific differences in the metabolism of palmitate and linoleate were analysed using an independent sample t-test and two-way ANOVA where appropriate. Statistical significance was set at $p<0.05$.

200 **Results**

201 *Participant characteristics*

202 Twenty-four healthy individuals (12 males and 12 females) participated in the study and
203 their characteristics are shown in Table 1. By design, there was no difference in age or
204 BMI between males and females, but males had a significantly (p<0.05) higher
205 bodyweight and lean mass but significantly (p<0.05) lower amount of body fat compared
206 to females (Table 1).

207

208 **Table 1**. Participant characteristics.

	Males	**Females**
n	12	12
Age (years)	41±2	46 ± 3
Weight (kg)	83.8±2.9	67.7 ± 2.9*
BMI (kg/m^2)	25.8±0.8	25.4 ± 0.9
Waist circumference (cm)	90.0 ± 2.8	85.8 ± 2.5
Body fat (%)	22.6±2.8	35.9±2.0*
Lean mass (kg)	60.9±1.8	38.4±3.4*

209 Data presented are means ± SEM. * p<0.05 significantly different between males and
210 females. Abbreviations: BMI, body mass index.

211

212 *Fasting and postprandial plasma biochemistry*

213 Fasting and postprandial biochemical parameters were similar on both study days (Table 2,
214 Figure 1A-E).

215 **Table 2**. Fasting and postprandial biochemical and metabolic parameters.

	[U^{13}C]palmitate	**[U^{13}C]linoleate**
Fasting plasma biochemical parameters		
Glucose (mmol/L)	4.8±0.1	4.8±0.1
Insulin (mU/L)	7.4±0.7	7.3±0.7
HOMA-IR	1.6±0.2	1.5±0.2
NEFA (μmol/L)	629±67	532±49
TAG (mmol/L)	0.8±0.1	0.9±0.1
3OHB (μmol/L)	150±23	115±14
Total cholesterol (mmol/L)	4.1±0.2	4.2±0.2
HDL cholesterol (mmol/L)	1.3±0.1	1.2±0.1
Non-HDL cholesterol (mmol/L)	2.9±0.1	2.9±0.1
Indirect calorimetry		
Fasting REE (kcal)	1306±58	1299±46
120 min REE (kcal)	1435±57	1458±55
360 min EE (kcal)	1408±60	1449±61
Fasting RQ	0.70±0.02	0.75±0.01
120 min RQ	0.77±0.02	0.78±0.01
360 min RQ	0.73±0.02	0.71±0.01
Net FA oxidation (g/min)	0.08±0.01	0.07±0.01

216 Data are presented as means ± SEM.

217 Abbreviations: HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NEFA,
218 non-esterified fatty acids; TAG, triacylglycerol; 3OHB, β-hydroxybutyrate; HDL, high-
219 density lipoprotein; REE, resting energy expenditure; RQ, respiratory quotient; FA, fatty
220 acid.

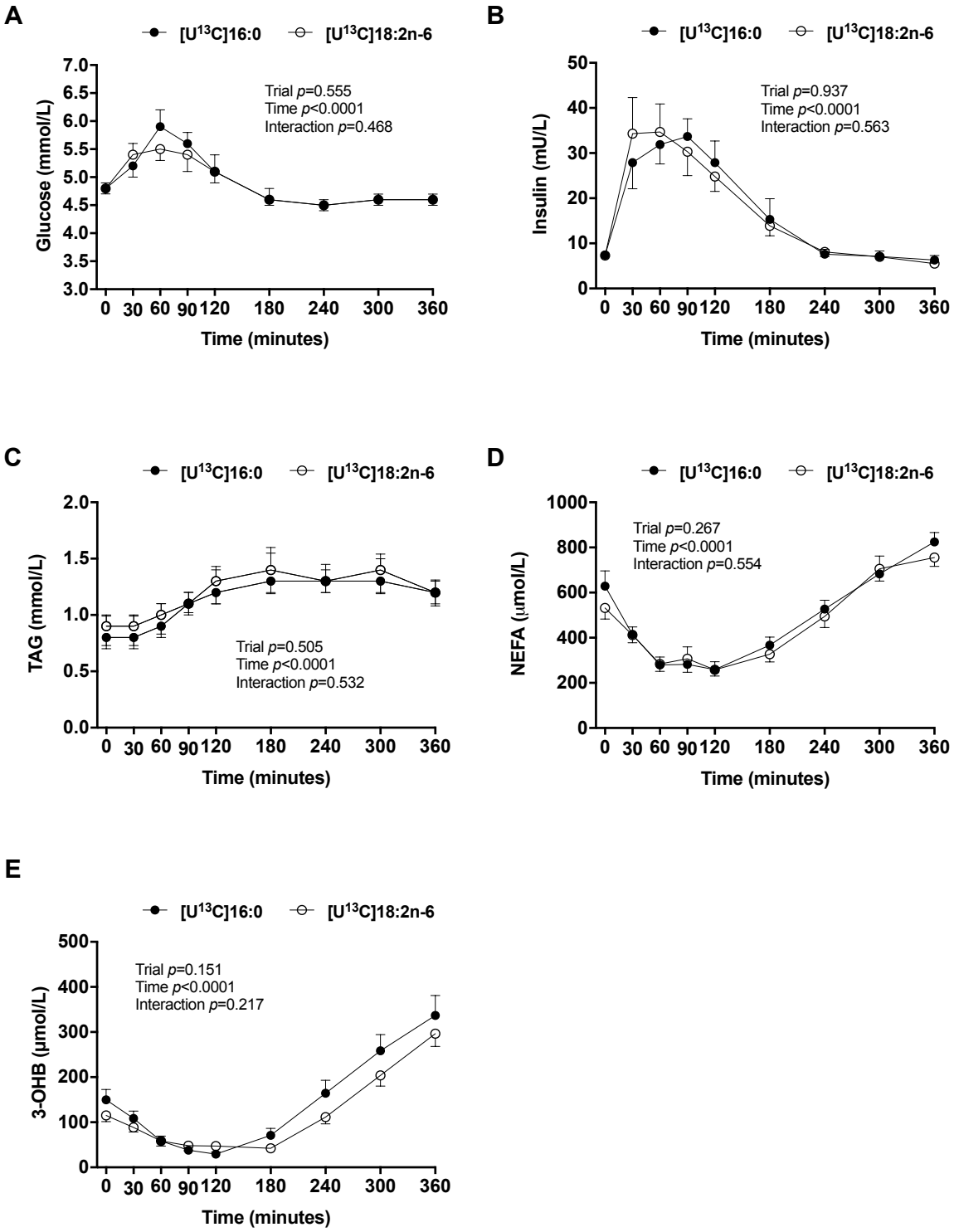


Figure 1. Systemic plasma concentrations of (**A**) plasma glucose, (**B**) insulin, (**C**) triacylglycerol (TAG), (**D**) non-esterified fatty acid (NEFA), and (**E**) β-hydroxybutyrate (3-OHB), following a standardized test meal containing either [U^{13}C]palmitate ([U^{13}C]16:0) or [U^{13}C]linoleate ([U^{13}C]18:2n-6). Data presented are means ± SEM. n = 24.

226

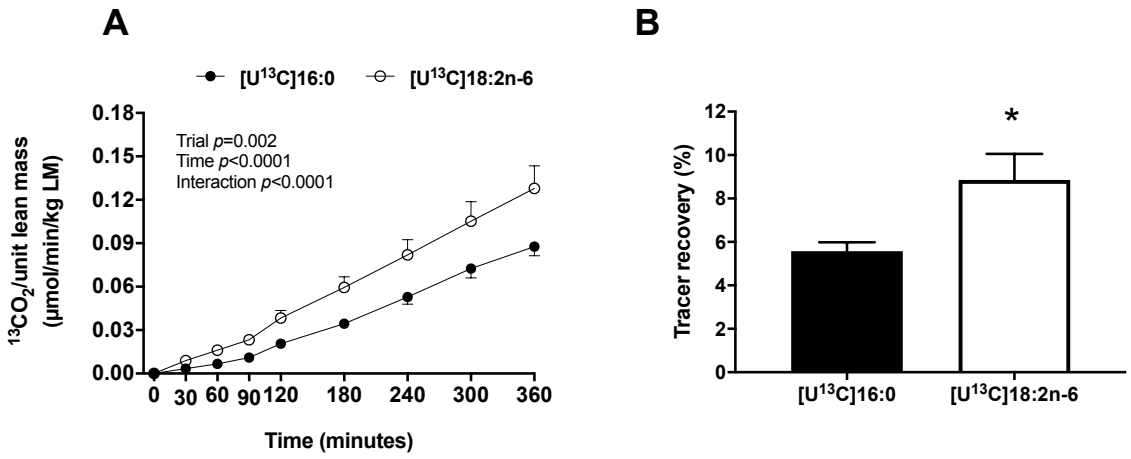
227 *Indirect calorimetry*

228 Fasting and postprandial RQ and REE were similar on both study days (Table 2). We also
229 calculated postprandial net FA oxidation rates [23], and found no significant difference in FA
230 oxidation rates between the two study days (Table 2).

231

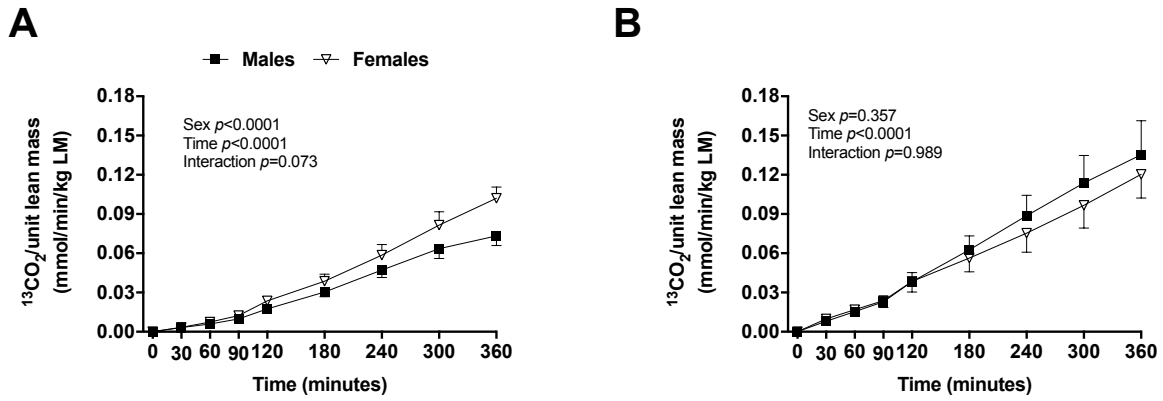
232 *Whole-body FA oxidation*

233 The appearance of ^{13}C in expired CO_2 was significantly ($p<0.05$) greater after consumption of
234 the meal containing [U^{13}C]linoleate compared to the meal containing [U^{13}C]palmitate (Figure
235 2A), as was the recovery of tracer given (Figure 2B). As we have previously found
236 differences in dietary FA oxidation between females and males [18] we investigated if sex-
237 differences in the oxidation of the specific FAs (i.e. palmitate or linoleate) occurred. In-line
238 with our previous finding when using [^{13}C]palmitate, we found that the appearance of ^{13}C in
239 expired CO_2 from [U^{13}C]palmitate was significantly ($p<0.05$) greater in females compared to
240 males (Figure 3A). Conversely, the appearance of ^{13}C in expired CO_2 from [U^{13}C]linoleate
241 was similar between males and females (Figure 3B).



242

243 **Figure 2**. (**A**) Expired $^{13}CO_2$ over time and (**B**) 6-hour tracer recovery, following a
244 standardized test meal containing either [U^{13}C]palmitate ([U^{13}C]16:0) or [U^{13}C]linoleate
245 ([U^{13}C]18:2n-6). Data presented are means ± SEM. n = 24. * p<0.05 for [U^{13}C]palmitate *vs*.
246 [U^{13}C]linoleate



247

248 **Figure 3.** Expired $^{13}CO_2$ in males and females following a standardized test meal containing
249 (**A**) [U^{13}C]palmitate ([U^{13}C]16:0) or (**B**) [U^{13}C]linoleate ([U^{13}C]18:2n-6). Data presented are
250 means ± SEM. n = 12 males and 12 females.

251

252 *Tracer incorporation into plasma lipid fractions*

253 The incorporation of ^{13}C from [U^{13}C]palmitate and [U^{13}C]linoleate into specific plasma lipid
254 fractions was measured. Across the 6-hour postprandial period, the incorporation of ^{13}C was
255 ~36% ($p < 0.05$) greater in plasma TAG and ~53% greater ($p < 0.05$) in plasma NEFA for
256 [U^{13}C]palmitate compared with [U^{13}C]linoleate, with incorporation being similar between
257 males and females for all plasma lipid fractions (Figure 4A and 4B). In contrast to plasma
258 TAG and NEFA, ^{13}C incorporation into plasma phospholipid, was ~5-fold higher for
259 [U^{13}C]linoleate compared with [U^{13}C]palmitate (Figure 4C). Sexual dimorphism was also
260 apparent for the incorporation of [U^{13}C]palmitate into plasma PL, as females had an
261 enrichment that was approximately double (p<0.05) that of males (Figure 4C).

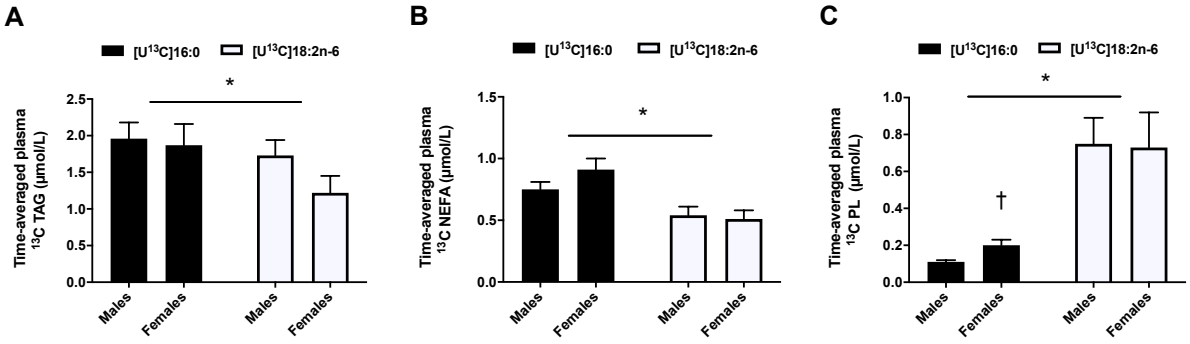


Figure 4. Time-averaged AUC concentrations (µmol/L) of ^{13}C palmitate or linoleate in (**A**) plasma triacylglycerol (TAG), (**B**) non-esterfied fatty acids (NEFA) and, (**C**) phospholipid (PL) in males and females following a standardized test meal containing either [U^{13}C]palmitate ([U^{13}C]16:0) or [U^{13}C]linoleate ([U^{13}C]18:2n-6). Data presented are means ± SEM. n = 24 (12 males and 12 females). $^{*}p<0.05$ for [U^{13}C]16:0 *vs*. [U^{13}C]18:2n-6; $^{\dagger}p<0.05$ for males *vs*. females.

Discussion

It has been hypothesized that dietary PUFA are partitioned into oxidation, rather than esterification pathways to a greater extent than SFA. This has been consistently demonstrated in rodents [6-12]; however, human data is limited. In this study we utilized stable-isotope tracers to assess the appearance of ^{13}C in expired CO_2 as a measure of whole-body oxidation of palmitate and linoleate, in 24 healthy participants after consumption of a single mixed composition meal. We found the appearance of ^{13}C in expired CO_2 was significantly greater after consumption of [U^{13}C]linoleate compared to [U^{13}C]palmitate, suggesting that dietary PUFA may preferentially enter oxidation pathways compared to dietary SFA. We also observed sexual dimorphism in this response, with ^{13}C appearance in expired CO_2 greater in females compared to males following the meal containing [U^{13}C]palmitate, whereas $^{13}CO_2$ production from [U^{13}C]linoleate was similar between males and females. There were also differences in the incorporation of ^{13}C in plasma lipid fractions; [U^{13}C]palmitate incorporation into plasma TAG and NEFA was greater than that of [U^{13}C]linoleate, whereas the opposite was observed in PL.

Our findings extend those of previous human studies and confirm that dietary FA oxidation is influenced by FA saturation. Jones *et al.*, [15] used ^{13}C tracers to compare oleate, linoleate and stearate oxidation in six healthy males and found the 9-hour recovery of tracer was ~15%, ~10% and ~3%, respectively. Similarly Schmidt *et al.*, [16] reported that the oxidation of oleate, was significantly greater than palmitate in ten healthy adults fed frequent small meals over a 7-hour period. Conversely, in a small (n=4 healthy males) study DeLany *et al.*, [17] found no significant differences when comparing the 9-hour recovery of ^{13}C oleate, linoleate and palmitate in breath after feeding standardized test meals. Although it is not possible to directly compare tracer recoveries across studies due to methodological

297 differences, taken together it would appear dietary unsaturated FAs enter oxidation pathways
298 to a greater extent than dietary SFAs. This is in-line with the observation that individuals
299 consuming diets enriched in unsaturated fat demonstrate a reduced ectopic fat deposition
300 when compared to those consuming a diet enriched in SFA [3-5].
301
302 Differences in the appearance of labelled FA into expired CO_2 may be mediated by three
303 processes: i) dietary FA absorption, ii) FA transport across cellular membranes, and iii)
304 intracellular FA partitioning. Following ingestion, within enterocytes FA are esterified to
305 TAG and then incorporated into chylomicrons for distribution to metabolic tissues [24]. We
306 did not isolate chylomicron-TAG, rather we measured the ^{13}C enrichment in plasma TAG,
307 which reflects both chylomicron and very low-density lipoprotein (VLDL)-TAG in the
308 postprandial period, and found TAG ^{13}C enrichment was greater after the meal containing
309 [U^{13}C]palmitate. Thus, we cannot exclude the possibility that there may have been
310 differences in absorption of the FA tracers. However, it has previously been shown that the
311 incorporation of labelled palmitate and linoleate into chylomicron-TAG is similar in humans
312 [25, 26]. If differences in absorption were apparent, it could be expected that ^{13}C
313 incorporation in plasma TAG would mirror the differences in $^{13}CO_2$ (i.e. greater enrichment
314 after [U^{13}C]linoleate ingestion), which is not the case. Thus, it is unlikely that differences in
315 absorption explain our findings. However, it is plausible the difference in plasma TAG
316 observed here is due to the partitioning of [^{13}C]linoleate into other lipid fractions, such as
317 plasma phospholipids; we found ~5-fold greater ^{13}C enrichment in plasma phospholipids
318 following the meal containing [U^{13}C]linoleate compared to [U^{13}C]palmitate.
319
320 In the postprandial period, circulating chylomicron-TAG are hydrolyzed by lipoprotein lipase
321 (LPL) in the capillaries of metabolic tissues [27]. The liberated FA are then transported into

cells via diffusion across the plasma membrane, or by FA transporter proteins and FA translocase/cluster of differentiation 36 which are found on the surface of cells, including hepatocytes, adipocytes and myocytes [28]. Although it remains unclear whether the affinity of these proteins is influenced by FA saturation and/or chain length, human arterio-venous difference studies have reported that the uptake of palmitate and linoleate by liver and skeletal muscle are similar in the fasting state [29, 30], in contrast a recent study in a porcine model found hepatic uptake of linoleate was markedly higher than palmitate [31]. When investigating adipose tissue uptake of specific FAs using the arterio-venous difference technique, Summers *et al*., report no difference in the postprandial uptake or LPL-mediated hydrolysis of palmitate and linoleate [32]. Although studies comparing the postprandial uptake of palmitate and linoleate in skeletal muscle and liver are sparse, findings from animal studies suggest that LPL-activity in heart- and skeletal muscle is higher after an n-6 PUFA compared to SFA diet [33, 34]. The greater appearance of ^{13}C in the plasma NEFA pool after the meal containing [U^{13}C]palmitate compared to [U^{13}C]linoleate may reflect differences in "spillover" and chylomicron-TAG hydrolysis [35]; we did not directly assess chylomicron-TAG hydrolysis or chylomicron-derived spillover.

In humans, skeletal muscle and liver are major contributors to whole-body FA oxidation. Once within hepatocytes or myocytes, FAs are rapidly converted to fatty acyl-coenzyme A (Acyl-CoA) which are then partitioned into oxidation or storage pathways. Carnitine palmitoyl transferase I (CPT-1) transports Acyl-CoA into the mitochondria and represents a rate-limiting step in FA oxidation. Evidence from isolated rat liver mitochondria found that CPT-1 is activated by lower concentrations of linoleate than palmitate, which would facilitate more rapid transfer into mitochondria for β-oxidation [36]. This would suggest that differences intracellular metabolism might mediate the differences in palmitate and linoleate

oxidation. We were unable to determine which metabolic tissues were primarily responsible for the differences in whole-body FA oxidation however a recent murine study using ^{13}C isotope tracers demonstrated that hepatic oxidation of linoleate was ~3 fold higher than palmitate [31].

By studying males and females, matched for age and BMI, we investigated sex-specific differences in the oxidation of dietary palmitate and dietary linoleate. We found the appearance of ^{13}C in expired CO_2 was greater in females compared to males following the meal containing [U^{13}C]palmitate, which is in-line with previous work from our group [18]. However, this difference was not evident for linoleate; the observation that sexual dimorphism in FA oxidation may be specific to individual FAs has not previously been demonstrated. The reasons for the differences in the oxidation of specific FA between sexes remain to be elucidated. However as we have previously reported increased chylomicron-derived spillover in females compared to males following a test meal containing [U-^{13}C]palmitate [37] it could be speculated that this may increase palmitate flux to non-adipose tissues such as liver and skeletal muscle in females, where they may enter oxidation pathways. This would be in line with the increased ^{13}C enrichment in plasma NEFA we observed in females following the meal containing [U^{13}C]palmitate, which was approximately 17% greater than males. We have not previously investigated linoleate metabolism in regards to chylomicron metabolism, specifically chylomicron-derived spillover and it would be of interest to do so. There is currently limited work which has investigated sex-specific differences in FA metabolism and given the notable difference in the prevalence of NAFLD between males and females [38], it would be of interest to elucidate the sex-specific mechanisms underpinning our observations. Based on our

observations, it is clear that the choice of FA tracer may influence results when comparing FA oxidation between sexes.

This study is not without limitations. Firstly, it is a single meal feeding study and the test meal composition may not completely replicate the ratios of FAs typically consumed by the individuals. Additionally, although diets were standardized for 3-days before each study visit, we did not control participant's habitual diets. Others have fed unsaturated or SFA rich diets for longer periods (28 days) and found that increased dietary palmitate resulted in reduced FA oxidation and daily energy expenditure (assessed by indirect calorimetry) [39]. Evidence also suggests that postprandial net FA oxidation, measured by indirect calorimetry, is influenced acutely by meal FA composition [40]. Whether dietary or meal FA composition influences the oxidation of specific FA remains to be determined. We traced palmitate as a marker of SFA, and linoleate as a marker of PUFA, and it is unclear if our findings can be generalized to all SFA and PUFA. Additionally, we are unable to account for the endogenous production of palmitate in this study, which may influence the TTR following the meal containing [U^{13}C]palmitate. As linoleate is an essential FA, accounting for endogenous production was not an issue.

In conclusion, we demonstrate that whole-body oxidation of linoleate is comparatively greater than whole-body oxidation of palmitate in humans consuming a mixed test meal. These observations, may help, in part to explain the increased ectopic fat deposition seen when feeding SFA enriched diets compared to PUFA enriched diets.

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Statement of authorship

LH designed research and gained research funding; LH, SAP, FR, TC, and AB conducted the research; SAP, FR, TC, and AB analyzed data; SAP, FR, and LH wrote the paper; LH had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of Interest

All authors declare no conflict of interest.

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514 **Figure Legends**

515

516 **Figure 1**. Systemic plasma concentrations of (**A**) plasma glucose, (**B**) insulin, (**C**)
517 triacylglycerol (TAG), (**D**) non-esterified fatty acid (NEFA), and (**E**) β-hydroxybutyrate (3-
518 OHB), following a standardized test meal containing either [U^{13}C]palmitate ([U^{13}C]16:0) or
519 [U^{13}C]linoleate ([U^{13}C]18:2n-6). Data presented are means ± SEM. n = 24.

520

521 **Figure 2**. (**A**) Expired $^{13}CO_2$ over time and (**B**) 6-hour tracer recovery, following a
522 standardized test meal containing either [U^{13}C]palmitate ([U^{13}C]16:0) or [U^{13}C]linoleate
523 ([U^{13}C]18:2n-6). Data presented are means ± SEM. n = 24. * p<0.05 for [U^{13}C]palmitate *vs*.
524 [U^{13}C]linoleate

525

526 **Figure 3.** (**A**) Expired $^{13}CO_2$ in males and females following a standardized test meal
527 containing [U^{13}C]palmitate ([U^{13}C]16:0) or (**B**) [U^{13}C]linoleate ([U^{13}C]18:2n-6). Data
528 presented are means ± SEM. n = 12 males and 12 females.

529

530 **Figure 4**. ^{13}C enrichment in (**A**) plasma triacylglycerol (TAG), (**B**) non-esterfied fatty acids
531 (NEFA) and, (**C**) phospholipid (PL) in males and females following a standardized test meal
532 containing either [U^{13}C]palmitate ([U^{13}C]16:0) or [U^{13}C]linoleate ([U^{13}C]18:2n-6). Data
533 presented are means ± SEM. n = 24 (12 males and 12 females). $^{*}p$<0.05 for [U^{13}C]palmitate
534 *vs*. [U^{13}C]linoleate, $^{\dagger}p$<0.05 for males *vs*. females.

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