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BRIEF COMMUNICATION

Intake of palmitic acid and its association with metabolic flexibility in middle-aged individuals: a preliminary study

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This paper forms part of a special collection based on the 2023 Oxygen Club of California (OCC) Workshop, part of Healthy Ageing Week 2023. The collection was led by Editor-in-Chief Professor Giuseppe Poli, University of Torino at San Luigi Hospital, Turin, Italy, and Guest Editors Professor Enrique Cadenas, University of Southern California, CA, USA, and Dr Ettore Bologna, Ferrero Foundation, Alba, Italy.

Abstract

Objective: This study aimed to assess the relationship between dietary palmitic acid (PA) intake and its association with body fat deposition and metabolic flexibility (MF) in middle-aged healthy individuals.
Methods: Fifteen healthy participants ($n = 15$; 6 males, 9 females) with a mean age of 54 were enlisted. They were subjected to graded exercise tests using a cycle ergometer coupled with a calorimeter. Respiratory gas exchange was evaluated to determine two MF parameters. First, the MF index was derived by multiplying peak fatty acid oxidation (PFO) per kg of fat-free mass (FFM) with the percentage of VO_2max at PFO. The second parameter, peak energy substrates' oxidation (aka PESO), was computed by aggregating the kilocalories from PFO and peak carbohydrate oxidation, normalized per kg FFM. Dietary intake was gauged using a 7-day dietary record. Spearman's regression was employed to analyze the association between dietary intake of specific fat classes, PA, MF parameters, and body fat percentage.
Results: Preliminary results demonstrate that dietary saturated fatty acids (SFA) within physiological limits correlate with enhanced substrate oxidation capacity. This suggests augmented MF in middle-aged subjects. Among dietary SFA, PA was identified as the primary factor in this favorable correlation.
Conclusions: Our initial observations, even though preliminary, strongly suggest a beneficial association between PA intake, MF, and body fat percentage. This underscores the potential nutritional importance of PA in promoting MF.

Keywords

- palmitic acid
- metabolic flexibility

Significance Statement

Disparities in data regarding the health effects of dietary fats may arise from the distinct roles various dietary fatty acids play in MF.

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Introduction

Multiple epidemiological investigations have demonstrated a correlation between increased dietary fat consumption and elevated body mass index (BMI) in humans (Bray & Popkin 1998). The predominant American diet sources approximately 35–40% of its caloric intake from fats (Reeves 1997). This exceeds the

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recommended threshold of 30% of total energy derived from fats (Hooper *et al.* 2020) as also recently pointed out by WHO (World Health Organization 2023) even though strongly criticized (Ludwig *et al.* 2023). An increase in obesity rates in the USA has coincided with a decline in fat and calorie intake, a phenomenon termed the 'American paradox' (Heini & Weinsier 1997). A recent comprehensive review examining dietary patterns in relation to non-communicable disease onset found an inverse correlation between animal fat consumption and these diseases (Lee *et al.* 2022). The existing disparities in data on dietary fat could be attributed to the varied roles that dietary fatty acids (FA) exert, especially in modulating inflammatory pathways and insulin resistance (IR). Additionally, bioactive metabolites originating from FA can influence lipid and energy metabolism through the peroxisome proliferator-activated receptor alpha (PPAR-α) and endocannabinoid (EC) systems (Banni & Di Marzo 2010, Tsuboi *et al.* 2018).

A high intake of saturated fatty acids (SFA) correlates with elevated endogenous cholesterol production and blood lipoprotein cholesterol concentrations, potentially resulting in lipotoxicity, cellular impairment, and the onset of metabolic syndrome. However, some randomized controlled trials did not identify a relationship between diminished SFA consumption and reduced overall or cardiovascular-related mortality (Astrup *et al.* 2020). As such, dietary fats, especially palmitic acid (PA), appear to profoundly impact lipid and energy metabolism and, subsequently, metabolic adaptability under various pathophysiological contexts.

Our recent findings suggest that in obese rats dietary PA enhances metabolic flexibility (MF), pointing to a unique nutritional influence exerted by PA (Carta *et al.* 2023).

In the present study, our objective was to determine if dietary PA, in ostensibly healthy individuals, correlates with changes in body fat deposition and MF. The latter was evaluated using two novel parameters identified through metabolometric analysis and previously studied in middle-aged athletes with varied exercise intensities (submitted for publication): (i) the metabolic flexibility index (MFI): computed as the product of peak FA oxidation (PFO) normalized for kg of fat-free mass (FFM) and the percentage of VO_2max at PFO, reflecting the relative PFO as the ability to utilize FA as substrates increases with the incremental %VO_2max and (ii) peak energy substrates' oxidation (PESO): determined by adding the kilocalories from PFO and peak carbohydrate oxidation (PCO), normalized per kg of FFM, representing the utmost capability to oxidize both primary energy substrates.

Materials and methods

Population and recruitment

The present study was conducted according to the Declaration of Helsinki and all procedures involving individuals were approved by the ethical committee of A.O.U.CA (Azienda Ospedaliera Universitaria di Cagliari). The trial was registered at ClinicalTrials.gov (protocol no. NCT06012227). Written informed consent was obtained from all subjects.

Healthy individuals ($n=15$, six males and nine females) were recruited from the Coronary Intensive Care Unit (UTIC) of the University Hospital of Cagliari; see Table 1 for anthropometric characteristics. No subjects reported metabolic dysfunction nor were on medication.

Metabolometric analysis

Following an overnight fast of at least 12 h, volunteers undergo a maximal cardiopulmonary exercise test (CPET) during a cyclo-ergometer (Ergoline Bosch 500, Berlin, Germany) incremental test. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were collected by a breath-by-breath analysis (Ergostik, Geratherm Medical AG, Geschwenda, Germany) and heart rate was continuously recorded by a 12-lead electrocardiogram (Mortara X-Scribe stress test, Milwaukee, WI, USA) under the supervision of a cardiologist. Blood pressure measurements were carried out at rest, during exercise, and during recovery.

Table 1 Anthropometric characteristics. Values are presented as mean ± S.E.M.

Characteristics	Values
Total *n*	15
Males, *n*	6
Females, *n*	9
Age	53.65 ± 4.64
Body weight, kg	56.12 ± 5.39
BMI	20.95 ± 1.82
Free fat mass	
kg	46.38 ± 3.31
%	77.66 ± 2.15
Fat mass	
kg	12.72 ± 1.05
%	22.23 ± 2.17

BMI, body mass index.

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After a 3-min resting period the subjects started pedaling at 60 rpm with the workload progressively increased to 15 up or 30 watts every 5 min (to allow the achievement of a metabolic steady state) until volitional exhaustion, indicated by the subject's inability to maintain a pedaling rate of at least 50 rpm.

Maximal oxygen consumption

Along testing, the gas-analyzer provided averaged data every 5–7 s. The acquired data were collected and analyzed by averaging the amount of the last 1 min of each step. The results obtained were then interfaced with the relative power held by the athlete and reported on Cartesian graphs, which showed the workload on the abscissa and the oxygen consumption on ordered axis. The calculated VO2max was considered valid if it met the achievement of these two criteria: (i) respiratory exchange ratio >1.10 and (ii) an HR ± 10 beats/min (bpm) of predicted maximum HR calculated as 220 minus the age (Howley *et al.* 1995).

Fatty acid and carbohydrate oxidation rate analyses

For the measurement of total FAO and CHO, stoichiometric equations were applied according to the methodology described by Frayn (Frayn 1983).

We assessed two measurable parameters of MF (methodology under publication): (i) the metabolic flexibility index (MFI) calculated by the product between $\%VO_2$max at PFO and PFO, normalized per kg of FFM. MFI could be considered to represent a unifying numerically measurable parameter for MF, displaying the ability to use FA as energy substrate during incremental exercise; (ii) by evaluating the combination of PFO and PCO levels, we calculated the PESO, expressed in kilocalories (kcal) and obtained by the sum between PFO and PCO, normalized per kg of FFM.

Bioelectrical impedance analysis

BIA was performed by Handy 3000 (DS Medica S.r.l., Milano, Italy). A constant current source at a frequency of 50 kHz and 100 kHz and 0.8 mÅ was applied to estimate body composition.

Food diary

A comprehensive analysis of food consumption was conducted by implementing a 7-day dietary recording method. Participants were directed to meticulously record their daily dietary consumption in a dedicated diary and present it in a tabular format categorized into five primary daily meals. The dietary data recorded in the food diary were then analyzed using the WinFood software, Medimatica S.u.r.l., Colonnella (T.E.).

Statistical analysis

Data are expressed as mean ± S.D. Data were analyzed using the GraphPad Prism 8.0.1 software. Correlations between PESO and the intake of different lipid classes, SFA, monounsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) were assessed using the Spearman's correlation coefficient with a 95% CI.

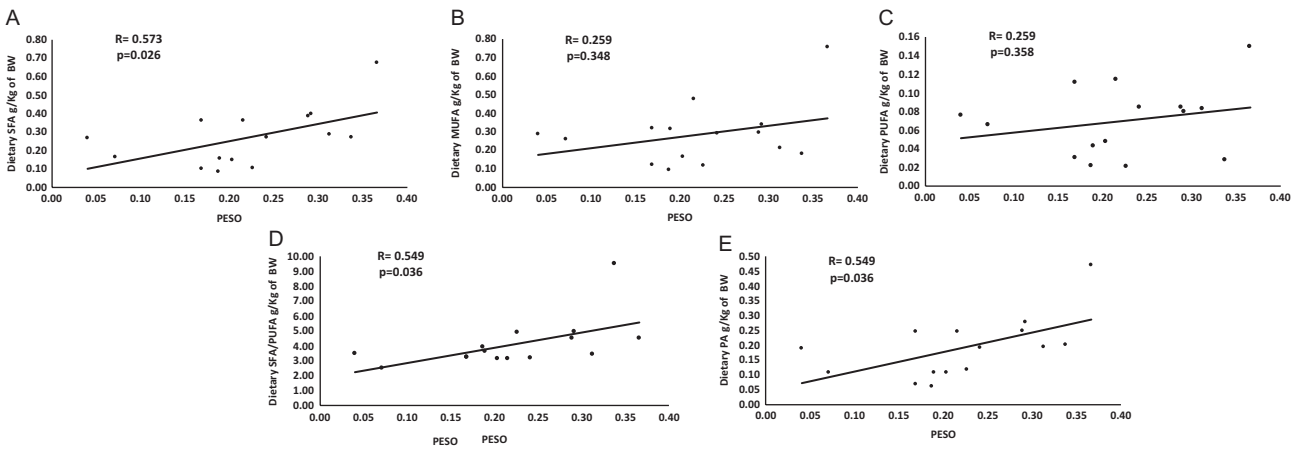

Figure 1
Spearman's regression analysis between PESO and intake of different lipid classes in participants: (A) with saturated fatty acids, SFA; (B) with monounsaturated fatty acids, MUFA; (C) with polyunsaturated fatty acids, PUFA; (D) with SFA/PUFA; (E) with palmitic acid (PA). Statistical significance: $P < 0.05$.

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Statistical significances were indicated as follows: *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Results

Spearman's regression analysis between PESO and the intake of different lipid classes, SFA, MUFA, and PUFA (Fig. 1), showed a positive correlation between PESO and dietary SFA ($R = 0.573$, $P = 0.026$), PESO and dietary PA ($R = 0.549$, $P = 0.036$). Quite interesting was the correlation between PESO and the dietary SFA/PUFA ratio ($R = 0.549$, $P = 0.036$).

However, no significant correlation was found between dietary intake of total fat or any of the macronutrients and PESO (Fig. 2).

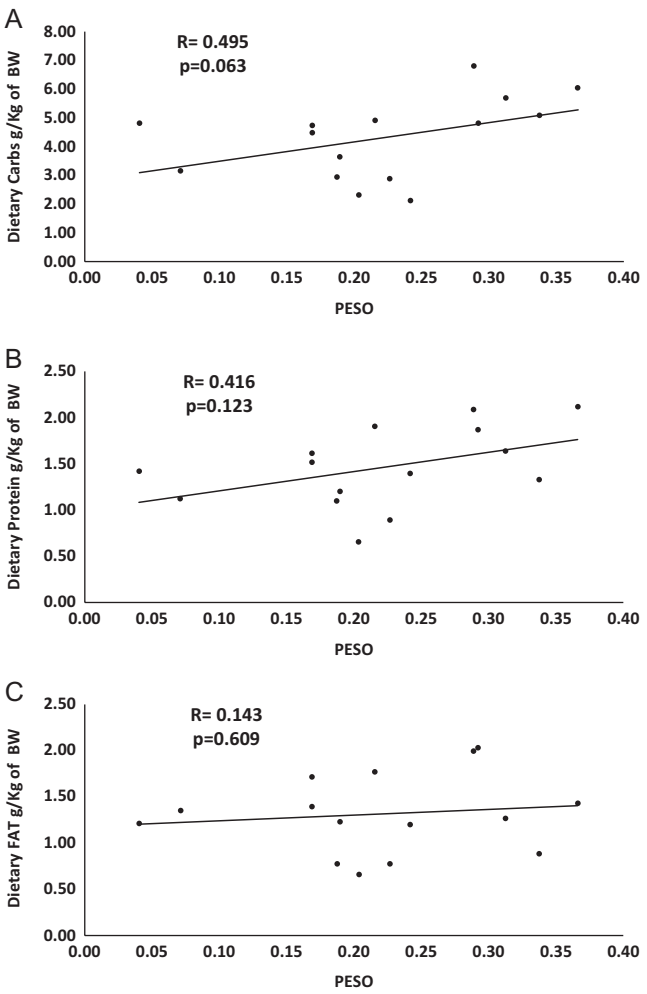

Figure 2
Spearman's regression analysis between PESO and intake of different macronutrients in participants: (A) with carbohydrate; (B) with protein; (C) with lipids. Statistical significance: $P < 0.05$.

We therefore evaluated whether dietary fat classes, SFA, MUFA, and PUFA, were correlated to body fat % (Fig. 3). Data showed an inverse correlation with dietary SFA ($R = -0.566$, $P = 0.030$), PA and MUFA ($R = -0.601$, $P = 0.017$) but not with PUFA ($R = -0.340$, $P = 0.214$).

Figure 3E clearly shows an inverse correlation between PESO and % of body fat.

On the other hand, we did not find any significant correlation between MFI and dietary fat classes or PA intake (data not shown).

Discussion

Metabolic flexibility is defined as the organism's ability to adeptly adjust to variations in metabolic or energy requirements based on prevailing conditions or activities (Olson *et al.* 2016). This flexibility can also be conceptualized as the capacity to alternate between energy substrates – FA and carbohydrates – contingent on metabolic conditions, training status, and nutrient presence. The primary impetus behind this substrate alternation is the transition between catabolic and anabolic processes, facilitating efficient energy storage in skeletal muscle, adipose tissue, and hepatic tissue.

Our initial findings indicate that dietary SFA within a physiological threshold correlate with a superior ability for substrate oxidation, which in turn implies enhanced MF in middle-aged individuals. Among dietary SFA, PA appears to be the principal contributor to this positive correlation. MF represents the capacity of organisms to discern, preserve, and utilize energy substrates based on availability and requirement (Kelley 2005). Consequently, by enhancing optimal fat deposition, MF might elucidate its association with body fat percentage. Indeed, it provides not only a window into efficient energy substrate use but also reflects the proficiency in their storage. This ensures metabolic equilibrium post demanding activities, fostering synergistic interactions between adipose and muscular tissues, mitigating dynapenia (muscular atrophy), and promoting optimal body composition.

Remarkably, both SFA and MUFA displayed an inverse relationship with body fat percentage. One could postulate a pivotal role of certain metabolites derived from these FA, specifically palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), in bolstering MF. The enhancement in MF could stem from the activation of particular receptors such as PPAR-α, amplifying FA beta-oxidation and, subsequently, more efficient glucose utilization. This might elucidate the observed

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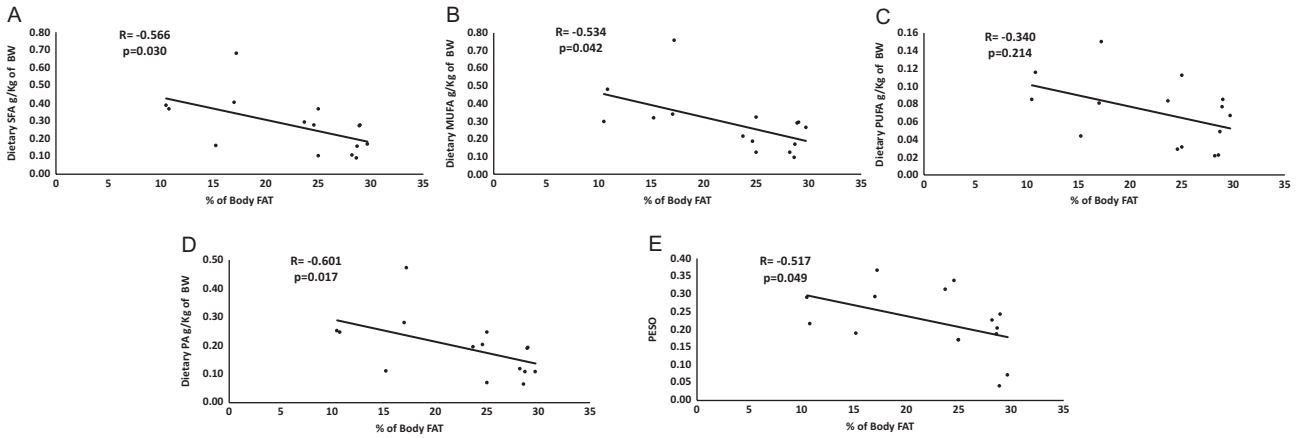

Figure 3
Spearman's regression analysis between % of body fat and intake of different lipid classes in participants: (A) with saturated fatty acids, SFA; (B) with monounsaturated fatty acids, MUFA; (C) with polyunsaturated fatty acids, PUFA; (D) with palmitic acid (PA); (E) with PESO. Statistical significance: $P < 0.05$.

inverse correlation between PESO and body mass percentage. Notably, our recent observations indicated that augmented dietary PA led to a rise in PEA and OEA levels in the muscular tissues of obese rodents, which corresponded with enhanced MF (Carta *et al.* 2023).

Contrary to expectations, PUFA did not demonstrate a correlation with either MF or body fat percentage. Future research endeavors should prioritize assessing, via intervention studies, if altering the PUFA omega-6-to-omega-3 ratio might influence this trend favorably toward MF. In previous studies, we established that krill oil intake, abundant in PUFA omega-3 eicosapentaenoic acid (20:5n3, EPA) and docosahexaenoic acid (22:6n3, DHA) in phospholipid form, can ameliorate glucose and lipid metabolism by suppressing EC biosynthesis in both animals and humans (Batetta *et al.* 2009, Banni *et al.* 2011, Murru *et al.* 2013).

Conclusion

In summary, our preliminary findings indicate a positive correlation between PA, MF, and body fat percentage, underscoring the potential nutritional importance of PA in promoting MF.

However, it is crucial to emphasize that such correlations do not necessarily establish causation. Future studies should explore the potential of modulating MF via dietary PA in a physiological context in humans as also potential determinant of a balanced intake of different classes of dietary lipid. Further investigations should also address its implications for body composition, particularly in individuals aged 45–65, when the incidence of most noncommunicable chronic diseases intensifies.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this commentary.

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Author contribution statement
All authors contributed equally to this work.

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References

Astrup A, Magkos F, Bier DM, Brenna JT, De Oliveira Otto MC, Hill JO, King JC, Mente A, Ordovas JM, Volek JS, *et al.* 2020 Saturated fats and health: a reassessment and proposal for food-based recommendations. *Journal of the American College of Cardiology* **76** 844–857. (https://doi.org/10.1016/j.jacc.2020.05.077)

Banni S & Di Marzo V 2010 Effect of dietary fat on endocannabinoids and related mediators: consequences on energy homeostasis, inflammation and mood. *Molecular Nutrition and Food Research* **54** 82–92. (https://doi.org/10.1002/mnfr.200900516)

Banni S, Carta G, Murru E, Cordeddu L, Giordano E, Sirigu AR, Berge K, Vik H, Maki KC, Di Marzo V, *et al.* 2011 Krill oil significantly decreases 2-arachidonoylglycerol plasma levels in obese subjects. *Nutrition and Metabolism* **8** 7. (https://doi.org/10.1186/1743-7075-8-7)

Batetta B, Griinari M, Carta G, Murru E, Ligresti A, Cordeddu L, Giordano E, Sanna F, Bisogno T, Uda S, *et al.* 2009 Endocannabinoids May Mediate the Ability of (n-3) Fatty Acids to Reduce ectopic Fat and inflammatory mediators in Obese Zucker Rats. *Journal of Nutrition* **139** 1495–1501. (https://doi.org/10.3945/jn.109.104844)

Bray GA & Popkin BM 1998 Dietary fat intake does affect obesity! *American Journal of Clinical Nutrition* **68** 1157–1173. (https://doi.org/10.1093/ajcn/68.6.1157)

Carta G, Murru E, Trinchese G, Cavaliere G, Manca C, Mollica MP & Banni S 2023 Reducing dietary polyunsaturated to saturated fatty acids ratio improves lipid and glucose metabolism in obese Zucker rats. *Nutrients* **15** 4761. (https://doi.org/10.3390/nu15224761)

Frayn KN 1983 Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology* **55** 628–634. (https://doi.org/10.1152/jappl.1983.55.2.628)

Heini AF & Weinsier RL 1997 Divergent trends in obesity and fat intake patterns: the American paradox. *American Journal of Medicine* **102** 259–264. (https://doi.org/10.1016/S0002-9343(96)00456-1)

Hooper L, Abdelhamid AS, Jimoh OF, Bunn D & Skeaff CM 2020 Effects of total fat intake on body fatness in adults. *Cochrane Database of Systematic Reviews* **6** CD013636. (https://doi.org/10.1002/14651858.CD013636)

Howley ET, Bassett DR & Welch HG 1995 Criteria for maximal oxygen uptake: review and commentary. *Medicine and Science in Sports and Exercise* **27** 1292–1301. (https://doi.org/10.1249/00005768-199509000-00009)

Kelley DE 2005 Skeletal muscle fat oxidation: timing and flexibility are everything. *Journal of Clinical Investigation* **115** 1699–1702. (https://doi.org/10.1172/JCI25758)

Lee JH, Duster M, Roberts T & Devinsky O 2022 United States dietary trends since 1800: lack of association between saturated fatty acid consumption and non-communicable diseases. *Frontiers in Nutrition* **8** 748847. (https://doi.org/10.3389/fnut.2021.748847)

Ludwig DS, Hu FB, Lichtenstein AH & Willett WC 2023 Low-fat diet Redux at WHO. *American Journal of Clinical Nutrition* **118** 849–851. (https://doi.org/10.1016/j.ajcnut.2023.09.006)

Murru E, Banni S & Carta G 2013 Nutritional properties of dietary Omega-3-enriched phospholipids. *BioMed Research International* **2013** 965417. (https://doi.org/10.1155/2013/965417)

Olson KA, Schell JC & Rutter J 2016 Pyruvate and metabolic flexibility: illuminating a path toward selective cancer therapies. *Trends in Biochemical Sciences* **41** 219–230. (https://doi.org/10.1016/j.tibs.2016.01.002)

Reeves PG 1997 Components of the AIN-93 diets as improvements in the AIN-76A diet. *Journal of Nutrition* **127**(Supplement) 838S–841S. (https://doi.org/10.1093/jn/127.5.838S)

Tsuboi K, Uyama T, Okamoto Y & Ueda N 2018 Endocannabinoids and related N-acylethanolamines: biological activities and metabolism. *Inflammation and Regeneration* **38** 28. (https://doi.org/10.1186/s41232-018-0086-5)

World Health Organization 2023 *Total Fat Intake for the Prevention of Unhealthy Weight Gain in Adults and Children: WHO guideline*. World Health Organization, Department of Nutrition and Food Safety, Geneva, Switzerland.

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