STATEMENT

Statement on additional scientific evidence in relation to the essential composition of total diet replacement for weight control

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to revise the Opinion on the essential composition of total diet replacements for weight control (TDRs) regarding the minimum content of linoleic acid (LA) and alpha-linolenic acid (ALA) and the maximum content of magnesium (Mg). Through a comprehensive literature search, human studies were retrieved reporting on LA and ALA concentrations in adipose tissue (AT), on weight loss and gallstone formation following TDR consumption and on diarrhoea after supplemental Mg intake. The distribution of the amount of LA and ALA release from AT during weight loss when consuming TDRs was estimated using statistical simulations. Using the fifth percentile, the coverage of the adequate intake (AI) for both FA was estimated. For the risk of developing diarrhoea when consuming TDRs with an Mg content of 350 mg/day, four cross-over studies using 360–368 mg Mg/day were reviewed. The Panel concludes that (1) there is no need to add LA to TDRs, as the amount released from AT during weight loss when consuming TDRs is sufficient to cover the AI for LA; (2) a minimum of 0.8 g/day ALA is needed in TDRs in order to meet the AI for ALA; (3) the minimum fat content of TDRs of 20 g/day as derived in the Panel's previous opinion is proposed to be maintained until the availability of further evidence, given the considerable uncertainty as to the amount of fat required for reducing the risk of gallstone formation; and (4) the likelihood that Mg-induced diarrhoea occurs at a severity that may be considered of concern for overweight and obese individuals consuming TDRs is low when the total maximum Mg content in TDRs is 350 mg/day.

Requestor: European Commission

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Commission delegated Regulation (EU) 2017/1798 repeals Directive 96/8/EC and revises the rules applicable to total diet replacement for weight control (TDR), taking into account the scientific opinion of the European Food Safety Authority (EFSA) of 2015.

EFSA's Scientific Opinion on the essential composition of TDR includes a recommendation on the minimum linoleic acid (LA) and alpha-linolenic acid (ALA) content of the products. It states that TDR should provide at least 11 g LA and 1.4 g ALA per day. As explained in the opinion, the values were proposed on the basis of the Adequate Intake for linoleic acid and alpha-linolenic acid established for energy-adequate diets. It is further noted that '*this recommendation is based on the consideration that the fatty acid content of adipose tissue and the rate of adipose tissue loss may vary between individuals; thus, there is considerable uncertainty as to whether body stores can completely cover requirements*'.

The opinion also includes a recommendation on the magnesium content of TDR, proposing a range of 150–250 mg/day of magnesium in these products. As regards the maximum magnesium content threshold, the EFSA Panel noted that '*as most of the magnesium in total diet replacements for weight control would be easily dissociable magnesium salts, the UL of 250 mg/day applies*'.

On 7 November 2019, Total Diet & Meal Replacements Europe (TDMR) sent a letter to the Commission requesting the revision of the Commission delegated Regulation (EU) 2017/1798[1], in particular the abovementioned requirements laid down therein. In its letter, TDMR referred to six scientific publications,[2,3,4,5,6,7] which, according to TDMR, provide sufficient evidence for a review of the minimum values for LA and ALA and for a review of the maximum level of magnesium in TDR.

1.1.2. Terms of Reference as provided by the mandate requestor

In accordance with Article 29 of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to assess the scientific evidence provided by TDMR together with any other relevant new scientific evidence available and, if necessary, to update the conclusions of the former Scientific Opinion on the essential composition of TDR as regards the minimum values for LA and ALA as well as the maximum level of magnesium in TDR.

1.2. Interpretation of the Terms of Reference

The NDA Panel understands that it is expected to provide advice on:

A) the amount of LA and ALA that is released from adipose tissue (AT) of overweight or obese adults[8] during weight loss;

B) to what extent the minimum content of LA and ALA in TDRs proposed by the Panel (EFSA NDA Panel, 2015b) could be reduced based on the outcome of the review of the scientific evidence related to point (A);

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[1] Commission Delegated Regulation (EU) 2017/1798 of 2 June 2017 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for total diet replacement for weight control.

[2] Bork CS, Venø SK and Lundbye-Christensen S, 2018. Adipose tissue content of alpha-linolenic acid and the risk of ischemic stroke and ischemic stroke subtypes: a Danish case-cohort study. PloSOne, 13, e0198927. 10.1371/journal.pone.0198927

[3] Christensen P, Bliddal H and Riecke BF, 2011. Comparison of a low-energy diet and a very low energy diet in sedentary obese individuals: a pragmatic randomised controlled trial. Clinical Obesity, 1, 31–40.

[4] Christensen P, Meinert Larsen T and Westerterp-Plantenga M, 2018. Men and women respond differently to rapid weight loss: metabolic outcomes of a multi-centre intervention study after a low energy diet in 2500 overweight, individuals with pre-diabetes (PREVIEW). Diabetes, Obesity & Metabolism, 20, 2840–2851.

[5] Lean ME, Leslie WS and Barnes AC, 2017. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. Lancet, 391, 541–51.

[6] Lefebvre P, Letois F and Sultan A, 2014. Nutrient deficiencies in patients with obesity considering bariatric surgery: a cross-sectional study. Surg Obes Relat Dis, 10, 540–546. https://doi.org/10.1016/j.soard.2013.10.003. Epub 2013 Oct 14.

[7] Venø SK, Bork CS and Jakobsen MU, 2018. Linoleic acid in adipose tissue and development of ischemic stroke: a Danish Case-Cohort Study. Journal of American Heart Association, 7, pii: e009820. https://doi.org/10.1161/JAHA.118.009820

[8] BMI $\geq$ 25 kg/m^2.

C) to what extent the minimum fat content in TDRs proposed by the Panel (EFSA NDA Panel, 2015b), that was derived from the proposed minimum content of LA and ALA in TDRs, is to be revised together with the minimum energy content that was based on the sum of the energy provided by macronutrients;

D) whether a maximum magnesium (Mg) content in TDRs of 350 mg/day, as proposed by TDMR, would give rise to concerns with respect to an increased risk of diarrhoea. In this context, the Panel is not expected to revise the tolerable upper intake level (UL) of the Scientific Committee on Food (SCF) (2001) for Mg salts.

1.3. Context of the assessment

TDRs are 'food specially formulated for use in energy restricted diets for weight reduction which, when used as instructed by the food business operator, replaces the whole daily diet'.[9] The compositional and information requirements for TDRs are laid down in Commission delegated Regulation (EU) 2017/1798[1]. This delegated Regulation is supplementing Regulation (EU) No 609/2013[8], which repealed Commission Directive 96/8/EC[10]. Commission delegated Regulation (EU) 2017/1798 shall apply from 27 October 2022. The compositional criteria laid down in this Regulation were informed by an Opinion of the NDA Panel on the essential composition of TDRs published in 2015 (EFSA NDA Panel, 2015b).

TDMR, the European trade body for manufacturers and distributors of formula diet products, including TDRs, provided the European Commission with additional scientific evidence related to the content of the fatty acids (FAs), LA and ALA, and the maximum content of Mg in TDRs that the NDA Panel is expected to assess together with any other relevant new scientific evidence available.

Briefly, in its previous Opinion (EFSA NDA Panel, 2015b), based on a narrative review of the evidence, the Panel proposed that TDRs should provide at least 11 g LA (C 18:2, n-6) per day and 1.4 g ALA (C 18:3, n-3) per day. This was based on the adequate intake (AI) for LA and ALA for healthy individuals (i.e. 4% of energy intake (E%) and 0.5 E%, respectively (EFSA NDA Panel, 2010)), considering a 40-year-old male reference subject with a physical activity level of 1.6 (moderate activity) and an average requirement (AR) for energy of 10.7 MJ/day (2,500 kcal/day) (EFSA NDA Panel, 2013). It was also based on the '*consideration that the FA concentration of AT and the rate of AT loss may vary between individuals; thus, there is considerable uncertainty as to whether body stores can completely cover requirements*'. This statement was based on a review of the FA concentration in AT by Seidelin (1995). The author had noted that, in particular, the proportion of FAs in the diet influences the proportion of FAs in AT and the proportion of LA in AT varied between different countries/ ethnicities. The influence of age, sex, sampling site, body weight (for which the author considered that there was no relationship with FA composition of AT), hypertension, smoking, diabetes mellitus, serum lipids, atherosclerosis, coronary artery disease and a number of other disorders/diseases was also discussed in this review.

Regarding fat content of TDRs, the Panel noted that '*it has been suggested that the increased risk of gallstone formation during rapid weight loss could be partly explained by a low fat content of the diet, in particular in [very-low calorie diets] VLCDs, and that a certain minimum amount of fat is needed in a diet to prevent or reduce the incidence of gallstone formation*'. After reviewing the evidence, the Panel concluded that '*the incidence of gallstones is reduced with increasing fat and energy content of the diet. However, the available evidence does not allow a precise cut-off value to be defined above which the risk for gallstone formation would be reduced*'. Therefore, the minimum fat content in TDRs (i.e. 20 g/day) was proposed to be based on the amount of oils needed to supply LA and ALA in the necessary above-mentioned amounts, considering that these oils have a maximum LA and ALA content of 55–75% of total FA (FA%).

Finally, the minimum energy content in TDRs was derived from the minimum macronutrient content of such diets.

With respect to Mg, the Panel proposed that TDRs should provide a maximum of 250 mg Mg per day. This was based on the UL for readily dissociable Mg salts (but not Mg normally present in foods and beverages), derived by SCF (2001) from data on mild diarrhoea. After the publication of the

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[9] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009.

[10] Commission Directive 96/8/EC of 26 February 1996 on foods intended for use in energy-restricted diets for weight reduction.

Opinion of the Panel on the essential composition of TDRs (EFSA NDA Panel, 2015b), AIs for Mg have been set for various population groups, including 350 mg/day for men and 300 mg/day for women (EFSA NDA Panel, 2015a). In this context, TDMR proposed to align the maximum Mg content in TDRs with the new AIs for Mg.

2. Data and methodologies

2.1. Data

For this scientific assessment, a protocol has been developed in line with existing methodology (EFSA, 2020) (see Appendix D).

This Statement is based on the data provided by TDMR as well as on data retrieved through comprehensive literature searches in Embase and PubMed performed by EFSA in June 2020 (08 June: Q1 on FA concentration of AT and release from AT (see Section 2.1.1), 02 June: Q2 on weight loss when consuming TDRs (see Section 2.1.2), 22 June: Q3 on Mg intake and risk of diarrhoea (see Section 2.1.3) and on 09 December 2020 (Q4 on fat content of energy-restricted diets and risk of gallstone formation (see Section 2.1.4)). No limitations on publication date were applied, except for the search for Q4 that included only studies published from 2014 onwards, i.e. studies not yet considered by EFSA NDA Panel (2015b). The searches were restricted to publications in English.

The title and abstract screening was carried out in duplicate in Distiller SR® (Evidence Partners, Ottawa, Canada), while the full texts were screened by a single reviewer (protocol amendment 1). Data were extracted in tabular format in Microsoft Excel® by one EFSA staff member and validated by another. The data extraction forms were further customised following the study selection to the data needs and available evidence. As no references were retrieved for the search on fat content of energy-restricted diets and risk of gallstone formation, the prespecified extraction list was not applied. Authors were contacted to clarify questionable values reported in two publications (see Section 3.1.1).

The following sections shortly summarise the evidence retrieval divided according to the four main literature searches.

The PRISMA flow charts of the searches are included as Appendix A.

The search strings and the eligibility criteria in the protocol are reported as Appendices D and E.

2.1.1. Linoleic acid and alpha-linolenic acid concentration and their release from adipose tissue during weight loss

Two searches were conducted to retrieve evidence 1) for the LA and ALA concentration of AT and 2) for the release of these FA from AT during weight loss. Owing to the high overlap between publications of the two searches, the data retrieved were combined and screened together (Q1).

Publications retrieved were human intervention or observational studies conducted in populations living in Europe investigating LA and ALA concentration in healthy normal weight adults or overweight or obese adults[8] either without apparent co-morbidity or with the following diseases that are frequently associated with obesity: type 2 diabetes mellitus (T2DM), hypertension, hyperlipidaemia. For the question on the release of FA from AT, studies were considered if they reported LA and/or ALA concentration of AT before and after weight loss or investigated release of these FA from AT using microdialysis of AT or tracer methods.

For the question on the release of these FA from AT during weight loss, hand search in the pertinent references was also performed from which relevant studies conducted in the US were taken into account (protocol amendment 2), given the limited references found in humans in Europe.

2.1.2. Range of weight loss when consuming total diet replacements for weight control

Publications retrieved were human intervention studies (including single arm trials) investigating the achieved range of weight loss following the consumption of TDRs alone (without consumption of other foodstuffs, e.g. vegetables, even if in limited amounts) without co-intervention (e.g. counselling, group sessions, physical activity programmes) within the permitted energy range of 2,510–5,020 kJ (600–1,200 kcal) per day, as laid down in Commission delegated Regulation (EU) 2017/1798. Study populations eligible were overweight or obese adults without apparent co-morbidity or those with T2DM, hypertension or hyperlipidaemia, for which weight was measured by investigators (and reported for any time point up to 8 weeks (i.e. the maximum duration of use of TDRs without medical

supervision)). Further, eligible studies were those reporting on compliance (both on how it was assessed (in order to judge reliability) and how many individuals were compliant).

2.1.3. Supplemental magnesium and risk of developing diarrhoea

Publications retrieved were human intervention studies reporting on the development of diarrhoea or on adverse effects following administration of two different interventions (i.e. placebo vs. Mg; or Mg vs. Mg at different doses) of readily dissociable Mg salts consumed by participants.

The initial eligibility considerations were to include only studies that reported the criteria used for the diagnosis of diarrhoea and applying the World Health Organisation (WHO) criteria (i.e. stool frequency of ≥ 3 loose or watery stools per day[11]). As this type of data was not retrievable, cross-over studies were considered to decrease uncertainty of the outcome assessment (i.e. diarrhoea) as subjects act as their own controls (protocol amendment 3). This is indicated as an additional full-text screening (step 2) in PRISMA flow chart for Q3 (Appendix A).

2.1.4. Fat content of energy-restricted diets and risk of gallstone formation

The Panel searched for publications on human intervention studies comparing the effect of different levels of dietary fat in energy-restricted diets on the risk of gallstone formation and of biliary sludge formation, as well as on gallbladder emptying. This search was considered relevant only in case of need for a reassessment of the minimum fat content in TDRs that was derived from the proposed minimum content of LA and ALA in TDRs.

2.2. Methodologies

As outlined in Section 1.1.2, the current scientific assessment was carried out to assess the additional available evidence related to the essential composition of TDRs. The methodology for proposing such an essential composition has been outlined in the previous Opinion of the EFSA NDA Panel (2015b): *'The guiding principle for providing advice on the essential composition of total diet replacements for weight control should be that products are safe and suitable when consumed as a sole source of nutrition, for several weeks to months, by overweight or obese adults in the context of energy-restricted diets for weight reduction. In order to avoid nutrient deficiencies, these products should, therefore, provide at least the PRI or AI for adults of all indispensable nutrients. As Dietary Reference Values (DRVs) are generally established for healthy normal-weight individuals, specific consideration will be given by the Panel in this opinion to whether there is an increase in nutrient requirements in the overweight or obese or whether (rapid) weight loss leads to an increased nutrient loss and thus a higher requirement. When advising on the macronutrient composition of total diet replacements, the Panel will also consider the extent to which different diet compositions impact on losses in fat free mass (FFM), and on other adverse effects of weight loss diets, such as gallstone formation. The effectiveness of a product in terms of extent of weight loss is not in itself an appropriate determinant for the necessary composition of total diet replacements for weight control.'*

2.3. Protocol amendments

1) The full-text screening was carried out by a single reviewer in Distiller SR® (Evidence Partners, Ottawa, Canada) (see Section 2.1).
2) Studies conducted in the United States (US) were included that investigated the release of LA and ALA from AT during weight loss (Q1) (Section 3.1.2).
3) For the evidence retrieval on Mg intake and risk of diarrhoea (Q3), only cross-over studies were included as no studies were identified reporting on the criteria for diagnosis of diarrhoea and applying the WHO criteria (Section 3.3.2).
4) Studies conducted in non-overweight/non-obese subjects with mild hypertension and not on medication were included as eligible evidence on Mg intake and risk of diarrhoea (Q3) (Section 3.3.2).
5) Observational and interventional studies either single-arm or with only one arm consuming a TDR without co-intervention were considered as supportive evidence in relation to gallstone formation, biliary sludge formation and/or gallbladder emptying (Q4) (Section 3.2).

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[11] https://www.who.int/en/news-room/fact-sheets/detail/diarrhoeal-disease

3. Assessment

3.1. Minimum content of linoleic acid and alpha-linolenic acid in total diet replacements for weight control

3.1.1. Linoleic acid and alpha-linolenic acid composition of adipose tissue

In the literature search that formed the basis of the present assessment, the Panel identified 82 studies that reported on the LA or ALA composition of AT in normal-weight, overweight or obese adults living in Europe, either without apparent co-morbidity or overweight or obese with T2DM, hypertension or hyperlipidaemia.

The two papers provided by TDMR to the European Commission on the FA concentrations in AT (Bork et al., 2018; Venø et al., 2018) reported on quantiles of LA and ALA concentrations in AT in the same cohort study (i.e. Danish Diet, Cancer and Health cohort). They did not provide summary data, i.e. means and measures of spread that allowed them to be combined with the other evidence that was retrieved through the search. Data from this cohort were included in the assessment through consideration of the publication by Dahm et al. (2011).

Two studies that originally met the inclusion criteria (Heemskerk et al., 2015; Sikorska-Wisniewska et al., 2020) were excluded from further review, as they reported implausibly low ALA concentrations in AT that could also not be explained by the authors upon contacting them.

Hence, a total of 80 studies were considered.

AT samples in the studies were obtained either via needle biopsy, a biopsy during surgery or during autopsy. Studies were available from 20 European countries. They were heterogeneous in terms of population (specific subgroup of a population or random samples from the general populations), weight status of individuals, sample size, sampling site of the body, method of FA extraction, method of FA derivatisation to FA methyl esters[12] and unit in which the FA concentration was reported (e.g. mol% of total FA, w% of total FA, w% of AT).

In the protocol, the Panel had predefined certain parameters that were intended to be investigated with respect to their potential influence on the amount of LA and ALA stored in AT. This included weight status, lifestyle factors (such as diet or physical activity), gender, age, ethnicity, AT sampling site, disease status (related to the diseases listed above) or medication use (related to the diseases indicated above).

Data analysis across the included studies: scatter plots for the predefined list of influencing factors

The overall data set was evaluated visually in the form of scatter plots, for each of the two FA LA and ALA, for the following potentially influencing factors for which enough data were available to create informative plots:

- gender (Appendices C.1 and C.10),
- weight status (i.e. overweight and obese, overweight, obese, normal weight, mixed, not reported; Appendices C.4, C.5, C.13, C.14),
- and sampling site (i.e. visceral (VC) or superficial subcutaneous (SC) AT from various sites; Appendices C.8 and C.17).

However, these graphical presentations did not reveal a pattern suggesting an association between the factors investigated and the reported LA and ALA concentrations in AT.

For other factors, such an evaluation was not feasible because (1) the data set did not allow categories to be created, e.g. for age owing to the broad age ranges of participants in the individual studies, or (2) too few studies were available that investigated the particular factor, i.e. ethnicity, diet and physical activity.

Comparisons, reported in individual publications, for the predefined list of influencing factors

In addition, individual papers that made direct comparisons between different potentially influencing factors in the same population were also considered and are described below.

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[12] i.e. the generation of volatile fatty acid methyl ester derivatives before fatty acid analysis by gas chromatography.

The majority of studies (six out of eight) that investigated site-specific difference in LA and/or ALA concentrations in AT did not observe such differences, both with respect to SC AT samples taken from different body areas (sample sizes ranging from 7 to 76 individuals), and when comparing SC and VC fat (Calder et al., 1992; Garaulet et al., 2001; Kotronen et al., 2010; Hernandez-Morante et al., 2011; Pinnick et al., 2012; Petrus et al., 2017). Only two studies reported site-specific differences in LA concentrations. Schäfer and Overvad (1990) found statistically significantly lower LA concentrations in abdominal SC AT than in gluteal SC AT (n = 6; mean difference 0.5 percentage points). Sledzinski et al. (2013) found lower LA concentrations in abdominal SC AT than in VC AT (n = 16; mean difference 2.3 percentage points).

Around half of the studies (7 out of 13) that investigated gender-specific differences in LA and ALA composition of AT did not report an association between gender and SC or VC AT composition with respect to these two FA (sample sizes ranged from 10 to 556 individuals per group) (Ruiz-Gutierrez et al., 1992; Tjonneland et al., 1993; Popp-Snijders and Blonk, 1995; Garaulet et al., 2001; Mamalakis et al., 2006, 2008; Dahm et al., 2011). The studies in which differences were observed reported mainly higher LA concentrations in women compared with men (sample sizes ranged from 14 to 2,308 per group) (Bolton-Smith et al., 1997; Mamalakis et al., 1998, 2002b; Petrova et al., 2011; Pinnick et al., 2012; Zatońska et al., 2012). Zatońska et al. (2012) reported the gender difference only in the rural, but not in the urban population. Except for Pinnick et al. (2012) who did not investigate ALA and for Zatońska et al. (2012) who found, in an unadjusted analysis, higher ALA concentrations in SC AT in rural women compared with rural men, ALA concentrations in SC AT were not different between men and women in the studies mentioned above.

With respect to the influence of the weight status (two studies), Garaulet et al. (2011) did not find significant differences in LA concentration of abdominal SC or VC AT between overweight (n = 20, body mass index (BMI) 25–29.9 kg/m^2), obese (n = 20, 30–39.9 kg/m^2) and morbidly obese (n = 20, $\geq$ 40 kg/m^2) individuals. Equally, Scazzocchio et al. (2020) found similar concentrations of LA in VC AT in 17 normal weight and 19 obese individuals.

With respect to the influence of age (three studies), Bolton-Smith et al. (1997) showed a significant inverse relationship between age and LA SC AT concentration of the outer upper arm in both men (n = 2,308) and women (n = 2,049), recruited from the general population. This was observed in a linear regression analysis after adjusting for height, weight, smoking, dietary polyunsaturated FAs, saturated FAs and monounsaturated FAs and total energy, plus menopausal status for women. Schäfer et al. (1989) found no significant difference in LA and ALA gluteal SC AT between women > 64 years (n = 28) and women < 36 years (n = 33) of age. Hodson et al. (2014) showed higher LA concentrations in gluteal SC AT in post-menopausal women (n = 23) than in premenopausal women (n = 19) (mean difference: 1.3 percentage points).

In the only study that looked at different ethnicities living in Europe, Cruz et al. (2001) did not find significant differences in LA and ALA abdominal SC AT concentrations of individuals from Latin America (n = 8), South Asia (n = 8) and Northern Europe (n = 9) who had been living in the UK > 1 year.

With respect to the influence of diet (two studies), Garaulet et al. (2001) observed a significant correlation between the dietary intake of LA and its concentration in omental AT and between dietary intake of ALA and its concentration in abdominal SC, perivisceral and omental AT in 84 obese individuals. Sanders et al. (1978) found significantly higher LA concentrations in SC AT of the triceps in 12 vegans compared with 12 omnivores (mean difference: 14.4 percentage points). Even though ALA concentrations were also somewhat higher (mean difference: 0.9 percentage points), this did not reach statistical significance.

In the only study that investigated the influence of physical activity/training, Danner et al. (1984) reported that LA concentrations in AT (site not reported) increased by around 2 percentage points in 15 rowers after 210-day intense training that followed a 4-month holiday without training (i.e. from mean (SD) 14.8 (2.4) to 16.9 (2.2) FA%). The increase was, however, not statistically significant. In control subjects without physical activity, no increase of the LA concentration in AT during this period was observed. The E% provided by polyunsaturated FA did not change, neither in the rowers nor in the control subjects, during the study. This was assessed by a 3-day dietary history.

There were no data to evaluate the influence of medication use.

Overall, the Panel considers that there is no evidence that the data set would need to be stratified for the factors listed above for the subsequent evaluation of data. The only exception was for age, for which stratification may have been relevant. However, in the absence of access to the individual data, only the age categories reported in the papers were available, which were too broad to create a meaningful stratification.

Data analysis across the included studies: scatter plots for other potential influencing factors

Additional potential influencing factors or parameters, not present in the predefined list, were further investigated visually in scatter plots, with regard to either LA or ALA concentration in AT. These factors were the combination of gender and country (Appendices C.2 and C.11) or gender and year of study conduct (Appendices C.3 and C.12), weight status and year of study conduct (Appendices C.6 and C.15), health status (i.e. patients, healthy subjects, general population) (Appendices C.7 and C.16) and unit of measurement of the FA concentration in AT (Appendices C.9 and C.18).

In order to attribute a year of study conduct to a study reported in a publication, the year in which the last AT sample was obtained was taken as the year of study conduct. If this information was not reported, the year was approximated based on the publication year minus two. The inspection of the scatter plots revealed that studies conducted before around 1990 showed generally higher ALA AT concentrations and lower LA AT concentrations than the ones conducted thereafter. The Panel assumed that this might be due to more refined analytical methods of FA analysis that have become available in more recent years. Therefore, the Panel decided to use in the following modelling exercise (described further down) only studies conducted following and including the year 1990 (as classified using the above-described criteria) in order to ensure comparability of results. Also, only results from a single sampling site per individual were included. This concerned the studies by Garaulet et al. (2001), Kotronen et al. (2010), Hernandez-Morante et al. (2011), Pinnick et al. (2012), Petrus et al. (2017) and Sledzinski et al. (2013), for which results from abdominal SC AT (arbitrary choice) were included. For the study by Calder et al. (1992), the averaged FA composition over 14–15 sampling sites that was reported in the article was used.

The Panel also decided to include studies irrespective of the unit in which the FA concentration was provided (i.e. w% of AT, w% of total fat, w% of FA, mol% of total AT, w% of FA in triglycerides, w% of FA methyl esters or unclear). The impact of the diversity in units of measurement on the overall conclusion was considered negligible, having in mind the overall heterogeneity of the included data and the fact that the scatter plots did not reveal an association between the unit of measurements used and the reported values of the FA concentration of AT.

The selection of studies conducted in 1990 and thereafter resulted in the inclusion of 54 studies in total. Out of these, 53 studies reported on LA and 42 on ALA.

The list of studies and their results that were finally included in the assessment are given as forest plots in Appendix B.

Data analysis of the finally included data set after visual inspection

The majority of studies reported on mean concentrations of LA and ALA in AT without providing information on the overall distribution of individual data. In three studies conducted in three different European countries (Calder et al., 1992; Leichsenring et al., 1992; Boué et al., 2000), information on the range of LA and ALA concentrations in AT was available. For three other studies (Simonsen et al., 1998; Andersen et al., 1999; Sjögren et al., 2012), including seven European countries, the interquartile range was provided. Out of the studies mentioned above, Leichsenring et al. (1992), Boué et al. (2000) and Sjögren et al. (2012) also reported on the mean concentrations.

Therefore, the Panel decided to use in further calculations the mean concentrations of LA and ALA in AT, as this information was available for most studies. The fifth percentiles (P5) of the empirical distributions of the mean LA and ALA concentrations in AT were, however, only based on data from two (LA) and three (ALA) countries and on 156 and 249 subjects, respectively. Considering the uncertainties around the robustness of the values for P5 derived from a limited number of countries and subjects, the Panel decided to explore the use of statistical modelling or resampling (i.e. bootstrapping) techniques in order to increase the confidence in the values observed in the lower tails of the empirical distributions. The decision to fit the most appropriate probability distribution to the mean values of LA and ALA concentrations in AT reported in the included studies (using the R package 'rriskDistributions') instead of bootstrapping was taken based on a simulation exercise done in another context by EFSA (internal data). This exercise showed that fitting theoretical distributions to empirical data rather than using bootstrapping from empirical data led to results closer to the true values, especially in situations in which the tails of a distribution should be reliably estimated. Even though the most evident distribution for data that are bounded by 0 and 1, such as data on a proportion (e.g. the FA concentration of AT), is the beta distribution, the Panel considered other distributions that were not limited to 1 at the upper bound if they fitted the data but were restricted by 0 at the lower bound.

This was done as the aim of the assessment was to estimate the lower rather than the upper tail of the distribution and to obtain the best fit for the lower tail.

For LA concentrations in AT, the following distributions were identified through 'rriskDistributions' as potentially compatible with the empirical data: beta, gamma, lognormal. For ALA concentrations in AT, the distributions were: beta, gamma, lognormal, Weibull and Gompertz.

The identification of the distribution best fitting the data was based on the Akaike information criterion (AIC; i.e. the lower the criterion, the better the fit). For LA concentrations in AT, the lognormal distribution was identified as the best fitting distribution (AIC = −344.00), followed by the gamma distribution (AIC = −338.34) and the beta distribution (AIC = −335.72). For ALA concentrations in AT, the gamma distribution showed the best fit (AIC = −634.31), the beta distribution resulted in an AIC of −634.29 and the lognormal in an AIC of −634.28. Because the AICs of the gamma, beta and lognormal distributions for ALA concentrations were close to each other, the Panel decided to perform a sensitivity analysis on the impact on the final outcome of using these three distributions (see Section 3.1.4). At the same time, sensitivity analyses were conducted for the three potentially fitting distributions for LA concentrations in AT, even though, from the AIC, there was clearly less support for the gamma and beta distribution than for the lognormal distribution, but they were less than 10 units away from the best AIC. Figure 1 depicts the fit of those distributions that were, in principle, compatible with the empirical data (each circle representing a mean FA concentration reported for a given population in a given study). Figure 2 shows how well the best fitting distribution relates to the empirical data, which is then used further in the assessment (i.e. the lognormal distribution for LA and the gamma distribution for ALA concentrations in AT). The P5 of the mean LA and ALA concentrations in AT derived from these theoretical distributions was 8.5% and 0.36%, respectively (in comparison to the P5 of the empirical data: 9.7% and 0.36%, respectively).

(a) LA   (b) ALA
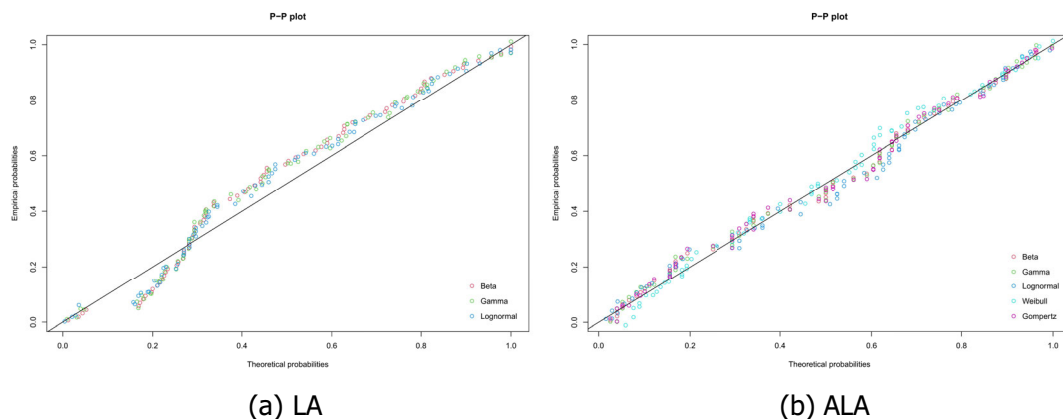
Figure 1: Probability-probability (P-P) plots of different theoretical probability distributions compatible with the empirical distributions of mean (a) linoleic acid (LA) and (b) alpha-linolenic acid (ALA) concentrations in adipose tissue (AT)

(a) LA − lognormal distribution (b) ALA − gamma distribution
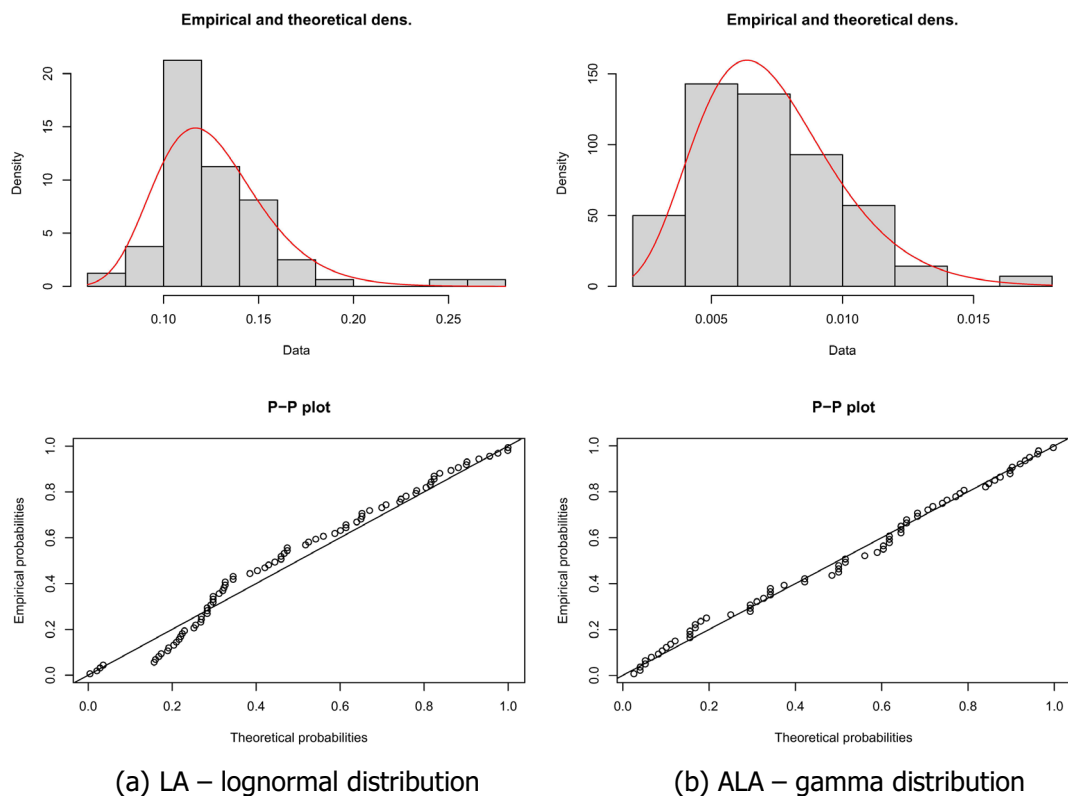
Figure 2: Comparison of empirical distributions of (a) mean LA AT concentrations (given as proportion) with a theoretical lognormal distribution and (b) mean ALA AT concentrations (given as proportion) with a theoretical gamma distribution (density and P-P plots, theoretical distribution represented as a red curve in the density graph)

3.1.2. Release of fatty acids from adipose tissue

Through the literature search, only two pertinent studies were retrieved (Kunešová et al., 2002, 2012) that met the original inclusion criteria (i.e. human studies in healthy volunteers or overweight or obese individuals with T2DM, hypertension or hyperlipidaemia conducted in Europe; see Section 2.1.2 and protocol in Appendix D). In addition, from hand search, the Panel retrieved two studies from the US (Phinney et al., 1990; Tang et al., 1993).

Phinney et al. (1990), in the US, studied FA composition in AT before and after a diet that provided between 1,800 and 2,500 kJ/day (420–600 kcal/day). The study included 12 and 21 obese females (group 1 and 2, respectively) in two different study sites. AT biopsies were taken from the buttock in group 1 and from the lateral thigh in group 2. The diets were continued until 18 or 27 kg, respectively, were lost. In group 2, it took 140 days, while the duration of energy restriction in group 1 was not reported. Two and seven women, respectively, did not finish the intervention. In both groups, women either consumed a formula-based TDR or an energy-restricted diet consisting of self-selected foods. In both groups, a significant reduction in AT ALA concentrations (16 and 17%, respectively) was observed. In group 1, ALA concentrations declined in all subjects and in group 2 in 12 out of 14 participants. Considering individual subject data, no consistent pattern was observed for LA.

Tang et al. (1993), as a follow-up of the study conducted by Phinney et al. (1990), examined the effect of supplementing a TDR with either canola oil providing 1.6 g/day ALA or linseed oil providing 1.1 g/day ALA on the ALA concentration in AT. The calorie contents of the TDRs ranged from 1,882 to 2,927 kJ/day (450–700 kcal/day). Five obese women received a formula-based diet for 4–5 months and lost on average 23.9 kg (group 1). Six women consumed a diet based on self-selected foods for 2–3 months (group 2). Average weight loss in group 2 was 17.4 kg. AT biopsies were taken from the thigh. Weight loss led to a significant reduction of AT ALA concentrations by 20% in group 1 and by 17% in group 2. LA concentrations reduced non-significantly by 1% and 7%, respectively.

Kunešová et al. (2002) investigated FA concentrations on 11 pairs of obese monozygotic twins (i.e. n = 22; age: 23–48 years, BMI: 24.8–41.2 kg/m^2) with a stable body weight in the 6 months before the study. Participants underwent an institutionalised weight reduction programme that consisted in a 1-week run-in period with a eucaloric diet and a 4-week weight reduction programme on a TDR with 1,600 kJ/day (383 kcal/day). During this time, participants were also subjected to light to moderate physical activity. Abdominal AT biopsies were obtained at the end of the run-in period, and at the end of the weight stabilisation period at the institution. During the TDR period, subjects lost on average 8.27 kg of weight. ALA concentrations in AT were reduced by around 11.4% and LA concentrations by around 3.8% following the TDR consumption, but the reductions did not reach statistical significance.

Kunešová et al. (2012) examined the effect on AT composition of FA in subjects participating to the Diet, Obesity and Genes (DIOGENES) study, a multi-centre intervention study in eight European countries. This study recruited families with at least one overweight and obese parent that underwent an 8-week TDR intervention with 3,200 kJ/day (766 kcal/day), supplemented with 400 g/day of vegetables, before randomisation into one of five energy ad libitum diets. Data on abdominal SC AT composition are presented for 195 subjects (out of 773 adults randomised). During the TDR period, subjects lost on average 11 kg of weight. ALA concentrations were statistically significantly reduced by around 13% during this period. No changes were observed for LA.

The Panel notes that the limited data available indicate that the relative release of ALA from AT during weight loss is higher than of LA.

The Panel considers that the available evidence does not allow to establish whether a correction factor needs to be applied to the LA and ALA concentrations of AT in order to derive the amount of these FAs that is released from AT during weight loss. Therefore, the Panel will assume in the following a proportionate release of both LA and ALA during weight loss.

3.1.3. Range of weight loss when consuming total diet replacements for weight control

A total of 12 studies were identified through the literature search. The target had been to identify trials reporting on the consumption of TDRs providing between 2,510 and 5,020 kJ (600 and 1,200 kcal) of energy per day and weight loss at time points up to 8 weeks. Two trials did not fully comply with these two requirements. Wing et al. (1995) administered TDRs with a calorie content of 2,467 kJ (590 kcal) and 2,484 kJ (594 kcal) per day and Yang et al. (1981) reported numeric average weight loss data only for the time point of 9 weeks and not for other intermediate time points (for which only graphic representations were available). These two studies were included as they were considered to provide useful information, in particular, in the light of the limited data that were available.

In eight studies (Yang et al., 1981; Hill et al., 1987; Phinney et al., 1988; Davies et al., 1989; Stanko et al., 1992a,b; Vazquez and Kazi, 1994; Wing et al., 1995), participants were confined in metabolic wards for the duration of the studies. Results of these studies therefore include only data from fully compliant subjects. In addition, Lee et al. (2010) dropped all subjects from analysis that were not compliant. Behary et al. (2019) report that, on average, 3,442 kJ/day (823 kcal)/day were consumed by participants, the target being 3,346 kJ/day (800 kcal/day) and Van Dale et al. (1987) described an average daily energy intake of 2,785 kJ/day (666 kcal/day) with the target of 2,844 kJday (680 kcal/day) per day. Compliance can, therefore, also be considered as high. Finally, Gils Contreras et al. (2018) classified 94% of individuals as being compliant (> 80% of sachets consumed) based on counts of returned empty packages, which was 96.7 ± 6.8 (SD)% of sachets distributed.

Most of the included studies were on obese subjects. Only two studies (Van Dale et al., 1987; Lee et al., 2010) also included overweight participants in addition to obese ones. Energy contents of diets ranged from 2,467 kJ (590 kcal) to 4,266 kJ (1,020 kcal) per day. Study durations were from 3 to 9 weeks. Average weekly weight loss in the included studies ranged from 1.18 to 2.58 kg per week.

In two studies (Hill et al., 1987; Van Dale et al., 1987), loss of fat mass was investigated by hydrostatic underwater weighing, which is considered as a valid methodology for body composition analysis. Hill et al. (1987) found that fat loss amounted to around 57% of weight loss in a sedentary dieting group and Van Dale et al. (1987) observed that fat loss amounted to around 76% of weight loss. Other studies, in which loss of fat mass (Stanko et al., 1992b; Lee et al., 2010; Gils Contreras et al., 2018; Behary et al., 2019) was assessed by bioelectrical impedance analysis were not taken into account, as the Panel considers that this method is generally not appropriate to assess small changes in body fat when used alone, particularly in obese subjects and/or when significant changes in body water compartments occur. The results for loss of fat mass in the study by Lee et al. (2010) assessed

by air displacement plethysmography were the same as for weight loss and were therefore considered implausible by the Panel. Hence, the results of this study with respect to loss of fat mass were not taken into account.

The percentage of weight that is lost as fat was estimated by the Panel as average of the values cited above (i.e. 57 and 76%) and rounding up to the closest 10% (i.e. 66.5% rounded up to 70%). Weekly fat loss in all included studies was therefore calculated by multiplying by 0.7 the weekly mean weight loss extracted from these studies. To these data (15 data points in total[13]), a theoretical distribution was fitted, as was done for LA and ALA concentrations in AT tissue (Section 3.1). The best fit was observed for the normal distribution (Figure 3), based on the lowest value for AIC (215.84). The P5 of this theoretical distribution for fat mass loss was 863 g/week, as compared with the P5 of the empirical distribution of 949 g/week.
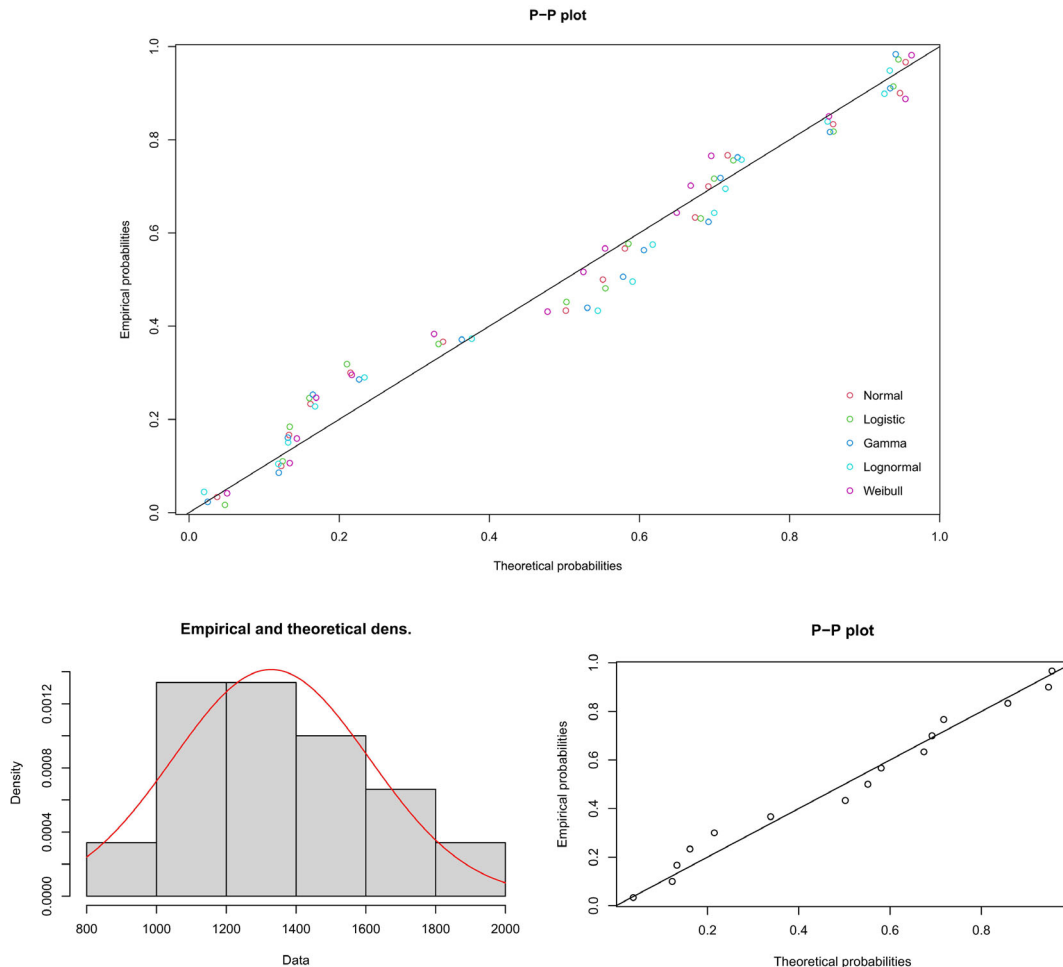
Figure 3: Probability-probability (P-P) plot of different theoretical probability distributions compatible with the empirical distribution of mean fat mass loss (g/week) (upper graph) and comparison of the empirical distribution with a theoretical normal distribution (density graph, theoretical distribution represented as a red curve in the density graph) (lower graphs)

3.1.4. Linoleic acid and alpha-linolenic acid release from adipose tissue during weight loss in subjects consuming TDR

In total, 10,000 data points were generated in the R software following the theoretical distributions with the best fit to the data, i.e. the lognormal distribution for LA, the gamma distribution for ALA and the normal distribution for fat loss. Values, taken randomly by R from the lognormal distribution for LA and the gamma distribution for ALA, were multiplied by random values taken from the normal

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[13] Three studies investigated two different TDRs with different macronutrient compositions.

distribution for fat loss. This thus generated an overall distribution of weekly release of LA and ALA from AT during weight loss in overweight or obese subjects consuming TDRs. The resulting distributions are depicted in Figure 4.

From these distributions, the P5 was taken as a conservative estimate of the weekly LA and ALA release from AT during weight loss in overweight and obese subjects consuming TDRs, i.e. 92.1 g/week for LA (13.2 g/day) and 4.2 g/week for ALA (0.60 g/day).
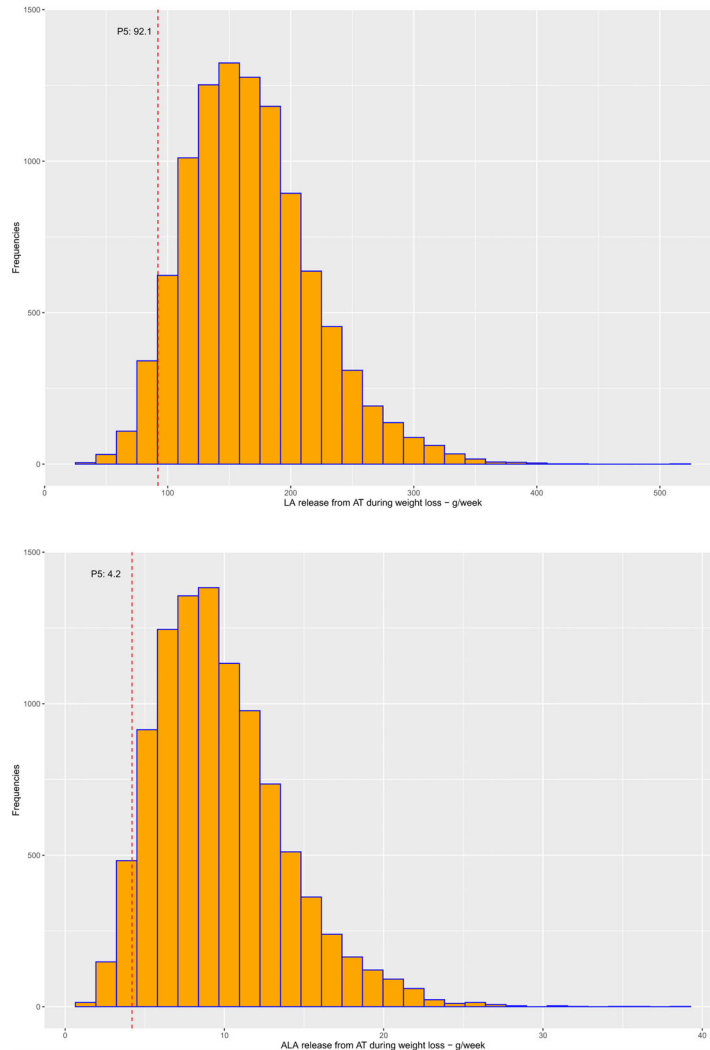
Figure 4: Distribution of weekly release of linoleic acid (LA) and alpha-linolenic acid (ALA) from adipose tissue (AT) during weight loss in overweight and obese individuals consuming total diet replacements for weight control (TDRs) (P5 indicated as red dotted line)

Sensitivity analyses were conducted using the second and third best fitting distributions to the data on LA and ALA concentration in AT and multiplying random values drawn from these distributions with random values from the normal distribution of fat loss. The use of a gamma distribution (AIC = −338.34) to the data on LA concentration in AT resulted in a P5 of LA release from AT during weight loss of 92.3 g/week (13.2 g/day), and the use of a beta distribution (AIC = −335.72) in a P5 of 89.9 g/week (12.8 g/day). For ALA, the use of the beta distribution (AIC = −634.29) and lognormal distribution (AIC = −634.28) resulted in P5 of 4.14 and 4.28 g/week, respectively (0.59 and 0.61 g/day).

Sensitivity analyses were also conducted for data on fat mass loss with the second and third best fitting distributions (i.e. gamma and Weibull distributions). The best fitting distributions for LA (i.e. lognormal distribution) and ALA (i.e. gamma distribution) concentration in AT were kept. The use of gamma and Weibull distributions (both AIC = 215.90) to the data on LA concentration in AT resulted in a P5 of LA release from AT during weight loss of 94.0 g/week (13.4 g/day) and 91.0 g/week (13.0 g/day), respectively. For ALA, this resulted in a P5 of 4.3 g/week (0.61 g/day) and 4.0 g/week (0.57 g/day).

The Panel notes the uncertainties that are associated with the derived values for the following reasons: (1) the modelling was based on means and not on individual data, (2) variation among individuals may be greater than variation among means, (3) weight status and the extent of obesity before weight loss may have an impact on the percentage of weight that is lost as fat, which could not be taken into account in the modelling due to the missing data, (4) there is no information to judge whether the LA and ALA concentration in AT used in the modelling is representative of the FA AT concentration of individuals consuming TDRs. These uncertainties may lead either to an under- or an overestimation of the release of FA from AT during weight loss.

3.1.5. Conclusions on the minimum content of linoleic acid and alpha-linolenic acid in total diet replacements for weight control

Even taking into account these uncertainties, the Panel concludes that the amount of LA released from AT during weight loss in overweight or obese subjects consuming TDRs is sufficient to cover the AI for this FA in the reference subject, as explained in Section 1.3. Therefore, there is no need to add LA to TDRs.

The Panel also concludes that around 40% (i.e. 0.6 g/day) of the AI of ALA could be provided by the release of ALA from AT during weight loss in overweight or obese individuals consuming TDRs. Therefore, a minimum of 0.8 g/day ALA needs to be supplied by TDRs in order to meet the AI for this FA in the reference subject.

3.2. Minimum fat content in total diet replacements for weight control

In its previous Opinion (EFSA NDA Panel, 2015b), the Panel concluded that '*the available evidence is insufficient to establish a minimum fat content in total diet replacements for weight control beyond their content of [essential fatty acids] EFA*'. The minimum content of LA and ALA proposed for TDRs in the previous opinion led to a minimum fat content in TDRs of around 20 g/day, under the assumption that oils used to supply these FAs have a maximum EFA content of 55–75 FA%.

Consistent with this consideration, 1.5 g of oil with an ALA content of 55% supplies 0.8 g ALA and would be equivalent to the minimum fat content in TDRs, if no other fats are added. The Panel notes that such a low fat content in TDRs has been associated with an increased risk of gallstone formation, as reviewed in the Panel's previous opinion (EFSA NDA Panel, 2015b) and further described below.

The comprehensive literature search for intervention studies investigating the effect of different fat contents in energy-restricted diets on gallstone formation, biliary sludge formation and/or gallbladder emptying did not yield any results. Therefore, the Panel relied on the four intervention studies that had already been reviewed in its previous Opinion (Stone et al., 1992; Gebhard et al., 1996; Festi et al., 1998; Vezina et al., 1998). As supporting evidence, the Panel also considered data from observational studies or intervention studies that were either single-arm or in which only one arm consumed a TDR without further co-intervention, even if they did not meet the initial inclusion criteria of the search (protocol amendment 5). These studies were retrieved by an additional non-systematic search of the literature not foreseen in the protocol. This was done in an attempt to complement the limited data from two-arm intervention studies. It was undertaken in February 2021, in PubMed. The five publications retrieved are described further down.

Table 1 provides an overview on the evidence, considered in this Statement, on the incidence of gallstone formation and on gallbladder emptying following the consumption of TDRs with differing fat content.

Among the four originally considered studies, there was only one randomised study that investigated gallstone formation on two isocaloric TDRs (2,238–2,414 kJ/day (535–577 kcal/day)) with varying fat contents in obese subjects (Festi et al., 1998). This study showed that consumption for 90 days of a TDR that provided 3 g/day fat led to the development of asymptomatic gallstones in six of 11 subjects (55%), while none of the 11 individuals on the TDR with 12.2 g/day developed gallstones. None of the subjects had gallstones at the beginning of the study.

A non-randomised intervention study (Vezina et al., 1998) found that, in obese individuals, gallstone formation was similar in the groups that consumed a TDR (providing 3,766 kJ/day (900 kcal/day)) for 13 weeks with 16 g/day fat (n = 16 out of 94 developed gallstones; 17%) and the group that consumed a TDR with the same energy content, but with 30 g/day fat (n = 20 out of 178 developed gallstones; 11.2%). None of the subjects had gallstones at the beginning of the study. However, diets differed not only in the fat but also in dietary fibre content (11 and 15 g/day in the 16 g and 30 g/day fat groups, respectively), which could also have had an effect on the outcome.

Gebhard et al. (1996) reported the development of asymptomatic gallstones in four out of six individuals (67%) who consumed a TDR providing 2,176 kJ/day (520 kcal/day) with < 2 g of fat per day after having consumed the TDR for 12 weeks. None of the participants in the group who consumed a TDR (n = 7) with 3,766 kJ/day (900 kcal/day) and 30 g fat per day developed gallstones. None of the subjects had gallstones at the beginning of the study.

One non-randomised (Stone et al., 1992) and one randomised intervention study previously described (Gebhard et al., 1996) found that gallbladder emptying was similar to gallbladder emptying at maximal stimulating conditions when a meal with a minimum of 10 g fat was consumed. This was not the case for meals with fat contents between 0 and 4 g. No other fat content per meal (between 4 and 10 g) was tested. Gallbladder stasis resulting from a reduced stimulus for gallbladder emptying has been proposed as one of the mechanisms by which diets inducing rapid weight loss may lead to gallstone formation (Festi et al., 2000).

Supportive evidence

From a non-comprehensive literature search, the Panel retrieved the five publications described below. The Panel notes that there is uncertainty whether these publications represent the totality of observational or single-arm intervention studies on TDRs reporting on gallstone formation/biliary sludge formation/gallbladder emptying.

In a matched cohort study, Johansson et al. (2014) investigated gallstone formation in individuals consuming a TDR providing 2,091 kJ/day (500 kcal/day, n=3320) and 8 g fat per day for 6–10 weeks. This was compared to a diet in which two meals were replaced by a meal replacement product; the diet providing 5,018–6,273 kJ/day (1,200–1,500 kcal/day, n = 3,320). No information on the approximate fat content of this overall diet was available. In total, 48 cases with gallstones that required hospital care per 6,361 person-years occurred in the TDR group and 14 in the meal replacement group.

Broomfield et al. (1988), Liddle et al. (1989), Shiffman et al. (1995) and Yang et al. (1992) studied TDRs with a fat content of 1–3 g/day and an energy content of around 2,176 kJ/day (520 kcal/day). In these studies, 5 out of 19 subjects (26%, 3 weeks), 13 out of 51 (26%, 8 weeks), 57 out of 202 (28%, 16 weeks) and 27 out of 248 (11%, 16 weeks) newly developed gallstones after having consumed the TDR, respectively. Subjects with gallstones at the beginning of the study were either excluded from the studies (Broomfield et al., 1988; Liddle et al., 1989; Shiffman et al., 1995) or data were presented separately (Yang et al., 1992).

Table 1: Summary of studies reporting on incidence of gallstone formation and on gallbladder emptying following the consumption of total diet replacements for weight control (TDRs), supportive evidence shaded in grey

Publication	Duration (weeks)	N	kcal/d	Fat g/d	Fat g/meal	Gallstones incidence %	Gallbladder emptying
Studies on gallstone formation							
Broomfield et al. (1988)	3	19	520	1		26	n/a
Liddle et al. (1989)	8	51	520	1		26	n/a
Yang et al. (1992)	16	248	520	1		11	n/a
Gebhard et al. (1996)	12	6	520	< 2		67	n/a
Shiffman et al. (1995)	16	202	520	1–3		28	n/a
Festi et al. (1998)	12	11	535	3		55	n/a
Johansson et al. (2014)	6–10	3,320	500	8		1.5[a]	n/a
Festi et al. (1998)	12	11	577	12.2		0	n/a
Vezina et al. (1998)	13	94	900	16		17	n/a
Vezina et al. (1998)	13	178	900	30		11.2	n/a

Publication	Duration (weeks)	N	kcal/d	Fat g/d	Fat g/meal	Gallstones incidence %	Gallbladder emptying
Gebhard et al. (1996)	12	7	900	30		0	n/a
Studies on gallbladder emptying							
Stone et al. (1992)	n/a	14			0	n/a	Below maximal
Gebhard et al. (1996)	n/a	6			< 1	n/a	Below maximal
Stone et al. (1992)	n/a	14			4	n/a	Below maximal
Stone et al. (1992)	n/a	14			10	n/a	Maximal
Gebhard et al. (1996)	n/a	7			10	n/a	Maximal
Stone et al. (1992	n/a	14			20	n/a	Maximal

d: day; g: gram; n: number of subjects in the study population; n/a: not applicable.
(a): Gallstones requiring hospital care.

The Panel notes that, in the only randomised study that investigated gallstone formation on two isocaloric TDRs (Festi et al., 1998), gallstones did not occur when a TDR with 12.2 g fat/day was consumed while 55% of subjects developed gallstones with a TDR with a fat content of 3 g/day. However, this observation is based on only 11 subjects per group and gallstones have been observed in another study at fat contents higher than 12.2 g/day (i.e. 16 and 30 g/day) (Vezina et al., 1998).

The Panel also notes that two small intervention studies focussing on gallbladder emptying (which limitations have been described above) indicate that around 10 g fat per meal is needed to reach gallbladder emptying equal to maximal stimulating conditions (Stone et al., 1992; Gebhard et al., 1996). With a total daily fat content in a TDR of 12.2 g/day, this will not be achieved under the assumption that TDRs are consumed in at least three portions per day.

The Panel considers that a minimum content of 1.5 g fat in a TDR as derived from the minimum content of ALA (see Section 3.1.5) is associated with an increased risk of gallstone formation. Hence, the minimum fat content in a TDR cannot be derived only from the minimum content of ALA proposed by the Panel. The Panel, however, considers that the limited available data do not allow to establish a minimal fat content in TDRs for the prevention of gallstone formation that is attributable to a too low fat content of the diet. There is some evidence that 10 g fat per meal is required for maximal gallbladder emptying. The Panel assumes a consumption of TDR in three meals per day and finally proposes, until further scientific evidence becomes available, to maintain the minimum fat content in TDRs at a level of (at least) 20 g/day, as in the Panel's previous opinion (EFSA NDA Panel, 2015b).

3.3. Maximum magnesium content in total diet replacements for weight control

3.3.1. Potential mechanisms of action

Mg absorption takes place in the distal small intestine, mainly through a paracellular process that is driven by electrochemical gradients and solvent drag. Transcellular absorption is a saturable process and seems to be significant only at low dietary intakes. At usual intakes, Mg absorption is generally considered to be 40–50%, but figures from 10 to 70% have also been reported (EFSA NDA Panel, 2015a). Fractional Mg absorption diminishes with increasing Mg intake in a non-linear manner. The non-linearity has been suggested to be the result of the transcellular absorption mechanism reaching its absorption maximum at around 120–145 mg of Mg intake per bolus, while the paracellular absorption mechanism continues with absorption at a rate of around 7% (Fine et al., 1991b). The part of Mg that remains unabsorbed, e.g. at high doses when the transcellular absorption mechanism reaches saturation, acts as an osmotic agent and may cause diarrhoea (Fine et al., 1991a,b). Mg-induced diarrhoea is characterised by high faecal Mg output and may be distinguished from diarrhoea from other causes by measuring faecal Mg concentrations (Fine et al., 1991a). Fine et al. (1991a) proposed as an upper bound of 'normal' 355 mg (14.6 mmol) Mg per day in faeces and observed that, for each 24 mg (1 mmol)/day increase in faecal Mg above the upper bound of normal, stool weight augmented by around 7.3 g.

3.3.2. Supplemental magnesium and risk of developing diarrhoea

The Panel originally set out to retrieve intervention studies in which (i) different doses of Mg were administered (including a zero (placebo) dose), (ii) in populations without apparent co-morbidities, or in overweight or obese individuals with T2DM, hypertension or hyperlipidaemia and (iii) which reported on the incidence of diarrhoea (or the absence of adverse effects) as well as (iv) on the criteria that were used to diagnose diarrhoea. However, no such studies were retrieved through the search.

The Panel notes that studies in which the incidence of diarrhoea is self-reported by participants without using predefined diagnostic criteria are at high risk of bias, because of the interindividual differences in the perception of what constitutes diarrhoea. Among those studies at high risk of bias, cross-over studies are more reliable than parallel studies, as bowel habits are described in both periods by the same person and reports are subject to the same subjective interpretations. Therefore, in the absence of studies in which diarrhoea was diagnosed according to objective criteria, the Panel decided to base its evaluation on results of cross-over studies in which the incidence of diarrhoea was self-reported (protocol amendment 3).

In addition, the Panel also considered studies conducted in non-overweight/non-obese individuals with mild to moderate hypertension treated with diet only that would have not met the original inclusion criteria of healthy non-overweight/non-obese individuals (protocol amendment 4). As these individuals were not under medication, the study population was considered appropriate.

The criteria applied in the current evaluation excluded a number of studies that were originally taken into account by the SCF (2001) in order to set the UL for Mg.

The 11 studies that met the inclusion criteria for the present evaluation are listed in Table 2.

The Mg form, the doses (150–3,000 mg/day), the dosage forms and patterns and the duration of administration (single dose to 90-day administration) in these studies were diverse. The Panel notes that the differences in Mg forms, the dosage forms and patterns investigated and the duration of administration, together with the uncertainties associated with the diagnosis of diarrhoea in the available studies, do not permit the derivation of an Mg content in TDRs below which diarrhoea is not expected to occur. Nor do these differences and uncertainties allow an evaluation of whether different Mg forms or the consumption of Mg with or without a meal have differential effects.

In its proposal to the European Commission, TDMR suggested to raise the maximum Mg content in TDRs to 350 mg/day. In the absence of data that allows the Panel to conclude on a content in TDRs below which diarrhoea is not expected to occur, the Panel will, however, comment on the likelihood of occurrence of Mg-induced diarrhoea when TDRs contain a maximum Mg dose of 350 mg/day.

The three articles highlighted by TDMR (Christensen et al., 2011, 2018; Lean et al., 2018) in support of their request did not report on the Mg doses used in these parallel studies nor did they report on the criteria used to diagnose diarrhoea. The Panel notes that these studies cannot be used to determine a content of Mg in TDRs that does not cause diarrhoea.

Table 2: Cross-over studies on differing magnesium doses reporting on the incidence of diarrhoea or the absence of adverse effects (ordered by increasing Mg daily dose)

Study study location	Study population: health status, gender (females %), age [years]	Total N (reference population for N with diarrhoea)	Mg form Mg vehicle	Mg dose [mg/d]	Mg duration (washout) [d]	n with diarrhoea in Mg group (%)*	n with diarrhoea in placebo group (%)
Kato et al. (2004) Japan	Healthy Males Mean: 31	25 (25)	Magnesium oxide Tablets (after meals)	150	7 (7–10)	0 (0)	0 (0)
Navarrete-Cortes et al. (2014) Mexico	NIDDM/mean BMI (SD) 30.55 (5.72) kg/m^2 Mixed (64) Mean (SD): 53 (8)	98 (56)	Magnesium lactate Tablets (with a meal)	360	90 (90)	0 (0)	0 (0)
Cappuccio et al. (1985) United Kingdom	Hypertension not on medication Mixed (47) Mean (range): 52 (33–66)	17 (17)	Magnesium-aspartate hydrochloride Tablets	365	30 (0)	0 (0)	0 (0)
Widman et al. (1993) Sweden	Mild hypertension not on medication Mixed (12) Mean (SD): 50 (6)	17 (16)	Magnesium hydroxide Tablets	365	21 (21)	0 (0)	0 (0)
Cosaro et al. (2014) Italy	Healthy Males Mean (SD): 260 (3)	16 (14)	Magnesium pidolate Powder (empty stomach before meal)	368	56 (28)	2 (14)	0 (0)
Purvis et al. (1994) United States	NIDDM/mostly obese Mixed (86) Mean (SD): 54 (13)	33 (28)	Magnesium chloride Tablets	384	42 (14)	4[1] (14)	2[1] (7)
Marken et al. (1989) United States	HealthyMixed (46)Range: 18–65	50 (47)	Magnesium oxideCapsules	476	60 (0)	5 (11)	1 (2)
Kishimoto et al. (2010) Japan	HealthyMalesMean (SE): 420 (3)	16 (16)	Magnesium chlorideFood	500	Single doses (≥ 6)	0 (0)	0 (0)
Shechter et al. (2012) Israel	Healthy Mixed (49) Mean (SD): 53 (8)	41 (40)	Magnesium oxide Tablets	520	30 (30)	5 (13)	3 (8)[2]

Study study location	Study population: health status, gender (females %), age [years]	Total N (reference population for N with diarrhoea)	Mg form Mg vehicle	Mg dose [mg/d]	Mg duration (washout) [d]	n with diarrhoea in Mg group (%)*	n with diarrhoea in placebo group (%)
Widman et al. (1993) Sweden	Mild hypertension not on medication Mixed (12) Mean (SD): 50 (6)	17 (16)	Magnesium hydroxide Tablets	972	21 (21)	0 (0)	0 (0)
Dolberg et al. (2017) Denmark	Healthy Males Mean (range): 22 (20-24)	10 (10)	Magnesium hydroxide Tablets (with 150 mL water)	1,094	Single doses ($\geq$ 6)	0 (0)	0 (0)
Vu et al. (2000) The Netherlands	Healthy Mixed (50) Mean (SE): 21 (3)	8 (8)	Magnesium sulfate Overnight fasting $\geq$ 10 h	3,000	Single doses (NR)	3 (38)	NR
Vu et al. (2000) The Netherlands	Healthy Mixed (50) Mean (SE): 21 (3)	8 (8)	Magnesium sulfate With a meal	3,000	Single doses (NR)	8 (100)	NR

d: day(s); h: hours; Mg: magnesium; N: number of subjects; NIDDM: non-insulin-dependent diabetes mellitus; NR: not reported; SD: standard deviation; SE: standard error.

*: Calculated % of cases with diarrhoea from the reference population when not reported in the paper (rounded numbers).

1: Reported as increased frequency of stools or diarrhoea.

2: Comparator being magnesium citrate given in a dose of 296 mg/day.

In the following, the five cross-over studies that investigated Mg doses between 300 and 400 mg/day are described.

Navarrete-Cortes et al. (2014) evaluated, in 56 subjects with T2DM not receiving insulin (mean BMI 30.55 (SD 5.72) kg/m^2), the effect of consuming 360 mg/day Mg in the form of Mg lactate supplementation in tablets, divided into two doses per day taken after meals, vs. placebo. Mg and placebo were administered for 3 months each, with a 3-month washout period in between. It is reported that no adverse effects occurred during both periods. Even though this study does not specifically report on diarrhoea, the absence of adverse effects may indicate that no severe diarrhoea occurred.

Cappuccio et al. (1985) administered 365 mg/day Mg given in the form of Mg-aspartate hydrochloride divided into three doses per day for a month to 17 hypertensive individuals not on blood pressure lowering medication or thiazide diuretics. This was compared with a 1-month placebo period that followed the Mg period without a washout. Diarrhoea did not occur in any of the subjects during the study periods.

Widman et al. (1993) analysed 16 individuals with mild hypertension not on blood pressure lowering medication or any medication that could interfere with Mg administration. They received 365 mg/day Mg given in the form of Mg hydroxide and placebo, presumably as a single daily dose, for 3 weeks each, with a 3-week washout period in between. Diarrhoea did not occur in any of the subjects during the study periods.

Cosaro et al. (2014) studied 14 males without hypertension, diabetes, obesity, kidney or liver disease, and previous cardiovascular events and not taking lipid-lowering medication or continuous therapy with non-steroidal anti-inflammatory medication. Placebo and Mg pidolate powder were taken twice a day before meals for 8 weeks with a 4-week washout period in between. Mg was administered in a daily dose equivalent to 368 mg elemental Mg. Two participants developed mild diarrhoea (not further defined) in the Mg period. The diarrhoea disappeared when the dose of Mg was reduced for a few days (extent and duration of reduction not reported).

Purvis et al. (1994) assessed 28 subjects with T2DM not receiving insulin (mean BMI 32.2 (SD 7.1) kg/m^2) treated by diet only or on stable doses of oral hypoglycaemics. Mg chloride was administered in a sustained release form as a single dose in an amount of 384 mg/day for 6 weeks and the effect was compared with placebo. The washout period was 2 weeks. Four subjects during the Mg period and two during the placebo period reported an increased frequency of stools or diarrhoea. It is not indicated whether the two participants reporting gastrointestinal effects during the placebo period overlapped with those reporting effects during the Mg period. It is also not reported how many individuals experienced diarrhoea and how many an increased frequency of stools. Also, there is no report on the severity of the diarrhoea that was observed. The Panel notes that no conclusions can be drawn from this study with respect to the risk of developing diarrhoea following Mg consumption, as the incidence of diarrhoea is not reported separately from the occurrence of an increased stool frequency, the latter not being an adverse effect.

The Panel notes that, in three out of four studies in which Mg was administered in doses of 360–368 mg/day and from which conclusions could be drawn, no adverse effects or diarrhoea were reported. The Panel also notes that, in the fourth study, the diarrhoea that developed in two out of 14 participants was self-reported as mild and disappeared when the dose of Mg was reduced for a few days. There was no consistent pattern in the data that allows conclusions to be drawn on whether dividing the Mg administration into multiple daily doses reduces the risk of developing diarrhoea as compared with a single daily dose. The Panel also notes that, in the studies in which diarrhoea was observed, the aetiology has not been verified.

3.3.3. Conclusions on the maximum magnesium content in total diet replacements for weight control

Taking into account that

1) in three out of four studies that investigated supplemental Mg doses of 360–368 mg/day, no adverse effects or diarrhoea were reported, and that

2) in one study in which diarrhoea was observed, the diarrhoea occurred only in two participants and was classified as mild,

the Panel considers that the likelihood that Mg-induced diarrhoea occurs at a severity that may be considered of concern for overweight and obese individuals consuming TDRs (who more often suffer

from constipation than diarrhoea (Christensen et al., 2011; Lean et al., 2018)) is low when the total maximum Mg content in TDRs is 350 mg/day.

4. Conclusions

The Panel concludes that

- there is no need to add LA to TDRs, as the amount released from AT during weight loss when consuming TDRs is sufficient to cover the AI for this FA;
- the release of ALA from AT during weight loss when consuming TDRs is not sufficient to cover the AI and a minimum of 0.8 g/day ALA needs to be supplied by TDRs in order to meet the AI for ALA;
- the minimum fat content of TDRs of 20 g/day as derived in the previous opinion is proposed to be maintained until the availability of further evidence, given the considerable uncertainty as to the amount of fat required for reducing the risk of gallstone formation;
- the likelihood that Mg-induced diarrhoea occurs at a severity that may be considered of concern for overweight or obese individuals consuming TDRs is low when the total maximum Mg content in TDRs is 350 mg/day.

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Abbreviations

AI	adequate intake
AIC	Akaike Information Criterion
ALA	alpha-linolenic acid
AR	average requirement
AT	adipose tissue
BE	Belgium
BG	Bulgaria
BMI	body mass index
CH	Switzerland
CI	confidence interval
CZ	Czechia
DASH	Dietary Approaches to Stop Hypertension (study)
DE	Germany
DIOGENES	Diet, Obesity and Genes (study)
DK	Denmark
DRV	dietary reference value
E%	percentage of energy intake
EFA	essential fatty acids
EKE	expert knowledge elicitation
ES	Spain
FA	fatty acid(s)

FFM	fat free mass
FI	Finland
FR	France
FSG	food for specific groups
FSMP	food for special medical purposes
GDR	German Democratic Republic
GR	Greece
IE	Ireland
ISO	International Organization for Standardization
LA	linoleic acid
LCD	low calorie diet
LT	Lithuania
Mg	magnesium
MRI	magnetic resonance imaging
N	number of subjects
NDA Panel	Panel on Nutrition, Novel Foods and Food Allergens
NIDDM	non-insulin dependent diabetes mellitus
NL	Netherlands
NO	Norway
NR	not reported
P5	fifth percentile
P-P	probability-probability
PL	Poland
PRI	population reference intake
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PT	Portugal
Q	question
RCT	randomised controlled trial
RU	Russia Federation
SC	subcutaneous
SCF	Scientific Committee on Food
SD	standard deviation
SE	standard error
SE	Sweden (Appendices B and C)
SEM	standard error of the mean
T2DM	type 2 diabetes mellitus
TDMR	Total Diet & Meal Replacements Europe
TDR	total diet replacement for weight control
TG	triglycerides
ToR	terms of reference
UK	United Kingdom
UL	tolerable upper intake level
US	United States
VC	visceral
VLCD	very low calorie diet
VKM	Norwegian Scientific Committee on Food Safety
WHO	World Health Organisation

Appendix A – PRISMA flow charts for the literature searches

PRISMA flow chart for Q1

Aimed at identifying data on the linoleic acid (LA) and alpha-linolenic acid (ALA) concentrations in from adipose tissue (AT) and their release from AT during weight loss.

```
┌─────────────────────────────┐
│ Records identified through  │
│    database searching       │
│        N = 13322            │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│   Records after duplicates  │
│          removed            │
│         N = 9518            │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐        ┌─────────────────────────────┐
│     Records screened        │───────▶│      Records excluded       │
│        N = 9518             │        │         N = 8904            │
└─────────────────────────────┘        └─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐        ┌─────────────────────────────┐
│  Full-text articles assessed│───────▶│ Full-text articles excluded │
│       for eligibility       │        │         N = 532             │
│         N = 614             │        └─────────────────────────────┘
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│      Studies included       │
│          N = 82             │
└─────────────────────────────┘
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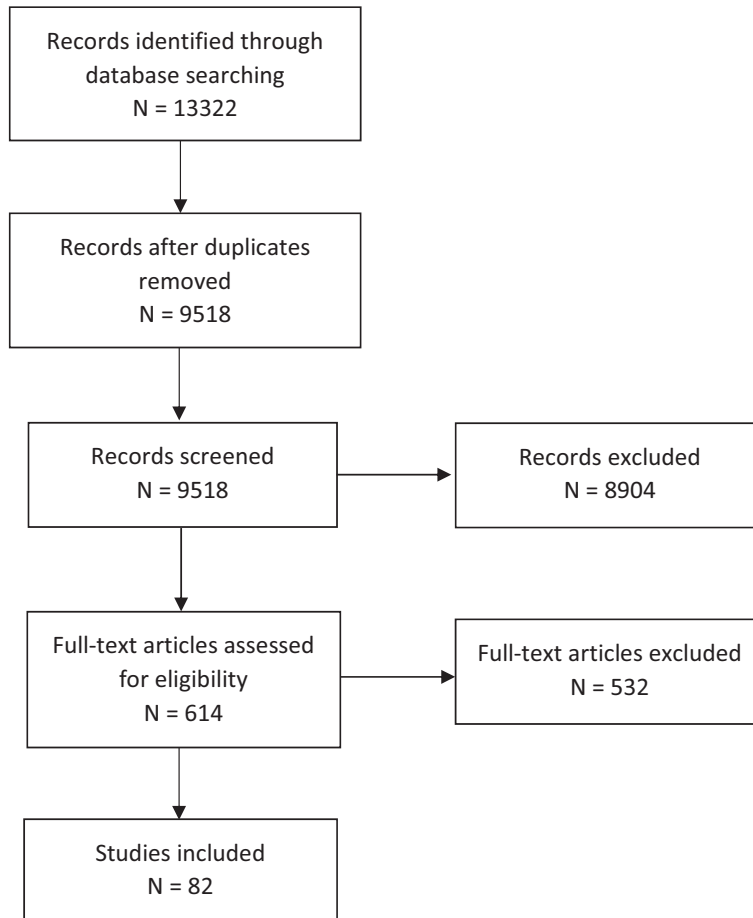
PRISMA flow chart for Q2

Aimed at identifying data on the weight loss achieved when consuming total diet replacements for weight control.

```
┌─────────────────────────────┐
│ Records identified through  │
│    database searching       │
│        N = 6353             │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│  Records after duplicates   │
│          removed            │
│        N = 4867             │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐        ┌─────────────────────────────┐
│     Records screened        │───────▶│     Records excluded        │
│        N = 4867             │        │        N = 4186             │
└─────────────────────────────┘        └─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐        ┌─────────────────────────────┐
│  Full-text articles assessed│───────▶│ Full-text articles excluded │
│       for eligibility       │        │        N = 669              │
│        N = 681              │        └─────────────────────────────┘
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│      Studies included       │
│         N = 12              │
└─────────────────────────────┘
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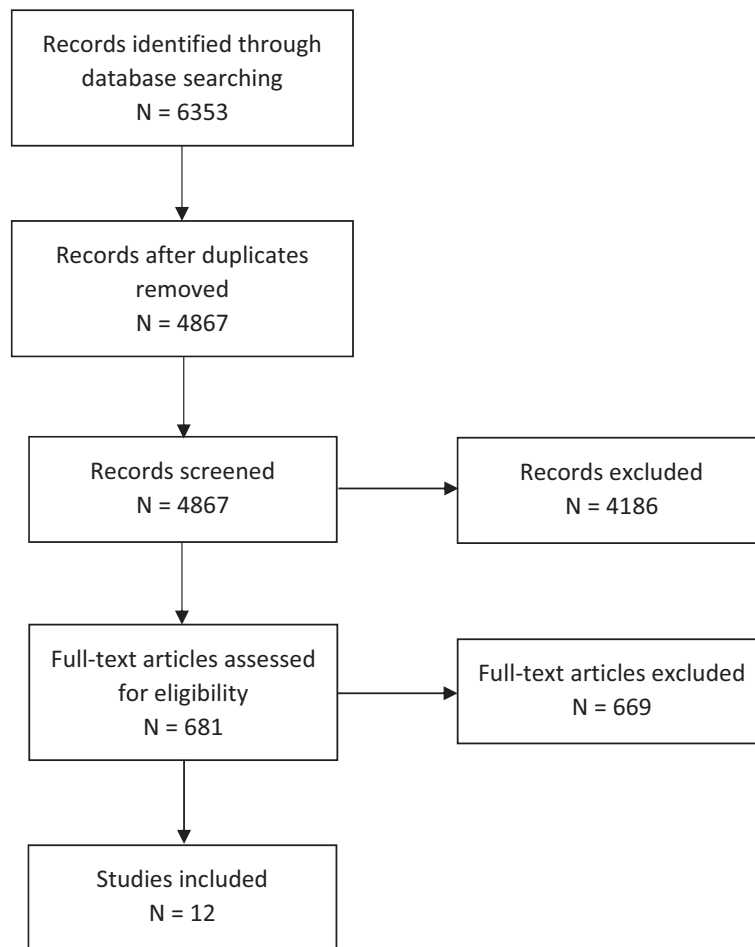
PRISMA flow chart for Q3

Aimed at identifying data on supplemental Mg intake and risk of developing diarrhoea.

```
┌─────────────────────────┐
│ Records identified      │
│ through database        │
│ searching               │
│ N = 10098               │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│ Records after duplicates│
│ removed                 │
│ N = 7726                │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐        ┌─────────────────────────┐
│ Records screened        │───────▶│ Records excluded        │
│ N = 7726                │        │ N = 7216                │
└─────────────────────────┘        └─────────────────────────┘
            │
            ▼
┌─────────────────────────┐        ┌─────────────────────────┐
│ Step 1. Full-text       │───────▶│ Full-text articles      │
│ articles assessed for   │        │ excluded                │
│ eligibility             │        │ N = 435                 │
│ N = 510                 │        └─────────────────────────┘
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐        ┌─────────────────────────┐
│ Step 2. Full-text       │───────▶│ Full-text articles      │
│ articles assessed for   │        │ excluded                │
│ eligibility             │        │ N = 64                  │
│ N = 75                  │        └─────────────────────────┘
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│ Studies included        │
│ N = 11                  │
└─────────────────────────┘
```
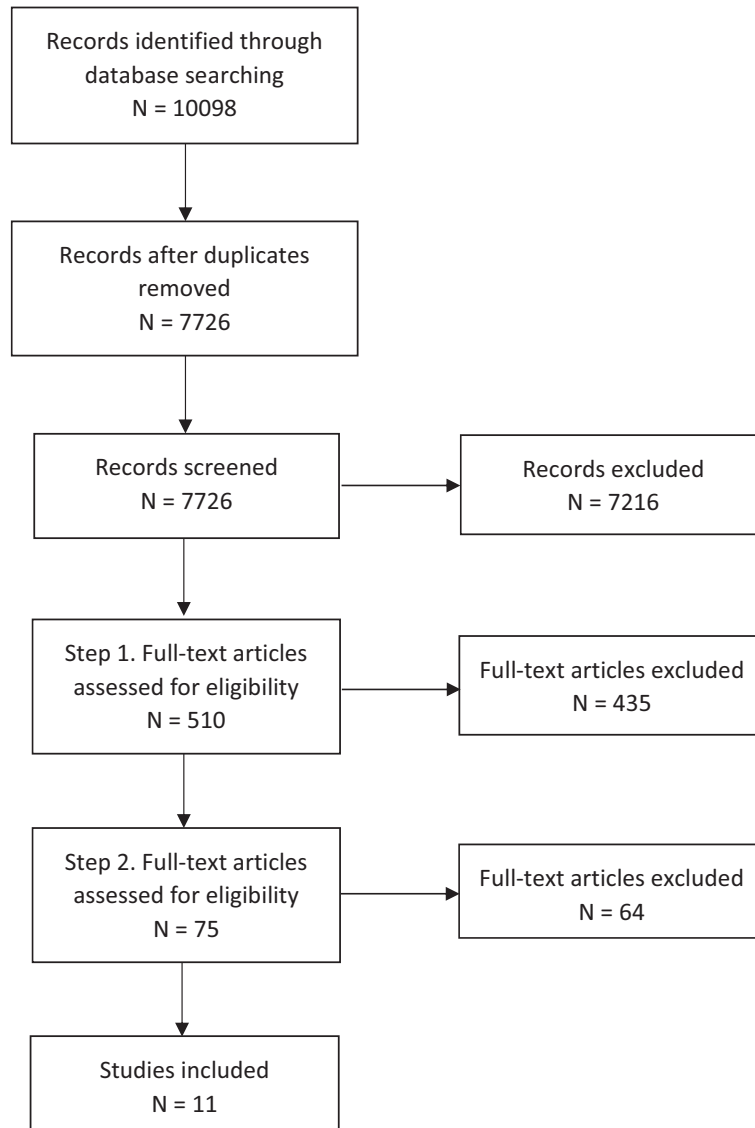
PRISMA flow chart for Q4

Aimed at identifying data on intake of dietary fat in energy-restricted diets and risk of gallstone/biliary sludge formation and gallbladder emptying.

```
┌─────────────────────────┐
│ Records identified through│
│   database searching     │
│        N = 81            │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│ Records after duplicates │
│        removed           │
│        N = 66            │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐        ┌─────────────────────────┐
│   Records screened       │───────▶│   Records excluded       │
│        N = 66            │        │        N = 64           │
└─────────────────────────┘        └─────────────────────────┘
            │
            ▼
┌─────────────────────────┐        ┌─────────────────────────┐
│ Full-text articles assessed│──────▶│ Full-text articles excluded│
│     for eligibility      │        │        N = 2            │
│        N = 2            │         └─────────────────────────┘
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│   Studies included       │
│        N = 0            │
└─────────────────────────┘
```
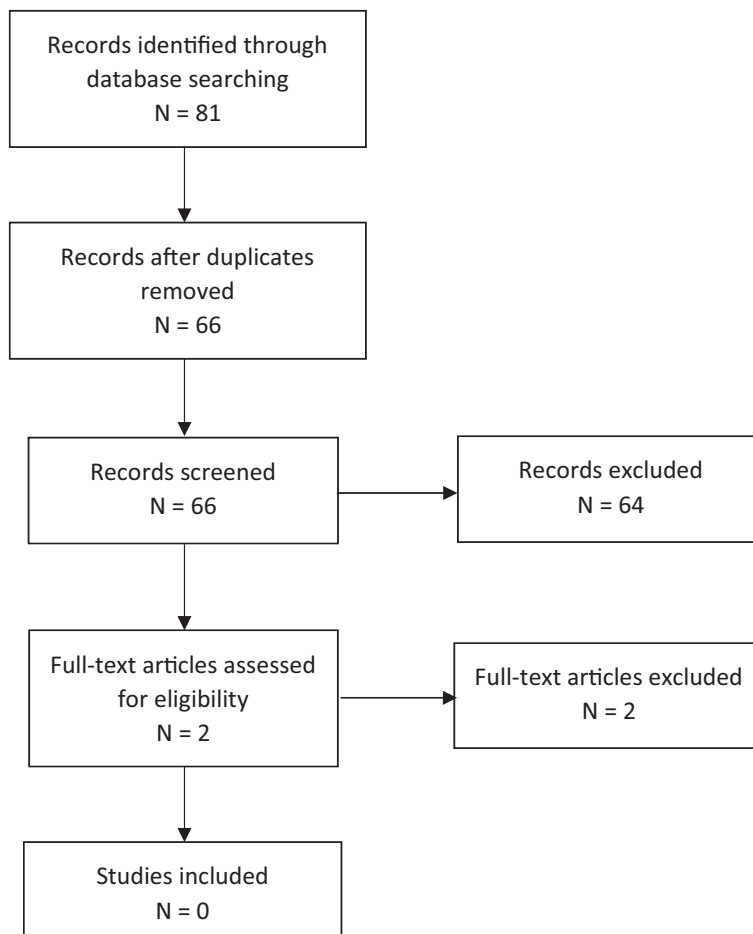
Appendix B – Studies included in the assessment of the linoleic and alpha-linolenic acid concentrations in adipose tissue

The following two forest plots are graphical presentations of the available data on the linoleic acid (LA) and alpha-linolenic acid (ALA) concentrations in adipose tissue. Data are presented for studies published from the year 1990 onwards.

The studies are sorted by ascending point estimates. Each point estimate constitutes the mean fatty acid (FA) concentration for the specific study population, which is also indicated numerically (as percentage) in the seventh column. The 95% confidence interval (CI) around each point estimate is presented by a horizontal line (also indicated in the eight column).

B.1. Linoleic acid concentrations in adipose tissue – studies from 1990

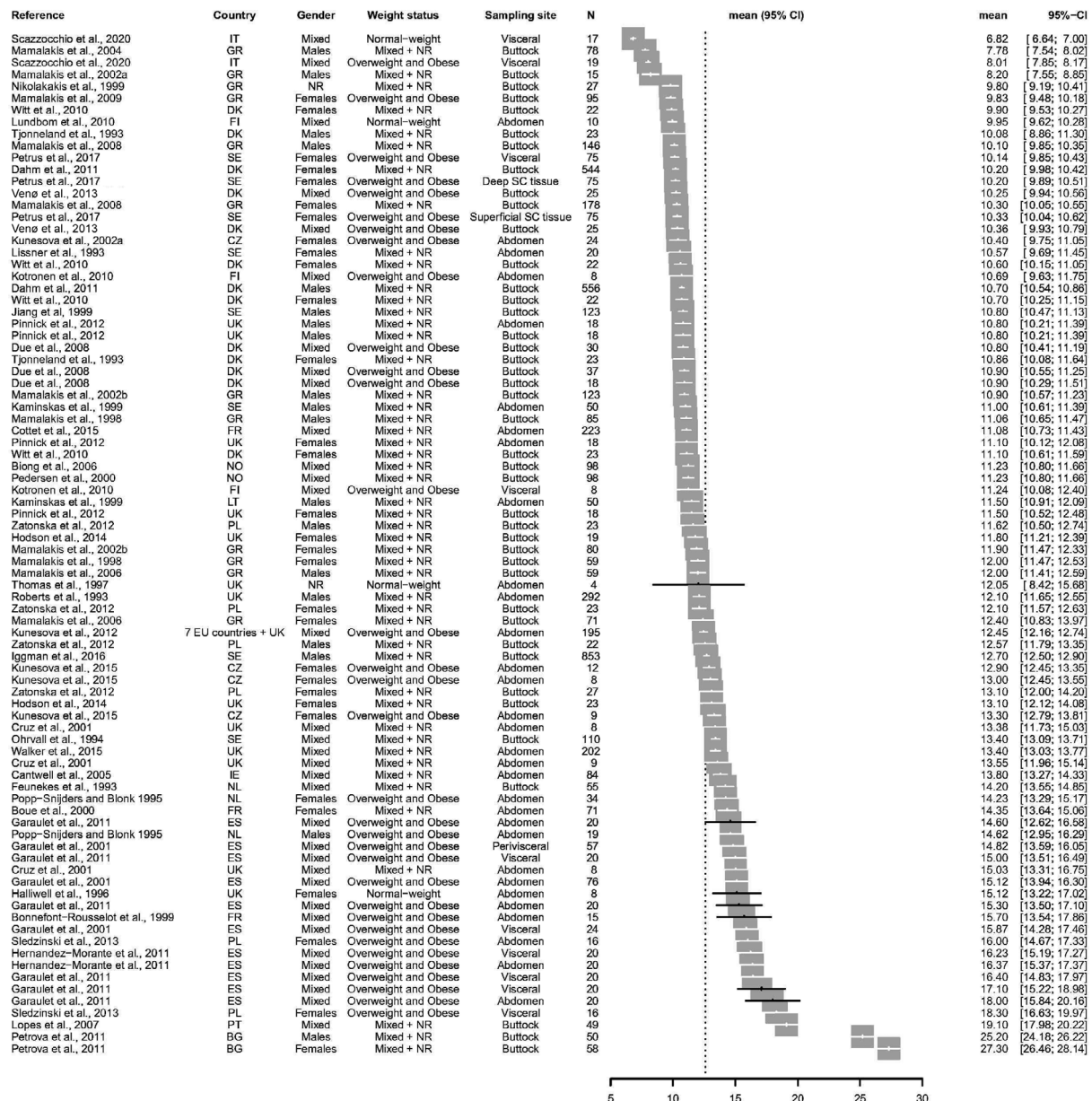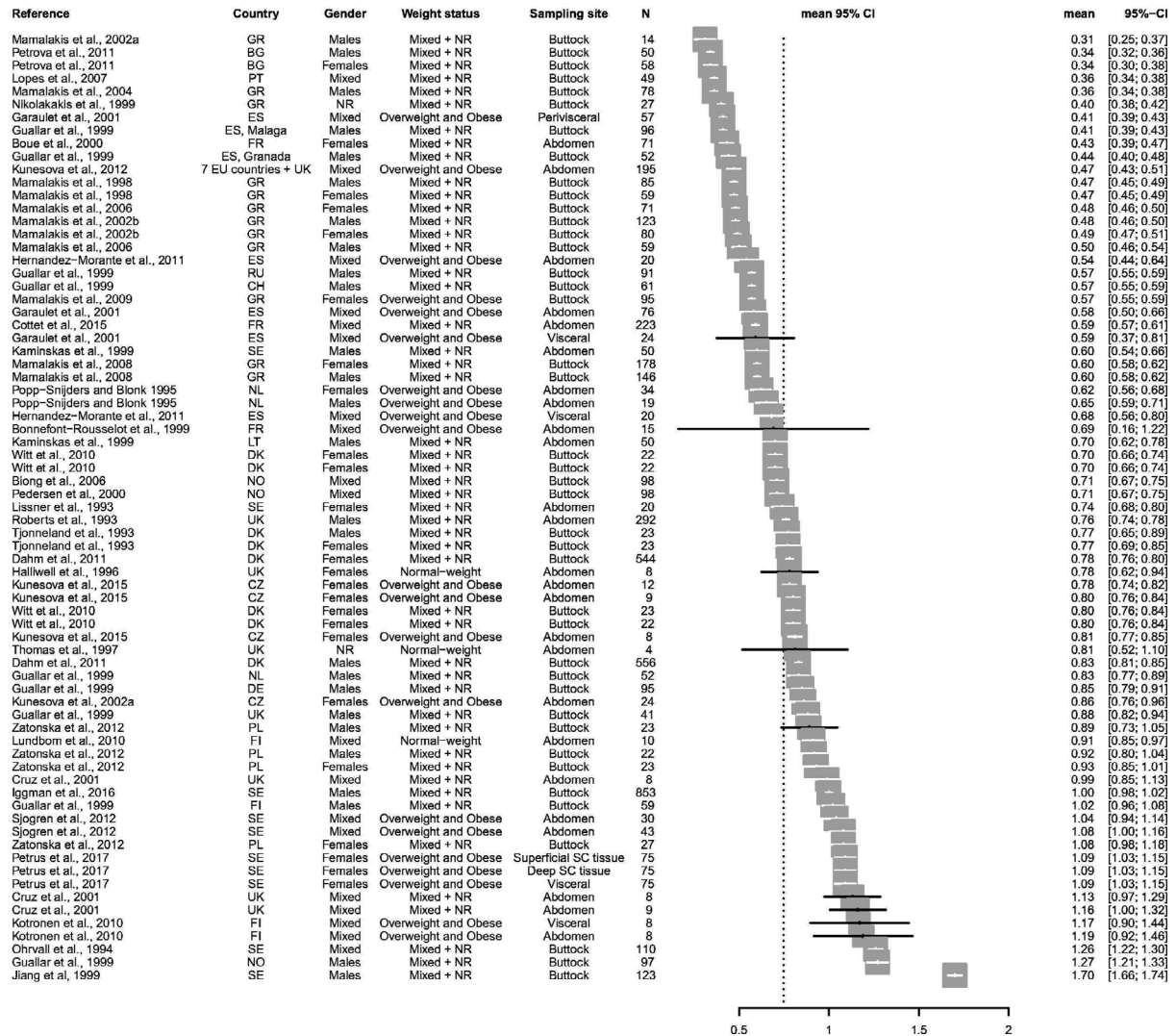| Reference	Country	Gender	Weight status	Sampling site	N	mean	95%-CI
Scazzocchio et al., 2020	IT	Mixed	Normal-weight	Visceral	17	6.82	[6.64; 7.00]
Mamalakis et al., 2004	GR	Males	Mixed + NR	Buttock	78	7.78	[7.54; 8.02]
Scazzocchio et al., 2020	IT	Mixed	Overweight and Obese	Visceral	19	8.01	[7.85; 8.17]
Mamalakis et al., 2002a	GR	Males	Mixed + NR	Buttock	15	8.20	[7.55; 8.85]
Nikolakakis et al., 1999	GR	NR	Mixed + NR	Buttock	27	9.80	[9.19; 10.41]
Mamalakis et al., 2009	GR	Females	Overweight and Obese	Buttock	95	9.83	[9.48; 10.18]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	22	9.90	[9.53; 10.27]
Lundbom et al., 2010	FI	Mixed	Normal-weight	Abdomen	10	9.95	[9.62; 10.28]
Tjonneland et al., 1993	DK	Males	Mixed + NR	Buttock	23	10.08	[8.86; 11.30]
Mamalakis et al., 2008	GR	Males	Mixed + NR	Buttock	146	10.10	[9.85; 10.35]
Petrus et al., 2017	SE	Females	Overweight and Obese	Visceral	75	10.14	[9.85; 10.43]
Dahm et al., 2011	DK	Females	Mixed + NR	Buttock	544	10.20	[9.98; 10.42]
Petrus et al., 2017	SE	Females	Overweight and Obese	Deep SC tissue	75	10.20	[9.89; 10.51]
Venø et al., 2013	DK	Mixed	Overweight and Obese	Buttock	25	10.25	[9.94; 10.56]
Mamalakis et al., 2008	GR	Females	Mixed + NR	Buttock	178	10.30	[10.05; 10.55]
Petrus et al., 2017	SE	Females	Overweight and Obese	Superficial SC tissue	75	10.33	[10.04; 10.62]
Venø et al., 2013	DK	Mixed	Overweight and Obese	Buttock	25	10.36	[9.93; 10.79]
Kunesova et al., 2002a	CZ	Females	Overweight and Obese	Abdomen	24	10.40	[9.75; 11.05]
Lissner et al., 1993	SE	Females	Mixed + NR	Abdomen	20	10.57	[9.69; 11.45]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	22	10.60	[10.15; 11.05]
Kotronen et al., 2010	FI	Mixed	Overweight and Obese	Abdomen	8	10.69	[9.63; 11.75]
Dahm et al., 2011	DK	Males	Mixed + NR	Buttock	556	10.70	[10.54; 10.86]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	22	10.70	[10.25; 11.15]
Jiang et al, 1999	SE	Males	Mixed + NR	Buttock	123	10.80	[10.47; 11.13]
Pinnick et al., 2012	UK	Males	Mixed + NR	Abdomen	18	10.80	[10.21; 11.39]
Pinnick et al., 2012	UK	Males	Mixed + NR	Buttock	18	10.80	[10.21; 11.39]
Due et al., 2008	DK	Mixed	Overweight and Obese	Buttock	30	10.80	[10.41; 11.19]
Tjonneland et al., 1993	DK	Females	Mixed + NR	Buttock	23	10.86	[10.08; 11.64]
Due et al., 2008	DK	Mixed	Overweight and Obese	Buttock	37	10.90	[10.55; 11.25]
Due et al., 2008	DK	Mixed	Overweight and Obese	Buttock	18	10.90	[10.29; 11.51]
Mamalakis et al., 2002b	GR	Males	Mixed + NR	Buttock	123	10.90	[10.57; 11.23]
Kaminskas et al., 1999	SE	Males	Mixed + NR	Abdomen	50	11.00	[10.61; 11.39]
Mamalakis et al., 1998	GR	Males	Mixed + NR	Buttock	85	11.06	[10.65; 11.47]
Cottet et al., 2015	FR	Mixed	Mixed + NR	Abdomen	223	11.08	[10.73; 11.43]
Pinnick et al., 2012	UK	Females	Mixed + NR	Abdomen	18	11.10	[10.12; 12.08]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	23	11.10	[10.61; 11.59]
Biong et al., 2006	NO	Mixed	Mixed + NR	Buttock	98	11.23	[10.80; 11.66]
Pedersen et al., 2000	NO	Mixed	Mixed + NR	Buttock	98	11.23	[10.80; 11.66]
Kotronen et al., 2010	FI	Mixed	Overweight and Obese	Visceral	8	11.24	[10.08; 12.40]
Kaminskas et al., 1999	LT	Males	Mixed + NR	Abdomen	50	11.50	[10.91; 12.09]
Pinnick et al., 2012	UK	Females	Mixed + NR	Buttock	18	11.50	[10.52; 12.48]
Zatonska et al., 2012	PL	Males	Mixed + NR	Buttock	23	11.62	[10.50; 12.74]
Hodson et al., 2014	UK	Females	Mixed + NR	Buttock	19	11.80	[11.21; 12.39]
Mamalakis et al., 2002b	GR	Females	Mixed + NR	Buttock	80	11.90	[11.47; 12.33]
Mamalakis et al., 1998	GR	Females	Mixed + NR	Buttock	59	12.00	[11.47; 12.53]
Mamalakis et al., 2006	GR	Males	Mixed + NR	Buttock	59	12.00	[11.41; 12.59]
Thomas et al., 1997	UK	NR	Normal-weight	Abdomen	4	12.05	[8.42; 15.68]
Roberts et al., 1993	UK	Males	Mixed + NR	Abdomen	292	12.10	[11.65; 12.55]
Zatonska et al., 2012	PL	Females	Mixed + NR	Buttock	23	12.10	[11.57; 12.63]
Mamalakis et al., 2006	GR	Females	Mixed + NR	Buttock	71	12.40	[10.83; 13.97]
Kunesova et al., 2012	7 EU countries + UK	Mixed	Overweight and Obese	Abdomen	195	12.45	[12.16; 12.74]
Zatonska et al., 2012	PL	Males	Mixed + NR	Buttock	22	12.57	[11.79; 13.35]
Iggman et al., 2016	SE	Males	Mixed + NR	Buttock	853	12.70	[12.50; 12.90]
Kunesova et al., 2015	CZ	Females	Overweight and Obese	Abdomen	12	12.90	[12.45; 13.35]
Kunesova et al., 2015	CZ	Females	Overweight and Obese	Abdomen	8	13.00	[12.45; 13.55]
Zatonska et al., 2012	PL	Females	Mixed + NR	Buttock	27	13.10	[12.00; 14.20]
Hodson et al., 2014	UK	Females	Mixed + NR	Buttock	23	13.10	[12.12; 14.08]
Kunesova et al., 2015	CZ	Females	Overweight and Obese	Abdomen	9	13.30	[12.79; 13.81]
Cruz et al., 2001	UK	Mixed	Mixed + NR	Abdomen	8	13.38	[11.73; 15.03]
Ohrvall et al., 1994	SE	Mixed	Mixed + NR	Buttock	110	13.40	[13.09; 13.71]
Walker et al., 2015	UK	Mixed	Mixed + NR	Abdomen	202	13.40	[13.03; 13.77]
Cruz et al., 2001	UK	Mixed	Mixed + NR	Abdomen	9	13.55	[11.96; 15.14]
Cantwell et al., 2005	IE	Mixed	Mixed + NR	Abdomen	84	13.80	[13.27; 14.33]
Feunekes et al., 1993	NL	Mixed	Mixed + NR	Buttock	55	14.20	[13.55; 14.85]
Popp-Snijders and Blonk 1995	NL	Females	Overweight and Obese	Abdomen	34	14.23	[13.29; 15.17]
Boue et al., 2000	FR	Females	Mixed + NR	Abdomen	71	14.35	[13.64; 15.06]
Garaulet et al., 2011	ES	Mixed	Overweight and Obese	Abdomen	20	14.60	[12.62; 16.58]
Popp-Snijders and Blonk 1995	NL	Males	Overweight and Obese	Abdomen	19	14.62	[12.95; 16.29]
Garaulet et al., 2001	ES	Mixed	Overweight and Obese	Perivisceral	57	14.82	[13.59; 16.05]
Garaulet et al., 2011	ES	Mixed	Overweight and Obese	Visceral	20	15.00	[13.51; 16.49]
Cruz et al., 2001	UK	Mixed	Mixed + NR	Abdomen	8	15.03	[13.31; 16.75]
Garaulet et al., 2001	ES	Mixed	Overweight and Obese	Abdomen	76	15.12	[13.94; 16.30]
Halliwell et al., 1996	UK	Females	Normal-weight	Abdomen	8	15.12	[13.22; 17.02]
Garaulet et al., 2011	ES	Mixed	Overweight and Obese	Abdomen	20	15.30	[13.50; 17.10]
Bonnefont-Rousselot et al., 1999	FR	Mixed	Overweight and Obese	Abdomen	15	15.70	[13.54; 17.86]
Garaulet et al., 2001	ES	Mixed	Overweight and Obese	Visceral	24	15.87	[14.28; 17.46]
Sledzinski et al., 2013	PL	Females	Overweight and Obese	Abdomen	16	16.00	[14.67; 17.33]
Hernandez-Morante et al., 2011	ES	Mixed	Overweight and Obese	Visceral	20	16.23	[15.19; 17.27]
Hernandez-Morante et al., 2011	ES	Mixed	Overweight and Obese	Abdomen	20	16.37	[15.37; 17.37]
Garaulet et al., 2011	ES	Mixed	Overweight and Obese	Visceral	20	16.40	[14.83; 17.97]
Garaulet et al., 2011	ES	Mixed	Overweight and Obese	Visceral	20	17.10	[15.22; 18.98]
Garaulet et al., 2011	ES	Mixed	Overweight and Obese	Abdomen	20	18.00	[15.84; 20.16]
Sledzinski et al., 2013	PL	Females	Overweight and Obese	Visceral	16	18.30	[16.63; 19.97]
Lopes et al., 2007	PT	Mixed	Mixed + NR	Buttock	49	19.10	[17.98; 20.22]
Petrova et al., 2011	BG	Males	Mixed + NR	Buttock	50	25.20	[24.18; 26.22]
Petrova et al., 2011	BG	Females	Mixed + NR	Buttock	58	27.30	[26.46; 28.14]

B.2. Alpha-linolenic acid concentrations in adipose tissue – studies from 1990

Reference	Country	Gender	Weight status	Sampling site	N	mean 95% CI	mean	95%–CI
Mamalakis et al., 2002a	GR	Males	Mixed + NR	Buttock	14		0.31	[0.25; 0.37]
Petrova et al., 2011	BG	Males	Mixed + NR	Buttock	50		0.34	[0.32; 0.36]
Petrova et al., 2011	BG	Females	Mixed + NR	Buttock	58		0.34	[0.30; 0.38]
Lopes et al., 2007	PT	Mixed	Mixed + NR	Buttock	49		0.36	[0.34; 0.38]
Mamalakis et al., 2004	GR	Males	Mixed + NR	Buttock	78		0.36	[0.34; 0.38]
Nikolakakis et al., 1999	GR	NR	Mixed + NR	Buttock	27		0.40	[0.38; 0.42]
Garaulet et al., 2001	ES	Mixed	Overweight and Obese	Perivisceral	57		0.41	[0.39; 0.43]
Guallar et al., 1999	ES, Malaga	Males	Mixed + NR	Buttock	96		0.41	[0.39; 0.43]
Boue et al., 2000	FR	Females	Mixed + NR	Abdomen	71		0.43	[0.39; 0.47]
Guallar et al., 1999	ES, Granada	Males	Mixed + NR	Buttock	52		0.44	[0.40; 0.48]
Kunesova et al., 2012	7 EU countries + UK	Mixed	Overweight and Obese	Abdomen	195		0.47	[0.43; 0.51]
Mamalakis et al., 1998	GR	Males	Mixed + NR	Buttock	85		0.47	[0.45; 0.49]
Mamalakis et al., 1998	GR	Females	Mixed + NR	Buttock	59		0.47	[0.45; 0.49]
Mamalakis et al., 2006	GR	Females	Mixed + NR	Buttock	71		0.48	[0.46; 0.50]
Mamalakis et al., 2002b	GR	Males	Mixed + NR	Buttock	123		0.48	[0.46; 0.50]
Mamalakis et al., 2002b	GR	Females	Mixed + NR	Buttock	80		0.49	[0.47; 0.51]
Mamalakis et al., 2006	GR	Males	Mixed + NR	Buttock	59		0.50	[0.46; 0.54]
Hernandez–Morante et al., 2011	ES	Mixed	Overweight and Obese	Abdomen	20		0.54	[0.44; 0.64]
Guallar et al., 1999	RU	Males	Mixed + NR	Buttock	91		0.57	[0.55; 0.59]
Guallar et al., 1999	CH	Males	Mixed + NR	Buttock	61		0.57	[0.55; 0.59]
Mamalakis et al., 2009	GR	Females	Overweight and Obese	Buttock	95		0.57	[0.55; 0.59]
Garaulet et al., 2001	ES	Mixed	Overweight and Obese	Abdomen	76		0.58	[0.50; 0.66]
Cottet et al., 2015	FR	Mixed	Mixed + NR	Abdomen	223		0.59	[0.57; 0.61]
Garaulet et al., 2001	ES	Mixed	Overweight and Obese	Visceral	24		0.59	[0.37; 0.81]
Kaminskas et al., 1999	SE	Males	Mixed + NR	Abdomen	50		0.60	[0.54; 0.66]
Mamalakis et al., 2008	GR	Females	Mixed + NR	Buttock	178		0.60	[0.58; 0.62]
Mamalakis et al., 2008	GR	Males	Mixed + NR	Buttock	146		0.60	[0.58; 0.62]
Popp–Snijders and Blonk 1995	NL	Females	Overweight and Obese	Abdomen	34		0.62	[0.56; 0.68]
Popp–Snijders and Blonk 1995	NL	Males	Overweight and Obese	Abdomen	19		0.65	[0.59; 0.71]
Hernandez–Morante et al., 2011	ES	Mixed	Overweight and Obese	Visceral	20		0.68	[0.56; 0.80]
Bonnefont–Rousselot et al., 1999	FR	Mixed	Overweight and Obese	Abdomen	15		0.69	[0.16; 1.22]
Kaminskas et al., 1999	LT	Males	Mixed + NR	Abdomen	50		0.70	[0.62; 0.78]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	22		0.70	[0.66; 0.74]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	22		0.70	[0.66; 0.74]
Biong et al., 2006	NO	Mixed	Mixed + NR	Buttock	98		0.71	[0.67; 0.75]
Pedersen et al., 2000	NO	Mixed	Mixed + NR	Buttock	98		0.71	[0.67; 0.75]
Lissner et al., 1993	SE	Females	Mixed + NR	Abdomen	20		0.74	[0.68; 0.80]
Roberts et al., 1993	UK	Males	Mixed + NR	Abdomen	292		0.76	[0.74; 0.78]
Tjonneland et al., 1993	DK	Males	Mixed + NR	Buttock	23		0.77	[0.65; 0.89]
Tjonneland et al., 1993	DK	Females	Mixed + NR	Buttock	23		0.77	[0.69; 0.85]
Dahm et al., 2011	DK	Females	Mixed + NR	Buttock	544		0.78	[0.76; 0.80]
Halliwell et al., 1996	UK	Females	Normal–weight	Abdomen	8		0.78	[0.62; 0.94]
Kunesova et al., 2015	CZ	Females	Overweight and Obese	Abdomen	12		0.78	[0.74; 0.82]
Kunesova et al., 2015	CZ	Females	Overweight and Obese	Abdomen	9		0.80	[0.76; 0.84]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	23		0.80	[0.76; 0.84]
Witt et al., 2010	DK	Females	Mixed + NR	Buttock	22		0.80	[0.76; 0.84]
Kunesova et al., 2015	CZ	Females	Overweight and Obese	Abdomen	8		0.81	[0.77; 0.85]
Thomas et al., 1997	UK	NR	Normal–weight	Abdomen	4		0.81	[0.52; 1.10]
Dahm et al., 2011	DK	Males	Mixed + NR	Buttock	556		0.83	[0.81; 0.85]
Guallar et al., 1999	NL	Males	Mixed + NR	Buttock	52		0.83	[0.77; 0.89]
Guallar et al., 1999	DE	Males	Mixed + NR	Buttock	95		0.85	[0.79; 0.91]
Kunesova et al., 2002a	CZ	Females	Overweight and Obese	Abdomen	24		0.86	[0.76; 0.96]
Guallar et al., 1999	UK	Males	Mixed + NR	Buttock	41		0.88	[0.82; 0.94]
Zatonska et al., 2012	PL	Males	Mixed + NR	Buttock	23		0.89	[0.73; 1.05]
Lundbom et al., 2010	FI	Mixed	Normal–weight	Abdomen	10		0.91	[0.85; 0.97]
Zatonska et al., 2012	PL	Males	Mixed + NR	Buttock	22		0.92	[0.80; 1.04]
Zatonska et al., 2012	PL	Females	Mixed + NR	Buttock	23		0.93	[0.85; 1.01]
Cruz et al., 2001	UK	Mixed	Mixed + NR	Abdomen	8		0.99	[0.85; 1.13]
Iggman et al., 2016	SE	Males	Mixed + NR	Buttock	853		1.00	[0.98; 1.02]
Guallar et al., 1999	FI	Males	Mixed + NR	Buttock	59		1.02	[0.96; 1.08]
Sjogren et al., 2012	SE	Mixed	Overweight and Obese	Abdomen	30		1.04	[0.94; 1.14]
Sjogren et al., 2012	SE	Mixed	Overweight and Obese	Abdomen	43		1.08	[1.00; 1.16]
Zatonska et al., 2012	PL	Females	Mixed + NR	Buttock	27		1.08	[0.98; 1.18]
Petrus et al., 2017	SE	Females	Overweight and Obese	Superficial SC tissue	75		1.09	[1.03; 1.15]
Petrus et al., 2017	SE	Females	Overweight and Obese	Deep SC tissue	75		1.09	[1.03; 1.15]
Petrus et al., 2017	SE	Females	Overweight and Obese	Visceral	75		1.09	[1.03; 1.15]
Cruz et al., 2001	UK	Mixed	Mixed + NR	Abdomen	8		1.13	[0.97; 1.29]
Cruz et al., 2001	UK	Mixed	Mixed + NR	Abdomen	9		1.16	[1.00; 1.32]
Kotronen et al., 2010	FI	Mixed	Overweight and Obese	Visceral	8		1.17	[0.90; 1.44]
Kotronen et al., 2010	FI	Mixed	Overweight and Obese	Abdomen	8		1.19	[0.92; 1.46]
Ohrvall et al., 1994	SE	Mixed	Mixed + NR	Buttock	110		1.26	[1.22; 1.30]
Guallar et al., 1999	NO	Males	Mixed + NR	Buttock	97		1.27	[1.21; 1.33]
Jiang et al, 1999	SE	Males	Mixed + NR	Buttock	123		1.70	[1.66; 1.74]

Axis: 0.5 1 1.5 2

Footnotes to Appendices B.1 and B.2. BG = Bulgaria; CH = Switzerland; CI = confidence interval; CZ = Czechia; DE = Germany; DK = Denmark; ES = Spain; EU = European Union; FI = Finland; FR = France; GR = Greece; IE = Ireland; IT = Italy; LT = Lithuania; Mixed = study populations consisting of mixtures of weight statuses; N = number of subjects in the study population; NL = Netherlands; NO = Norway; NR = not reported; PL = Poland; PT = Portugal; RU = Russia Federation; SC = subcutaneous; SE = Sweden; UK = United Kingdom.

Appendix C − Scatter plots of fatty acid concentrations in adipose tissue

The following scatter plots depict how the linoleic acid (LA) and alpha-linolenic acid (ALA) concentrations in adipose tissue are affected by a number of variables, i.e. gender, country, year of study conduct, weight status, health status, sampling site, unit of measurement and some combinations of these criteria.
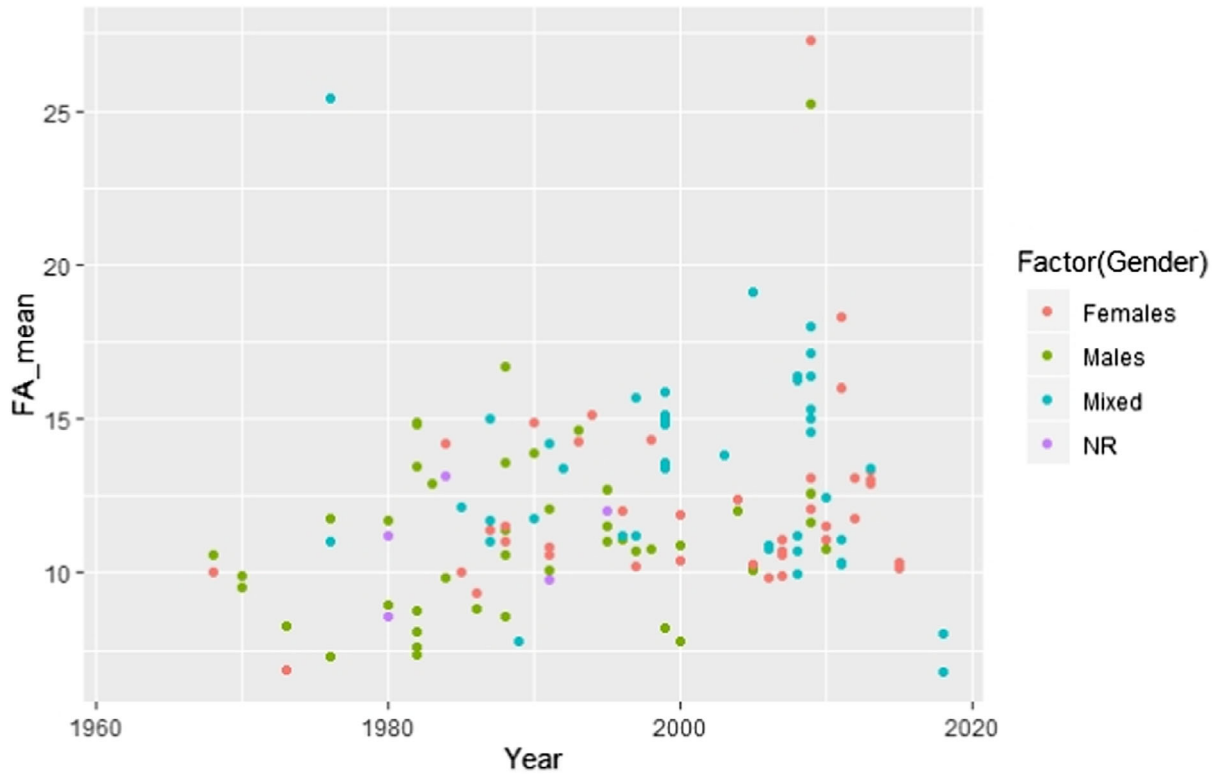
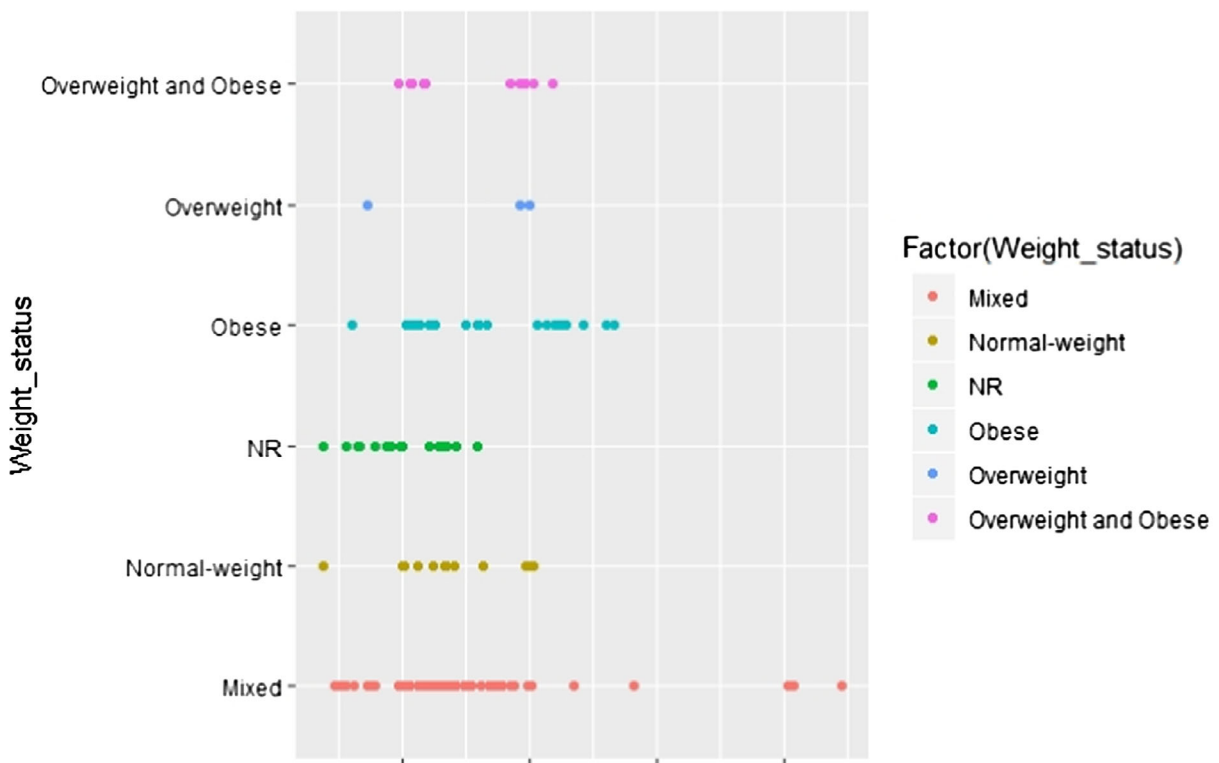### C.1. Mean linoleic acid concentration in adipose tissue by gender
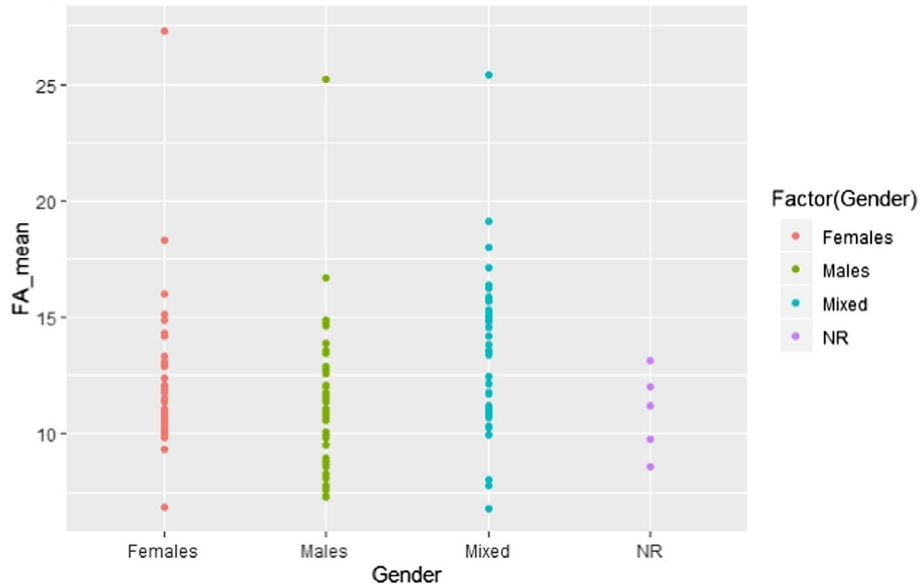
C.2. Mean linoleic acid concentration in adipose tissue by gender and country
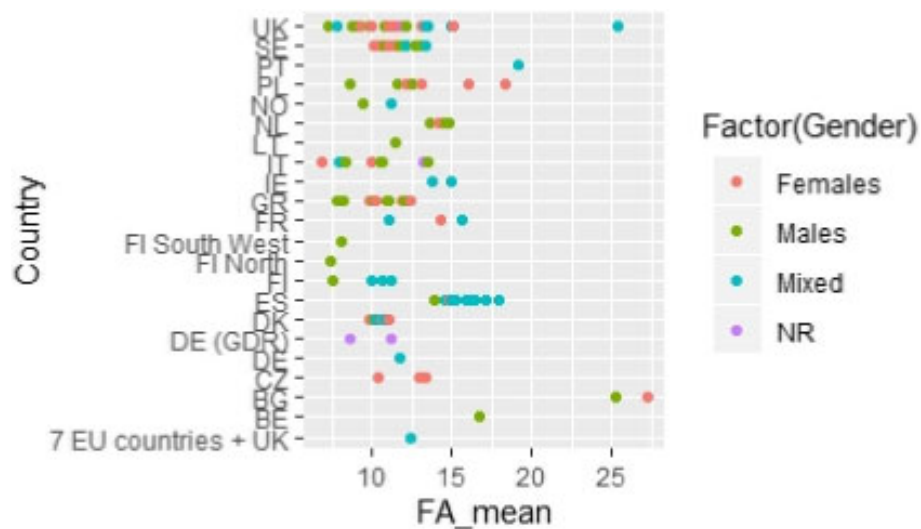
C.3. Mean linoleic acid concentration in adipose tissue by gender and year of study conduct

C.4. Mean linoleic acid concentration in adipose tissue by weight status

C.5. Mean linoleic acid concentration in adipose tissue by weight status collapsed into three categories (called in the following simplified)
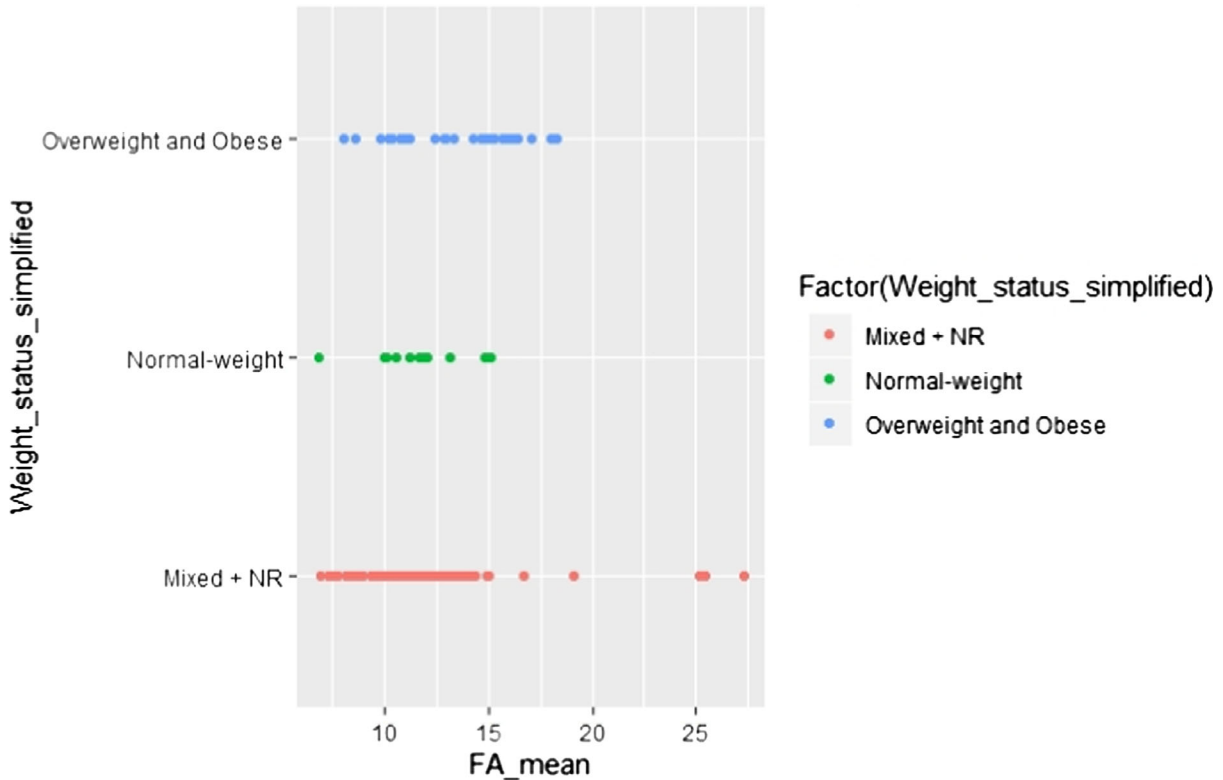
C.6. Mean linoleic acid concentration in adipose tissue by weight status (simplified) and year of study conduct
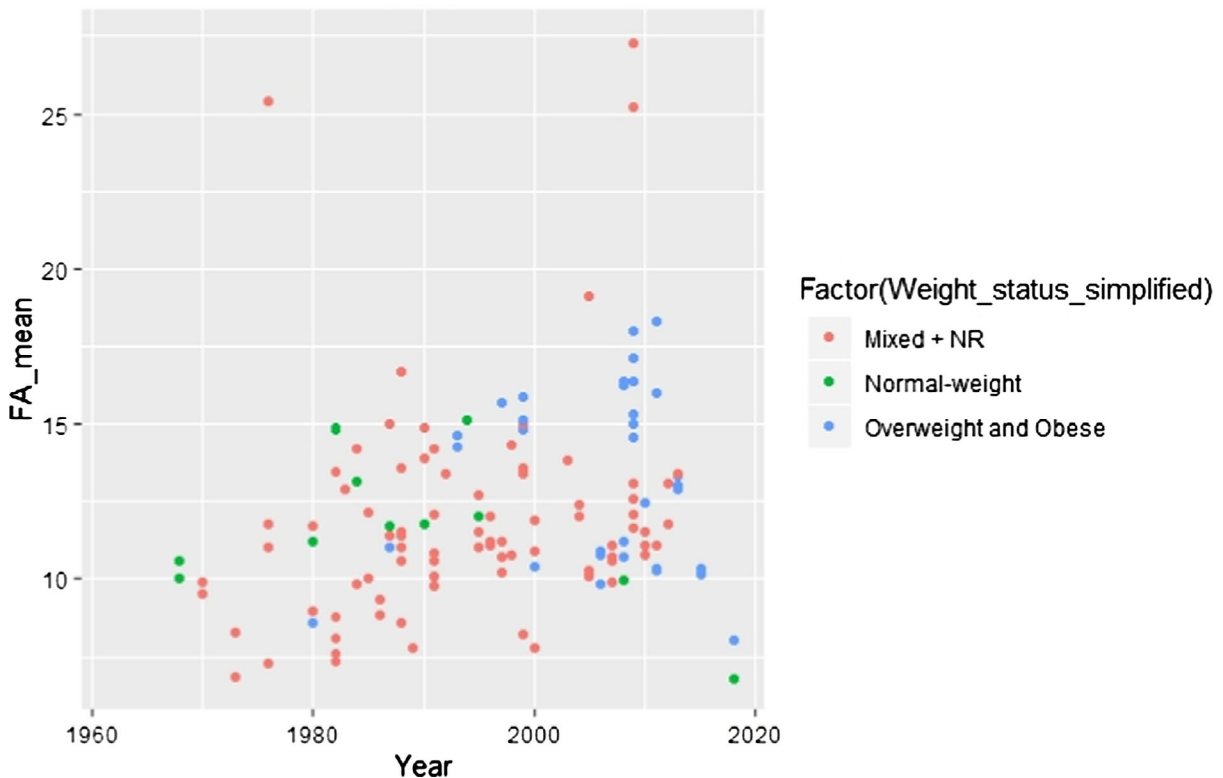
C.7. Mean linoleic acid concentration in adipose tissue by health status
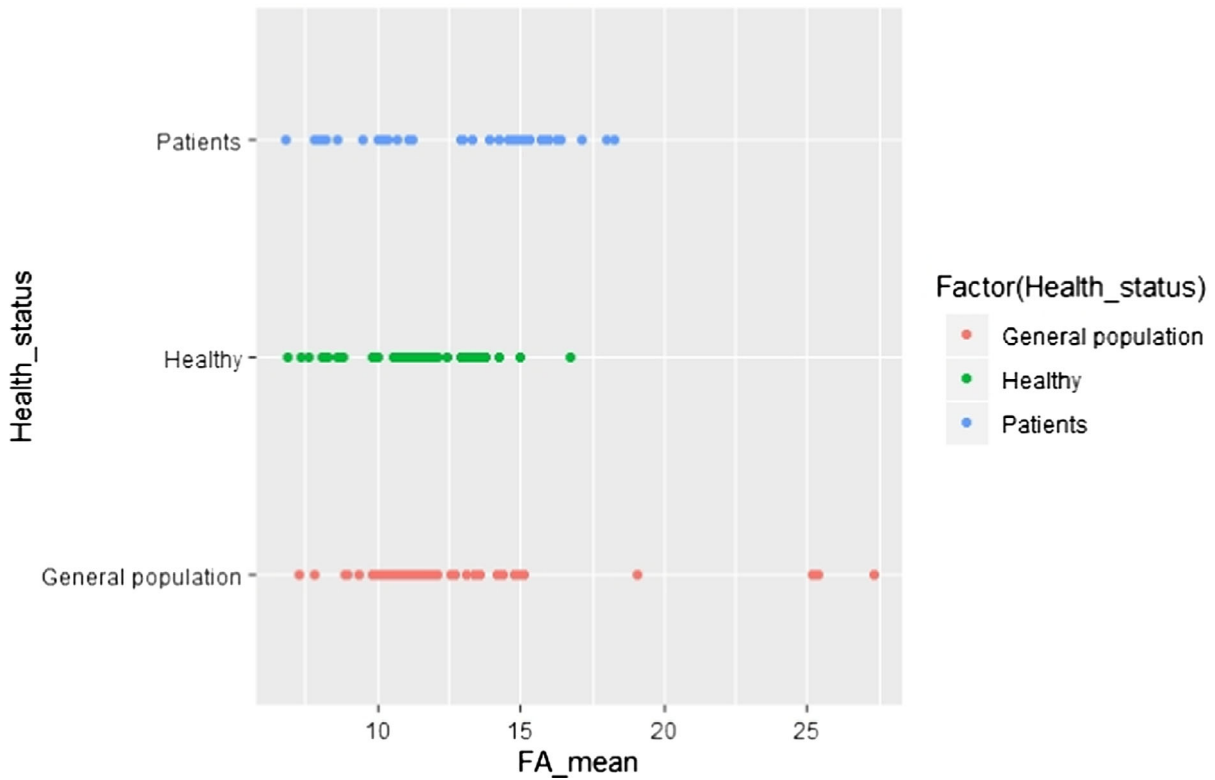
C.8. Mean linoleic acid concentration in adipose tissue by sampling site
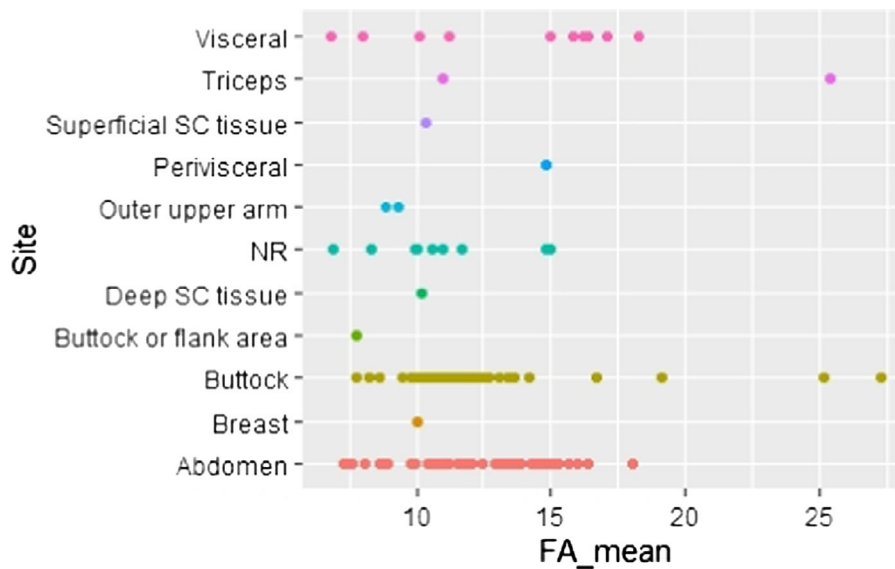
C.9. Mean linoleic acid concentration in adipose tissue by unit of measurement
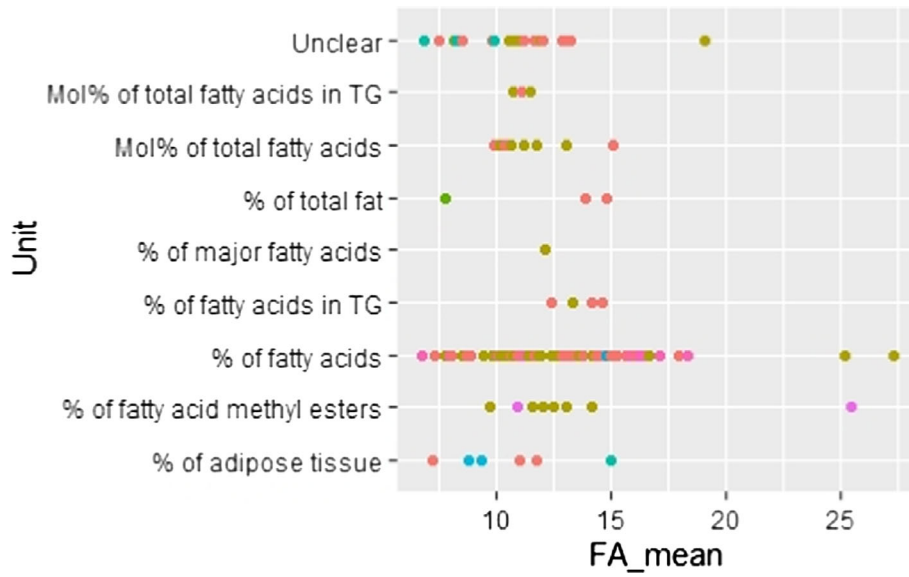
C.10. Mean alpha-linolenic acid concentration in adipose tissue by gender
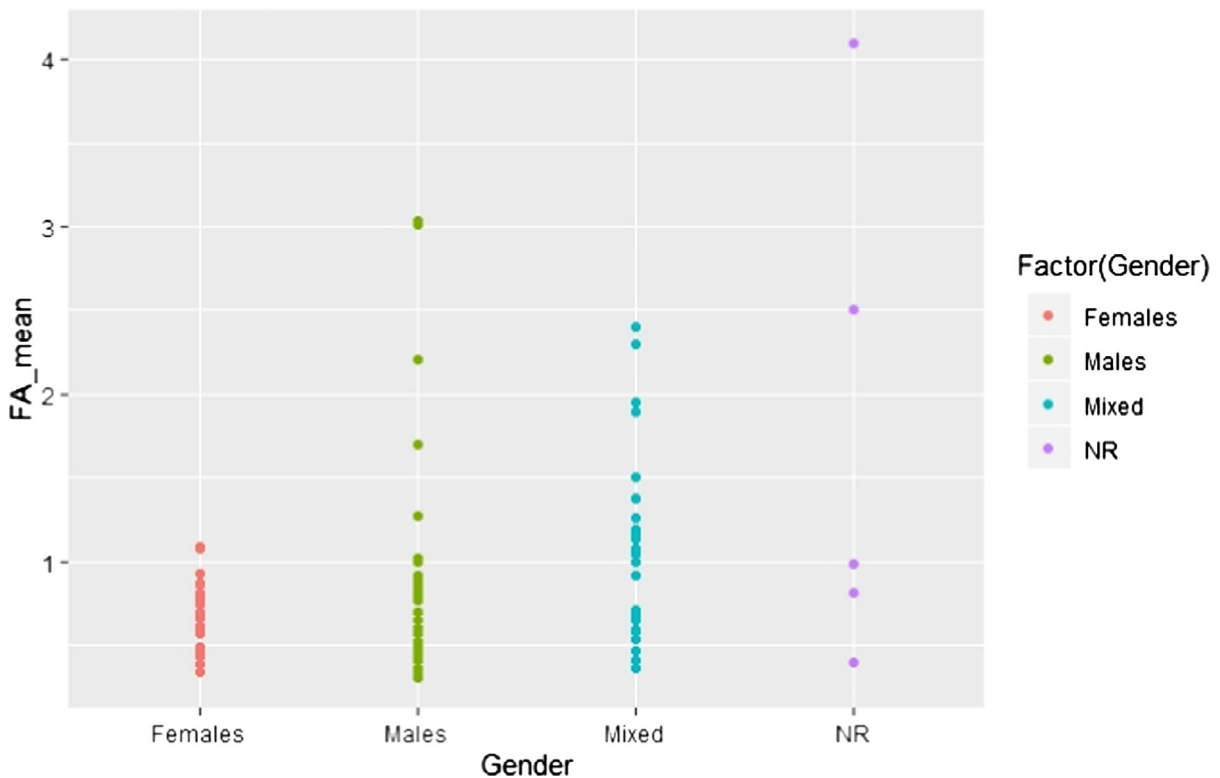
C.11. Mean alpha-linolenic acid concentration in adipose tissue by gender and country
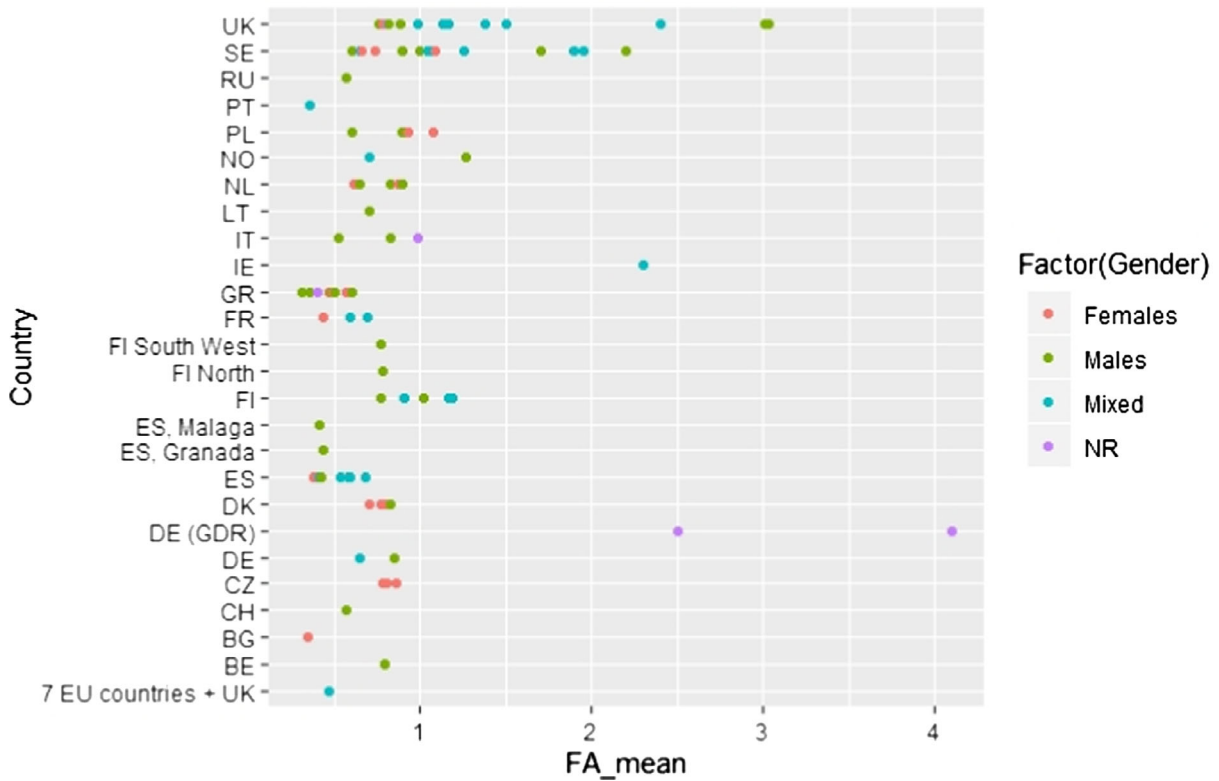
C.12. Mean alpha-linolenic acid concentration in adipose tissue by gender and year of study conduct
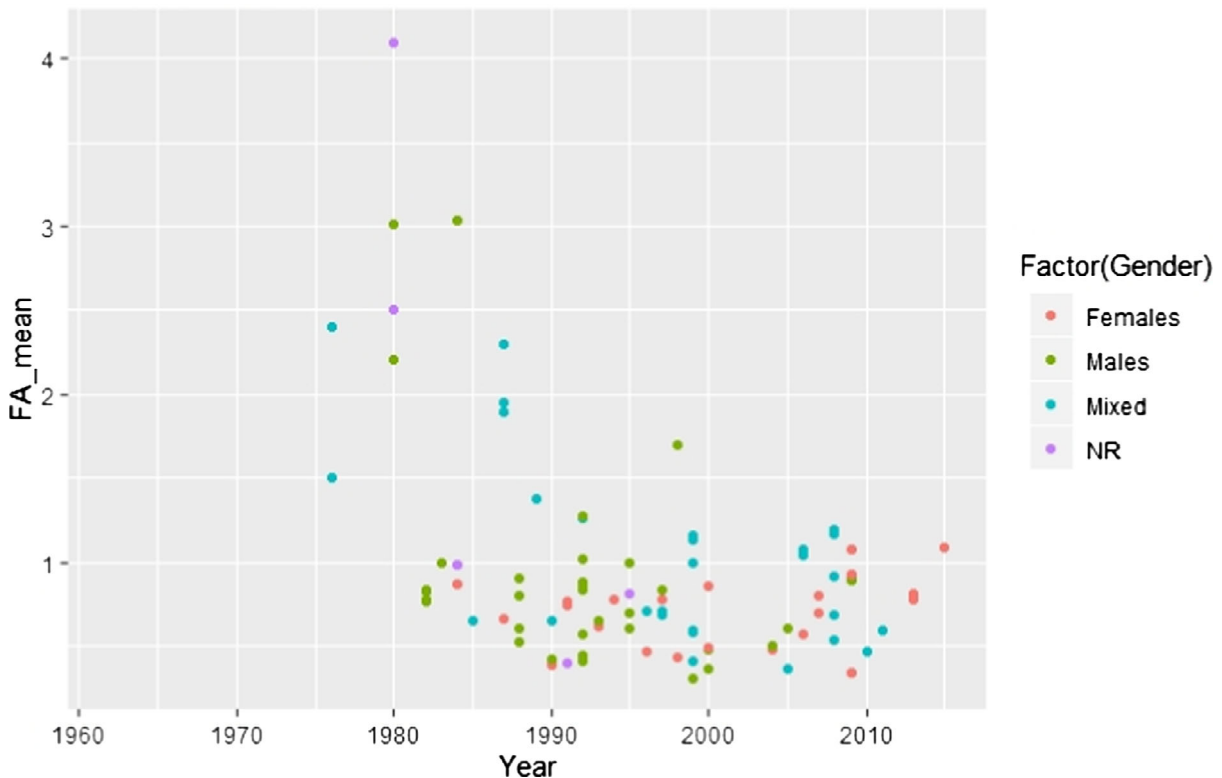
C.13. Mean alpha-linolenic acid concentration in adipose tissue by weight status
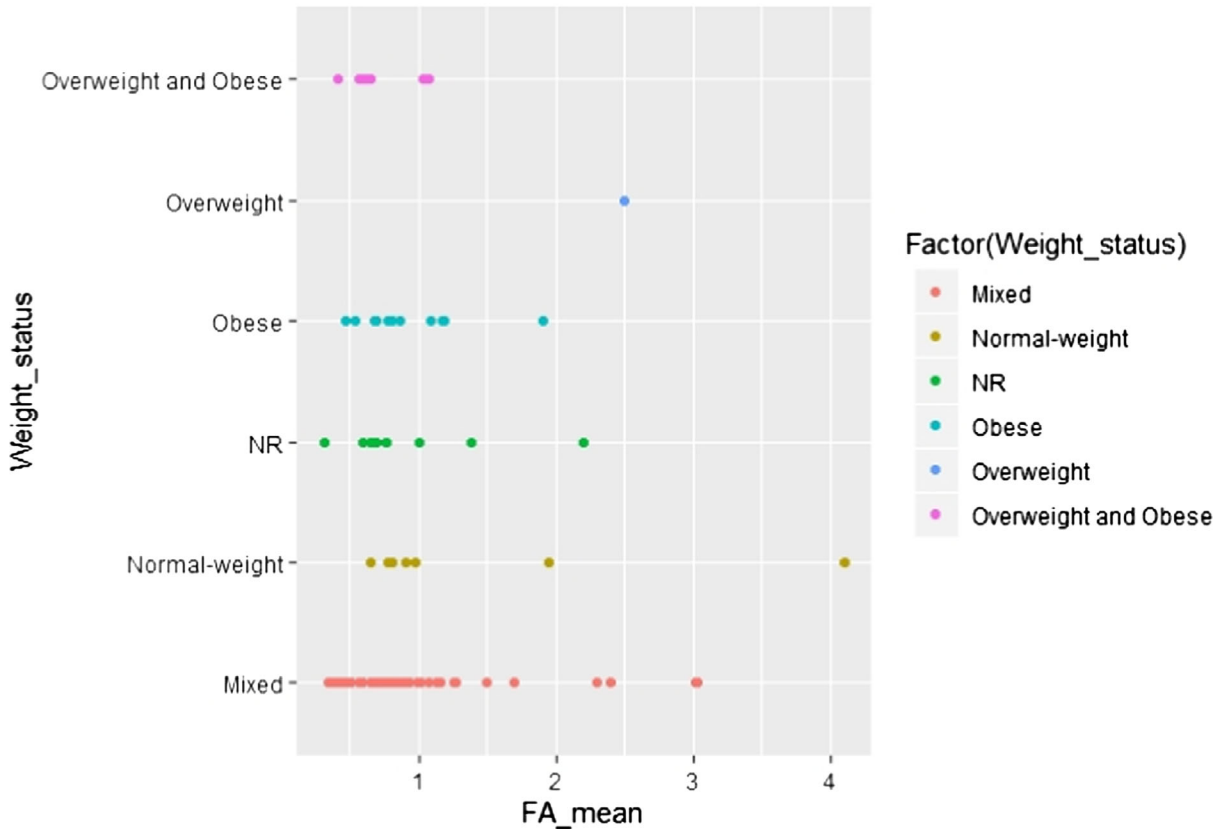
C.14. Mean alpha-linolenic acid concentration in adipose tissue by weight status (simplified)
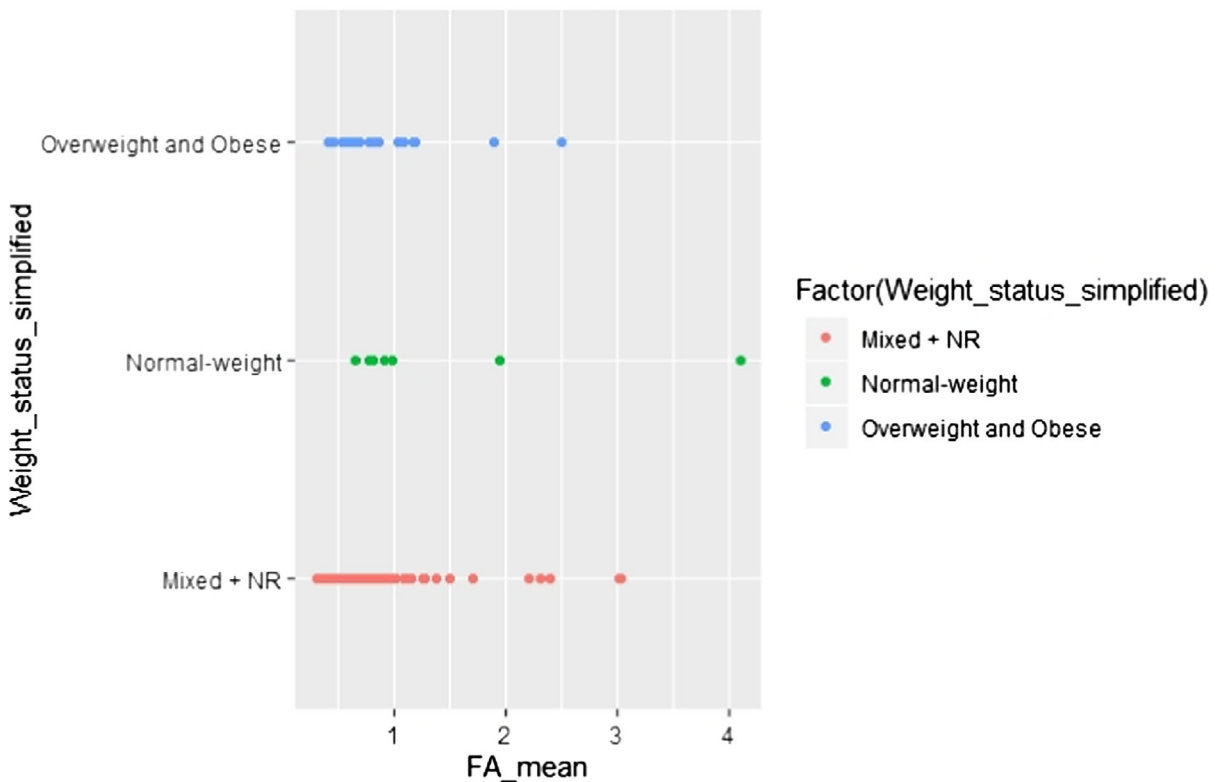
C.15. Mean alpha-linolenic acid concentration in adipose tissue by weight status (simplified) and year of study conduct

C.16. Mean alpha-linolenic acid concentration in adipose tissue by health status
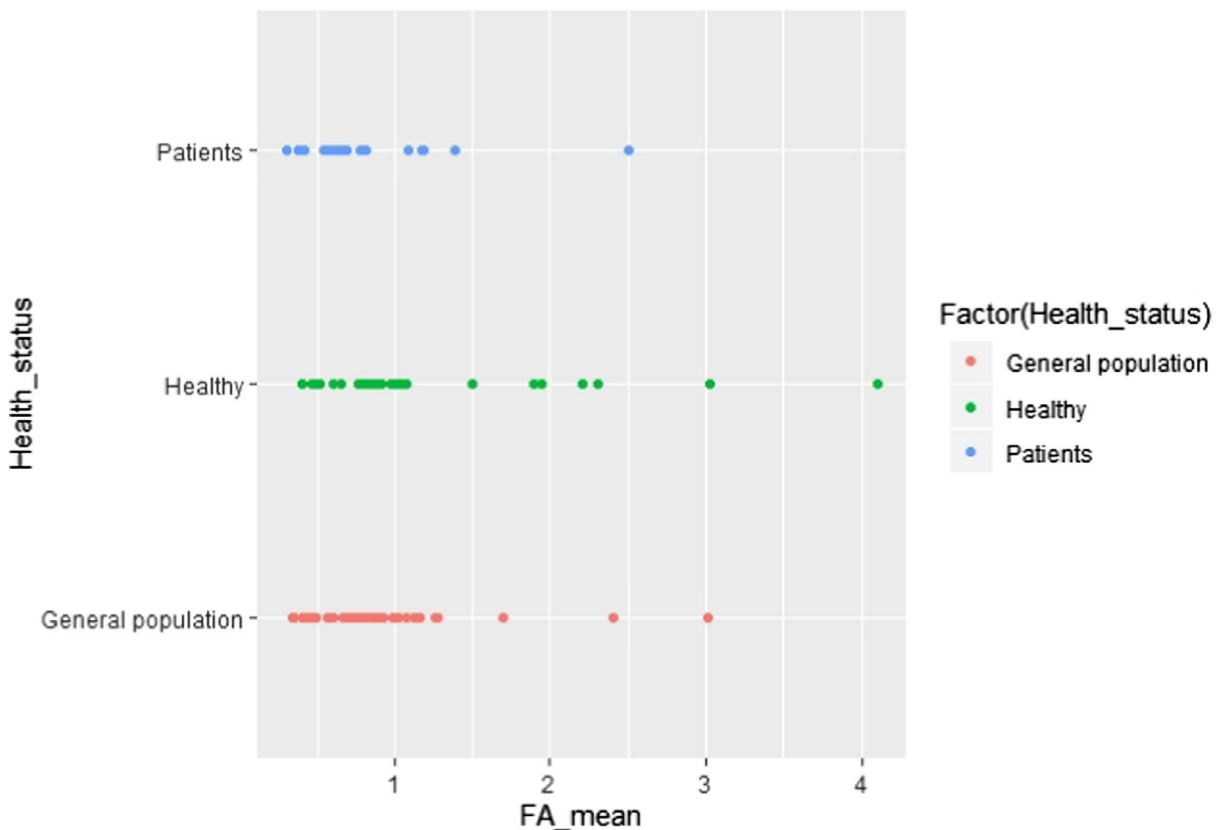
C.17. Mean alpha-linolenic acid concentration in adipose tissue by sampling site
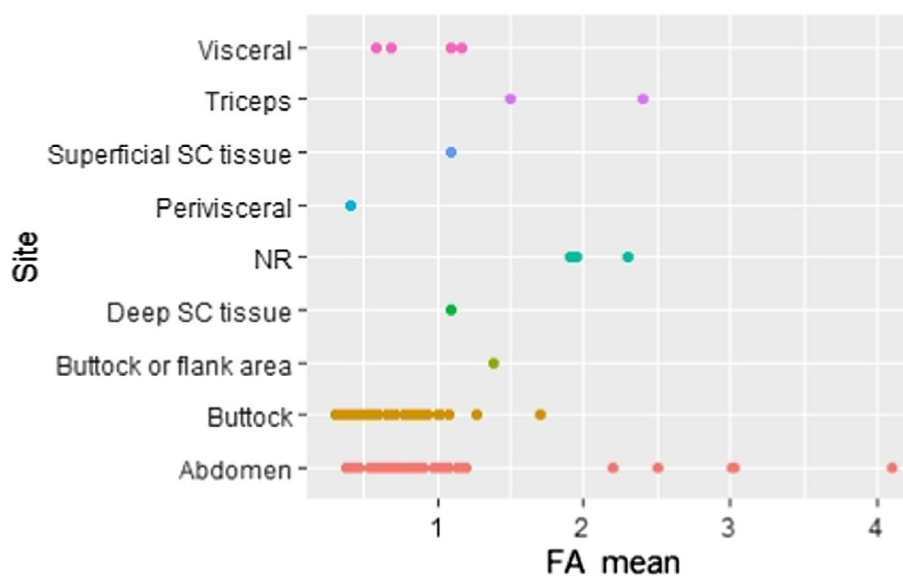
C.18. Mean alpha-linolenic acid concentration in adipose tissue by unit of measurement
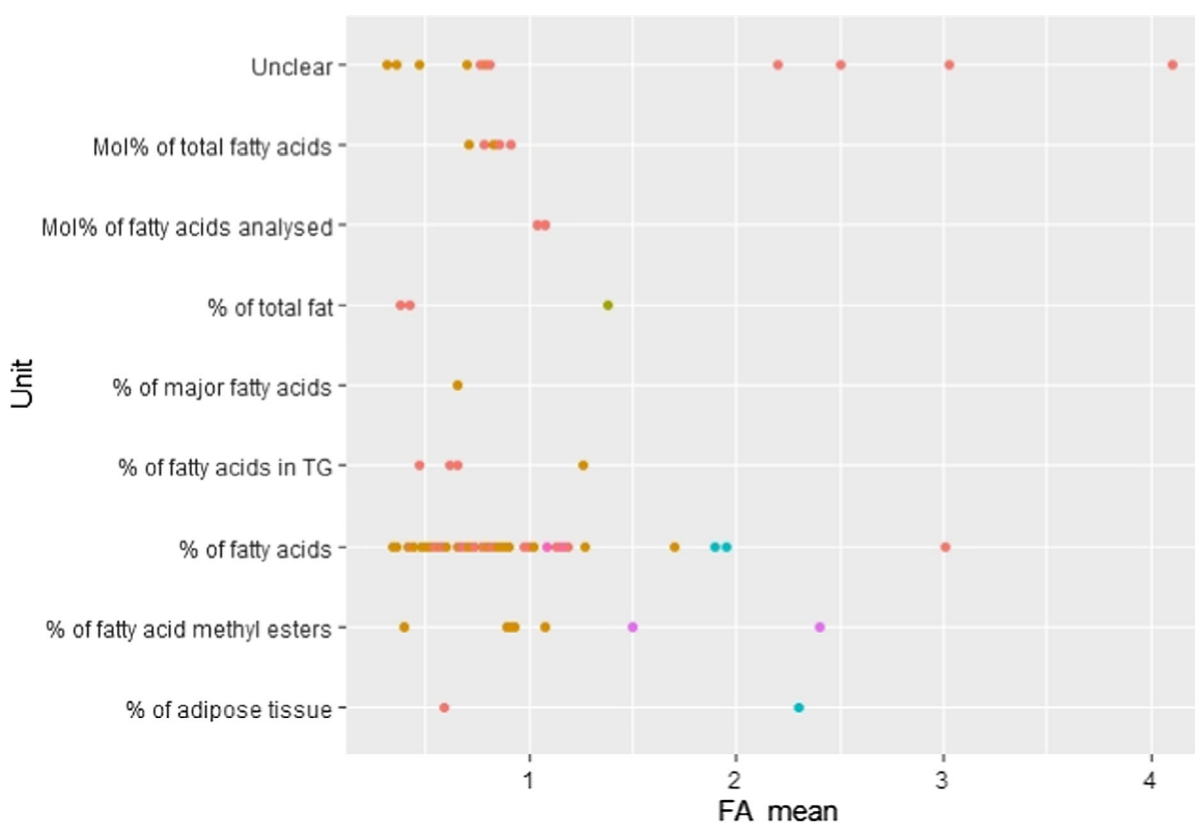
Abbreviations to Appendices C. BE = Belgium; BG = Bulgaria; CH = Switzerland; CZ = Czechia; DE = Germany; DK = Denmark; ES = Spain; FA = fatty acids; FI = Finland; FR = France; GDR = German Democratic Republic; GR = Greece; IE = Ireland; IT = Italy; LT = Lithuania; Mixed (Gender) = males and females; NL = Netherlands; NO = Norway; NR = not reported; PL = Poland; PT = Portugal; RU = Russia Federation; SC = subcutaneous; SE = Sweden; TG = triglycerides; UK = United Kingdom.

Appendix D – Protocol for the assessment of additional scientific evidence in relation to the composition of total diet replacements for weight control with respect to linoleic acid and alpha-linolenic acid as well as magnesium

Assessment questions and subquestions

The following protocol has been developed in line with current EFSA methodology (EFSA, 2020). As described in the 2020 EFSA 'Draft framework for protocol development for EFSA's scientific assessments', the extent of planning ahead of the assessment is determined as 'low' in order to accommodate the deadline (February 2021) and resources available, although this does not affect the complexity of the subject and of the methodologies to be applied.

In order to answer the Terms of Reference (ToR) as interpreted in Section 1.2 of the statement, the Panel considers that the following questions and subquestions need to be answered.

For point A (Section 1.2):

A.1. What is the amount of linoleic acid (LA) and alpha-linolenic acid (ALA) stored in adipose tissue (AT) in overweight and obese individuals living in Europe?

In order to identify the scientific evidence relevant for the assessment, the following subquestions may need to be answered:

- Do the methods for fat store measurement influence the results and, if so, what are the effects?
- Is the LA and ALA concentration in AT proportionally different between normal weight, overweight and obese individuals?
- Are the LA and ALA concentrations/proportions in AT different between different body sites (i.e. does the sampling site influence the assessment of the data), if so, what would be appropriate sampling sites in overweight and obese (not normal weight)?
- What are the fat stores that are released first during weight loss?
- What is the amount of LA and ALA that is released from AT during weight loss?
- How is the proportion of LA and ALA in AT affected by:

  - lifestyle factors (i.e. diet, physical activity)
  - gender,
  - age,
  - ethnicity,
  - and/or diseases (i.e. hypertension, type 2 diabetes mellitus (T2DM), hyperlipidaemia, cardiovascular diseases) in the overweight and obese? If so, which disease(s)? In this context, it should also be considered that overweight and obese individuals consuming total diet replacements for weight control (TDRs) under medical supervision may not only be healthy individuals.

- Does medication intake for the diseases listed above influence the LA and ALA concentration of AT?
- To what extent are LA and ALA released from AT during weight loss reutilised?

A.2. Is there preferential release of certain fatty acids (FA) from AT during weight loss?

In addition to all potential influencing factors listed above that could also influence the release of LA and ALA from AT, the following sub-questions may need to be answered to identify the relevant scientific evidence:

- Does the release of LA and ALA from AT depend on the duration of energy restriction and/or intensity of weight loss?
- Is there preferential release from certain fat depots?
- What are appropriate study designs? Do subjects necessarily need to have lost weight or is a fasting state sufficient? What are the appropriate techniques to measure FA release from AT?
- What is the expected range of weight loss in overweight and obese individuals consuming TDRs?

For point C (Section 1.2):

C.1. What is the lowest fat content consumed in an energy-restricted diet that does not give rise to concern with respect to an increased risk of gallstone formation?

For points D (Section 1.2):

D.1. What is the amount of supplemental magnesium (Mg) consumed in the context of a diet (i.e. not on an empty stomach) that does not give rise to concern with respect to an increased risk of diarrhoea?

D.2. Is this amount different from the amount of supplemental Mg consumed on an empty stomach that does not give rise to concern with respect to an increased risk of diarrhoea?

For points B and E (Section 1.2):

These questions will be answered based on the outcome of the comprehensive literature searches for point A) and D), respectively.

Method for the study selection for inclusion/exclusion

The questions listed above will be answered based on a comprehensive literature search. The literature screening will be done in parallel and duplicates (first come first served basis) using DistillerSR® (Evidence Partners, Ottawa, Canada). Conflicts that may arise will be discussed and resolved internally during the screening process. Following the screening, the references will be exported to EndNote®. Reasons for exclusion at full-text level will be recorded.

The comprehensive literature searches will be conducted by EFSA's information specialist in Embase and PubMed. No limitations on publication date (except for the search on gallstone formation) will be applied but limited to publications in English due to translation constraints.

Search strings are provided in Appendix E with numbers of items identified prior to deduplication. Hand search will be undertaken through the comprehensive reviews and meta-analyses excluded during the screening but identified (with a label in Distiller) as potentially useful for this assessment. In addition, data that could be provided spontaneously by stakeholders and interested parties will be screened for their eligibility. Regarding Mg, previously published scientific reports of officially recognised scientific bodies, and the studies therein, e.g. SCF (2001) and VKM (2016), may be considered.

Planned approach towards the evidence retrieval from the scientific literature

The following sections will summarise the approach taken for the evidence retrieval to answer the predefined subquestions (Section 1.1).

Linoleic acid and alpha-linolenic acid concentrations in adipose tissue and their release from adipose tissue during weight loss

References for these two questions will be screened jointly (to limit duplicates; Q1).

Eligible studies are:

Human intervention (clinical trials) or observational studies in adults not on an intentionally modified diet (irrespective of weight status) on the FA composition of human AT or FA release from human AT:

- Studies in study populations living in Europe.
- Studies in either healthy subjects or in overweight or obese individuals with hypertension, hyperlipidaemia or T2DM, but no other disease. Medication use should be restricted to medicines related to these diseases.
- At title and abstract screening,

  - studies on FA composition of subcutaneous (SC) AT in which samples were taken by biopsies or from visceral (VC) AT taken during surgery or autopsy (incl. subsequent gas chromatography or other methods of sample analysis),
  - or studies on FA composition of SC AT before and after weight loss,
  - or studies on FA release during weight loss or in the fasting state via microdialysis of the tissue of interest or using isotopes to study the fate of the FA in metabolism.

- To be finally included,

- articles providing quantitative data on LA (18:2 (n-6)) and/or ALA (18:3 (n−3)) concentration of AT. Preferably, full-text papers should report on the study population characteristics (including age, sex), diet, weight and health status to be included. For large observational studies, this information may be provided in other publications on the same studies.

Not eligible are:

- Systematic or narrative reviews and meta-analyses. Although these may be used to provide background information to the general sections of the statement or for hand search of relevant references.
- Protocols (as no results are reported), commentaries, editorials, letters to the editor, grey literature (PhD theses, extended abstracts, conference proceedings, etc.), or other publication types not peer-reviewed (except contributions from stakeholders and interested parties).
- Animal studies, *in vitro* studies and studies on cells.
- Studies on infants, children and adolescents, pregnant and lactating women.
- Studies on patients except for diseases listed above, as they may have an impact on the outcome of interest,
- Studies on subjects on medication except related to the diseases listed above.
- Studies on dietary FA/FA intake only.
- Studies on FA concentration not measured in AT, e.g. in liver, muscle, brain, bone marrow, etc.
- Studies on plasma free FA only.
- Studies on individuals living outside of Europe.
- Additional reasons at full-text screening:

    - studies not reporting on LA and/ALA quantitatively in SC AT or VC AT.
    - inappropriate method of measurement.

Range of weight loss when consuming TDR

Eligible studies are:

Human intervention studies (i.e. clinical trials, including single arm trials) in overweight or obese[8] adults, in which TDRs with an energy content between 600 and 1,200 kcal (i.e. 2,510–5,020 kJ/day) are administered (i.e. within the permitted range as laid down in Commission delegated Regulation (EU) 2017/1798[1]) and that report on weight loss (Q2):

- As the terms 'total diet replacement' are rarely present in the abstracts, studies on 'total meal replacement', 'very low calorie diet' or 'low calorie diet' will be included at title and abstract screening if the diet investigated is in the energy range as specified in the Regulation.
- TDRs may have been consumed with or without added/supplemental vitamins and/or minerals (considering vitamins and/or minerals will not have an impact on weight loss).
- TDRs could have been consumed before bariatric surgery.
- Overweight or obese subjects without apparent co-morbidity, or with hypertension, hyperlipidaemia, T2DM, but no other disease. Medication use should be restricted to medicines related to the disease listed.
- For practical reasons during the title and abstract screening, as the abstracts often do not provide information in a harmonised and/or detailed way, the following criteria will be applied:

    - the maximal duration of the studies included at title and abstract level should be less than 6 months (no minimal duration expected).
    - abstracts of studies only mentioning the daily energy restriction/deficit (compared to the initial diet) will be included for full-text screening if this deficit is of at least -800 kcal/day (-3,347 kJ/day), is expressed in absolute amount, or at least -40% energy/day if expressed as a percentage.
    - if the abstracts report the energy intake per kg body weight, the 'rule of thumb' to decide if the inclusion or exclusion at the next step is to multiply by 60 kg to calculate the calories/day.

- For full-text screening, additional criteria are:

- studies in which body weight (or fat mass) is measured by investigators (i.e. no self-reported body weight) using validated and standardised methods, before and immediately after energy restriction.
- studies that report on the amount of weight loss, the caloric content of the TDR administered and the duration of the energy restriction.
- studies that report on weight loss up to and including 2 months.
- studies that report on compliance and how compliance was assessed.

Preferably, full-text publications included at title and abstract screening should report on the study population characteristics (including age, sex), diet, weight and health status.

Not eligible are:

- Studies on weight loss following diets not based on specifically designed foods, e.g. Atkins, DASH,[14] Paleolithic, Mediterranean, 'low carb', 'low fat', vegetarian, ketogenic (if not with specially designed foods), low glycaemic diet (if not with specially designed foods/TDR) or 'low calorie'/'low energy'/'hypocaloric'/'energy-restricted' diets using usual foods e.g. almonds, dairy, liquorice.
- Studies on intermittent fasting.
- Studies undertaken after bariatric surgery.
- Studies on subjects with catabolic diseases (such as cancer) should be excluded, as these diseases may have an impact on the outcome of interest).
- Studies in which TDR/energy-restricted diets was consumed in combination with a medication for weight loss in all groups or with training/physical activity/exercise, or counselling/group sessions or with other foods (even if in limited amounts) in all groups (i.e. no arm with TDR/ energy-restricted diet and without co-intervention).
- Observational studies (e.g. surveys, case reports, case-series, prospective cohort, case-control, retrospective).
- Systematic or narrative reviews and meta-analyses. Although, these may be used to provide background information to the general sections of the statement or for hand search of relevant references.
- Protocols (as no results are reported), commentaries, editorials, letters to the editor, grey literature (PhD theses, extended abstracts, conference proceedings, etc.), or other publication types not peer-reviewed except contributions from stakeholders and interested parties.
- Studies on infants, children and adolescents, pregnant and lactating women, normal weight/ lean subjects (i.e. BMI below 25).
- Animal studies, *in vitro* studies.
- Studies on meal replacements (also called 'partial meal replacement'), as these are not covered by Commission delegated Regulation (EU) 2017/1798 and Regulation (EU) 609/2013.
- Studies not using a specifically formulated product (i.e. TDRs) or products with an energy content outside the range as specified in the Regulation.

Magnesium and risk of developing diarrhoea

Eligible studies are:

Human intervention studies (clinical trials) that report on the risk of developing diarrhoea associated with supplemental Mg or Mg intentionally added to food in adults (Q3):

- The intervention studies should have at least two arms and compare different levels of supplemental/added Mg (possibly including a placebo) administered orally to adults.
- Trials in which only the amount of Mg consumed between groups differ.
- Studies in subjects either healthy or defined as overweight/obese presenting with hypertension, hyperlipidaemia or T2DM.
- Studies reporting on adverse effects (including diarrhoea) associated with Mg administration. Studies included at title and abstract level may report on adverse effects only at full-text level. Studies for which the full texts report on various adverse effects but not diarrhoea will be also considered as relevant for inclusion.

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[14] Dietary Approaches to Stop Hypertension.

- Studies that report on the definition of diarrhoea (applying the World Health Organization (WHO) criteria). For the full-text screening, trials reporting on Mg administration and (the severity of) diarrhoea should preferably report on the frequency and consistency of the stools using validated assessment methods and documenting diarrhoea by the frequency of ≥ 3 loose or watery stools per day.[11] Hence, a ranking of the final included studies may be made, distinguishing those with a reliable outcome assessment from those with an unreliable outcome assessment or improper reporting.
- After full-text screening, included studies should specify (among other parameters) the Mg dose and duration of administration in all groups.

Not eligible are:

- Systematic or narrative reviews and meta-analyses. However, these may be used to provide background information to the general sections of the statement or for hand search of relevant references.
- Observational studies, animal studies, *in vitro* studies, studies in cells, protocols (as no results are reported), commentaries, editorials, letters to the editor, grey literature (PhD theses, extended abstracts, conference proceedings, etc.), or other publication types not peer-reviewed except contributions from stakeholders and interested parties.
- Studies in infants, children and adolescents, pregnant and lactating women, patients with diseases not mentioned above.
- Studies comparing similar doses of different forms of Mg.
- Single arm studies.
- Studies that investigate the effect of Mg in combination with other substances
- Studies on Mg naturally present in food, as the UL for Mg set by SCF only applies to readily dissociable Mg salts.
- Studies in which Mg is not provided orally (e.g. injection, perfusion, infusion, nebulisation, intramuscular, intrathecal, intravenous or intraperitoneal administration, transdermal administration, bioresorbable devices, placental transfer), including those with an initial parenteral Mg administration.
- Studies not on the subject of interest, e.g. on Mg content of foods or breast milk, on Mg deficiency/insufficiency/hypomagnesaemia, or on Mg status/biomarkers/adequacy.
- At full-text screening, publications not reporting on the Mg dose and duration of consumption.
- At full-text screening, publications not reporting on adverse events.

Dietary fat and risk of gallstone formation

Eligible studies are:

Human intervention studies investigating the relationship between low-fat energy-restricted diets and the risk of gallstone/biliary sludge formation or gallbladder emptying (Q4):

- Studies published from the year 2014 and onwards, i.e. starting from the conclusion of the scientific assessment by EFSA NDA Panel (2015b).
- Human intervention studies in adults comparing at least two different fat contents of energy-restricted diets or investigating the effect of at least two test meals with varying fat content (on gallbladder emptying).
- Studies that report on the fat and caloric content of the diet per day and the duration of the dietary intake.
- Studies reporting on gallstone formation or biliary sludge formation or gallbladder emptying.

Preferably, full-text papers of studies included at title and abstract screening should report on the study population characteristics (including age, sex), diet, weight and health status to be included.

Not eligible are:

- Systematic or narrative reviews and meta-analyses. Although these may be used to provide background information to the general sections of the statement or for hand search of relevant references.
- Observational studies, animal studies, *in vitro* studies, protocols (as no results are reported), commentaries, editorials, letters to the editor, or other publication types not peer-reviewed,

grey literature (PhD theses, extended abstracts, conference proceedings, etc.) except contributions from stakeholders and interested parties.

- Studies on infants, children and adolescents, pregnant and lactating women and studies on patients with diseases not listed above.

Method for data extraction from included studies

Data will be extracted in prespecified forms in Microsoft Excel$^®$ by one reviewer and a second reviewer will serve as validator. The data extraction will be divided according to the main literature searches, i.e. LA and ALA concentration of and release from human adipose tissue during weight loss (Q1), range of weight loss when consuming TDRs (Q2), Mg administration and risk of diarrhoea (Q3) and dietary fat and risk of gallstone formation (Q4). Missing data will only be collected by contacting corresponding authors in cases where clarification is critical for the interpretation of results or for the decision to include a study.

Method for appraising evidence

Appraisal of included studies is not foreseen.

Preliminary identification of sources of uncertainty and methods for prioritising them

Uncertainty analysis of the scientific assessment, i.e. identifying possible limitations in scientific knowledge and assessing their implications for scientific conclusions, will be discussed briefly, based on the EFSA guidance document on uncertainty (EFSA Scientific Committee, 2018). This implies, in particular, identifying the sources of uncertainty affecting the assessment, prioritising these sources based on their expected influence on the outcome/results and final overall discussions and planning how the uncertainty analysis will be handled. It is expected that the following identified sources of uncertainties will be discussed qualitatively (descriptive method) and no quantitative analysis will be undertaken.

The following uncertainties have been preliminary identified:

- Uncertainty in the method and precision of measurement.
- Exposure/compliance to TDR regimen/to Mg consumption.
- Reporting/assessment of the adverse effect 'diarrhoea' by investigators
- Publication bias.
- Language (only English language considered).
- Representativity/relevance for the EU population.

Uncertainties will be assessed by expert judgement.

Methods for analysing uncertainties individually and combined

Uncertainties will be identified at each step of the assessment, but no formal uncertainty assessment is foreseen.

Methods for synthesising evidence

The method for synthesising the evidence for points A–E (Section 1.2 of the statement) will be primarily semiquantitative.

A qualitative approach will be followed for those subquestions belonging to point A (Section 1.2 of the statement) answering to the influence of the methods of measurement and the extent of reutilisation of LA and ALA upon release from AT.

A conservative approach will be taken when assessing the evidence to cover the entire population that could potentially consume TDRs.

Expert knowledge elicitation (EKE) might be needed, taking into account the relevant EFSA guidance (EFSA, 2014),[15] if updated composition requirements for ALA, LA and Mg are set.

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[15] http://www.efsa.europa.eu/en/efsajournal/pub/3734

Appendix E – Search strings

E.1. Linoleic acid and alpha-linolenic acid concentrations in adipose tissue and their release from adipose tissue (Q1)

Date of search: 8 June 2020

Linoleic acid and alpha-linolenic acid concentrations in adipose tissue
Embase

Search	Query	Items identified
#8	#6 NOT #7	3,690
#7	#3 AND #4 AND ([editorial]/lim OR [letter]/lim)	16
#6	#5 AND [humans]/lim AND [english]/lim	3,704
#5	#3 AND #4	10,416
#4	#1 OR #2	85,376
#3	'adipose tissue'/exp OR 'adipose tissue cell'/exp OR adipocyte*:ti,ab,kw OR (((adipose OR fat OR fatty) NEAR/3 (tissue* OR body)):ti,ab,kw) OR ((fat NEAR/3 (abdominal OR cell OR cells OR intraabdominal OR pad OR pads OR subcutaneous OR visceral)):ti,ab,kw)	285,640
#2	('fatty acid'/de OR 'essential fatty acid'/exp OR 'unsaturated fatty acid'/exp OR ffa:ti,ab,kw OR ffas:ti,ab,kw OR efa:ti,ab,kw OR efas:ti,ab,kw OR pufa:ti,ab,kw OR pufas:ti,ab,kw OR ufa:ti,ab,kw OR ufas:ti,ab,kw OR ((fatty OR 'alpha linolenic' OR alphalinolenic OR linolic OR linoleicOR 'n 3' OR 'n 6') NEAR/3 (acid OR acids)) OR linolate:ti,ab,kw OR linoleate:ti,ab,kw OR linolenate:ti,ab,kw OR 'omega 3':ti,ab,kw OR 'omega 6':ti,ab,kw OR omega3:ti,ab,kw OR omega6:ti,ab,kw OR ω3:ti,ab,kw OR ω6:ti,ab,kw OR '18 3ω3':ti,ab,kw OR '183omega3':ti,ab,kw OR '18 3omega3':ti,ab,kw) AND 'lipid composition'/exp	19,889
#1	((ffa OR ffas OR efa OR efas OR pufa OR pufas OR ufa OR ufas OR 'fatty acid' OR 'fatty acids' OR 'alpha linolenic acid' OR 'alpha linolenic acids' OR 'alphalinolenic acid' OR 'alphalinolenic acids' OR 'linoleic acid' OR 'linoleic acids' OR 'linolic acid' OR 'linolic acids' OR linolateOR linoleate OR linolenate OR 'n 3 acid' OR 'n 3 acids' OR 'n 6 acid' OR 'n 6 acids' OR 'omega 3' OR 'omega 6' OR omega3 OR omega6 OR ω3 OR ω6 OR '18 3ω3' OR '183omega3' OR '18 3omega3') NEAR/5 (amount* OR biops* OR composition OR concentration* OR constituent* OR content* OR distribution* OR level OR levels OR pattern* OR percentage* OR profile*)):ti,ab,kw	78,162

PubMed

Search	Query	Items identified
#13	Search: (((((((Amount[tiab] OR biops*[tiab] OR composition[tiab] OR concentration*[tiab] OR constituent[tiab] OR content*[tiab] OR distribution*[tiab] OR level[tiab] OR levels[tiab] OR pattern*[tiab] OR percentage*[tiab] OR profile[tiab]) AND ("Fatty Acids"[Mesh: NoExp] OR "Fatty Acids, Unsaturated"[Mesh] OR "Fatty Acids, Essential"[Mesh] OR Fatty acid[tiab] OR fatty acids[tiab] OR FFA[tiab] OR FFAs[tiab] OR EFA[tiab] OR EFAs[tiab] OR PUFA[tiab] OR PUFAs[tiab] OR UFA[tiab] OR UFAs[tiab] OR Alpha linolenic acid[tiab] OR Alpha linolenic acids[tiab] OR Alphalinolenic acid[tiab] OR Alphalinolenic acids[tiab] OR Linoleic acid[tiab] OR Linoleic acids[tiab] OR Linolic acid[tiab] OR Linolic acids[tiab] OR linolate[tiab] OR linoleate[tiab] OR Linolenate[tiab] OR "omega 3"[tiab] OR "omega 6"[tiab] OR omega3[tiab] OR omega6[tiab] OR (("n 3"[tiab] OR "n 6"[tiab]) AND (acid[tiab] OR acids[tiab])) OR "183ω3"[tiab] OR "18 3ω3"[tiab] OR "183omega3"[tiab] OR "18 3omega3"[tiab] OR ω3[tiab] OR ω6[tiab])) AND ("Adiposity"[Mesh] OR "Adipose Tissue"[Mesh] OR "Adipocytes"[Mesh] OR adipocyte*[tiab] OR Adipose tissue*[tiab] OR Adipose body[tiab] OR Abdominal fat[tiab] OR Body fat[tiab] OR Fat cell[tiab] OR Fat cells[tiab] OR Fat pad[tiab] OR Fat pads[tiab] OR Fat tissue*[tiab] OR Fatty tissue*[tiab] OR Intraabdominal fat[tiab] OR Subcutaneous fat[tiab] OR Visceral fat[tiab]) NOT	6,512

Search	Query	Items identified
	(("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])))) NOT ((rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti]))) AND ("english"[Language])) NOT (("editorial"[Publication Type]) OR ("letter"[Publication Type])) Sort by: Most Recent	
#12	Search: ("editorial"[Publication Type]) OR ("letter"[Publication Type]) Sort by: Most Recent	1,610,464
#11	Search: ((((((Amount[tiab] OR biops*[tiab] OR composition[tiab] OR concentration*[tiab] OR constituent[tiab] OR content*[tiab] OR distribution*[tiab] OR level[tiab] OR levels[tiab] OR pattern*[tiab] OR percentage*[tiab] OR profile[tiab]) AND ("Fatty Acids"[Mesh:NoExp] OR "Fatty Acids, Unsaturated"[Mesh] OR "Fatty Acids, Essential"[Mesh] OR Fatty acid[tiab] OR fatty acids[tiab] OR FFA[tiab] OR FFAs[tiab] OR EFA[tiab] OR EFAs[tiab] OR PUFA[tiab] OR PUFAs[tiab] OR UFA[tiab] OR UFAs[tiab] OR Alpha linolenic acid[tiab] OR Alpha linolenic acids[tiab] OR Alphalinolenic acid[tiab] OR Alphalinolenic acids[tiab] OR Linoleic acid[tiab] OR Linoleic acids[tiab] OR Linolic acid[tiab] OR Linolic acids[tiab] OR linolate[tiab] OR linoleate[tiab] OR Linolenate[tiab] OR "omega 3"[tiab] OR "omega 6"[tiab] OR omega3[tiab] OR omega6[tiab] OR (("n 3"[tiab] OR "n 6"[tiab]) AND (acid[tiab] OR acids[tiab])) OR "183ω3"[tiab] OR "18 3ω3"[tiab] OR "183omega3"[tiab] OR "18 3omega3"[tiab] OR ω3[tiab] OR ω6[tiab])) AND ("Adiposity"[Mesh] OR "Adipose Tissue"[Mesh] OR "Adipocytes"[Mesh] OR adipocyte*[tiab] OR Adipose tissue*[tiab] OR Adipose body[tiab] OR Abdominal fat[tiab] OR Body fat[tiab] OR Fat cell[tiab] OR Fat cells[tiab] OR Fat pad[tiab] OR Fat pads[tiab] OR Fat tissue*[tiab] OR Fatty tissue*[tiab] OR Intraabdominal fat[tiab] OR Subcutaneous fat[tiab] OR Visceral fat[tiab]) NOT (("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])))) NOT ((rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti]))) AND ("english"[Language]) Sort by: Most Recent	6,532
#10	Search: "english"[Language] Sort by: Most Recent	26,364,599
#9	Search: (((((Amount[tiab] OR biops*[tiab] OR composition[tiab] OR concentration*[tiab] OR constituent[tiab] OR content*[tiab] OR distribution*[tiab] OR level[tiab] OR levels[tiab] OR pattern*[tiab] OR percentage*[tiab] OR profile[tiab]) AND ("Fatty Acids"[Mesh:NoExp] OR "Fatty Acids, Unsaturated"[Mesh] OR "Fatty Acids, Essential"[Mesh] OR Fatty acid[tiab] OR fatty acids[tiab] OR FFA[tiab] OR FFAs[tiab] OR EFA[tiab] OR EFAs[tiab] OR PUFA[tiab] OR PUFAs[tiab] OR UFA[tiab] OR UFAs[tiab] OR Alpha linolenic acid[tiab] OR Alpha linolenic acids[tiab] OR Alphalinolenic acid[tiab] OR Alphalinolenic acids[tiab] OR Linoleic acid[tiab] OR Linoleic acids[tiab] OR Linolic acid[tiab] OR Linolic acids[tiab] OR linolate[tiab] OR linoleate[tiab] OR Linolenate[tiab] OR "omega 3"[tiab] OR "omega 6"[tiab] OR omega3[tiab] OR omega6[tiab] OR (("n 3"[tiab] OR "n 6"[tiab]) AND (acid[tiab] OR acids[tiab])) OR "183ω3"[tiab] OR "18 3ω3"[tiab] OR "183omega3"[tiab] OR "18 3omega3"[tiab] OR ω3[tiab] OR ω6[tiab])) AND ("Adiposity"[Mesh] OR "Adipose Tissue"[Mesh] OR "Adipocytes"[Mesh] OR adipocyte*[tiab] OR Adipose tissue*[tiab] OR Adipose body[tiab] OR Abdominal fat[tiab] OR Body fat[tiab] OR Fat cell[tiab] OR Fat cells[tiab] OR Fat pad[tiab] OR Fat pads[tiab] OR Fat tissue*[tiab] OR Fatty tissue*[tiab] OR Intraabdominal fat[tiab] OR Subcutaneous fat[tiab] OR Visceral fat[tiab]) NOT (("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])))) NOT ((rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti])) Sort by: Most Recent	6,828
#8	Search: (rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti]) Sort by: Most Recent	2,007,584

Search	Query	Items identified
#7	Search: (((Amount[tiab] OR biops*[tiab] OR composition[tiab] OR concentration*[tiab] OR constituent[tiab] OR content*[tiab] OR distribution*[tiab] OR level[tiab] OR levels [tiab] OR pattern*[tiab] OR percentage*[tiab] OR profile[tiab]) AND ("Fatty Acids"[Mesh: NoExp] OR "Fatty Acids, Unsaturated"[Mesh] OR "Fatty Acids, Essential"[Mesh] OR Fatty acid[tiab] OR fatty acids[tiab] OR FFA[tiab] OR FFAs[tiab] OR EFA[tiab] OR EFAs[tiab] OR PUFA[tiab] OR PUFAs[tiab] OR UFA[tiab] OR UFAs[tiab] OR Alpha linolenic acid[tiab] OR Alpha linolenic acids[tiab] OR Alphalinolenic acid[tiab] OR Alphalinolenic acids[tiab] OR Linoleic acid[tiab] OR Linoleic acids[tiab] OR Linolic acid[tiab] OR Linolic acids[tiab] OR linolate[tiab] OR linoleate[tiab] OR Linolenate[tiab] OR "omega 3"[tiab] OR "omega 6"[tiab] OR omega3[tiab] OR omega6[tiab] OR (("n 3"[tiab] OR "n 6"[tiab]) AND (acid [tiab] OR acids[tiab])) OR "183ω3"[tiab] OR "18 3ω3"[tiab] OR "183omega3"[tiab] OR "18 3omega3"[tiab] OR ω3[tiab] OR ω6[tiab])) AND ("Adiposity"[Mesh] OR "Adipose Tissue"[Mesh] OR "Adipocytes"[Mesh] OR adipocyte*[tiab] OR Adipose tissue*[tiab] OR Adipose body[tiab] OR Abdominal fat[tiab] OR Body fat[tiab] OR Fat cell[tiab] OR Fat cells[tiab] OR Fat pad[tiab] OR Fat pads[tiab] OR Fat tissue*[tiab] OR Fatty tissue*[tiab] OR Intraabdominal fat[tiab] OR Subcutaneous fat[tiab] OR Visceral fat[tiab])) NOT (("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh]))) Sort by: Most Recent	7,767
#6	Search: ("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])) Sort by: Most Recent	4,705,782
#5	Search: ((Amount[tiab] OR biops*[tiab] OR composition[tiab] OR concentration*[tiab] OR constituent[tiab] OR content*[tiab] OR distribution*[tiab] OR level[tiab] OR levels[tiab] OR pattern*[tiab] OR percentage*[tiab] OR profile[tiab]) AND ("Fatty Acids"[Mesh: NoExp] OR "Fatty Acids, Unsaturated"[Mesh] OR "Fatty Acids, Essential"[Mesh] OR Fatty acid[tiab] OR fatty acids[tiab] OR FFA[tiab] OR FFAs[tiab] OR EFA[tiab] OR EFAs[tiab] OR PUFA[tiab] OR PUFAs[tiab] OR UFA[tiab] OR UFAs[tiab] OR Alpha linolenic acid[tiab] OR Alpha linolenic acids[tiab] OR Alphalinolenic acid[tiab] OR Alphalinolenic acids[tiab] OR Linoleic acid[tiab] OR Linoleic acids[tiab] OR Linolic acid[tiab] OR Linolic acids[tiab] OR linolate[tiab] OR linoleate[tiab] OR Linolenate[tiab] OR "omega 3"[tiab] OR "omega 6"[tiab] OR omega3[tiab] OR omega6[tiab] OR (("n 3"[tiab] OR "n 6"[tiab]) AND (acid [tiab] OR acids[tiab])) OR "183ω3"[tiab] OR "18 3ω3"[tiab] OR "183omega3"[tiab] OR "18 3omega3"[tiab] OR ω3[tiab] OR ω6[tiab])) AND ("Adiposity"[Mesh] OR "Adipose Tissue"[Mesh] OR "Adipocytes"[Mesh] OR adipocyte*[tiab] OR Adipose tissue*[tiab] OR Adipose body[tiab] OR Abdominal fat[tiab] OR Body fat[tiab] OR Fat cell[tiab] OR Fat cells[tiab] OR Fat pad[tiab] OR Fat pads[tiab] OR Fat tissue*[tiab] OR Fatty tissue*[tiab] OR Intraabdominal fat[tiab] OR Subcutaneous fat[tiab] OR Visceral fat[tiab]) Sort by: Most Recent	16,638
#4	Search: "Adiposity"[Mesh] OR "Adipose Tissue"[Mesh] OR "Adipocytes"[Mesh] OR adipocyte*[tiab] OR Adipose tissue*[tiab] OR Adipose body[tiab] OR Abdominal fat[tiab] OR Body fat[tiab] OR Fat cell[tiab] OR Fat cells[tiab] OR Fat pad[tiab] OR Fat pads[tiab] OR Fat tissue*[tiab] OR Fatty tissue*[tiab] OR Intraabdominal fat[tiab] OR Subcutaneous fat[tiab] OR Visceral fat[tiab] Sort by: Most Recent	193,456
#3	Search: "Fatty Acids"[Mesh:NoExp] OR "Fatty Acids, Unsaturated"[Mesh] OR "Fatty Acids, Essential"[Mesh] OR Fatty acid[tiab] OR fatty acids[tiab] OR FFA[tiab] OR FFAs[tiab] OR EFA[tiab] OR EFAs[tiab] OR PUFA[tiab] OR PUFAs[tiab] OR UFA[tiab] OR UFAs[tiab] OR Alpha linolenic acid[tiab] OR Alpha linolenic acids[tiab] OR Alphalinolenic acid[tiab] OR Alphalinolenic acids[tiab] OR Linoleic acid[tiab] OR Linoleic acids[tiab] OR Linolic acid [tiab] OR Linolic acids[tiab] OR linolate[tiab] OR linoleate[tiab] OR Linolenate[tiab] OR "omega 3"[tiab] OR "omega 6"[tiab] OR omega3[tiab] OR omega6[tiab] OR (("n 3"[tiab] OR "n 6"[tiab]) AND (acid[tiab] OR acids[tiab])) OR "183ω3"[tiab] OR "18 3ω3"[tiab] OR "183omega3"[tiab] OR "18 3omega3"[tiab] OR ω3[tiab] OR ω6[tiab] Sort by: Most Recent	434,755
#2	Search: Amount[tiab] OR biops*[tiab] OR composition[tiab] OR concentration*[tiab] OR constituent[tiab] OR content*[tiab] OR distribution*[tiab] OR level[tiab] OR levels[tiab] OR pattern*[tiab] OR percentage*[tiab] OR profile[tiab] Sort by: Most Recent	8,394,012
#18	#8 AND #16 AND [english]/lim	1,764
#17	#8 AND #16	1,806
#16	#9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15	5,460,152

Search	Query	Items identified
#15	((data NEAR/1 (synthes* OR extraction* OR abstraction*)):ti,ab) OR handsearch*:ti,ab OR 'hand search':ti,ab OR 'hand searches':ti,ab OR 'hand searching':ti,ab OR 'mantel haenszel':ti,ab OR peto:ti,ab OR 'der simonian':ti,ab OR dersimonian:ti,ab OR 'fixed effect':ti,ab OR 'fixed effects':ti,ab OR 'latin square':ti,ab OR 'latin squares':ti,ab OR 'meta analysis':ti,ab OR 'meta analyses':ti,ab OR 'met analysis':ti,ab OR 'met analyses':ti,ab OR metaanaly*:ti,ab OR metanaly*:ti,ab OR 'meta regression':ti,ab OR 'meta regressions':ti,ab OR metaregression*:ti,ab OR medline:ti,ab OR cochrane:ti,ab OR pubmed:ti,ab OR medlars:ti,ab OR embase:ti,ab OR cinahl:ti,ab OR cochrane:jt OR 'evidence report':jt OR ((comparative NEAR/3 (efficacy OR effectiveness)):ti,ab) OR 'outcomes research':ti,ab OR 'relative effectiveness':ti,ab OR (((indirect OR 'indirect treatment' OR 'mixed treatment') NEAR/3 comparison):ti,ab)	457,229
#14	((systematic* NEAR/3 (review* OR overview*)):ti,ab) OR ((methodologic* NEAR/3 (review* OR overview*)):ti,ab) OR ((quantitative NEAR/3 (review* OR overview* OR synthes*)):ti,ab) OR ((research NEAR/3 (integrati* OR overview*)):ti,ab) OR ((integrative NEAR/3 (review* OR overview*)):ti,ab) OR ((collaborative NEAR/3 (review* OR overview*)):ti,ab) OR ((pool* NEAR/3 analy*):ti,ab)	273,284
#13	'meta analysis'/exp OR 'meta analysis (topic)'/exp OR 'systematic review'/exp OR 'systematic review (topic)'/exp OR 'biomedical technology assessment'/exp	402,070
#12	'crossover procedure'/exp OR (((crossover OR 'cross over') NEAR/10 (study OR studies OR design* OR method* OR procedure OR comparison)):ti,ab)	93,441
#11	((singl* OR doubl* OR trebl* OR tripl*) NEAR/10 (mask* OR blind* OR dumm*)):ti,ab	249,336
#10	'clinical trial (topic)'/exp OR 'double blind procedure'/exp OR 'single blind procedure'/exp OR 'triple blind procedure'/exp	532,576
#9	'clinical trial'/exp OR 'randomization'/exp OR randomized:ti,ab OR randomised:ti,ab OR placebo:ti,ab OR randomly:ti,ab OR trial:ti,ab OR groups:ti,ab OR ('group 1':ti,ab,kw AND 'group 2':ti,ab,kw)	4,883,034
#8	#6 NOT #7	5,091
#7	(rat:ti OR rats:ti OR mouse:ti OR mice:ti OR swine:ti OR porcine:ti OR murine:ti OR sheep:ti OR lambs:ti OR pigs:ti OR piglets:ti OR rabbit:ti OR rabbits:ti OR cat:ti OR cats:ti OR dog:ti OR dogs:ti OR cattle:ti OR bovine:ti OR monkey:ti OR monkeys:ti OR trout:ti OR marmoset*:ti) AND 'animal experiment'/de	1,064,962
#6	#4 NOT #5	5,185
#5	'animal experiment'/de NOT ('human experiment'/de OR 'human'/de)	2,250,451
#4	#1 AND #2 AND #3	8,320
#3	'body weight change'/exp OR (((weight OR fat OR 'adipose tissue') NEAR/3 (decreas* OR loss OR losing OR reduc*)):ti,ab,kw) OR ((weightNEAR/3 management):ti,ab,kw) OR ((chang* NEAR/3 ('adipose tissue' OR 'fat mass' OR 'fat tissue' OR weight)):ti,ab,kw)	419,800
#2	'fatty acid'/de OR 'essential fatty acid'/exp OR 'unsaturated fatty acid'/exp OR ffa:ti,ab,kw OR ffas:ti,ab,kw OR efa:ti,ab,kw OR efas:ti,ab,kw OR pufa:ti,ab,kw OR pufas:ti,ab,kw OR ufa:ti,ab,kw OR ufas:ti,ab,kw OR ((fatty OR 'alpha linolenic' OR alphalinolenic OR linolic OR linoleicOR 'n 3' OR 'n 6') NEAR/3 (acid OR acids)) OR linolate:ti,ab,kw OR linoleate:ti,ab,kw OR linolenate:ti,ab,kw OR 'omega 3':ti,ab,kw OR 'omega 6':ti,ab,kw OR omega3:ti,ab,kw OR omega6:ti,ab,kw OR ω3:ti,ab,kw OR ω6:ti,ab,kw OR '18 3ω3':ti,ab,kw OR '183omega3':ti,ab,kw OR '18 3omega3':ti,ab,kw	423,778
#1	'adipose tissue'/exp OR 'adipose tissue cell'/exp OR adipocyte*:ti,ab,kw OR (((adipose OR fat OR fatty) NEAR/3 (tissue* OR body)):ti,ab,kw) OR ((fat NEAR/3 (abdominal OR cell OR cells OR intraabdominal OR pad OR pads OR subcutaneous OR visceral)):ti,ab,kw)	285,922

PubMed

Search	Query	Items identified
#14	Search: "english"[Language] AND #13 Sort by: Most Recent	1,356
#13	Search: #12 AND #9 Sort by: Most Recent	1,387
#12	Search: #10 OR #11 Sort by: Most Recent	3,747,938
#11	Search: systematic[sb] OR meta-analysis[pt] OR meta-analysis as topic[Mesh] OR meta-analysis[Mesh] OR meta analy*[tw] OR metanaly*[tw] OR metaanaly*[tw] OR met analy*[tw] OR integrative research[tiab] OR integrative review*[tiab] OR integrative overview*[tiab] OR research integration*[tiab] OR research overview*[tiab] OR collaborative review*[tiab] OR collaborative overview*[tiab] OR systematic review*[tiab] OR comparative efficacy[tiab] OR comparative effectiveness[tiab] OR outcomes research [tiab] OR indirect comparison*[tiab] OR Embase*[tiab] OR Cinahl*[tiab] OR systematic overview*[tiab] OR methodological overview*[tiab] OR methodologic overview*[tiab] OR methodological review*[tiab] OR methodologic review*[tiab] OR quantitative review* [tiab] OR quantitative overview*[tiab] OR quantitative synthes*[tiab] OR pooled analy* [tiab] OR Cochrane[tiab] OR Medline[tiab] OR Pubmed[tiab] OR Medlars[tiab] OR handsearch*[tiab] OR hand search*[tiab] OR meta-regression*[tiab] OR metaregression* [tiab] OR data synthes*[tiab] OR data extraction[tiab] OR data abstraction*[tiab] OR mantel haenszel[tiab] OR peto[tiab] OR der-simonian[tiab] OR dersimonian[tiab] OR fixed effect*[tiab] OR "Cochrane Database Syst Rev"[Journal:__jrid21711] Sort by: Most Recent	421,910
#10	Search: "clinical trial"[pt] OR "Random Allocation"[Mesh] OR randomized[tiab] OR randomised[tiab] OR placebo[tiab] OR randomly[tiab] OR trial[tiab] OR groups[tiab] OR ("group 1"[tiab] AND "group 2"[tiab]) OR "Clinical Trials as Topic"[Mesh] OR "Double-Blind Method"[Mesh] OR "Single-Blind Method"[Mesh] OR ((singl*[tiab] OR doubl*[tiab] OR trebl*[tiab] OR tripl*[tiab]) AND (mask*[tiab] OR blind*[tiab] OR dumm*[tiab])) OR "Cross-Over Studies"[Mesh] OR ((crossover[tiab] OR "cross over"[tiab]) AND (study[tiab] OR studies[tiab] OR design*[tiab] OR method*[tiab] OR procedure[tiab] OR comparison [tiab])) Sort by: Most Recent	3,484,152
#9	Search: #7 NOT #8 Sort by: Most Recent	4,775
#8	Search: (rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti]) Sort by: Most Recent	2,010,568
#7	Search: #5 NOT #6 Sort by: Most Recent	5,629
#6	Search: ("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])) Sort by: Most Recent	4,711,847
#5	Search: #1 AND #3 AND #4 Sort by: Most Recent	12,817
#4	Search: "Fatty Acids"[Mesh:NoExp] OR "Fatty Acids, Unsaturated"[Mesh] OR "Fatty Acids, Essential"[Mesh] OR Fatty acid[tiab] OR fatty acids[tiab] OR FFA[tiab] OR FFAs[tiab] OR EFA[tiab] OR EFAs[tiab] OR PUFA[tiab] OR PUFAs[tiab] OR UFA[tiab] OR UFAs[tiab] OR Alpha linolenic acid[tiab] OR Alpha linolenic acids[tiab] OR Alphalinolenic acid[tiab] OR Alphalinolenic acids[tiab] OR Linoleic acid[tiab] OR Linoleic acids[tiab] OR Linolic acid [tiab] OR Linolic acids[tiab] OR linolate[tiab] OR linoleate[tiab] OR Linolenate[tiab] OR "omega 3"[tiab] OR "omega 6"[tiab] OR omega3[tiab] OR omega6[tiab] OR (("n 3"[tiab] OR "n 6"[tiab]) AND (acid[tiab] OR acids[tiab])) OR "183ω3"[tiab] OR "18 3ω3"[tiab] OR "183omega3"[tiab] OR "18 3omega3"[tiab] OR ω3[tiab] OR ω6[tiab] Sort by: Most Recent	435,798
#3	Search: "Body Weight Changes"[Mesh] OR (("adipose tissue"[tiab] OR fat[tiab] OR weight[tiab]) AND (decreas*[tiab] OR chang*[tiab] OR loss[tiab] OR losing[tiab] OR reduc*[tiab])) OR "body weight"[tiab] OR "body weights"[tiab] Sort by: Most Recent	645,747
#1	Search: "Adiposity"[Mesh] OR "Adipose Tissue"[Mesh] OR "Adipocytes"[Mesh] OR adipocyte*[tiab] OR Adipose tissue*[tiab] OR Adipose body[tiab] OR Abdominal fat[tiab] OR Body fat[tiab] OR Fat cell[tiab] OR Fat cells[tiab] OR Fat pad[tiab] OR Fat pads[tiab] OR Fat tissue*[tiab] OR Fatty tissue*[tiab] OR Intraabdominal fat[tiab] OR Subcutaneous fat[tiab] OR Visceral fat[tiab] OR Intraabdominal fats[tiab] OR Subcutaneous fats[tiab] OR Visceral fats[tiab] Sort by: Most Recent	194,140

E.2. Range of weight loss when consuming total diet replacements for weight control (Q2)

Date of search: 02 June 2020

Embase

Search	Query	Items identified
#10	#9 AND [english]/lim	3,547
#9	#7 NOT #8	3,685
#8	(rat:ti OR rats:ti OR mouse:ti OR mice:ti OR swine:ti OR porcine:ti OR murine:ti OR sheep:ti OR lambs:ti OR pigs:ti OR piglets:ti OR rabbit:ti OR rabbits:ti OR cat:ti OR cats:ti OR dog:ti OR dogs:ti OR cattle:ti OR bovine:ti OR monkey:ti OR monkeys:ti OR trout:ti OR marmoset*:ti) AND 'animal experiment'/de	1,062,853
#7	#5 NOT #6	3,690
#6	'animal experiment'/de NOT ('human experiment'/de OR 'human'/de)	2,247,064
#5	#3 AND #4	3,943
#4	'clinical trial'/exp OR 'randomization'/exp OR randomized:ti,ab OR randomised:ti,ab OR placebo:ti,ab OR randomly:ti,ab OR trial:ti,ab OR groups:ti,ab OR 'clinical trial (topic)'/exp OR 'double blind procedure'/exp OR 'single blind procedure'/exp OR 'triple blind procedure'/exp OR (((singl* OR doubl* OR trebl* OR tripl*) NEAR/10 (mask* OR blind* OR dumm*)):ti,ab) OR 'crossover procedure'/exp OR (((crossoverOR 'cross over') NEAR/10 (study OR studies OR design* OR method* OR procedure OR comparison)):ti,ab) OR 'meta analysis'/exp OR 'meta analysis (topic)'/exp OR 'systematic review'/exp OR 'systematic review (topic)'/exp OR 'biomedical technology assessment'/exp OR ((systematic* NEAR/3 (review* OR overview*)):ti,ab) OR ((methodologic* NEAR/3 (review* OR overview*)):ti,ab) OR ((quantitative NEAR/3 (review* OR overview* OR synthes*)):ti,ab) OR ((research NEAR/3 (integrati* OR overview*)):ti,ab) OR ((integrative NEAR/3 (review* OR overview*)):ti,ab) OR ((collaborative NEAR/3 (review* OR overview*)):ti,ab) OR ((pool* NEAR/3 analy*):ti,ab) OR ((data NEAR/1 (synthes* OR extraction* OR abstraction*)):ti,ab) OR handsearch*:ti,ab OR 'hand search':ti,ab OR 'hand searches':ti,ab OR 'hand searching':ti,ab OR 'mantel haenszel':ti,ab OR peto:ti,ab OR 'der simonian':ti,ab OR dersimonian:ti,ab OR 'fixed effect':ti,ab OR 'fixed effects':ti,ab OR 'latin square':ti,ab OR 'latin squares':ti,ab OR 'meta analysis':ti,ab OR 'meta analyses':ti,ab OR 'met analysis':ti,ab OR 'met analyses':ti,ab OR metaanaly*:ti,ab OR metanaly*:ti,ab OR 'meta regression':ti,ab OR 'meta regressions':ti,ab OR metaregression*:ti,ab OR medline:ti,ab OR cochrane:ti,ab OR pubmed:ti,ab OR medlars:ti,ab OR embase:ti,ab OR cinahl:ti,ab OR cochrane:jt OR 'evidence report':it OR ((comparativeNEAR/3 (efficacy OR effectiveness)):ti,ab) OR 'outcomes research':ti,ab OR 'relative effectiveness':ti,ab OR (((indirect OR 'indirect treatment' OR 'mixed treatment') NEAR/3 comparison):ti,ab)	5,425,604
#3	#1 AND #2	7,629
#2	'body weight loss'/exp OR (((weight OR fat OR 'adipose tissue') NEAR/3 (decreas* OR loss OR losing OR reduc*)):ti,ab,kw) OR ((weightNEAR/3 management):ti,ab,kw) OR ((chang* NEAR/3 ('adipose tissue' OR 'fat mass' OR 'fat tissue' OR weight)):ti,ab,kw)	332,351
#1	'low calorie diet'/exp/mj OR 'very low calorie diet'/exp/mj OR 'elimination diet'/exp/mj OR 'liquid diet'/exp/mj OR ((low NEAR/3 (calori* OR energy OR elimination) NEAR/5 (diet OR diets)):ti,ab,kw) OR vlcd:ti,ab,kw OR vled:ti,ab,kw OR lcd:ti,ab,kw OR (((ketogenic OR replacementOR 'meal substitute' OR liquid) NEAR/5 (diet OR diets)):ti,ab,kw) OR 'reducing diet':ti,ab,kw OR 'reducing diets':ti,ab,kw OR (((calori* OR energ*) NEAR/3 (reduc* OR restrict*) NEAR/5 (diet OR diets)):ti,ab,kw)	25,963

PubMed

Search	Query	Items identified
#14	Search: #12 AND #13 Sort by: Most Recent	2,806
#13	Search: ENGLISH[Language] Sort by: Most Recent	26,345,924
#12	Search: #11 Sort by: Most Recent	2,928
#11	Search: #10 and #7 Sort by: Most Recent	2,928
#10	Search: #8 OR #9 Sort by: Most Recent	3,717,566
#9	Search: Systematic[sb] OR meta-analysis[pt] OR meta-analysis as topic[Mesh] OR meta-analysis[Mesh] OR meta analy*[tw] OR metanaly*[tw] OR metaanaly*[tw] OR met analy*[tw] OR integrative research[tiab] OR integrative review*[tiab] OR integrative overview*[tiab] OR research integration*[tiab] OR research overview*[tiab] OR collaborative review*[tiab] OR collaborative overview*[tiab] OR systematic review*[tiab] OR comparative efficacy[tiab] OR comparative effectiveness[tiab] OR outcomes research[tiab] OR indirect comparison*[tiab] OR Embase*[tiab] OR Cinahl*[tiab] OR systematic overview*[tiab] OR methodological overview*[tiab] OR methodologic overview*[tiab] OR methodological review*[tiab] OR methodologic review*[tiab] OR quantitative review*[tiab] OR quantitative overview*[tiab] OR quantitative synthes*[tiab] OR pooled analy*[tiab] OR Cochrane[tiab] OR Medline[tiab] OR Pubmed[tiab] OR Medlars[tiab] OR handsearch*[tiab] OR hand search*[tiab] OR meta-regression*[tiab] OR metaregression*[tiab] OR data synthes*[tiab] OR data extraction[tiab] OR data abstraction*[tiab] OR mantel haenszel[tiab] OR peto[tiab] OR der-simonian[tiab] OR dersimonian[tiab] OR fixed effect*[tiab] OR "Cochrane Database Syst Rev"[Journal:__jrid21711] Sort by: Most Recent	417,908
#8	Search: "clinical trial"[pt] OR "Random Allocation"[Mesh] OR randomized[tiab] OR randomised[tiab] OR placebo[tiab] OR randomly[tiab] OR trial[tiab] OR groups[tiab] OR "Clinical Trials as Topic"[Mesh] OR "Double-Blind Method"[Mesh] OR "Single-Blind Method"[Mesh] OR ((singl*[tiab] OR doubl*[tiab] OR trebl*[tiab] OR tripl*[tiab]) AND (mask*[tiab] OR blind*[tiab] OR dumm*[tiab])) OR "Cross-Over Studies"[Mesh] OR ((crossover[tiab] OR "cross over"[tiab]) AND (study[tiab] OR studies[tiab] OR design*[tiab] OR method*[tiab] OR procedure[tiab] OR comparison[tiab])) Sort by: Most Recent	3,456,468
#7	Search: #5 NOT #6 Sort by: Most Recent	5,493
#6	Search: (rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti]) Sort by: Most Recent	2,006,904
#5	Search: #3 NOT #4 Sort by: Most Recent	5,732
#4	Search: ("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])) Sort by: Most Recent	4,704,526
#3	Search: ("Caloric Restriction"[Mesh] OR "Diet, Ketogenic"[Mesh] OR (("caloric reduction"[tiab] OR "calorie reduction"[tiab] OR "calories reduction"[tiab] OR "caloric restriction"[tiab] OR "calorie restriction"[tiab] OR "calories restriction"[tiab] OR "energy reduction"[tiab] OR "energy restriction"[tiab] OR "low caloric"[tiab] OR "low calorie"[tiab] OR "low calories"[tiab] OR "low energy"[tiab] OR "meal replacement"[tiab] OR "meal substitute"[tiab] OR "total replacement"[tiab]) AND (diet[tiab] OR diets[tiab])) OR "diet replacement"[tiab] OR "ketogenic diet"[tiab] OR "ketogenic diets"[tiab] OR "liquid diet"[tiab] OR "liquid diets"[tiab] OR "reducing diet"[tiab] OR "reducing diets"[tiab] OR ("total diet"[tiab] AND replacement[tiab]) OR VLED[tiab] OR VLCD[tiab] OR LCD[tiab]) AND ("Body Weight Changes"[Mesh:NoExp] OR "Weight Loss"[Mesh] OR (("adipose tissue"[tiab] OR fat[tiab] OR weight[tiab]) AND (decreas*[tiab] OR chang*[tiab] OR loss[tiab] OR losing[tiab] OR reduc*[tiab]))) Sort by: Most Recent	7,949
#2	Search: "Body Weight Changes"[Mesh:NoExp] OR "Weight Loss"[Mesh] OR (("adipose tissue"[tiab] OR fat[tiab] OR weight[tiab]) AND (decreas*[tiab] OR chang*[tiab] OR loss[tiab] OR losing[tiab] OR reduc*[tiab])) Sort by: Most Recent	553,343

Search	Query	Items identified
#1	Search: "Caloric Restriction"[Mesh] OR "Diet, Ketogenic"[Mesh] OR (("caloric reduction"[tiab] OR "calorie reduction"[tiab] OR "calories reduction"[tiab] OR "caloric restriction"[tiab] OR "calorie restriction"[tiab] OR "calories restriction"[tiab] OR "energy reduction"[tiab] OR "energy restriction"[tiab] OR "low caloric"[tiab] OR "low calorie"[tiab] OR "low calories"[tiab] OR "low energy"[tiab] OR "meal replacement"[tiab] OR "meal substitute"[tiab] OR "total replacement"[tiab]) AND (diet[tiab] OR diets[tiab])) OR "diet replacement"[tiab] OR "ketogenic diet"[tiab] OR "ketogenic diets"[tiab] OR "liquid diet"[tiab] OR "liquid diets"[tiab] OR "reducing diet"[tiab] OR "reducing diets"[tiab] OR ("total diet"[tiab] AND replacement[tiab]) OR VLED[tiab] OR VLCD[tiab] OR LCD [tiab] Sort by: Most Recent	20,854

E.3. Supplemental magnesium intake and risk of developing diarrhoea (Q3)

Date of search: 22 June 2020

Embase

Search	Query	Items identified
#24	#14 AND #22 AND [english]/lim	5,340
#23	#14 AND #22	5,792
#22	#15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21	5,441,679
#21	((data NEAR/1 (synthes* OR extraction* OR abstraction*)):ti,ab) OR handsearch*:ti,ab OR 'hand search':ti,ab OR 'hand searches':ti,ab OR 'hand searching':ti,ab OR 'mantel haenszel':ti,ab OR peto:ti,ab OR 'der simonian':ti,ab OR dersimonian:ti,ab OR 'fixed effect':ti,ab OR 'fixed effects':ti,ab OR 'latin square':ti,ab OR 'latin squares':ti,ab OR 'meta analysis':ti,ab OR 'meta analyses':ti,ab OR 'met analysis':ti,ab OR 'met analyses':ti,ab OR metaanaly*:ti,ab OR metanaly*:ti,ab OR 'meta regression':ti,ab OR 'meta regressions':ti,ab OR metaregression*:ti,ab OR medline:ti,ab OR cochrane:ti,ab OR pubmed:ti,ab OR medlars:ti,ab OR embase:ti,ab OR cinahl:ti,ab OR cochrane:jt OR 'evidence report':jt OR ((comparative NEAR/3 (efficacy OR effectiveness)):ti,ab) OR 'outcomes research':ti,ab OR 'relative effectiveness':ti,ab OR (((indirect OR 'indirect treatment' OR 'mixed treatment') NEAR/3 comparison):ti,ab)	457,245
#20	((systematic* NEAR/3 (review* OR overview*)):ti,ab) OR ((methodologic* NEAR/3 (review* OR overview*)):ti,ab) OR ((quantitative NEAR/3 (review* OR overview* OR synthes*)):ti,ab) OR ((research NEAR/3 (integrati* OR overview*)):ti,ab) OR ((integrative NEAR/3 (review* OR overview*)):ti,ab) OR ((collaborative NEAR/3 (review* OR overview*)):ti,ab) OR ((pool* NEAR/3 analy*):ti,ab)	273,287
#19	'meta analysis'/exp OR 'meta analysis (topic)'/exp OR 'systematic review'/exp OR 'systematic review (topic)'/exp OR 'biomedical technology assessment'/exp	402,087
#18	'crossover procedure'/exp OR (((crossover OR 'cross over') NEAR/10 (study OR studies OR design* OR method* OR procedure OR comparison)):ti,ab)	93,442
#17	((singl* OR doubl* OR trebl* OR tripl*) NEAR/10 (mask* OR blind* OR dumm*)):ti,ab	249,335
#16	'clinical trial (topic)'/exp OR 'double blind procedure'/exp OR 'single blind procedure'/exp OR 'triple blind procedure'/exp	532,591
#15	'clinical trial'/exp OR 'randomization'/exp OR randomized:ti,ab OR randomised:ti,ab OR placebo:ti,ab OR randomly:ti,ab OR trial:ti,ab OR groups:ti,ab OR (group1:ti,ab AND group 2:ti,ab)	4,864,364
#14	#12 NOT #13	16,546
#13	(rat:ti OR rats:ti OR mouse:ti OR mice:ti OR swine:ti OR porcine:ti OR murine:ti OR sheep:ti OR lambs:ti OR pigs:ti OR piglets:ti OR rabbit:ti OR rabbits:ti OR cat:ti OR cats:ti OR dog:ti OR dogs:ti OR cattle:ti OR bovine:ti OR monkey:ti OR monkeys:ti OR trout:ti OR marmoset*:ti) AND 'animal experiment'/de	1,064,998
#12	#10 NOT #11	16,569

Search	Query	Items identified
#11	'animal experiment'/de NOT ('human experiment'/de OR 'human'/de)	2,250,526
#10	#3 OR #6 OR #8 OR #9	18,100
#9	('magnesium'/exp OR 'magnesium salt'/exp OR magnesium:ti,ab,kw) AND ('dietary supplement'/exp OR 'fortified food'/exp OR 'diet'/de) OR ((magnesium NEAR/3 (intak* OR supplement* OR therap* OR diet*;ti,ab,kw)):ti,ab,kw) OR 'mg supplement*': ti,ab,kw OR 'mg fortified':ti,ab,kw OR 'magnesium intake'/exp OR 'magnesium'/mj/dd_dt OR 'magnesium salt'/mj/dd_dt	9,906
#8	#6 OR #7	6,767
#7	'magnesium'/exp/dd_ae,dd_to OR 'magnesium salt'/exp/dd_ae,dd_to	1,092
#6	#4 AND #5	5,886
#5	'adverse event'/de OR 'adverse drug reaction'/exp OR 'intoxication'/de OR 'drug intoxication'/exp OR 'drug overdose'/exp OR 'toxicity'/exp OR 'contraindication'/exp OR 'risk'/de OR 'risk factor'/exp OR 'risk assessment'/exp OR 'safety'/de OR 'patient safety'/exp OR 'food safety'/exp OR 'drug safety'/exp OR adverse:ti,ab,kw OR (((undesirable OR harm* OR serious OR negative OR side OR unwanted OR untoward) NEAR/5 (effect* OR reaction* OR event* OR interaction* OR outcome* OR response* OR sequala* OR sequela*)):ti,ab, kw) OR intoxic*:ti,ab,kw OR toxic*:ti,ab,kw OR overdose*:ti,ab,kw OR ((risk* NEAR/5 (assessment* OR factor*)):ti,ab,kw) OR contraindication*:ti,ab,kw OR 'contra indication': ti,ab,kw OR 'contra indications':ti,ab,kw OR safe:ti,ab,kw OR safety:ti,ab,kw	5,724,197
#4	'magnesium'/exp/mj OR 'magnesium salt'/exp/mj OR 'magnesium intake'/exp/mj OR magnesium:ti	44,385
#3	#1 AND #2	4,041
#2	'tolerability'/exp OR 'diarrhea'/exp OR diarrhe*:ti,ab,kw OR diarrhoe*:ti,ab,kw OR dysenter*:ti,ab,kw OR 'feces'/exp OR feces:ti,ab,kw OR faec*:ti,ab,kw OR fecal:ti, kw OR laxative:ti,ab,kw OR stool:ti,ab,kw OR stools:ti,ab,kw OR tolerab*:ti,ab,kw OR ((osmotic NEAR/3 (effect OR effects)):ti,ab,kw)	636,059
#1	'magnesium'/exp OR 'magnesium intake'/exp OR 'magnesium salt'/exp OR magnesium:ti, ab,kw	127,141

PubMed

Search	Query	Items identified
#19	Search: "english"[Language] AND #18 Sort by: Most Recent	4,751
#18	Search: #17 AND #14 Sort by: Most Recent	5,231
#17	Search: #16 OR #15 Sort by: Most Recent	3,731,983
#16	Search: systematic[sb] OR meta-analysis[pt] OR meta-analysis as topic[Mesh] OR meta-analysis[Mesh] OR meta analy*[tw] OR metanaly*[tw] OR metaanaly*[tw] OR met analy*[tw] OR integrative research[tiab] OR integrative review*[tiab] OR integrative overview*[tiab] OR research integration*[tiab] OR research overview*[tiab] OR collaborative review*[tiab] OR collaborative overview*[tiab] OR systematic review*[tiab] OR comparative efficacy[tiab] OR comparative effectiveness[tiab] OR outcomes research [tiab] OR indirect comparison*[tiab] OR Embase*[tiab] OR Cinahl*[tiab] OR systematic overview*[tiab] OR methodological overview*[tiab] OR methodologic overview*[tiab] OR methodological review*[tiab] OR methodologic review*[tiab] OR quantitative review* [tiab] OR quantitative overview*[tiab] OR quantitative synthes*[tiab] OR pooled analy* [tiab] OR Cochrane[tiab] OR Medline[tiab] OR Pubmed[tiab] OR Medlars[tiab] OR handsearch*[tiab] OR hand search*[tiab] OR meta-regression*[tiab] OR metaregression* [tiab] OR data synthes*[tiab] OR data extraction[tiab] OR data abstraction*[tiab] OR mantel haenszel[tiab] OR peto[tiab] OR der-simonian[tiab] OR dersimonian[tiab] OR fixed effect*[tiab] OR "Cochrane Database Syst Rev"[Journal:__jrid21711] Sort by: Most Recent	421,144

Search	Query	Items identified
#15	Search: "clinical trial"[pt] OR "Random Allocation"[Mesh] OR randomized[tiab] OR randomised[tiab] OR placebo[tiab] OR randomly[tiab] OR trial[tiab] OR groups[tiab] OR (group 1[tiab] AND group 2[tiab]) OR "Clinical Trials as Topic"[Mesh] OR "Double-Blind Method"[Mesh] OR "Single-Blind Method"[Mesh] OR ((singl*[tiab] OR doubl*[tiab] OR trebl*[tiab] OR tripl*[tiab]) AND (mask*[tiab] OR blind*[tiab] OR dumm*[tiab])) OR "Cross-Over Studies"[Mesh] OR ((crossover[tiab] OR "cross over"[tiab]) AND (study[tiab] OR studies[tiab] OR design*[tiab] OR method*[tiab] OR procedure[tiab] OR comparison [tiab])) Sort by: Most Recent	3,468,659
#14	Search: #12 NOT #13 Sort by: Most Recent	16,416
#13	Search: (rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti]) Sort by: Most Recent	2,009,802
#12	Search: #10 NOT #11 Sort by: Most Recent	16,693
#11	Search: ("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])) Sort by: Most Recent	4,709,914
#10	Search: #3 OR #8 OR #9 Sort by: Most Recent	21,965
#9	Search: (("Magnesium"[Mesh] OR Magnesium[tiab]) AND ("Dietary Supplements"[Mesh:noexp] OR "Food, Fortified"[Mesh] OR "Diet"[Mesh:NoExp] OR diet* [tiab] OR intak*[tiab] OR supplement*[tiab] OR therap*[tiab])) OR "Mg supplement*"[tiab] OR "Mg fortified"[tiab] OR "Magnesium/therapeutic use"[Mesh] Sort by: Most Recent	19,040
#8	Search: (("Magnesium"[Majr] OR magnesium[ti]) AND ("Drug-Related Side Effects and Adverse Reactions"[Mesh] OR "Poisoning"[Mesh] OR "Drug Hypersensitivity"[Mesh] OR "Drug Overdose"[Mesh] OR "Contraindications"[Mesh] OR "Risk"[Mesh] OR "Risk Factors"[Mesh] OR "Risk Assessment"[Mesh:NoExp] OR "Safety"[Mesh:NoExp] OR "Patient Safety"[Mesh] OR "Food Safety"[Mesh] OR adverse[tiab] OR contraindication* [tiab] OR "contra indication*"[tiab] OR safe[tiab] OR safety[tiab] OR risk assessment* [tiab] OR risk factor*[tiab] OR ((undesirable[tiab] OR harm*[tiab] OR serious[tiab] OR negative[tiab] OR side[tiab] OR unwanted[tiab] OR untoward[tiab]) AND (effect*[tiab] OR reaction*[tiab] OR event*[tiab] OR interaction*[tiab] OR outcome*[tiab] OR response*[tiab] OR sequala*[tiab] OR sequela*[tiab])))) OR ("magnesium/adverse effects"[MeSH Terms] OR "magnesium/poisoning"[MeSH Terms] OR "magnesium/ toxicity"[MeSH Terms]) Sort by: Most Recent	3,954
#7	Search: "magnesium/adverse effects"[MeSH Terms] OR "magnesium/poisoning"[MeSH Terms] OR "magnesium/toxicity"[MeSH Terms] Sort by: Most Recent	580
#6	Search: (("Magnesium"[Majr] OR magnesium[ti]) AND ("Drug-Related Side Effects and Adverse Reactions"[Mesh] OR "Poisoning"[Mesh] OR "Drug Hypersensitivity"[Mesh] OR "Drug Overdose"[Mesh] OR "Contraindications"[Mesh] OR "Risk"[Mesh] OR "Risk Factors"[Mesh] OR "Risk Assessment"[Mesh:NoExp] OR "Safety"[Mesh:NoExp] OR "Patient Safety"[Mesh] OR "Food Safety"[Mesh] OR adverse[tiab] OR contraindication* [tiab] OR "contra indication*"[tiab] OR safe[tiab] OR safety[tiab] OR risk assessment* [tiab] OR risk factor*[tiab] OR ((undesirable[tiab] OR harm*[tiab] OR serious[tiab] OR negative[tiab] OR side[tiab] OR unwanted[tiab] OR untoward[tiab]) AND (effect*[tiab] OR reaction*[tiab] OR event*[tiab] OR interaction*[tiab] OR outcome*[tiab] OR response*[tiab] OR sequala*[tiab] OR sequela*[tiab]))) Sort by: Most Recent	3,497
#5	Search: "Drug-Related Side Effects and Adverse Reactions"[Mesh] OR "Poisoning"[Mesh] OR "Drug Hypersensitivity"[Mesh] OR "Drug Overdose"[Mesh] OR "Contraindications"[Mesh] OR "Risk"[Mesh] OR "Risk Factors"[Mesh] OR "Risk Assessment"[Mesh:NoExp] OR "Safety"[Mesh:NoExp] OR "Patient Safety"[Mesh] OR "Food Safety"[Mesh] OR adverse[tiab] OR contraindication*[tiab] OR "contra indication*"[tiab] OR safe[tiab] OR safety[tiab] OR risk assessment*[tiab] OR risk factor* [tiab] OR ((undesirable[tiab] OR harm*[tiab] OR serious[tiab] OR negative[tiab] OR side [tiab] OR unwanted[tiab] OR untoward[tiab]) AND (effect*[tiab] OR reaction*[tiab] OR event*[tiab] OR interaction*[tiab] OR outcome*[tiab] OR response*[tiab] OR sequala* [tiab] OR sequela*[tiab])) Sort by: Most Recent	3,758,191

Search	Query	Items identified
#4	Search: "Magnesium"[Majr] OR magnesium[ti] Sort by: Most Recent	34,053
#3	Search: ("Magnesium"[Mesh] OR magnesium[tiab]) AND ("Diarrhea"[Mesh] OR diarrhe* [tiab] OR diarrho*[tiab] OR dysenter*[tiab]OR "Feces"[Mesh] OR feces[tiab] OR fecal [tiab] OR faec*[tiab] OR laxative[tiab] OR stool*[tiab] OR osmotic effect*[tiab] OR tolerab*[tiab]) Sort by: Most Recent	1,978
#2	Search: "Diarrhea"[Mesh] OR diarrhe*[tiab] OR diarrho*[tiab] OR dysenter*[tiab]OR "Feces"[Mesh] OR feces[tiab] OR fecal[tiab] OR faec*[tiab] OR laxative[tiab] OR stool* [tiab] OR osmotic effect*[tiab] OR tolerab*[tiab] Sort by: Most Recent	370,900
#1	Search: "Magnesium"[Mesh] OR magnesium[tiab] Sort by: Most Recent	101,094

E.4. Fat content of energy-restricted diets and risk of gallstone formation (Q4)

Date for search: 9 December 2020

Embase

Search	Query	Items identified
#9	#8 AND [2013-2021]/py	67
#8	#7 AND [english]/lim	160
#7	#5 NOT #6	179
#6	(rat:ti OR rats:ti OR mouse:ti OR mice:ti OR swine:ti OR porcine:ti OR murine:ti OR sheep:ti OR lambs:ti OR pigs:ti OR piglets:ti OR rabbit:ti OR rabbits:ti OR cat:ti OR cats:ti OR dog:ti OR dogs:ti OR cattle:ti OR bovine:ti OR monkey:ti OR monkeys:ti OR trout:ti OR marmoset*:ti) AND 'animal experiment'/de	1,089,880
#5	#3 NOT #4	178
#4	'animal experiment'/de NOT ('human experiment'/de OR 'human'/de)	2,294,858
#3	#1 AND #2	183
#2	'biliary sludge'/exp OR 'cholelithiasis'/exp OR 'gallbladder'/exp OR 'gallbladder function'/ exp OR (((bile OR biliary) NEAR/3 (calculus OR calculi OR sludge*)):ti,ab,kw) OR cholelith*:ti,ab,kw OR cholecystolithias*:ti,ab,kw OR gallbladder*:ti,ab,kw OR 'gall bladder*':ti,ab,kw OR gallsludge*:ti,ab,kw OR 'gall sludge*':ti,ab,kw OR gallstone*:ti,ab, kw OR 'gall stone*':ti,ab,kw OR 'vesica biliaris':ti,ab,kw OR 'vesica fellea':ti,ab,kw	121,762
#1	'low calorie diet'/exp/mj OR 'very low calorie diet'/exp/mj OR 'elimination diet'/exp/mj OR 'liquid diet'/exp/mj OR ((low NEAR/3 (calori* OR energy OR elimination) NEAR/5 (diet OR diets)):ti,ab,kw) OR vlcd:ti,ab,kw OR vled:ti,ab,kw OR lcd:ti,ab,kw OR (((ketogenic OR replacement OR 'meal substitute' OR liquid) NEAR/5 (diet OR diets)):ti,ab,kw) OR 'reducing diet':ti,ab,kw OR 'reducing diets':ti,ab,kw OR (((calori* OR energ*) NEAR/3 (reduc* OR restrict*) NEAR/5 (diet OR diets)):ti,ab,kw)	26,839

Pubmed

Search	Query	Items identified
#9	(#8) AND (("2014"[Date - Publication]: "3000"[Date - Publication]))	14
#8	#7 AND ENGLISH[Language]	56
#7	#5 NOT #6	69
#6	(rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR swine[ti] OR porcine[ti] OR murine[ti] OR sheep[ti] OR lambs[ti] OR pigs[ti] OR piglets[ti] OR rabbit[ti] OR rabbits[ti] OR cat[ti] OR cats[ti] OR dog[ti] OR dogs[ti] OR cattle[ti] OR bovine[ti] OR monkey[ti] OR monkeys[ti] OR trout[ti] OR marmoset*[ti])	2,307,004
#5	#3 NOT #4	69
#4	("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh]))	4,762,683

Search	Query	Items identified
#3	#1 AND #2	80
#2	"Cholecystolithiasis"[Mesh] OR "Gallbladder"[Mesh] OR "Gallbladder Emptying"[Mesh] OR "Gallstones"[Mesh] OR "bile duct calcul*"[tiab] OR "bile duct sludge*"[tiab] OR "bile calcul*"[tiab] OR "bile sludge*"[tiab] OR "biliary calcul*"[tiab] OR "biliary sludge"[tiab] OR "biliary system calcul*"[tiab] OR "biliary system sludge*"[tiab] OR cholelith*[tiab] OR cholecystolithias*[tiab] OR gallbladder*[tiab] OR "gall bladder*"[tiab] OR gallsludge[tiab] OR "gall sludge*"[tiab] OR gallstone*[tiab] OR "gall stone*"[tiab] OR "vesica biliaris"[tiab] OR "vesica fellea"[tiab]	68,834
#1	"Caloric Restriction"[Mesh] OR "Diet, Ketogenic"[Mesh] OR (("caloric reduction"[tiab] OR "calorie reduction"[tiab] OR "calories reduction"[tiab] OR "caloric restriction"[tiab] OR "calorie restriction"[tiab] OR "calories restriction"[tiab] OR "energy reduction"[tiab] OR "energy restriction"[tiab] OR "low caloric"[tiab] OR "low calorie"[tiab] OR "low calories"[tiab] OR "low energy"[tiab] OR "meal replacement"[tiab] OR "meal substitute"[tiab] OR "total replacement"[tiab]) AND (diet[tiab] OR diets[tiab])) OR "diet replacement"[tiab] OR "ketogenic diet"[tiab] OR "ketogenic diets"[tiab] OR "liquid diet"[tiab] OR "liquid diets"[tiab] OR "reducing diet"[tiab] OR "reducing diets"[tiab] OR ("total diet"[tiab] AND replacement[tiab]) OR VLED[tiab] OR VLCD[tiab] OR LCD[tiab]	21,663