Abating abdominal adiposity: Modifiable lifestyle risk factors for visceral and liver fat deposition

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Dietary effects of macronutrients and macronutrient types on liver fat content in adults: a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Dietary macronutrient composition may affect hepatic liver content and its associated diseases, but the results from human intervention trials have been equivocal or underpowered. We aimed to assess the effects of dietary macronutrient composition on liver fat content by conducting a systematic review and meta-analysis of randomized controlled trials in adults. Four databases (PubMed, Embase, Web of Science and COCHRANE Library) were systematically searched for trials with isocaloric diets evaluating the effect of dietary macronutrient composition (energy percentages of fat, carbohydrates and protein, and their specific types) on liver fat content as assessed by magnetic resonance techniques, computed tomography or liver biopsy. Data on change in liver fat content were pooled by random or fixed-effects meta-analyses and expressed as standardized mean difference (SMD). We included 21 randomized controlled trials providing data for 25 comparisons on dietary macronutrient composition. A high-carbohydrate low-fat diet did not change liver fat content as compared with a low-carbohydrate high-fat diet (12 comparisons, SMD 0.01 (95% CI -0.36; 0.37)). Heterogeneity was substantial (I^2 67.8%, p<0.001). Unsaturated fat as compared with saturated fat reduced liver fat content (3 comparisons, SMD -0.75 (95% CI -1.11; -0.39)). A high-protein low-carbohydrate diet reduced liver fat content as compared with a low-protein high-carbohydrate diet (3 comparisons, SMD -0.32 (95% CI -0.58; -0.05)). Our meta-analyses showed that replacing carbohydrates with total fat on liver fat content was not effective, while replacing carbohydrates with proteins was. We showed that unsaturated fat consumption leads to less liver fat content compared with saturated fat consumption. Too few studies were included to perform separate meta-analyses on types of carbohydrates and proteins, and therefore more well-performed and well-described studies on the effect of types of carbohydrates and proteins on liver fat content are needed, especially studies comparing proteins with fats.

INTRODUCTION

Non-alcoholic fatty liver (NAFL) is clinically defined as a liver fat content of more than 5.6%, not due to excessive alcohol consumption [1]. It is a major cause of chronic liver disease worldwide, associated with an increased risk of liver- and cardiovascular disease-related mortality [2-5]. Moreover, obesity and other features of the metabolic syndrome such as dyslipidaemia, insulin resistance and diabetes mellitus, are associated with NAFL [6-10]. The prevalence of NAFL continues to rise [2, 3] and has been estimated at 25% in adults [2], and between 65% and 85% in adults with obesity [11].

Since NAFL is still reversible, adequate treatment is needed to prevent the development into more severe forms of hepatic fat storage such as non-alcoholic steatohepatitis (NASH)[12, 13]. Drug-based treatments are primarily recommended for patients with a later stage of NAFL, whereas lifestyle changes are a cornerstone in guidelines on treatment of NAFL, including weight loss, eating healthier, and increasing physical exercise [12]. To date, interventions on NAFL mainly focus on decreasing total body fat by recommending calorie restricted diets in overweight or obese patients [14-16]. However, besides diet quantity in the form of caloric restriction, macronutrient composition may be of importance, although evidence on this is scarce. Recent meta-analyses have shown that supplementation of omega-3 polyunsaturated fatty acids (PUFAs) is an effective intervention for reducing NAFL [17, 18].

Besides specific types of macronutrient such as omega-3 polyunsaturated fatty acids and fructose consumption, there are no meta-analyses on other macronutrients and other macronutrient types. In only one review on the effects of macronutrients on liver fat it has been described that a relatively high consumption of saturated fat increases the percentage of liver fat, whereas an increased consumption of refined sugars had no influence on liver fat [19]. However, the search of this review was limited and was not substantiated by a meta-analysis. Therefore, it remains unclear whether dietary macronutrients and their composition affect liver fat content. We aimed to assess the effect of dietary macronutrient composition on liver fat content, as measured by magnetic resonance imaging, proton magnetic resonance spectroscopy, computed tomography or liver biopsy, by performing a systematic review and meta-analysis of isocaloric randomized controlled trials in adults.

METHODS

This systematic review and meta-analysis on dietary macronutrient composition and liver fat content was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-) guidelines and the recommendations of the Cochrane Collaboration [20, 21]. The protocol is registered at PROSPERO with registry ID number 100356.

Eligibility criteria

Databases were systematically searched for eligible publications based on a priori determined eligibility criteria. We systematically searched for randomized controlled dietary intervention trials evaluating the effect of macronutrient composition on liver fat content in adults. Studies including healthy adults as well as patients with obesity, metabolic syndrome, (pre)diabetes, NAFL or NASH and/or cardiovascular disease, were considered eligible. Trials that included individuals with malignant diseases or with alcoholic, drug-induced, viral or genetic causes of liver injury, were excluded.

Both macronutrient comparisons (carbohydrates versus fat, carbohydrates versus protein, protein versus fat) and macronutrient types comparisons (types of fat, types of carbohydrates and types of protein) were assessed. Since several reviews and meta-analyses on omega-3 fatty acids and fructose have been published recently [17, 22-26], studies were excluded when the dietary intervention was primarily focused on these types of macronutrient comparisons. Studies that used hyper- or hypo-caloric interventions were only eligible when caloric intake was equal in both study arms. Furthermore, the interventions had to be provided for at least one week, since seven days of dietary intervention was deemed necessary to influence fat oxidation in the liver [27]. In addition, trials that involved co-interventions, such as exercise or other lifestyle interventions, were only included when similar in both arms of the trial. Trials solely providing their participants with dietary advice rather than food items, as well as trials presenting insufficient information on macronutrient composition were not eligible. Assessment methods of liver fat content were predefined: only trials in which liver fat content was measured by magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), computed tomography (CT) or liver biopsy were considered [28, 29].

Search strategy

We conducted a systematic search to identify eligible publications. In cooperation with a trained librarian (JWS), a detailed search strategy was composed for the four bibliographic databases: PubMed, Embase (OVID-version), Web of Science, and COCHRANE Library. The search query consisted of a combination of the following concepts: macronutrients (exposure terms), liver fat (outcome terms) and (randomized controlled) trials. The search strategy was adjusted for all consulted databases, taking into account the differences of the various controlled vocabularies as well the differences of database specific technical variations (e.g., the use of quotation marks). Case reports, animal-only studies and conference abstracts were excluded. No restrictions were made on language and publication year. The final search was performed on February 19th, 2018 and repeated on June 17, 2019. All search strings used can be found in the supplementary data.

Study selection process

First, duplicate publications were removed. Titles and abstracts of remaining identified publications were screened for eligibility by 6 reviewers (BdR, EvE, HP, IV, KR, MA) in preassembled pairs. Each reviewer of a pair independently screened and coded an assigned part of the articles 'include', 'unclear' or 'exclude'. Disagreements on inclusion were discussed in the pre-assembled pairs until consensus was reached. Subsequently, potentially relevant publications were independently assessed in full-text by three reviewers (BdR, IV, EvE). In case of multiple publications of a single trial, the first published version was included. Discrepancies on the eligibility of articles were resolved by discussion until consensus was reached. The selection of publications was managed by the Rayyan QCRI web application (Qatura Computing Research Institute, 2016) [30].

Data collection and extraction

Data extraction was independently performed by two reviewers (EvE and IV) using a predefined sheet in Microsoft Excel, Version 15.40. Extracted data were compared and discrepancies were resolved. Data were extracted on four categories following the recommendations of the Cochrane Collaboration; characteristics of the study (i.e., dietary comparison, location, design), the participants (i.e., number of randomized/analyzed participants, sex, mean age, mean body weight, mean BMI), the dietary interventions (i.e., compositions, follow-up time) and the outcomes per arm of the trial [21].

Risk of bias assessment

Two reviewers (EvE and IV) independently assessed the risk of bias for included studies, using the Cochrane 'Risk of bias' tool for randomized controlled trials [24]. This tool involved a classification of six different domains of bias (i.e., selection bias, performance bias, attrition bias, detection bias, reporting bias and (design-specific) other sources of bias) with seven corresponding domains: random sequence generation, allocation

concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and "other sources of bias". For detection of the "other sources of bias", reviewers were in particular alert to (self)reporting bias, compliance assessment and carry-over effects in cross-over trials, with trials lacking a wash-out period being at higher risk. Each domain was separately judged as having a "low", "high" or "unclear" risk of bias. In addition, a support for judgement was given and summarised following the criteria outlined by the Cochrane Collaboration [21]. Any discrepancies in bias coding were resolved by discussion.

Direct pairwise meta-analyses

To perform meta-analyses for continuous outcomes measured with different measuring instruments of liver fat on different scales (i.e., MRS/MRI (%) and CT-scans (Hounsfield Units)), effect estimates were expressed as standardized mean difference (SMD) with corresponding 95% confidence interval (95% CI). When studies only reported relative changes in liver fat, the absolute change based on the relative change and the baseline value was calculated. If trials presented medians and interquartile ranges (IQRs), values were converted into means and standard deviations according to the Cochrane Collaboration [24].

Intervention effects were pooled by performing standard pairwise meta-analyses for all comparisons that contained at least three comparisons between diets. A random-effects model was used (method of DerSimonian and Laird [31]) for the comparison between a low-carbohydrate high-fat and a high-carbohydrate low-fat diet and due to the limited number of included studies a fixed-effect model for the other two comparisons. For the study of Luukkonen et al.[32], two interventions (saturated fat and unsaturated fat) were compared against the same control group (carbohydrates). To correct for these multiple correlated comparisons the number of participants in the control arm was divided by the number of comparisons (i.e. two) thereby creating two (reasonably independent) comparisons (Cochrane handbook Chapter 16.5.4). We performed a sensitivity analysis in which the two groups with physical activity as a co-intervention from the study of Bozzetto et al were excluded to eliminate the potential effect of physical activity on the results. The diet that was expected to be beneficial, as described in the rationale of the included studies, was considered as the intervention arm (high unsaturated fat-low saturated fat, high protein-low carbohydrates and high-carbohydrates low-fat), and the other the control arm (saturated fat, high carbohydrates and high fat). As a result, a negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured. In case of an overfeeding design, a negative standardized mean difference represents a smaller increase in liver fat in the intervention arm compared to the control arm. A positive standardized mean difference indicates that the control arm is favoured. Guidelines state that an SMD of 0.2 can be considered small, 0.5 as medium and 0.8 as high [33].

Statistical heterogeneity was assessed using the I-squared statistic [37]. Heterogeneity was considered to be low if the I^2 value was under 40%, moderate if between 30% to 60%, substantial if between 50% to 90% and considerable when between 75% and 100% [24]. All statistical analyses were conducted using Stata statistical Software (Statacorp, College Station, Texas, USA) version 14.

Handling missing data

In case of unreported or incomplete data on mean changes (or SD) in liver fat content between baseline and follow-up, the original investigators were contacted and asked to provide missing data. When no response was received, we calculated mean differences using standard deviations based on the information that was provided (baseline or follow-up value with corresponding SD), as described in a previous meta-analysis [34]. Trials were not included when relevant data to calculate mean differences was not provided [21].

Small-study effects

A funnel plot was used for graphical examination of small-study effects [39, 40]. In addition, Egger's test was performed [24, 40] if more than 10 studies for a specific analysis were available [41].

RESULTS

Study selection

Of the 4.291 publications retrieved, a total of 3.320 unique publications were screened on title and abstract (Figure 1). Of those, 3.215 publications were excluded after screening of titles and abstracts for eligibility. A total of 105 articles were assessed for eligibility based on full text, of which 84 were excluded due to the following reasons: no dietary intervention (n=23), interventions not isocaloric (n=10), multiple publications from a single trial (n=4), no original research paper (n=7), co-interventions not equal in both arms (n=2), no adequate comparison (n=3), no MRI/MRS/CT/biopsy liver fat outcome (n=24), population younger than 18 years (n=3) or no RCT design (n=8), leaving a total of 21 included articles [32, 35-54] (**Figure 1**).

For one study, only two out of three arms were incorporated into the meta-analysis, as the diet in one arm contained less calories than the diet in the other two arms [49]. Ultimately, 25 eligible comparisons remained for analyses as three studies contained more than one comparison [32, 38, 39].

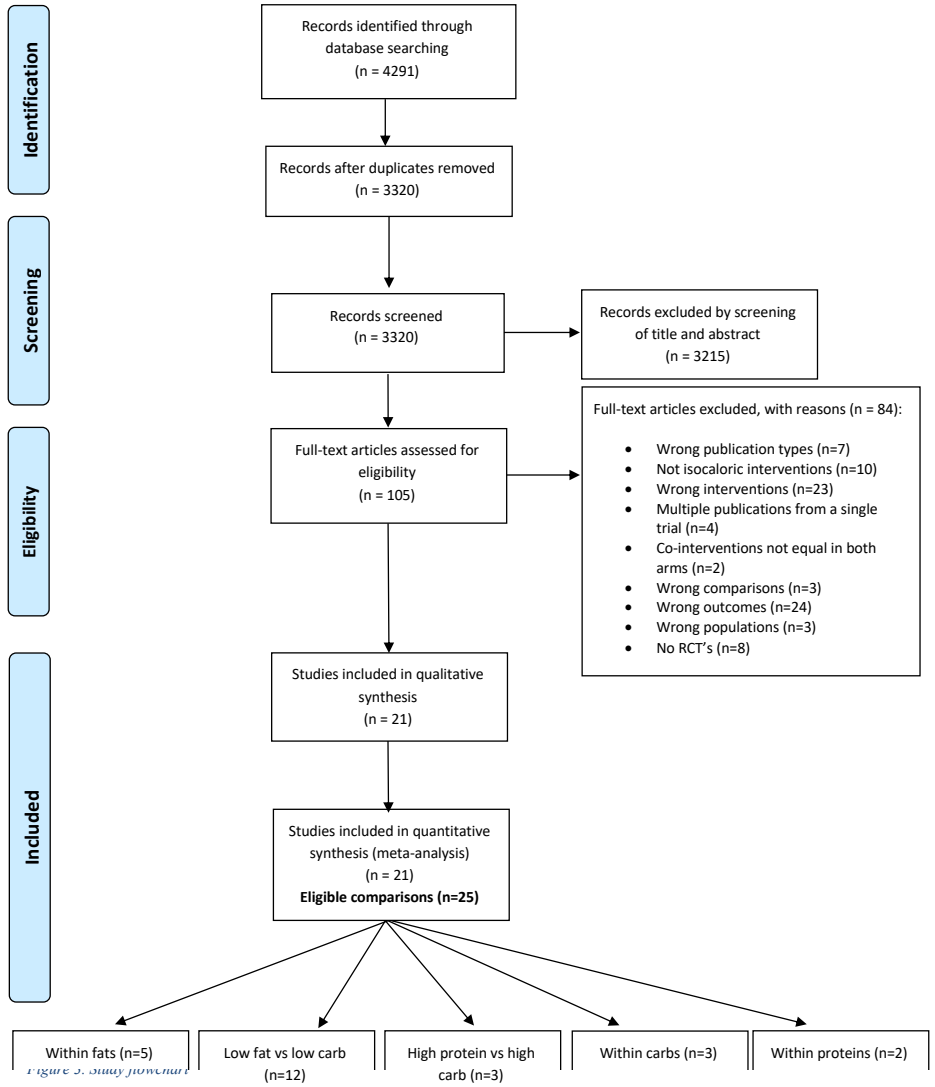

Figure 1. Flowchart of included randomized controlled trials in meta-analysis on dietary macronutrient composition in relation to liver fat

Study characteristics

Table 1 shows the characteristics of the 21 randomized controlled trials. Studies were published between 2002 and 2019 and the number of participants ranged from 7 to 166. The duration of the studies varied between 7 days and two years. With regard to the macronutrient comparisons, ten studies reported effects of a low-carbohydrate high-fat (LCHF)-diet compared with a high-carbohydrate low-fat (HCLF)-diet [32, 38-43, 51-53]. Three studies compared a low-protein high-carbohydrate (LPHC)-diet with a high-protein low-carbohydrate (HPLC)-diet [45, 48, 49]. There were no studies on the comparisons between fat and protein content of the diet.

The other studies performed comparisons between types of macronutrients. A total of five studies compared different types of dietary fat, of which three studies compared a diet high in saturated fatty acids (SFAs) with a diet high in unsaturated fatty acids (UFAs) [32, 37, 50], one study compared trans fatty acids with palm- and sunflower oil [36] and one study looked at replacement of long chain fatty acids with medium chain fatty acids [47]. In two studies dietary fibres were compared with other carbohydrates [35, 39], one study compared whole grain wheats with refined wheats [54] and in two studies diets containing animal protein was compared with diets containing plant/soy protein [44, 46].

In total, sixteen studies used a parallel design, whereas five had a cross-over design [35, 43, 46, 49, 52]. Two studies assessed the liver fat content using CT [47, 48], whereas all other studies used MRS/MRI. One study assessed liver fat content both with MRI and MRS, of which we chose to use the MRS results in the meta-analysis as this is considered the most reliable method [11]. Most studies mainly included participants with overweight or obesity, varying from adolescents to elderly, except for six studies that included lean participants [35, 43, 45, 47, 49, 50](Table 1). The amount of (macro)nutrients exchanged varies considerably between studies (**Supplemental table 1**). Additional information on the macronutrient composition per study arm can be found in Supplemental table 1.

Risk of bias

The risk of bias assessment for included studies can be found in **Table 2**. In six studies there was high risk of performance bias, in two studies there was high risk of detection bias, in four studies of attrition bias, in seven studies of reporting bias and in six studies there was a high risk of other bias.

The majority of the studies had an unclear risk of selection bias due to a lack of information on concealment of allocation. Overall, there was unclear risk of selection bias and detection bias, and substantial risk of performance, attrition, reporting and other types of bias.

Table 1. Characteristics of randomized controlled trials included in meta-analysis on association between dietary macronutrient composition and hepatic triglyceride content

Author, year	Study design	Length (days)	Run-in/ wash-out	Liver fat measure-ment	Men (%)	Age range or mean age (y)	BMI range or mean at baseline (kg/m²)	Intervention	N	Control	N
Bawden, 2016	Cross-over	7	No/Yes	^{1}H-MRS	100	20.1	23.0	LGI (high fiber)	7	HGI (low fiber)	7
Bendsen, 2011	Parallel	112	No/NA	^{1}H-MRS	0	45-70	25-32	Control	23	Trans fatty acids	23
Bjermo, 2012	Parallel	70	No/NA	^{1}H-MRS	34	30-65	30.8[1]	PUFA	28	SFA	28
Bozzetto, 2012[a]	Parallel	56	Yes/NA	^{1}H-MRS	75.0	35-70	29.1[1]	CHO/fiber	9	MUFA	8
Bozzetto, 2012[b]							30.5[1]	CHO/fiber (+ exercise)	10	MUFA (+ exercise)	9
Errazuriz, 2017	Parallel	84	Yes/NA	^{1}H-MRS	53.3	61.7	31.7[1]	Control	11	MUFA	15
Errazuriz, 2017								Fiber	13	Control	11
Haufe, 2017	Parallel	~180	No/NA	^{1}H-MRS	17.6		>25	Low fat	50	Low carbohydrates	52
Gepner, 2019	Parallel	~180	No/NA	MRI	85.4	47.7	30.9[1]	Low fat	79	Mediterranean/low carbohydrate	78
Herpen, 2011	Parallel	42	Yes/NA	^{1}H-MRS	100	55.2	28.8[1]	Low fat/high carbohydrates	9	High fat/ low carbohydates	9
Kirk, 2009	Parallel	42	No/NA	^{1}H-MRS	18.2	43.6	36.5	High carbohydrates	11	Low carbohydrates	11
Luukkonen, 2018	Parallel	21	No/NA	^{1}H-MRS	44.7	48.0	31.0	Carbohydrates	12	Unsaturated fat	12
Luukkonen, 2018								Carbohydrates	12	Saturated fat	14
Luukkonen, 2018								Unsaturated fat	12	Saturated fat	14
Marina, 2014	Cross-over	28	Yes/Yes	^{1}H-MRS	76.9	36.0	33.6	Low fat	10	High fat	10
Markova, 2017	Parallel	42	No/NA	^{1}H-MRS	64.9	49-78	30.2[1]	Animal protein	18	Plant protein	19
Martens, 2014	Parallel	84	No/NA	^{1}H-MRS	33.3	24.0	22.9	High protein/low carbohydates	7	Low protein/high carbohydrates	9
Nosaka, 2002	Parallel	28	No/NA	CT	100	27-51	23.1[1]	Long-chain triacylglycerols	11	Medium-chain triacylglycerol	11
Ooi, 2015	Parallel	~730	No/NA	CT	0	70-80	26.5[1]	Protein	82	Control	84
Rietman, 2014	Cross-over	14	Yes/No	^{1}H-MRS	70.4	22.8	21.5	Normal protein/ normal carbohydrates	17	High protein/ low carbohydrates	17
Rosqvist, 2014	Parallel	49	No/NA	MRI	70.3	20-38	20.3[1]	SFA	19	PUFA	18
Schutte, 2018	Parallel	84	Yes/NA	1H-MRS	62.0	45-70	27.81	Whole grain wheat	20	Refined wheats	18
Utzschneider, 2013	Parallel	28	No/NA	^{1}H-MRS	37.0	69.3	27.4[1]	Low SAT/LGI	20	High fat/HGI	15
Van Nielen, 2014	Cross-over	28	Yes/NA	^{1}H-MRS	0	61.0		Soy protein	10	Mixed protein	10
Westerbacka, 2005	Cross-over	14	Yes/Unclear	^{1}H-MRS	0	43	33.0	Low fat	10	High fat	10

[1]Weighted mean BMI based on mean baseline BMI values of separate arms. BMI, body mass index; CHO, carbohydrates; CT, computed tomography; ^{1}H-MRS, proton magnetic resonance spectroscopy; HGI, high glycaemic index; LGI, low glycaemic index; MRI, magnetic resonance imaging; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acids.

Table 2. Risk of bias of randomized controlled trials included in systematic review and meta-analysis on macronutrient and macronutrient types composition in relation to liver fat content in adults of 18 years and older

	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
First author	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Bawden, 2017	Unclear	Unclear	Unclear	Low	Unclear	Unclear	Low
Bendsen, 2011	Low	Low	Low	Low	Low	Unclear	Unclear
Bjermo, 2012	Unclear	Unclear	High	Low	High	Unclear	High
Bozzetto, 2012	Low	Low	Unclear	Low	Low	Unclear	High
Errazuriz, 2007	Unclear	Unclear	Unclear	Low	Low	High	Low
Haufe, 2017	Low	Low	High	Unclear	High	Unclear	Unclear
Gepner, 2019	Unclear	Unclear	High	Low	High	Unclear	High
Herpen, 2011	Low	Unclear	Unclear	Unclear	Low	High	High
Kirk, 2009	Unclear	Unclear	Unclear	Unclear	Low	Unclear	High
Luukkonen, 2018	Unclear	Unclear	High	Low	Low	Low	Low
Marina, 2014	Unclear	Unclear	Unclear	Low	High	High	High
Markova, 2017	Low	Unclear	High	High	Unclear	Low	Unclear
Martens, 2014	Low	Low	High	Unclear	High	High	Low
van Nielen, 2014	Unclear	Unclear	High	Low	Unclear	Low	Low
Nosaka, 2002	Low	Unclear	Low	Unclear	Low	Unclear	Unclear
Ooi, 2015	Low	Low	Low	Low	Low	High	Low
Rietman, 2014	Low	Low	Low	Unclear	Low	High	Unclear
Rosqvist, 2014	Low	Unclear	Low	Low	Unclear	Unclear	Unclear
Schutte, 2018	Unclear	Unclear	Low	Unclear	High	Unclear	Low
Utzschneider, 2012	Unclear	Unclear	Low	Low	Low	Unclear	Unclear

Effects of interventions

Table 3 provides a summary of findings for all included trials. It also shows the changes in liver fat content and corresponding SMDs for all studies individually. Based on all included trials, we were able to perform three meta-analyses, as described below. A total of 21 studies were included, comprising a total of 25 comparisons between different diets. As we decided to only perform a meta-analysis on exchanges that contained at least three comparisons between dietary intervention arms, we could not meta-analyse comparisons of trans fats with palm- and sunflower oil, long chain with medium chain fat, dietary fibre with other carbohydrates, whole grain wheats with refined wheats, and animal protein with plant protein. Due to the limited number of included trials, we were not able to perform subgroup analyses on disease state, sex, ethnicity or study duration. Moreover, as there were no studies comparing dietary protein with fat, we could not

perform a network meta-analysis in which all macronutrients could be compared both directly and indirectly [35, 36, 39, 44, 46, 47].

High-carbohydrate low-fat versus low-carbohydrate high-fat diets

Out of 12 comparisons for a low-carbohydrate high-fat with a high-carbohydrate low-fat diet, three comparisons favoured a low-carbohydrate high-fat diet over a high-carbohydrate low-fat diet [38, 39], while two other comparisons showed the opposite [41, 52] **(Figure 2).** The other studies showed no difference. Heterogeneity was substantial (67.8%). No small study effects seemed to be present (**Supplemental figure 1**) (*P*-value for Egger's test 0.58). The overall pooled effect of high-carbohydrate low-fat versus high-fat low-carbohydrate was: SMD 0.01, 95% CI -0.36; 0.37 (Figure 2).

After excluding the two groups with a co-intervention of physical exercise from the study of Bozzetto, results were similar (data not shown).

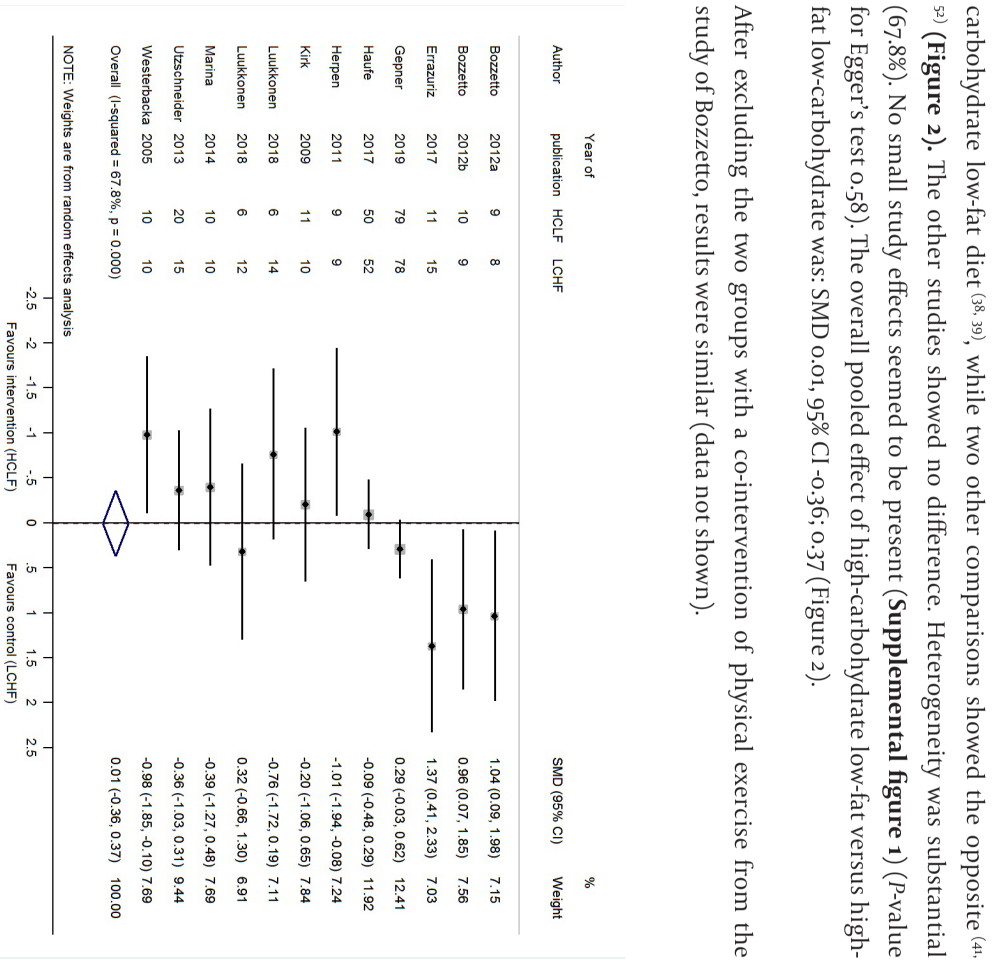

Figure 2. Difference between effects of a low-carbohydrate high-fat diet (LCHF) and a high-carbohydrate low-fat (HCLF) on liver fat content in studies included in meta-analysis: a random effects model. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms. A negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured.

Table 3. Standardized mean differences of randomized controlled trials included in meta-analysis on association between dietary macronutrient composition and hepatic triglyceride content

Author	Intervention	N	Change in liver fat after intervention (% or HU)	Control	N	Change in liver fat after intervention (% or HU)	Mean difference in change in liver fat between arms (intervention-control, % or HU)	Standard deviation of mean difference	Standardized mean difference
Bawden, 2016	Fibre	7	-0.4	Other carbs	7	1.3	-1.70	1.46	-1.16
Bendsen, 2011	Palm/sunflower oil	23	-0.6	Trans fatty acids	23	-0.8	0.20	4.10	0.05
Bjermo, 2012	PUFA	28	-0.9	SFA	28	0.3	-1.20	2.01	-0.60
Bozzetto, 2012[a]	Low fat	9	-1.6	Low carb	8	2.2	0.60	0.58	1.04
Bozzetto, 2012[b]	Low fat	10	0.1	Low carb	9	-2.5	2.60	2.70	0.96
Errazuriz, 2017	Low fat	11	0.7	Low carb	15	-1.7	2.40	1.75	1.37
Errazuriz, 2017	Fibre	13	-0.6	Other carbs	11	0.7	-1.30	1.33	-0.98
Gepner, 2019	Low fat	79	-5.8	Low carb	78	-7.3	1.5	5.31	0.29
Haufe, 2017	Low fat	50	-4.0	Low carb	52	-3.6	-0.40	4.31	-0.09
Herpen, 2011	Low fat	9	-0.52	Low carb	9	0.37	-0.89	0.88	-1.01
Kirk, 2009	Low fat	11	-4.98	Low carb	10	-4.71	-0.27	1.35	-0.20
Luukkonen, 2018	UFA	12	0.79	SFA	14	2.72	-1.93	1.76	-1.10
Luukkonen, 2018	Low fat	12	1.37	Low carb	14	2.72	-1.35	1.77	-0.76
Luukkonen, 2018	Low fat	12	1.37	Low carb	12	0.79	0.58	1.78	0.32
Marina, 2014	Low fat	10	-2.2	Low carb	10	-1.3	-0.90	2.28	-0.39
Markova, 2017	Plant protein	17	-6.8	Animal protein	15	-6.7	-0.10	8.96	-0.01
Martens, 2014	High protein	7	-0.03	High carb	9	0.05	-0.08	0.08	-1.05
Nosaka, 2002	Long chain FA	11	0.03	Medium chain FA	11	0.02	0.01	0.10	0.1
Ooi, 2015	High protein	82	0.00	High carb	84	0.04	-0.04	0.20	-0.2
Rietman, 2014	High protein	17	-0.05	High carb	17	0.11	-0.16	0.26	-0.62
Rosqvist, 2014	PUFA	18	0.04	SFA	19	0.56	-0.52	0.71	-0.73
Utzschneider, 2013	Low fat	20	-0.50	Low carb	15	0.4	-0.9	2.50	-0.36
Van Nielen, 2014	Soy protein	10	-0.4	Meat protein	10	-0.9	0.5	0.84	0.59
Schutte, 2018	Whole grain wheats	20	0.53	Refined grain wheats	18	2.00	-1.47	2.00	-0.73
Westerbacka, 2005	Low fat	10	-2.0	Low carb	10	3.5	-5.5	5.62	-0.98

FA, fatty acid; HU, Hounsfield Unit; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acids. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms.

Dietary saturated fat versus unsaturated fat

Only three studies examined the effect of unsaturated fat compared to saturated fat, of which all three found that an unsaturated fat diet reduces liver fat compared with saturated fat [32, 37, 50] **(Figure 3).** The overall effect showed that unsaturated fat as compared with saturated fat reduced liver fat to a large extent (SMD -0.75, 95% CI -1.11; -0.39. A funnel plot is shown in **Supplemental figure 2**; Egger's test was not performed due to an insufficient number of included studies.

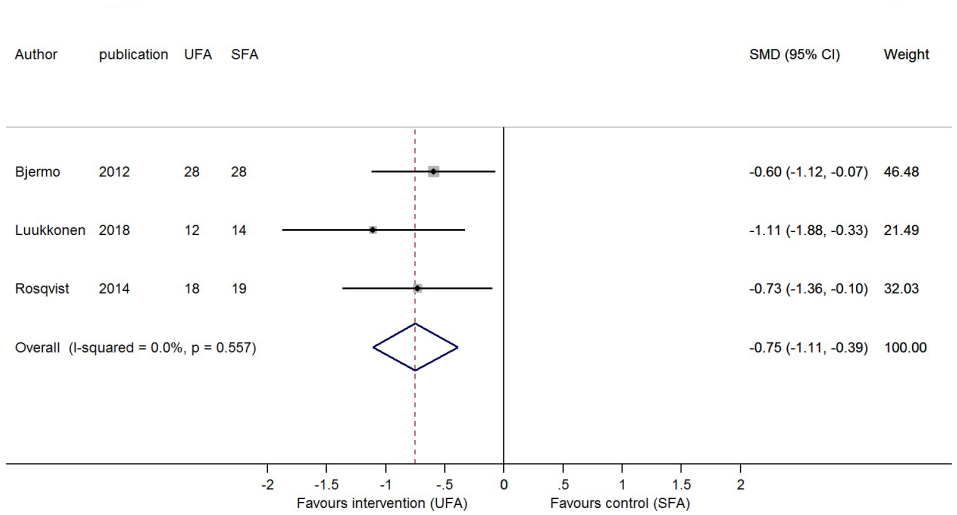

Figure 3. Difference between effects of a diet high in saturated fats (SFA) and a diet high in unsaturated fat (UFA) on liver fat content in studies included in meta-analysis: a fixed effects model. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms. A negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured.

High-protein low-carbohydrate versus low-protein high-carbohydrate diets

Three studies assessed the effect of a high protein-low carbohydrate compared to a low-protein high-carbohydrate diet on liver fat. One study found that a high-protein low-carbohydrate diet resulted in reduced liver fat content compared to a low-protein high-carbohydrate diet [45], whereas the other two studies did not find a difference [48, 49] (**Figure 4**). The overall pooled effect showed that a high-protein low-carbohydrate diet moderately reduced liver fat as compared to a low-protein high-carbohydrate diet (SMD -0.32, 95%CI -0.58; -0.05). A funnel plot is shown in **Supplemental figure 3**, Egger's test was not performed due to an insufficient number of included studies.

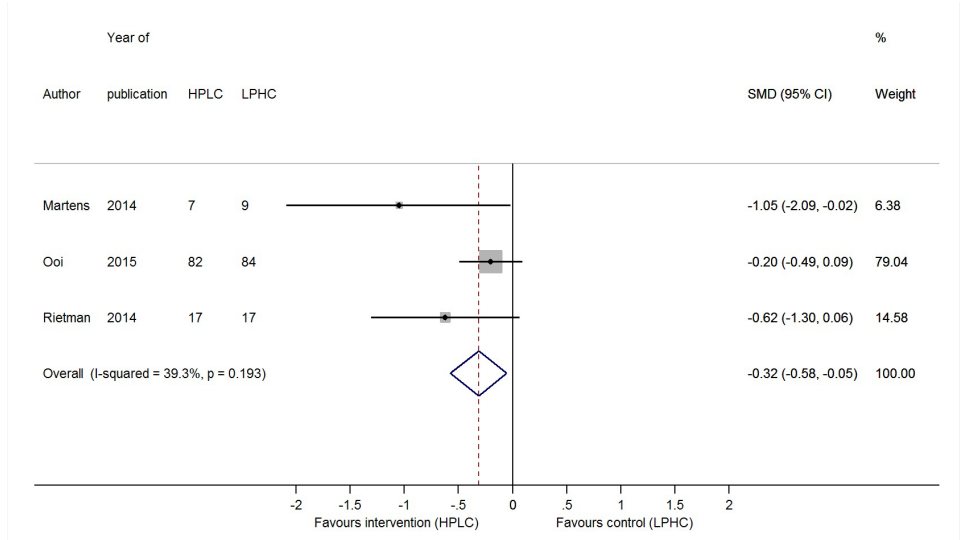

Figure 4. Difference between effects of a low-protein high-carbohydrate (LPHC) diet and a high-protein low-carbohydrate (HPLC) diet on liver fat content in studies included in meta-analysis: a fixed effects model. Standardized mean difference (SMD) was calculated by dividing the mean difference between the arms by the standardized deviation of the difference between the arms. A negative standardized mean difference can be interpreted as a decrease in liver fat in the intervention arm compared with the control arm, which means that the intervention arm is favoured.

DISCUSSION

With this systematic review and meta-analysis including randomized controlled trials we have provided a summary of the evidence on the effect of dietary macronutrient composition on the amount of liver fat, as assessed by ^{1}H-MRS, MRI or CT. Our results show that replacing dietary fat with carbohydrates did not result in changes in liver fat. Diets high in unsaturated fat lead to a larger decrease (or smaller increase in case of an overfeeding design) in liver fat content than diets high in saturated fat. A high-protein low-carbohydrate diet reduces liver fat as compared with a low-protein high-carbohydrate diet.

Our results focusing on liver fat content are in line with the review of Parry and Hodson, in which the authors describe that most studies suggest no influence on liver fat by diets that are high in carbohydrates in the form of free sugars [19]. The increase in liver fat observed in diets high in fat seems to be attributable to an increased saturated fat consumption, while increased consumption of mono- or polyunsaturated fat may reduce liver fat content [19], which supports the results of our meta-analysis. The beneficial effects of unsaturated fat on liver fat content compared to saturated fat were also reported in another recent review [55]. Additionally, results from this meta-analysis are in agreement with the findings from a meta-analysis on the effects of mutual exchanges of different dietary fats and carbohydrates on glucose-insulin homeostasis, an outcome strongly related to NAFL. The authors of this meta-analysis found that replacement of carbohydrates or saturated fat with polyunsaturated fat led to an improved insulin secretion capacity, lower fasting glucose, improved Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and lower haemoglobin A1C (HbA1c)[55]. The exchange of saturated fat for carbohydrates did not affect most outcomes, except for a decrease in fasting insulin [56].

Although the pathogenesis of liver fat accumulation is not completely elucidated yet, it is assumed that both high caloric intake and dietary composition influence liver fat content. Dietary intake of specific nutrients (e.g. fructose) may increase *de novo* lipogenesis, and together with increased lipolysis of visceral fat this may contribute to an increased flux of free fatty acids in the liver, leading to hepatic fat accumulation [10, 57]. Additionally, n-6 polyunsaturated fatty acids have been suggested to suppress lipogenic gene expression and could thereby decrease de novo lipogenesis and thereby decrease accumulation of liver fat [58], which is consistent with the findings of this meta-analysis showing that this holds true more generally for unsaturated fat and that exchanging saturated for unsaturated fat can lower liver fat.

A strength of this study is that it is the first comprehensive meta-analysis on the effect of macronutrient composition and macronutrient types on liver fat. The review process has been performed systematically and only studies in which liver fat was measured with either MRI, ^{1}H-MRS or CT were included. Moreover, we only included studies that performed a dietary intervention rather than only providing dietary advice.

This study also has some limitations. The first one is that comparing and meta-analysing data from different dietary intervention trials appeared challenging, as there was considerable heterogeneity in study duration and composition of the diets, percentages of macronutrients exchanged, and total amount of energy of provided diets (hyper-, hypo- or isocaloric). Firstly, whereas some studies specified which subtypes of dietary fats or carbohydrates were replaced, others did not, making the interpretation of the results difficult. As our results on exchanging unsaturated with saturated fat have shown, the fat type that is replacing the carbohydrates is likely relevant. Three randomized trials [32, 38, 39] replaced carbohydrates with unsaturated fats and show that a low-carbohydrate high-fat diet leads to less liver fat compared with a high-carbohydrate low-fat diet, whereas most other studies suggest that a high-carbohydrate low-fat diet leads to less liver fat. However, information on the type of fat used to replace carbohydrates in most studies lacking.

Secondly, this meta-analysis focused on the exchange between two macronutrient (subtypes) irrespective of the energy percentage derived from these specific macronutrients. Therefore, the studies show marked heterogeneity in the percentual energy contribution of the macronutrient subtypes that were exchanged. Studies with a larger exchanged energy percentage of macronutrients between the compared diets may have resulted in larger effect estimates than studies with smaller exchanges in energy percentages. However, the effect sizes of the studies were not proportional to the amount of energy percentage that was exchanged.

Thirdly, total caloric intake varied considerably between studies. Whereas some studies used an overfeeding design in which participants were instructed to consume more calories than their usual diet, other studies used an isocaloric or hypocaloric diet. Our only criterion regarding energy intake was that it should be equal in both study arms within a trial, regardless of whether energy intake was below, above or equal to the energy requirement of the participants. Therefore, mean caloric intake varied from 1.100 kilocalories per day [42] to over 3.400 kilocalories per day [49]. Although the number of included arms was too small to perform stratified analyses, the effect of macronutrient composition did not seem to be modified by caloric intake after visual inspection in the meta-analysis on dietary carbohydrates versus fat, which included the most comparisons. A second limitation of this review is that data of variance within the dietary arms of the included trials (e.g. variance of mean change in liver fat or variance of mean difference) were not always reported. Therefore, P-values of the mean differences in change in liver fat – that were converted to corresponding t-values – had to be used to calculate the standard deviations, standard error of the means and the 95% CIs of the mean differences in change in liver fat by Cochrane equations [59]. With these calculated values, mean differences could be converted to standardized mean differences and their corresponding 95% CIs. However, some studies did not present exact P-values of the mean difference, but exclusively presented the level of significance (e.g., $P < 0.05$ or $P < 0.01$). As

described by the Cochrane Handbook, the limits of the significance level were used for these trials as a conservative approach [59]. This approach may have caused imprecision of the variance for each trial, which is reflected in a larger confidence interval around the SMD and a decreased weight of the study [59].

As only a limited number of studies could be included in this meta-analysis, we recommend that more large randomized controlled dietary trials with a low risk of bias and of sufficient power are performed, in which complete and transparent reporting of results is of great importance in order to address this gap in knowledge. Especially trials in which proteins and fats are exchanged are warranted, as they were completely lacking, and then preferably with three arms to compare carbohydrates, fats and proteins in one study in which the sources and types of these macronutrients are specified. Bridging this gap in research is essential for the development of preventive strategies for fatty liver in the future.

In conclusion, this systematic review and meta-analysis of randomized controlled trials showed that replacing total carbohydrates with total fats has no effect on liver fat content. Replacing saturated fat with unsaturated fat resulted in a decrease or a smaller increase in liver fat content, and replacing carbohydrates with proteins also seems to lead to less liver fat. Only a limited number of eligible studies could be included, which supports an essential need for additional experimental studies on dietary macronutrient composition and liver fat content in order to provide optimal prevention and treatment for non-alcoholic fatty liver by dietary interventions.

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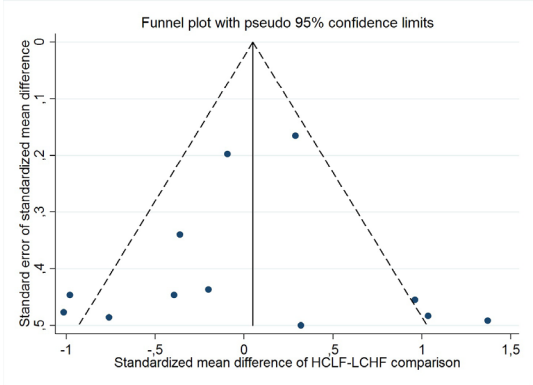
SUPPLEMENTARY FILES

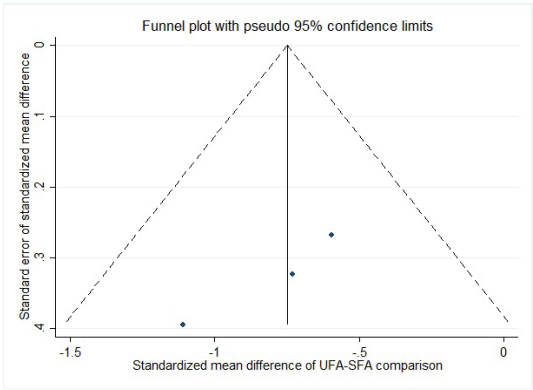
Supplemental table 1. Macronutrient composition per arm of randomized controlled trials included in meta-analysis on association between dietary macronutrient composition and hepatic triglyceride content

Author	Arm	%En (CHO/fat/protein)	Mean caloric intake (kcal)	Arm	%En (CHO/fat/protein)	Mean caloric intake of range (kcal)
Bawden, 2016	Fibre	71/14/14	2004	Other carbs	71/14/14	2003
Bendsen, 2011	Trans fatty acids	44.2/37.1/14.5	1982	Palm/ sunflower oil	44.0/33.9/16.0	1913
Bjermo, 2012	PUFA		2190	SFA		2170
Bozzetto, 2012	Low fat	53/28/19	1873	Low carb	40/42/18	2039
Bozzetto, 2012 (+ exercise)	Low fat	53/29/18	2037	Low carb	40/42/18	2480
Errazuriz, 2017	Low fat	–/34/17	2006	Low carb	–/46/14	2064
	Fibre	–/28/17	1889			
Gepner, 2019	Low fat		2852	Low carb		2839
Haufe, 2017	Low fat			Low carb		
Herpen, 2011	Low fat	56.0/21.7/16.3	2169	Low carb	34.0/49.3/15.2	2345
Kirk, 2009	Low fat	65/20/15	1100	Low carb	10/75/15	1100
Luukkonen, 2018	PUFA/MUFA	22.7/59.7/13.2	2883	SFA	25.9/58.9/15	2787
	Low fat	63.7/23.8/11.4	2902			
Marina, 2014	Low fat	61.7/20.2/18.1	3321	Low carb	27.4/54.8/17.8	3208
Markova, 2017	Plant protein	39.2/30.9/29.9		Animal protein	40.4/30.1/29.5	
Martens, 2014	High protein	35/35/30		High carb	60/35/0	
Van Nielen, 2014	High protein soy	49/27/22	2174	High protein no soy	52/26/21	2150
Nosaka, 2002	Long chain FA	58.4/27.0/12.8	2330	Medium chain FA	57.9/27.2/12.8	2320
Ooi, 2015	High protein	41.0/31.0/23.0	1757	High carb	46.0/31.0/18.0	1717
Rietman, 2014	High protein	36.6/37.7/25.7	3439	High carb	45.2/39.4/15.4	3463
Rosqvist, 2014	PUFA	43.3/40.3/11.8	3136	SFA	47.7/36.8/11.5	3035
Utzschneider, 2013	Low fat	57.3/23.0/17.3	2241	Low carb	37.9/43.0/16.4	2354
Westerbacka, 2005	Low fat	61/16/19		Low carb	31/56/13	

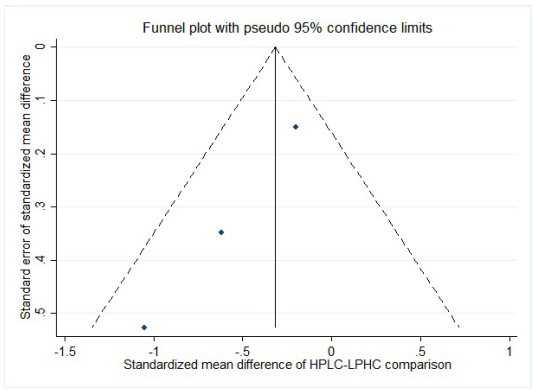
CHO, carbohydrates; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; SFA, saturated fatty acids.



Supplemental figure 1. Funnel plot of studies comparing a low-carbohydrate high-fat (LCHF) diet and a high-carbohydrate low-fat (HCLF) diet



Supplemental figure 2. Funnel plot of studies comparing a unsaturated fat (UFA) diet and a saturated fat (SFA) diet



Supplemental figure 3. Funnel plot of studies comparison a high-protein low-carbohydrate (HPLC) diet and a low-protein high-carbohydrate (LPHC) diet