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Abdollahi, S., Kazemi, A., de Souza, R. J., Clark, C. C. T. & Soltani, S.

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The Effect of Meal Frequency on Biochemical Cardio-Metabolic Factors: A Systematic Review and Meta-Analysis of Randomized Controlled Trials[1],[2]

Shima Abdollahi[1], Asma Kazemi[2], Cain C. T. Clark[3], Sepideh Soltani[4*]

[1]*Department of Nutrition and Public Health, School of Public Health, North Khorasan University of Medical Sciences, Bojnurd, Iran.*

[2]*Nutrition research center, Shiraz University of Medical Sciences, Shiraz, Iran.*

[3]*Centre for Intelligent Healthcare, Coventry University, Coventry, CV1 5FB, UK.*

[4]*Nutrition and food security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Corresponding author:

Sepideh Soltani, PhD in Nutritional Sciences
Nutrition and food security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Tel: +98 5832240571 Campus/internal phone number: 146
Fax: + 98 58- 32247281
Email: S.soltani1979@yahoo.com

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ABSTRACT

Background: Although several randomized controlled trials (RCTs) have supported the beneficial effects of higher meal frequency (MF) on cardio-metabolic parameters, the putative effects of higher MF remains inconclusive.

Objective: This study examined the effect of higher MF on lipid profile, glucose homeostasis, apolipoprotines, and adipokines, compared with lower MF, by conducting a systematic review and meta-analysis of RCTs.

Methods: PubMed, Scopus, and ISI Web of Science were searched up to May 2019 to retrieve relevant RCTs. A random effect model was used to calculate mean differences and 95 % CI for each outcome. The quality of studies and evidence was assessed through standard methods.

Results: Twenty RCTs (658 participants) were eligible for this meta-analysis. Overall results showed a significant improvement in total cholesterol [weighted mean difference (WMD) = -6.08 mg/dl; 95% CI: -10.68, -1.48; P = 0.01; I^2= 88], low-density cholesterol (LDL-C) (WMD= -6.82 mg/dl; 95% CI: -10.97, -1.59; P= 0.009; I^2= 85.7), and LDL-C to high-density cholesterol ratio (LDL-C: HDL-C) (WMD= 0.22; 95% CI: 0.07, 0.36; P= 0.003; I^2= 0.0), in higher MF vs. lower MF. However, no significant effects were found for glycemic indices. The main findings from subgroup analyses suggested that, when the intervention was >12 weeks, higher MF significantly reduced serum triglyceride (TG) and increased HDL-C, compared with lower MF. Moreover, in healthy participants, higher MF led to a significant decrease in serum level of TC and LDL-C,.

Conclusion: Our results revealed that increased MF may potentially be efficacious in lowering TC, LDL-C, and LDL/HDL ratio. It seems that long-term energy-deficit diets may confer an additional beneficial effect to higher MF in a healthy population. However, low or moderate credibility of the currently available evidence highlights the need for more high-quality studies in order to to reach a firm conclusion.

Keywords: Meals; Meta-analysis; blood glucose; diet; eating; cholesterol

INTRODUCTION

In the preceding decade, the prevalence of chronic diseases, such as diabetes and cardiovascular diseases, has increased in both developed and developing countries (1). Chronic diseases reportedly caused 33.7% of deaths in 1990, and progressed to 40.3% in 2016 (2). The upward trend of chronic diseases is largely driven by cardio-metabolic risk factors; including, among others, smoking, obesity, dyslipidemia, dysglycemia, and hypertension(3, 4). Dietary therapy is considered as a first line intervention for the management of this condition, and, in some cases, it may be utilized instead of pharmacological-based therapy (5, 6).

Although a large body of evidence from meta-analyses of epidemiologic studies purports the role of food groups and dietary patterns as a modifiable factor in the etiology of chronic diseases (7-10), additional dietary aspects are poorly understood. One contentious area, which seems to contribute to chronic disease risk factors, is eating habits, and, in particular, eating/meal frequency (11, 12).

An increased meal frequency is regarded as a dietary strategy that may be utilized to regulate cardio-metabolic profile. Such a hypothesis is driven from observational studies that suggest an inverse relationship between metabolic profile and meal frequency (13-16). It has been speculated that, when multiple small size meals eaten through the day, the incumbent lower stomach extension and lower nutrient transience in the intestine can lead to a lower insulin secretion response (17). Given that insulin plays an important role in lipogenesis and cholesterol synthesis, lowering insulin levels can conceivably improve lipid profile, as well as glycemic indices (18). Indeed, a prospective cohort study found an increased risk for cardiovascular diseases and metabolic syndrome in higher meal frequency compared with lower meal frequency, even after adjustment for confounders (19, 20).

With regards to lower meal frequency, which prolongs the fasting period between meals, there is evidence to suggest an improvement in cardio-metabolic factors, including lipid profile, glucose homeostasis, and some types of inflammatory and oxidative stress markers (21-24). Furthermore, when lower frequency meals are consumed, glycogen stores are depleted due to the extended time in fasting; thus, to supply energy, the adipose tissue breaks down and the serum levels of free fatty acids are increased (25). Increased levels of free fatty acids is considered an etiology of insulin resistance (26); indeed, some trials have reported beneficial effects of higher meal frequency on lipid profile and/or glycemic indices versus lower meal frequency (22, 27, 28). However, despite some beneficial effect reported in the literature, many studies have failed to detect any significant effect of manipulating meal frequency (29-31).

Therefore, the aim of the present study was to investigate the effect of meal frequency manipulation, in a eucaloric condition, on biochemical cardio-metabolic parameters and adipokines in adults, by conducting a systematic review and meta-analysis of RCTs.

METHODS

The protocol of this systematic review has been registered on the international prospective register of systematic reviews (PROSPERO) website (www.crd.york.ac.uk/PROSPERO). The present study was developed and conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (32).

Search Strategy and Study Selection

PubMed, Scopus, and Web of Science databases were searched up to 3-May-2019, using MeSH and non-MeSH terms, with no restriction in language or time. Reference lists of all identified

studies were also scanned for additional relevant studies. Details of search strategies implemented are presented in **supplementary Table 1**.

Two reviewers (AK and SS, separately) assessed the titles and abstracts of all primary articles, according to the following criteria, to determine eligible studies.

Studies that met following criteria were included: (a) open-label randomized clinical trial with concurrent control group (either parallel or cross-over design); (b) studies investigated the effect of meal frequency on one of the biochemical cardio-metabolic markers including serum levels of fasting blood glucose (FBS), insulin, hemoglobin A1c (HbA1c), glucagon, c-peptide, triglyceride (TG), total cholesterol (TC), high-density cholesterol (HDL-C), low-density cholesterol (LDL-C), APO-A1, APO-B, free fatty acid (FFA), adipokines, homeostatic model assessment of insulin resistance (HOMA-IR), and systolic and diastolic blood pressure (c) male and female participants ≥18y; (d) the diets of both intervention and control groups were identical (in calorie and macronutrient composition) except for the number of meals in the included studies; (e) number of meals were clearly defined in both intervention and control groups as a part of the study design. Meals were defined as any food consumption episodes (drink or solid meal) providing 200 kcal or more of energy.

Studies were excluded for the following reasons: (a) studies that included children, adolescents, pregnant or lactating women, patients with psychological or eating disorders, and critically ill patients; (b) studies that reported differences in calorie intake, macronutrients components or any other difference except the frequency of the meals between intervention and control groups; (c); studies that applied short term interventions (less than one week); and, (d) when meal frequency was not specified in the design of the study.

Data extraction

Three reviewers evaluated the full text of potentially relevant articles and extracted the following information (AK, SS, SA); 1) participant characteristics, including gender, age, and disease. 2) Study characteristics, including last name of the first author, country of origin, study design, publication year, duration of the study, and sample size in each group. 3) Intervention characteristics including number of meals in each group, any other intervention beside meal frequency, calorie distribution at meals (isocalorically vs. non-isocalorically distributed meals). 4) Means and standard deviations (SDs) at the baseline and end of the intervention as well as mean changes and the corresponding SDs for all of the outcomes also were extracted. If data were presented in graphical format only, it was extracted using Plot Digitizer software (http://plotdigitizer. sourceforge.net/). In cases where the required data was not provided in a study, corresponding authors were contacted to request the data needed for the purpose of meta-analysis. Any discrepancy was resolved by discussion to reach consensus.

Statistical analysis

Statistical analysis of data was performed using Stata software version 13 (StataCorp LP, College Station, TX, USA). The mean change for outcomes and their corresponding SDs between intervention and control groups were extracted from each study and was used to estimate the overall difference in mean change across all studies and was used as the effect size for analysis. The group with higher meal frequency was considered as the intervention and lower meal frequency group was considered as the control group. For the studies that did not report mean change, we calculated the mean changes, and also estimated SD for mean change, using correlation r using studies that reported baseline, after intervention and change values. The units of all of outcomes were unified prior to inclusion in the meta-analysis. The weighted mean difference (WMD) and its corresponding SD was calculated for each outcome using the

DerSimonian and Laird method. Statistical heterogeneity between studies was assessed using the Cochran's Q test and I^2 test. We sought to find the sources of heterogeneity by meta-regression and subgroup analysis for potential factors, including: age, gender, difference in meal frequency between the intervention and control group, distribution of calorie at meals (isocalorically vs. non-isocalorically distribution), any other intervention beside the meal frequency, health status of participants, duration of intervention and quality of studies. The potential for publication bias was assessed by visual examination of funnel plots, whilst the Begg rank correlation method and the Egger weighted regression method were also used for statistical assessment of publication bias (33). Sensitivity analysis was performed by excluding studies from the meta-analysis one by one to explore the extent to which the summary estimates might depend on any singular study (34). A p-value of 0.05 was considered to represent statistical significance.

Quality assessment

Quality assessments were performed using the Cochrane Collaboration's tool (35), based on the following parameters: random sequence generation, allocation concealment, incomplete outcome data and selective reporting. As blinding is almost impossible for dietary intervention trials, we did not consider the blinding of participants and investigators as a key domain to assess the overall risk of bias.

Quality of evidence

The GRADE (Grading of Recommendations, Assessment, Development, and Evaluations) approach was used to rate the certainty of the evidence for each outcome (36). Two reviewers (SA, SS) independently evaluated the certainty of evidence and any disagreements were resolved by discussion. RCTs start with high quality of evidence and may be downgraded based on risk of bias inconsistency (substantial unexplained heterogeneity, $I^2 > 40\%$; $p < 0.05$), indirectness,

imprecision (95% CI for effect size are wide or cross the minimally important difference), and evidence of small study effects.

RESULTS

As shown in **Figure 1**, the primary search yielded 13,135 studies. After excluding duplicate studies and screening the articles by the title and abstract, 63 studies remained and were evaluated based on reading full-texts. Excluded studies, as well as the reasons for exclusion, are presented in **Supplementary Table 2**. Finally, 20 studies were entered into the analyses; (n= 658 participants) (22, 27, 31, 37-53).

Study characteristics

Most of the included studies were conducted in Europe (n=8) (31, 42, 43, 47-49, 52, 53), whilst some other studies were from USA (n=7) (22, 27, 37, 38, 44, 46, 50), Asia (45, 51) and, Oceania continent countries (39-41). Across all the studies, 13 studies (14 publications) (22, 27, 31, 37, 39-41, 43, 46, 48-50, 52, 53) had cross-over study design, whilst eight had a parallel study design (38, 42, 44, 45, 47, 51). The duration of the intervention ranged from 2 to 48 weeks; most of the studies included both male and female participants (22, 27, 31, 38-42, 45, 48, 50-53), although some studies focused on female (37, 44, 49) or male (43, 46, 47), exclusively. Eight studies were conducted under a weight reduction condition (31, 37, 38, 42, 45, 47, 51, 53), while the remaining studies applied a weight maintenance diet (22, 27, 39-41, 43, 44, 46, 48, 49). Nine studies applied iso-caloric meals (22, 27, 31, 44-46, 50-52), although others did not consider this factor (37-43, 47-49, 53). Included studies were conducted on participants with overweight and obesity (37, 38, 42, 44, 45), diabetes mellitus (31, 40, 48, 51-53), polycystic ovary syndrome (PCOS) (49), hypercholesterolemia (39), whilst seven studies also recruited healthy participants

(22, 27, 41, 43, 46, 47, 50). **Table 1** details the characteristics of RCTs included in the meta-analysis.

Risk of bias

Most methodological domains of included studies were not met according the Cochrane criteria and rated as high risk of bias (22, 27, 37-46, 48-52). The randomization procedure was not described by most of the studies and was considered as the most common bias (22, 27, 37, 39-42, 44, 46, 52). Thirteen studies did not demonstrate adequate concealment of allocation prior to assignment (22, 27, 29, 37, 39-41, 43-46, 51, 52). Furthermore, a large number of studies did not report pre-specified primary outcomes (22, 27, 37, 39, 41, 43, 44, 48, 49). Although risk of bias due to incomplete outcome data was low in most of the studies (22, 27, 31, 37-49, 52, 53), the two remaining studies were classified as fair quality (31, 47), and only one study met all the Cochrane's standards for quality research designs (53) (**Supplementary Table 3**).

Quality of evidence

The GRADE assessment system showed that the quality of evidence was moderate for the effect of meal frequency on LDL-C, and LDL-C/HDL-C ratio, low for FBS, insulin, HbA1c, HOMA-IR, glucagon, c-peptide, TC, Apo A1, Apo B, FFA, HDL-C, and leptin, and very low for TG (**Supplementary Table 4**).

Meta-analysis

Effects of meal frequency intervention on glycemic indices

FBS. The pooled effect size based on 13 studies (27, 37, 39, 40, 42, 43, 46-49, 51-53) showed no significant effect of higher, compared with lower, meal frequency on FBS, with high

heterogeneity (n= 466 participants, WMD= -0.02 mmol/L; 95% CI: -0.20, 0.16; P= 0.98; I^2= 88.1; P-heterogeneity < 0.001) (**Table 2**)(**Figure 2 A**). Subgroup analysis based on gender and health status of participants reduced the heterogeneity; however, the results remained non-significant across subgroups (**Supplementary Table 5**).

Insulin. The pooled effect size based on 12 studies revealed no significant effect of higher, compared with lower, meal frequency on insulin levels; with significant between-study heterogeneity (n= 443 participants, WMD= -0.65 micro unit/ml; 95% CI: -1.74, 0.43; P= 0.23; I^2= 80.7; P-heterogeneity < 0.001) (**Table 2**) (**Figure 2 B**)(27, 37, 39, 40, 42, 43, 46-49, 51, 53). Subgroup analyses based on the gender, study design, and energy density of the diet could explain the heterogeneity, and showed that, in females, higher meal frequency resulted in a decrease in insulin levels compared with lower meal frequency (2 studies, n= 62 participants, WMD= -2.73 micro unit/ml; 95% CI: -5.28, -0.18; P= 0.03; I^2= 0.0; P-heterogeneity= 0.45) (**Supplementary Table 6**).

HbA1c. Five studies investigated the effect of meal frequency intervention on HbA1c (40, 48, 49, 51, 52). No significant effect was observed, with high heterogeneity (n= 176 participants, WMD= -0.12 %; 95% CI: -0.30, 0.06; P= 0.18; I^2= 90.9; P-heterogeneity <0.001) (**Table 2**) (**Figure 2 C**). However, the source of heterogeneity remained unclear due to the low number of studies.

HOMA-IR. Four studies investigated the effect of meal frequency intervention on HOMA-IR. No significant effect was observed, with high heterogeneity (27, 48, 49, 53). (n= 171 participants, WMD= -0.05 %; 95% CI: -0.55, 0.45; P= 0.84; I^2= 92.2; P-heterogeneity <0.001) (**Table 2**) (**Figure 2 D**). However, the source of heterogeneity remained unclear due to the low number of studies.

C-Peptide. Three studies investigated the effect of meal frequency intervention on C-peptide (39, 40, 46). One additional study was excluded because of inappropriate data (53). Our analysis showed no significant change in C-peptide levels, with no evidence of heterogeneity (3 studies, n= 36 participants, g= -0.15; 95% CI: -0.45, 0.16; P= 0.34; I^2= 0.0; P-heterogeneity= 0.72) (**Table 2**) (**Supplementary Figure 1**).

Effects of meal frequency intervention on lipid profile

TG. The pooled effect size based on 14 studies revealed no significant effect of higher, compared with lower, meal frequency on TG levels; with significant between-study heterogeneity (n= 504 participants, WMD= -2.24 mg/dl; 95% CI: -5.62, 1.14; P= 0.19; I^2= 72.9; P-heterogeneity <0.001) (**Table 2**) (**Figure 3 A**)(22, 37, 39-43, 45-47, 49, 51-53). Subgroup analysis also revealed that higher meal frequency significantly decreased serum levels of TG in long-term intervention (WMD= -11.25 mg/dl; 95% CI: -18.19, -4.31; P= 0.001; I^2= 31.2; P-heterogeneity= 0.21). In addition, higher meal frequency increased TG levels in men (WMD= 16.48 mg/dl; 95% CI: 7.75, 25.21; P< 0.001; I^2= 0.0; P-heterogeneity= 0.42), short-term intervention (WMD= 8.37 mg/dl; 95% CI: 1.40, 15.34; P= 0.01; I^2= 42.8; P-heterogeneity= 0.07) and studies that applied meal frequency intervention without energy restriction (WMD= 9.33 mg/dl; 95% CI: 1.15, 17.51; P = 0.02; I^2= 48.7; P-heterogeneity= 0.05), compared with lower meal frequency (**Supplementary Table 7**). The gender of participants was identified as the source of heterogeneity using subgroup analysis.

TC. Pooling data from 13 studies suggested beneficial effects of higher meal frequency on serum levels of total cholesterol; with evidence of high between-study heterogeneity (n= 462 participants, WMD= -6.08 mg/dl; 95% CI: -10.68, -1.48; P = 0.01; I^2= 88; P-heterogeneity<0.001) (**Table 2**) (**Figure 3 B**) (22, 37, 39-42, 44-46, 49, 51, 52, 54). In subgroup

analyses, we also found that higher meal frequency reduced TC in healthy individuals compared with lower meal frequency (WMD= -19.98 mg/dl; 95% CI: -30.73, -9.22; P< 0.001; I^2= 74.8; P-heterogeneity= 0.01) and, in studies with cross-over design (WMD= -5.47 mg/dl; 95% CI: -10.35, -0.59; P= 0.02; I^2= 89.6; P-heterogeneity< 0.001) (**Supplementary Table 8**), age and health status of participants were able to partially justify the observed heterogeneity.

LDL-C. The pooled effect size based on 12 studies revealed higher meal frequency significantly reduced serum levels of LDL-C compared with lower meal frequency (n= 454 participants, WMD= -6.82 mg/dl; 95% CI: -10.97, -1.59; P= 0.009; I^2= 85.7; P-heterogeneity< 0.001) (**Table 2**) (**Figure 3 C**)(22, 37, 39-42, 45, 46, 49, 51-53). Subgroup analysis also highlighted that higher meal frequency was associated with lower LDL-C levels in participants aged 45 or less (WMD= -12.63 mg/dl; 95% CI: -20.74, -4.51; P= 0.002; I^2= 87.1; P-heterogeneity< 0.001), healthy participants (WMD= -18.13 mg/dl; 95% CI: -29.38, -6.87; P= 0.002; I^2= 83.7; P-heterogeneity= 0.002), and when isocaloric meals were administered (WMD= -14.08 mg/dl; 95% CI: -24.71, -3.45; P= 0.009; I^2= 87.8; P-heterogeneity< 0.001). Furthermore, LDL-C levels decreased in studies where the difference of meal frequency between intervention and control groups was three or less (WMD= -10.60 mg/dl; 95% CI: -20.48, -0.72; P= 0.03; I^2= 87.2; P-heterogeneity< 0.001). It also seems that meal frequency interventions were more effective in parallel design studies (WMD= -11.17 mg/dl; 95% CI: -19.50, -2.85; P= 0.008; I^2= 47.5; P-heterogeneity= 0.14) compared with cross-over studies (WMD= -5.06 mg/dl; 95% CI: -10.05, -0.07; P= 0.04; I^2= 85.9; P-heterogeneity<0.001). However, heterogeneity was high across studies, and subgroup analysis identified gender, age, and health status of participants as sources of heterogeneity (**Supplementary Table 9**).

HDL-C. The pooled effect size based on 12 studies revealed no significant effect of meal frequency intervention on the serum levels of HDL-C, with considerable heterogeneity (n= 454 participants, WMD= -0.73 mg/dl; 95% CI: -2.24, 0.78; P= 0.34; I^2= 78.3; P-heterogeneity< 0.001) (**Table 2**) (**Figure 3 D**)(22, 37, 39-42, 45, 46, 49, 51-53). Subgroup analysis showed that higher meal frequency significantly increased HDL-C levels when parallel design was applied, with considerably reduced heterogeneity (WMD= 2.07 mg/dl; 95% CI: 1.31, 2.82; P< 0.001; I^2=0.0 ; P-heterogeneity= 0.69), duration of intervention was long (WMD= 1.46 mg/dl; 95% CI: 0.14, 2.78; P= 0.03; I^2= 61.8; P-heterogeneity= 0.03), and it decreased when the meal frequency intervention was not accompanied with energy restriction (WMD= -2.79 mg/dl; 95% CI: -4.04, -1.55; P< 0.001; I^2= 0.0; P-heterogeneity= 0.52). Moreover, we found that higher meal frequency decreased HDL-C levels in cross-over design (WMD= -1.98 mg/dl; 95% CI: -3.81, -0.15; P= 0.03; I^2= 69.1; P-heterogeneity= 0.001), and short-term duration (WMD= -2.77 mg/dl; 95% CI: -3.99, -1.55; P< 0.001; I^2= 0.0; P-heterogeneity= 0.57) subgroups (**Supplementary Table 10**).

LDL-C/HDL-C ratio. Three studies investigated the effect of meal frequency intervention on LDL/HDL ratio (39, 41, 52). Pooling data showed a significant reduction in LDL-C/HDL-C ratio following higher, compared with lower, meal frequency intervention (n= 45 participants, WMD= 0.22; 95% CI: 0.07, 0.36; P= 0.003; I^2= 0.0; P-heterogeneity= 0.38) (**Table 2**) (**Supplementary Figure 2**).

FFA. Four studies investigated the effect of meal frequency on serum levels of FFA (43, 46, 47, 52). However, no significant change was observed (n= 72 participants, WMD= 0.01 mmol/L; 95% CI: -0.02, 0.04; P= 0.59; I^2= 0.0; P-heterogeneity= 0.43) (**Table 2**) (**Supplementary Figure 3**).

Apo lipoproteins. We found that meal frequency interventions had no significant effect on either Apo-A1 (39, 41, 46) (3 studies, n= 42 participants, WMD= -3.00 mg/dl; 95% CI: -15.70, 9.70; P= 0.64; I^2= 4.5; P-heterogeneity= 0.35) (**Figure 4 A**), or Apo-B (4 studies, n= 55 participants, WMD= -1.63 mg/dl; 95% CI: -7.65, 4.39; P= 0.59; I^2= 0.0; P-heterogeneity= 0.42) (**Table 2**) (**Figure 4 B**) (39-41, 46).

Effects of meal frequency intervention on adipokines

Leptin. Pooling effect sizes from seven studies, we found no significant association between meal frequency and serum levels of leptin (n= 258 participants, WMD= -0.06 ng/dl; 95% CI: -0.94, 0.82; P= 0.89; I^2= 60.0; P-heterogeneity= 0.01) (**Table 2**) (**Figure 5**) (27, 31, 38, 43, 45, 47, 50). The low number of studies precluded the conducting of subgroup analyses, despite the presence of moderate heterogeneity.

Outcomes not included in the meta-analysis

Supplementary Table 11 shows the characteristics of studies which were not included in the meta-analysis, because of inappropriate or inadequate data.

Blood pressure. We found four eligible studies evaluating the effect of meal frequency on blood pressure, with contradictory results (22, 39, 50, 52). While Stote et al revealed that consuming one meal/day can lead to raised blood pressure ,compared with three meals/day (22), two studies found no significant change in blood pressure following intervention (39, 52). In another study, systolic blood pressure significantly dropped in the lower meal frequency condition, as compared to the higher meal frequency condition (50). However, these studies could not be included in the quantitative analysis because the data were not appropriate for the statistical approach utilized.

Glucagon. We identified three studies investigating the effect of meal frequency intervention on glucagon concentration (27, 47, 53). While two studies reported non-significant changes (27, 47), Kahleova reported fasting plasma glucagon decreased with the lower meal frequency regimen, and increased with the higher meal frequency regimen (53).

Other glycemic indicators. Three studies were found that investigated the effect of meal frequency on 2 hours post prandial blood glucose (2hPPBG) (39, 48, 51). However, just one of them reported a significant reduction in 2hPPBG in higher meal frequency *vs* lower meal frequency group (48). Three studies also reported results on insulin/glucose ratio without reaching statistically significant changes (39, 41, 52). We found other studies investigating the effect of meal frequency intervention on insulin sensitivity (27, 52) and glucose sensitivity (53), which and no significant difference between groups was evident. However, compared with higher meal frequency, the first phase of beta-cell function was significantly diminished when lower meal frequency was administered in one study (27).

Brain-derived neurotrophic factor. One study investigated the effect of meal frequency intervention on brain-derived neurotrophic factor (BDNF); however, only non-significant changes were observed (27).

Lipid profile. There were two studies that measured total cholesterol: HDL-C ratio (39, 41) and one study measured VLDL concentration (41) in response to meal frequency intervention. Accordingly, none of the studies reported any significant effect manifest following intervention.

Heart rate. Thomsen et al., examined the effect of meal frequency intervention on heart rate; highlighting no significant findings (52).

Sensitivity analysis and publication bias

Leave-one-out sensitivity analysis was conducted to assess the robustness of the overall effect. However, after excluding each trial from the study, effect estimates remained the same for all outcomes. Visual inspection of the funnel plots showed no sign of asymmetry in the meta-analyses of meal frequency intervention effects on TC, TG, LDL-C, HDL-C, FBS, and insulin. Indeed, Egger and Begg's tests, respectively, also confirmed these findings.

DISCUSSION

In the present systematic review and meta-analysis, we summarized the available data from twenty eligible RCTs focusing on the effect of meal frequency intervention on biochemical cardio-metabolic factors in adults. The main results of this study suggest that, compared with lower meal frequency, higher meal frequency significantly reduces TC, LDL-C and LDL-C/HDL-C ratio; without any significant effect on other lipid profile markers, glycemic indices, apolipoprotines, and leptin levels.

Subgroup analyses suggested that higher meal frequency, if not accompanied by calorie restriction, increases TG and decreases HDL-C, respectively. Beneficial effects of higher meal frequency were also observed when interventions lasted for 12 weeks or more. Furthermore, higher meal frequency intervention reduced TC and LDL-C levels in healthy participants, when compared with lower meal frequency. A greater reduction in LDL-C levels was also shown in the studies with a parallel vs a crossover study design. Alterations in cholesterol levels and glycemic indices are reportedly capable of impacting risk of cardio-vascular diseases (55-57); indeed, so previous studies reported that for each 1 mg/dl reduction in TC, LDL-C, TG, and 1 mg/dl increase in HDL-C, 2 to 3% reduction, respectively, in the risk of CVD is evident (55, 58, 59). Therefore, it seems that the higher meal frequency, with an approximate decrease of 6 mg/dl in TG and LDL, may lead to an effective improvement in reducing cardiovascular disease risk.

Changes in eating habits have significant ramifications on the regulation of metabolic status. Indeed, the findings of some observational studies have suggested an inverse relationship between meal frequency and cardio-metabolic risk factors (13-16). However, in clinical trials, contradictory results have been reported. Some studies have suggested beneficial effects of higher meal frequency on lipid profile (22, 45, 46), glycemic indices (48, 51) or appetite (22, 48, 60); however, there were no significant differences manifest between higher and lower meal frequency in other studies (39, 40). Accordingly, our analyses have demonstrated that higher meal frequency may improve plasma lipids. Moreover, our subgroup analyses showed that the intervention can be even more effective in long-term interventions or when energy restriction also applied.

The mechanisms by which higher meal frequency may yield beneficial effects on plasma lipids are not well understood. However, such observations may be explained by some putative mechanisms. Cholesterol reduction may be related to lower insulin levels; indeed, insulin mediates the cholesterol biosynthesis pathway through hydroxymethylglutaryl-CoA (HMGCoA) reductase activation (61). Moreover, enzymes that provide substrates for cholesterogenesis are insulin sensitive (62).; however, we observed no significant change in insulin levels, indicating that this mechanism may not suitably explain our results.

In this systematic review and meta-analysis, all of the included studies administered 30% or less of daily energy needs from fat. It is conceivable that many of the participants followed a high-fat diet before the intervention, and the modification in dietary fat intake has yielded improvement in lipid profile. However, most of these studies did not consider dietary intake before intervention, which precludes any consensus being made. Furthermore, this highlights the importance of considering dietary habits of participants.

We found that TG and HDL-C levels changed detrimentally when the intervention was not accompanied by energy restriction. This finding gives indicates that energy restriction may be a more effective factor in improving these biomarkers than the meal frequency. However, the reason for this is not apparent, and necessitates further investigation

We did not observe any significant change in glycemic indices. Indeed, one probable explanation for these null findings may be related to the varying meal composition and quantity of meals across studies, which axiomatically affects glycemic responses. Moreover, energy-deficit diets may lead to increase FFA levels, which are related to insulin resistance (63), however positive effects of energy-deficit diets would likely appear via weight loss, if the dietary intervention continues. As demonstrated in Pierre's study, meal frequency has no effect on either fasting glucose or insulin in the absence of weight loss (64).

Meal timing is another factor that may confound results, where, in the present study, ost of the included work did not control the time each meal was consumed. Meal timing can affect the circadian clock and glucose and lipids metabolism (65). Indeed, Paoli and collogues demonstrated that a regular eating pattern has potential positive effects on health outcomes, regardless of meal frequency, and the authors asserted that reduced meal frequency, following regular meal timing, may yield additional beneficial effects. (11). Further considerations include that wash-out periods in cross-over design studies may not be sufficient to remove the effect of the first period intervention. In this regard, we found a significant elevation in HDL-C levels in parallel studies; while the opposite result was observed in cross-over designs.

for a further explanation for our results is that the difference in number of meals between the intervention and control groups varies across studies. Indeed, Jenkin reports that large differences are likely needed to find significant associations (46). In some included studies, the

control group received one or two meals a day, which is comparable to a fasting diet. There is some evidence to suggest fasting may yield beneficial effects on cardiovascular risk factors (66). Whilst we found that LDL-C was significantly decreased when the difference in number of meals between groups was three or less, it is not clear how many meals per day is optimal for producing clinically beneficial effects.

Several meta-analyses have reported on the association between meal frequency and body weight (67-69), however, biochemical cardio-metabolic factors were not assessed in these analyses. To our knowledge, this is the first meta-analysis investigating the effect of meal frequency on biochemical cardio-metabolic factors. A further strength of our study is that, as we included RCTs for analysis, causal conclusions were able to be drawn, whilst a wide search strategy ensured we identified all relevant publications. Moreover, we evaluated the methodological quality, as well as quality of evidence, for included studies using standard approaches. Since total energy intake, in addition to diet composition, plays an important role in metabolism regulation, we only included studies where energy and macronutrient intakes were similar between groups.

Despite the aforementioned strengths some limitations also exist in our study that need to be considered. Implementing a dietary intervention trial faces multiple challenges; for instance, maintaining high compliance of participants, blinding the intervention, and controlling confounders (70). Indeed, such confounder are out of the operational control of any study, and as such, the results of our study have also been affected by these challenges. As emphasized previously, we considered some confounding factors in the sub-analyses we conducted, however, some other important variables, such as initial calorie intake and dietary habit, physical activity, medications, time of blood sampling, body weight changes, baseline values of biomarkers,

smoking, and meal times were not taken into account in most of the studies. Moreover, most of the included studies were performed over a relatively short period of time, and their sample sizes were relatively small, thus, it is conceivable that studies may have lacked sufficient statistical power to detect significant differences. Therefore, the quality of studies was graded as poor. Some risk factors for cardiovascular diseases were not measured in included studies, including C-reactive protein, and other inflammatory markers, so no inference can be made regarding the effect of meal frequency on said parameters. Finally, small number of studies also unable us to conduct subgroup analysis for some of our interested outcomes, and the source of heterogeneity remained unclear for these outcomes.

Conclusion

The findings of our study suggest that, in an isocaloric condition, high meal frequency may yield beneficial effects on total cholesterol, LDL-C and LDL-C: HDL-C ratio, as compared with lower meal frequency. Although increased meal frequency had no significant effect on glucose hemostasis markers, the subgroup analyses revealed that it may be more advantageous if higher meal frequency is accompanied with energy restriction, or if the intervention lasts for 12 weeks or more. However, the low quality of meta-evidence reduces the reliability of the results; thus, high-quality, larger-scale, and longer-term trials, controlled for confounders, are necessitated before any firm conclusions can be drawn.

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Author's contribution:

The authors' responsibilities were as follows— SS, AS conceived the study. SS, AS, Sh A carried out the literature search and data extraction. Sh A and SS conducted the quality of included studies, Data analysis and interpretation. All authors contributed to the study conception, design and drafting of the manuscript. SS, AS, Sh A: analysis and interpretation of data, Sh A, AS, CC: writing of the manuscript, critical revision of manuscript drafts for important intellectual content.

Conflict of interest

The authors have no potential financial or other conflicts of interest to disclose.

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Table 1. Characteristics of randomized controlled trials that investigated the effect of the meal frequency intervention on cardio-metabolic profile in adults and were eligible for inclusion in the meta-analysis

Author, year	Gender, participant[1]	Mean age (year)	Country	Health status of subjects	Study design	Outcome	Daily meal frequency (int/cont)	Energy restriction	Iso-caloric meals	Duration, weeks	Results
Alencar, 2015 (37)	F, 22	52	New Mexico	Obesity	Cross-over	HDL, TG, LDL, TC, insulin, FBS	6/2	Yes	No	2	Non-significant change: glucose, insulin, total-cholesterol, or LDL-C, TG Significant change: HDL improved in control group with no significant difference between groups
Arcerio, 2013 (38)	Both, 20	46	USA	Obesity	Parallel	Leptin	6/3	Yes	NM	4	Non-significant change: -- Significant change: leptin increased in intervention group
Arnold, 1993 (41)	Both, 19	32.1	New Zealand	Healthy	Cross-over	HDL, Apo-B, Apo-A1, LDL/HDL, TG, LDL, TC	9/3	No	No	4	Non-significant change: TG, Apo-A1, Apo B, LDL/HDL Significant change: TC, LDL, HDL improved in intervention group
Arnold, 1994 (39)	Both, 16	49.9	New Zealand	Hyperchol	Cross-over	HDL, Apo-B, Apo-A1, LDL/HDL, TG, LDL, TC, C-peptide, insulin, FBS	9/3	No	No	4	Non-significant change: TC, LDL, HDL, TG, Apo A1, Apo B, LDL/HDL, TG, insulin, C-peptide Significant change:--
Arnold, 1997 (40)	Both, 13	46-70	New Zealand	T2DM	Cross-over	HDL, Apo-B, TG, LDL, TC, C-peptide, insulin, HbA1c, FBS	9/3	No	No	8	Non-significant change: HDL, Apo-B, TG, LDL, TC, C-peptide, Insulin, HbA1c, FBS Significant change: --
Belinova, 2017 (31)	Both, 54	59.4	Czech Republic	T2DM	Cross-over	Leptin	6/2	Yes	Yes	12	Non-significant change: -- Significant change: leptin decreased in both groups.
Bertéus Forslund, 2008 (42)	Both, 93	41.8	Sweden	Obesity	Parallel	HDL, TG, LDL, TC, insulin, FBS	6/3	Yes	No	48	Non-significant change: TG, LDL, TC, Insulin, FBS Significant change: HDL improved in control group
Carlson, 2007 (27)	Both, 30	45	USA	Healthy	Cross-over	FBS, insulin, leptin, HOMA IR,	3/1	No	Yes	8	Non-significant change: insulin, glucagon, leptin, HOMA IR

											Significant change: higher FBS in control group compared with intervention
Chapelot, 2006 (43)	M, 24	19-25	France	Healthy	Cross-over	FFA, TG, FBS, insulin, leptin	4/3	No	No	4	Non-significant change: FFA, TG, FBS, insulin Significant change: leptin increased in control group compared with baseline. Glucose and insulin increased in intervention compared with control group.
Finkelstein, 1971 (44)	F, 8	20-22	USA	Overweight	Parallel	FBS, TC	6/3	No	Yes	8	Non-significant change: FBS, TC Significant change: --
Hatami Zargaran, 2014 (45)	Both, 84	36.99	Iran	Obesity	Parallel	HDL, TG, LDL, TC, leptin	6/5	Yes	Yes	12	Non-significant change: -- Significant change: TC, LDL, TG, leptin decreased and HDL increased in intervention compared with control group
Jenkins, 1989 (46)	M, 7	39.6	Canada	Healthy	Cross-over	HDL, FFA, Apo-B, Apo-A1, TG, LDL, TC, C-peptide, insulin, FBS	17/3	No	Yes	4	Non-significant change: FBS, FFA, TG, Apo-A Significant change: TC, LDL, Apo-B, decreased in intervention compared with control group. Insulin and C-peptide decreased in intervention group compared with baseline.
Kahleova, 2015 (53)	Both, 54	59.4	Czech Republic	T2DM	Cross-over	HDL, TG, LDL, TC, HOMA-IR, insulin, FBS, C-peptide	6/2	Yes	No	12	Non-significant change: TC, HDL Significant change: FBS and C-peptide levels decreased in both groups. Glucagon decreased in the control and increased in the intervention group.
Koopman, 2014 (43)	M, 31	22	Netherland	Healthy	Parallel	FFA, TG, insulin, FBS, leptin	6/3	Yes	No	6	Non-significant change: FFA, glucagon, FBS Significant change: Plasma leptin concentrations increased in all diet intervention groups. TG increased in 6 meals/day group.
Papakonstantinou, 2016 (49)	F, 40	27.6	Greece	PCOS	Cross-over	HDL, TG, LDL, TC, HOMA-IR,	6/3	No	No	12	Non-significant change: FBS, HOMA-IR, TC. HDL, LDL, TG

						insulin, HbA1c, FBS					Significant change: insulin level decreased and HbA1c marginally increased after 6 meals/day intervention.
Papakonstantinou, 2018 (48)	Both, 47	49.3	Greece	IGT and T2DM	Cross-over	HOMA-IR, insulin, HbA1c, FBS	6/3	No	No	12	Non-significant change: HOMA-IR Significant change: HbA1c decreased in 6 vs 3 meals/d group.
Perrigue, 2017 (50)	Both, 15	28.5	USA	Healthy	Cross-over	Leptin	8/3	No	Yes	3	Non-significant change: leptin Significant change: --
Salehi, 2014 (51)	Both, 66	51.8	Iran	T2DM	Parallel	HDL, TG, LDL, TC, insulin, HbA1c, FBS	6/5	Yes	Yes	12	Non-significant change: FBS, insulin, TC, LDL, HDL and TG Significant change: HbA1c decreased in 6 meals/day compared with 5 meals/day.
Stote, 2007 (22)	Both, 30	45	USA	Healthy	Cross-over	HDL, TG, LDL, TC	3/1	No	Yes	8	Non-significant change: TG Significant change: HDL, LDL and TC increased after 1 meal/day intervention
Thomsen, 1997 (66)	Both, 10	60	Iceland	T2DM	Cross-over	HDL, FFA, LDL/HDL, TG, LDL, TC, HbA1c, FBS	8/3	No	Yes	2	Non-significant change: FBS, FFA, HbA1c, TC, LDL, LDL/HDL, TG Significant change: HDL increased in 3 meals/day compared with 8 meals/day.

[1]number of participants who completed the study

Cont, Control; FBS, fasting blood sugar; F, Female; FFA, Free fatty acid; HbA1c, Hemoglobin A1C; HDL, High-density lipoprotein; HOMA-IR, Homeostatic model assessment for insulin resistance; IGT, Impaired glucose tolerance; Int, Intervention; LDL, Low-density lipoprotein; M, Male; NM, Not mentioned; PCOS, Polycystic ovary syndrome; TC, Total cholesterol; TG, Triglyceride; T2DM, Type 2 diabetes mellitus

Table 2. Meta-analysis showing the overall effect of meal frequency intervention on cardio-metabolic factors

		Meta-analysis		Heterogeneity		
Outcome	**Number of Studies**	**WMD[1] (95%CI)**	**P effect**	**Q statistic**	**P within group**	**I^2 (%)**
Glucose Homeostasis						
FBS (mmol/L)	13	-0.02 (-0.16, 0.20)	0.976	124.47	<0.001	88.1
Insulin (micro unit/ml)	12	-0.65 (-1.74, 0.43)	0.236	72.37	<0.001	80.7
HbA1c (%)	5	-0.12 (-0.30, 0.06)	0.184	65.76	<0.001	90.9
HOMA-IR (%)	4	-0.05 (-0.55, 0.45)	0.840	63.79	<0.001	92.2
C-Peptide[2]	3	-0.15 (-0.45, 0.16)	0.340	0.65	0.724	0.00
Lipid Profile						
TG (mg/dl)	14	-2.24 (-5.62, 1.14)	0.194	51.68	<0.001	72.9
Total Cholesterol (mg/dl)	13	-6.08 (-10.68, -1.48)	0.010	100.05	<0.001	88
LDL (mg/dl)	12	-6.82 (-10.97, -1.59)	0.009	77.19	<0.001	85.7
HDL (mg/dl)	12	-0.73 (-2.24, 0.78)	0.344	50.59	<0.001	78.3
LDL/HDL ratio	3	0.22 (0.07, 0.37)	0.003	1.89	0.389	0.00
Apo A1 (mg/dl)	3	-3.00 (-15.70, 9.70)	0.644	2.09	0.351	4.5
Apo B (mg/dl)	4	-1.63 (-7.65, 4.39)	0.594	2.78	0.426	0.00
FFA (mmol/L)	4	0.01 (-0.02, 0.04)	0.598	3.79	0.435	0.00
Adipokines						
Leptin (ng/dl)	7	-0.06 (-0.94, 0.82)	0.899	17.50	0.014	60

[1]WMD, Weighted mean difference

[2]Effect size presented as Hedges' g

Figure 1- Flow diagram for study selection process

Figure 2 - Forest plot of randomized controlled trials (RCTs) illustrating weighted mean differences in (A) serum glucose, (B) serum insulin, (C) HbA1c, and (D) HOMA-IR between higher meal frequency and lower meal frequency for all eligible studies. Analysis was conducted using a random effects model.

Figure 3 - Forest plot of randomized controlled trials (RCTs) illustrating weighted mean differences in (A) serum triglyceride, (B) serum Total Cholesterol, (C) serum LDL, and (D) serum HDL between higher meal frequency and lower meal frequency for all eligible studies. Analysis was conducted using a random effects model.

Figure 4 - Forest plot of randomized controlled trials (RCTs) illustrating weighted mean differences in (A) serum Apo-A1, and (B) serum Apo-B between higher meal frequency and lower meal frequency for all eligible studies. Analysis was conducted using a random effects model.

Figure 5 - Forest plot of randomized controlled trials (RCTs) illustrating weighted mean differences in serum leptin between higher meal frequency and lower meal frequency for all eligible studies. Analysis was conducted using a random effects model.