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Randomized Controlled Trial

Effects of almond consumption on metabolic function and liver fat in overweight and obese adults with elevated fasting blood glucose: A randomised controlled trial

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SUMMARY

Background: Almonds are a rich source of bioactive components. This study examined the effects of daily almond consumption on glycaemic regulation, liver fat concentration and function, adiposity, systemic inflammation and cardiometabolic health.

Methods: 76 adults with elevated risk of type 2 diabetes (T2D) or T2D (age: 60.7 ± 7.7 years, body mass index: 33.8 ± 5.6 kg/m^2) were randomly assigned to daily consumption of either 2 servings of almonds (AS:56 g/day) or an isocaloric, higher carbohydrate biscuit snack (BS) for 8 weeks. Glycosylated haemoglobin (HbA1c), glycaemic variability (GV), liver fat, serum aminotransferases, body weight and composition, markers of cardio-metabolic risk and systemic inflammation were assessed at baseline and week 8.

Results: No group differential effects were observed on HbA1c, GV, body weight and composition, liver fat and aminotransferases, cardio-metabolic health and inflammatory markers (all $P > 0.05$). For serum TC/HDL-C ratio a significant gender × treatment × time interaction occurred ($P < 0.01$), such that in women TC/HDL-C ratio was significantly reduced after AS compared to BS (−0.36 [0.26] mmol/L [n = 14] vs. −0.14 [0.32] mmol/L [n = 17]; $P = 0.05$), but not in men ($P = 0.52$).

Conclusions: Compared to BS, AS consumed between meals did not substantially alter glycaemic regulation, liver fat or function, adiposity, and metabolic health and inflammatory markers. Serum TC/HDL-C ratio improved in women, but not in men with AS; but as this sub-analysis was not defined *a priori* the results should be interpreted with caution. Further research should examine the longer-term health effects of regular almond consumption and differential gender responses.

Clinical trial registry number and website: Australia New Zealand Clinical Trial Registry: ACTRN12616000571471 (https://www.anzctr.org.au).

1. Introduction

Impaired glucose tolerance (IGT) prevalence is increasing worldwide. IGT elevates type 2 diabetes (T2D) and cardiovascular disease (CVD) risk and is strongly associated with visceral adipose tissue (VAT) [1]. Emerging evidence also suggests excess liver fat (LF) without consuming excessive alcohol (non-alcoholic fatty liver disease [NAFLD]) independently increases insulin resistance, T2D and CVD risk [2]. Dietary therapies remain a cornerstone in the management of these inter-related metabolic conditions. Nuts, including almonds, contain various bioactive components that affect multiple physiological and metabolic processes, and thus are an important component of healthy eating patterns. Previous

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evidence suggests regular almond consumption benefits weight management and lipid profiles [3–5]; however, few studies have examined their role in regulating daily glycaemic control or liver fat deposition in populations at risk of NAFLD, T2D and CVD [6–10].

High glycaemic variability (GV – the amplitude, frequency and duration of daily glucose fluctuations) and postprandial hyperglycaemia (PPG) are independent risk factors of cardiovascular complications in T2D [11,12]. Almonds are a rich source of protein, fibre and unsaturated fats, and are low in carbohydrate; nutritional attributes known to reduce gastric emptying rate and PPG response [12–15]. Polyphenolics and phytates in almond skins are also associated with reduced PPG responses [16–18]. Small, single-meal feeding trials have shown that the addition of almonds to a mixed-nutrient meal suppresses PPG responses in healthy and T2D individuals [8,19]. However, these acute single-meal trials that restrict assessment of PPG response to ≤6 h within a laboratory setting limit understanding of the effects of regular almond consumption on daily glucose patterns across consecutive days when consumed as part of a free-living diet.

Relatively small amounts of LF and VAT contribute significantly to insulin resistance [20–22], poor glycaemic control and T2D [2,22] and CVD [23,24]. Several human trials have demonstrated that foods or dietary patterns with high MUFA content can reduce LF [25]. High MUFA diets may also reduce serum alanine aminotransferase (ALT), an indicator of liver function [26]. High-dose vitamin E supplementation (≥800 IU vitamin E per day) improves ALT levels [27] and liver histology (steatosis, inflammation and cell injury) [28]. Furthermore, evidence is accumulating for the beneficial role of polyphenols on NAFLD progression through their antioxidant, anti-inflammatory, antifibrogenic and antilipogenic properties [29]. Since almonds are rich in these nutrients, it is possible that regular almond consumption may benefit liver health. To date no study has evaluated the effects of daily almond consumption on liver fat and function.

The objective of this study was to compare the short-term effects of replacing between meal snacks with either 2 servings of almonds (56 g/day) or an isocaloric, higher carbohydrate biscuit snack as part of individual's habitual *ad libitum* diet on daily glycaemic regulation and liver fat. Liver function, adiposity, systemic inflammation and cardio-metabolic health were assessed as secondary outcomes.

2. Methods

2.1. Participants

Ninety-five adults who were overweight or obese (age: 20–70 years; body mass index (BMI): ≥27 kg/m^2), with an elevated waist circumference (>88 cm females, >102 cm males), and elevated fasting plasma glucose (≥5.6 – <7.0 mmol/L) or T2D (confirmed by evidence of previous diagnosis) and not taking any diabetes medication and/or HbA1c >10%, and weight stable (i.e. <3 kg weight loss/gain in the past 2 months) were recruited via public advertisement. Exclusion criteria included use of medications and/or supplements that may affect primary outcomes (glycaemic control and liver fat), health conditions including gastrointestinal disease, kidney disease, liver disease, CVD, type 1 diabetes and cancer, and lifestyle factors that may affect the study outcomes or participant's health at the discretion of the overseeing physician; known allergies to nuts, dairy, gluten or not willing to consume test foods; history of heavy alcohol consumption (>4 standard drinks/day) [30]; current smoker or history of smoking during 6 month prior to the study; and, women attempting to become pregnant, pregnant or lactating.

2.2. Study design

This single-center, 8-week, randomized, controlled trial was conducted at the Commonwealth Scientific and Industrial Research Organization's (CSIRO) Clinical Research Unit in Adelaide, Australia between July 2016 and May 2017. Participants attended the research clinic at baseline (Week 0) and end of the intervention (Week 8) after an overnight fast for clinical assessments. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12616000571471 http://www.anzctr.org.au). The trial was approved by the CSIRO Human Ethics Committee. All participants provided written, informed consent prior to commencing the trial.

2.3. Random allocation and blinding

Eligible participants were randomly assigned by computer generation (http://www.randomization.com/) to either the almond snack group (AS) or the biscuit snack (BS) control group in a 1:1 ratio using stratified random assignment based on sex, diabetes status, age and BMI. Researchers allocating participants to interventions were blinded to the intervention codes, as were all staff involved in data collection, analysis and statistical analysis. Group allocations were revealed after completion of statistical analysis.

2.4. Dietary intervention, compliance and liking

The dietary intervention consisted of either 56 g of raw almonds/day (Almond Board of California), consumed as 28 g morning and afternoon snacks (AS group); or 72 g (energy matched) of a commercially available, sweet, nut- and seed-free biscuits/day (The Original Scotch Finger, Arnott's Biscuits, North Strathfield, Australia) consumed as 36 g morning and afternoon snacks (BS group) (Table 1). The control BS was selected because sweet biscuits are a common snack choice in Australia and other Western countries and the aim of this trial was to investigate the health impacts of replacing a commonly consumed snack with a healthier snack. Compared to the BS, the AS had ~4-fold less carbohydrate, sugar and SFA, and 3- to 4-fold more protein and fibre, respectively. It was hypothesized that compared to the BS, the nutritional attributes of the AS would promote favourable changes in the primary outcomes by lowering GV and LF concentration.

Prior to intervention commencement, participants were counselled by a research dietician on a 2-week 'nut and seed washout' period. At study commencement participants were instructed to replace usual between-meal snacks with their allocated intervention snack food; to continue abstaining from other sources of nuts and seeds, and to otherwise maintain their usual *ad libitum* diet and physical activity patterns. Detailed and individualised advice was

Table 1
Nutrient composition of test foods, consumed as 2 mid-meal snacks.

	Almond Snack[a]	Biscuit Snack[b]
Weight, g	56	72
Energy, kJ	1448	1448
Total fat, g	28.3	15.2
Saturated fat, g	2.11	8.8
Carbohydrate, g	12.7	46.8
Sugar, g	3.14	13.2
Protein, g	12.0	4.4
Fibre, g	7.34	1.6

[a] Almonds provided and analysed by the Almond Board of California.
[b] The Original Scotch Finger (sweet, shortbread biscuit), Arnotts Biscuits, 24 George Street, North Strathfield, 2137 Australia.

provided to minimise the caloric, nutritional and experiential impact of the intervention throughout the study. All intervention foods were supplied free of charge and a $200 AUD gift card was given to participants as an honorarium for their participation. Three day weighed food records were completed by participants during the week immediately preceding the assessment visits at baseline and week 8. These were analysed using Foodworks (Professional Edition, Version 8 TM, Xyris Software, Brisbane, Australia) to assess energy and nutrient intakes.

Intervention compliance was assessed using a daily, self-reported checklist and was expressed as the total number of days the test food was consumed compared to the total number of possible days of consumption. Plasma α-tocopherol and fatty acids were measured as objective biomarkers of AS and BS compliance.

'Liking' and 'desire to consume' the assigned snack food was measured at the end of the study to assess intervention acceptance. Responses were given on a 100-mm visual analogue scale, anchored with 'strong desire not to consume' or 'not liking' on the left (0 mm) to 'strong desire to consume' or 'strong liking' on the right, using standardised consumer testing methods previously published [31,32].

2.5. Outcome measures

2.5.1. Glycaemic control and variability

Dynamic glucose regulation profiling (GV) was assessed from diurnal glucose profiles with data collected using a continuous glucose monitoring device worn for 7-days (CGM-iPro 2 device, Medtronic) as previously described [12,33]. GV measures and outcomes subsequently computed included minimum, maximum and mean blood glucose; glucose range; overall SD of glucose ($SD_{Glucose}$), intraday standard deviation of blood glucose ($SD_{intraday}$); mean amplitude of glycaemic excursions (MAGEs - average of blood glucose excursions >1 SD of the mean blood glucose value); continuous overall net glycaemic action of observations 1 h apart (CONGA-1) and continuous overall net glycaemic action of observations 3 h apart (CONGA-3); mean of daily blood glucose differences (MODD – difference between paired blood glucose values during successive 24-h period); and glucose range.

2.5.2. Liver fat concentration

LF as intrahepatic lipid (IHL) was measured by localised proton magnetic resonance spectroscopy (^{1}H-MRS) using a 3.0 T Achieva whole-body system (Philips Medical Systems; Best, The Netherlands). Intrahepatic lipid concentration and composition were determined as detailed previously [34]. ^{1}H-MRS is routinely used in research settings for valid and reliable assessment of LF by differentiating between signals from protons in lipids and water within the liver. For hepatic lipid concentration and composition, a five resonance model was used including the "fat metabolite peaks" (allylic, methylene and methyl) which allowed for quantification of both LF percentage and composition [34]. Hepatic water signal amplitudes were measured from the non-water suppressed spectrum using Hankel Lanczos Squares Singular Values Decomposition.

2.5.3. Biochemical analyses

Serum ALT, aspartate transaminase (AST), plasma glucose, serum total cholesterol (TC), HDL-C, triacylglycerol (TAG) and high sensitivity C-reactive protein (hsCRP) were measured using commercial enzymatic kits on a Beckman AU480 clinical analyser (Beckman Coulter Inc, Brea, CA, USA). The Friedewald equation was used to calculate LDL-C levels [35]. Plasma insulin was measured using a multilabel plate reader (PerkinElmer, Massachusetts, USA) and commercial enzyme immunoassay kits (Mercodia AB, Uppsala, Sweden), HbA1c was measured using HPLC and serum interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) were measured using MILLIPLEX MAP Human High Sensitivity T Cell panel Multiplex Assay Kit (Merck, Darmstadt, Germany). Plasma α-tocopherol was measured using HPLC (Agilent 1260 Infinity, Agilent Technologies, Melbourne, Australia) with a Varian microsorb MV100-5 C18 250 4.6 mm Column (Agilent Technologies, Melbourne, Australia) and detectors from Agilent (DEAA300230 detector). Plasma fatty acids were analysed using gas chromatography–mass spectrometry analysis of fatty acid methyl esters as previously described [36].

2.5.4. Anthropometry

Height was measured using a stadiometer (SECA, Hamburg, Germany) and body weight using calibrated electronic digital scales (Mercury, AMZ 14, Tokyo, Japan). BMI was calculated as weight (kg)/height^2 (m^2). Total fat mass and fat free mass was assessed by BIA (Tanita SC330S BIA Scale).

Abdominal fat (including VAT and subcutaneous adipose tissue [SCAT]) was measured by MRI. Non-invasive and non-ionising MRI and computed tomography (CT) are gold standard techniques for VAT measurement, with coefficients of variation for repeated measures of ~2% [37]. Cross-sectional areas of both VAT and SCAT at L4-L5 were subsequently determined using automated software (Hippo Fat™) [38].

2.6. Sample size and power

The primary outcomes were daily GV and LF concentration. Prior acute meal studies have shown the addition of almonds to a single meal suppresses PPG response between 15 and 30% [16–18]. Based on these data, with an expected average PPG response rate of 22% in the AS group, the study had 80% power (alpha<0.05) to detect a statistically significant difference between the treatments for diurnal GV. There was also sufficient power to detect a minimum 2% differential change in liver lipid concentration between the treatments, which was a markedly smaller effect size than has been previously observed following a dietary intervention performed in energy balance over a similar duration [25].

2.7. Statistical methods

Data was examined for normality by generating Q-Q plots. Non-normally distributed data (including overall maximum, mean and SD of glucose, SD of daily glucose means, CONGA-1, CONGA-3, MAGE, MODD, HbA1c, serum hsCRP, IL-6, TNFα, plasma glucose, serum insulin, HbA1c, serum ALT, AST, TAG, HDL-C, TC/HDL-C, weight, fat/fat free and muscle masses, visceral and abdominal sucutaneous adipose tissue, liver fat % and saturation) were logarithmically transformed prior to analysis. Group differences in baseline characteristics, nutrient intake, likeness data and changes in plasma fatty acids and α-tocopherol were compared by independent *t* tests. The primary analysis to assess differential responses between groups were performed using linear mixed model analysis of variance. Each participant was treated as fixed effects in the repeated measures model, allowing each participant to have their own intercept and slopes that enables more precise modelling of changes over time. The model included all available data from the 76 participants who commenced the intervention, and an unstructured repeated covariance matrix structure was used. Treatment and time were included as fixed factors and analysed for main effects and treatment × time interaction effects. The following *a priori* defined confounding factors were included in the models: age, gender, BMI, baseline visceral adiposity (for liver fat content concentration only) and baseline LDL-C (for lipid outcomes only;

LDL-C <3.37 mmol/L vs ≥ 3.37 mmol/L). Confounding factors were retained in the model if they showed significant main effects and were accompanied by a change in time × treatment effects. In cases where significant confounding factor × treatment × time interactions were observed, and these interactions were accompanied by a significant time × treatment effect for the specific outcome variable, analysis were performed stratified for the interaction variable. Changes from baseline to end within treatments were analysed using paired samples T-test. Statistical analysis was performed using SPSS software version 23 (IBM Corporation, New York, USA). Results are presented as means and SD of the raw data. For all data analyses, statistical significance was determined at a p-value of <0.05.

3. Results

Ninety-four participants enrolled into the trial with 18 (AS = 8; BS = 10) withdrawing prior to commencement, Fig. 1. A total of 76 participants (AS = 39 [25 male, 14 female], age 60.8 ± 6.6 yrs, BMI 34.4 ± 6.2 kg/m^2; BS = 37 [20 male, 17 female], age 60.6 ± 8.8 yrs, BMI 33.2 ± 4.9 kg/m^2) commenced the trial after which two participants in the BS group withdrew. Two and four participants had T2D, and eight and twelve were on lipid lowering medication in the AS and BS groups, respectively.

3.1. Dietary intake, intervention compliance and liking

There were no significant between group differences in energy or nutrient intakes at baseline. The study test foods contributed an average of 16.2 (SD 4.18)% and 16.6 (4.46)% to total energy intake for the AS and BS, respectively, with no significant difference between groups for change in energy intake at the end of the intervention ($P = 0.57$, Table 2).

Inclusion of AS in the diet resulted in several nutrient intake changes compared to BS. Average fat intake was greater with AS compared to BS and fatty acid profiles were different between groups with lower intakes of SFA and greater intakes of PUFA and MUFA in AS compared to BS (Table 2). Carbohydrate, sugar and starch intakes decreased and protein, fibre, riboflavin, α-tocopherol, magnesium and phosphorous intakes increased with AS compared to BS (Table 2). These dietary changes were mostly due to significant improvements from baseline to end with AS ($P < 0.05$), whereas most dietary variables did not change from baseline to end with BS, except for SFA (%E) that increased and fibre intake that decreased.

Both groups reported at least 80% compliance to the assigned snack. This is similar to other short-term dietary interventions examining daily almond consumption [19,39]. Changes in plasma fatty acids reflected the changes in dietary fatty acid intakes, confirming compliance to the dietary interventions. Plasma total SFA decreased significantly with AS compared to BS due to decreases in myristic acid (C14:0) and palmitic acid (C16:0). Palmitoleic acid (C16:1), a metabolite of palmitic acid, also decreased with AS compared to BS. In addition, plasma linoleic acid (C18:2n-6), dominant PUFA in the diet and circulation, increased with AS compared to BS. Furthermore, plasma α-tocopherol increased with AS, but the increase was not significantly different from BS ($P = 0.26$) as levels also increased, albeit to a lesser extent with BS ($P = 0.05$), possibly due to α-tocopherol present in fats used to manufacture the biscuits (Table 2).

At week 8, compared to BS, AS reported a higher desire to consume the snack provided (AS: 73.3 ± 23.8 mm vs BS: 41.3 ± 34.1 mm, $P < 0.001$) and higher liking rating (AS: 84.1 ± 19.4 mm vs BS: 49.0 +/1 28.4 mm, $P < 0.001$).

3.2. Glycaemic control and variability

There was no significant effect of treatment on minimum, maximum or mean blood glucose, glucose range, $SD_{intraday}$, $SD_{interday}$, CONGA-1, MODD, HbA1c, fasting glucose or insulin ($P \geq 0.11$, Table 3). Compared to AS, BS achieved statistically significant greater reductions in CONGA-3 and MAGE ($P \leq 0.04$), however after controlling for BMI, sex and age as covariates in the statistical model, these effects were no longer statistically significant ($P \geq 0.21$, Table 3).

3.3. Anthropometry, liver fat concentration and serum aminotransferases

There were no significant effects of time ($P \geq 0.16$ for all), or time × treatment interactions ($P \geq 0.6$ for all), for any of the anthropometric, VAT, SCAT, liver fat concentration or serum aminotransferase outcomes (Table 4).

3.4. Lipid profiles and inflammatory markers

There was no statistically significant differential response between the groups for serum TC, LDL-C, HDL-C and TAG concentrations (Table 5). Serum TC/HDL-C ratio was significantly reduced by 4% in the AS group, but this reduction was not significantly different from the BS group. Gender, however, modified this response such that in women TC/HDL-C ratio reduced after the AS compared to the BS ($P < 0.05$), but not in men ($P = 0.52$). Serum inflammatory markers did not differ between diets (Table 5).

4. Discussion

High compliance was achieved as indicated by the dietary intake data and plasma α-tocopherol and fatty acid changes that were consistent with the differential nutrient contents of the interventions. Compared to BS, AS resulted in higher reported intakes of protein, unsaturated fat, fibre and vitamin E and lower intake of carbohydrate, sugars, and saturated fat. Despite these differential changes in nutrient intakes, no major differential changes in GV, liver fat, serum aminotransferases and cardio-metabolic markers were observed.

Previous studies have demonstrated pre-meal consumption of a protein-rich load reduces post-prandial blood glucose response at the subsequent meal [40,41], and that co-ingestion of almonds blunts the blood glucose response to carbohydrate-rich foods [8,19]. However, in the present study no differences in the majority of the GV markers we assessed were observed between groups. The specific reason for the absence of any effect is unclear, but may be explained by the protein/almond 'dose' used. Previous studies that observed postprandial lowering effects with protein-rich preloads used a protein bolus of 17-50 g of whey protein compared to either water and/or a low-energy solution [40–42]. Co-ingestion of almonds with a carbohydrate rich meal was also shown to lower peak postprandial glucose compared to the control with an almond dose of 90 g (~19 g as protein) but not 30 g or 60 g (~6 and 12 g as protein, respectively) [8]. The protein content of our almond snack (12 g) and the differential protein levels between the almond and biscuit snack (8 g) were also considerably smaller in the present study. Previous studies also provided either a protein pre-load food within 15-30 min of the subsequent meal or co-ingested almonds within a meal [8,19] whereas, in this study, the test foods were consumed at least 2 h before the next meal. Therefore, it is possible any pre-load effects were no longer sufficiently present to exert an influence on the next meal; and that differences in the choice of biscuit snack, almond/

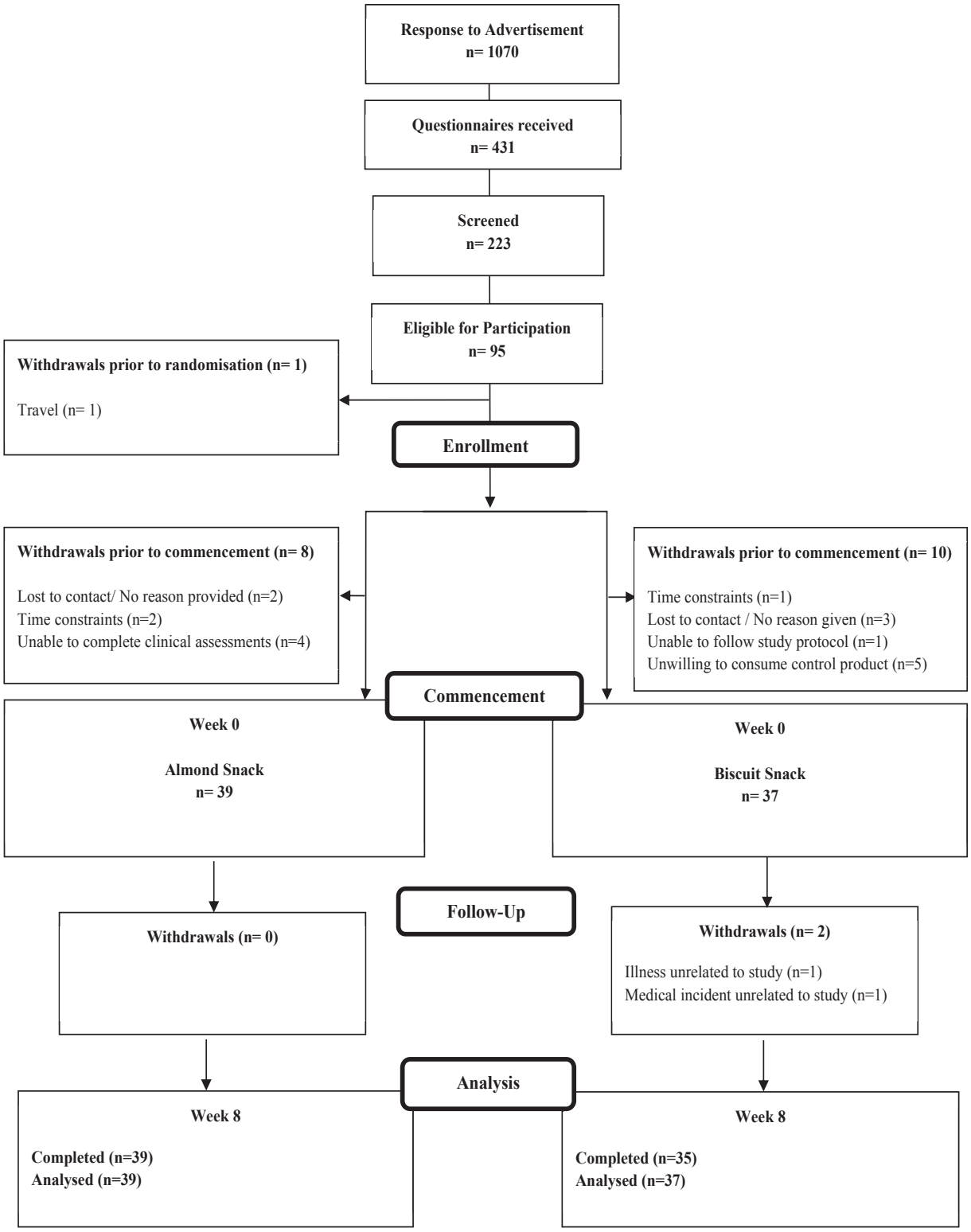

Fig. 1. Consort statement of participant flow.

protein dose and timing of consumption between the studies could explain the differential glycaemic lowering responses observed.

Interestingly, the BS group experienced significant greater reductions in CONGA-3 and MAGE compared to the AS group. This could suggest the BS promoted lower daily GV which seems counterintuitive. It is possible that the higher carbohydrate (particularly sugar) content of the BS promoted a sustained higher blood glucose response throughout the day thereby reducing the degree of blood glucose fluctuation. However, the lack of difference in the overall mean glucose and HbA1c levels between the groups does not support this. Notably, these differential changes between groups were no longer evident after controlling for BMI, sex and age

Table 2
Changes in nutrient intakes and plasma α-tocopherol and fatty acids during the study and comparisons between groups.

Variable	Almond Snack (n = 39)			Biscuit Snack (n = 37)			*P*-Value[a]
	Baseline	Week 8	Change	Baseline	Week 8	Change	
Energy (kJ)	8848 (2594)	9508 (2377)	660 (2195)	8520 (2088)	9420 (2697)	961 (2240)	0.57
Protein (%E)	19.3 (4.46)	19.7 (4.66)	0.35 (4.4)	20.5 (4.04)	18.2 (4.46)	−2.39 (3.65)	0.01
Total fat (%E)	32.9 (7.86)	39.5 (7.14)	6.68 (7.74)	33.3 (6.84)	36.2 (4.73)	2.57 (5.87)	0.01
SFA (%E)	13.6 (3.97)	11.9 (2.89)	−1.64 (3.64)	13.5 (3.59)	16.1 (2.42)	2.45 (3.69)	<0.001
PUFA (%E)	4.76 (2.59)	6.27 (2.04)	1.51 (2.66)	4.67 (2.01)	4.47 (1.31)	−0.2 (2.14)	0.004
MUFA (%E)	11.8 (3.4)	18.3 (4.49)	6.55 (4.89)	12.4 (2.64)	12.8 (2.32)	0.3 (2.12)	<0.001
Cholesterol (mg)	324 (162)	352 (198)	28.6 (182)	363 (149)	377 (181)	17.0 (193)	0.79
Carbohydrate (%E)	40 (10.3)	32.1 (9.68)	−7.86 (7.59)	38.8 (5.99)	39.3 (4.82)	0.86 (5.34)	<0.001
Sugars (%E)	17.74 (7.41)	15.3 (6.89)	−2.42 (5.16)	16.4 (5.34)	16.8 (5.12)	−0.03 (4.71)	0.04
Starch (%E)	22.7 (7.42)	17.2 (6.36)	−5.46 (5.59)	22.8 (6.03)	22.9 (4.34)	0.87 (5.06)	<0.001
Fibre (g)	24.3 (9.73)	28.4 (9.34)	4.11 (9.16)	26.8 (10.4)	21.7 (7.32)	−3.92 (9.42)	<0.001
Ribloflavin (mg)	2.14 (0.9)	2.79 (0.89)	0.66 (0.73)	2.42 (1.41)	1.97 (0.8)	−0.37 (1.53)	<0.001
α-Tocopherol (mg)	9.74 (6.42)	18.5 (4.39)	8.74 (7.86)	9.2 (3.67)	11.0 (5.67)	1.92 (5.64)	<0.001
Magnesium (mg)	361 (154)	470 (116)	109 (121)	364 (179)	312 (91.81)	−28.3 (125)	<0.001
Phosphorus (mg)	1592 (426)	1743 (391)	152 (333)	1661 (372)	1572 (428)	−77.4 (501)	0.02
P-α-Tocopherol (μg/ml)	11.1 (3.59)	11.9 (3.91)	0.86 (1.29)	10.8 (2.92)	11.3 (3.44)	0.49 (1.48)	0.26
P-Lauric acid (C12:0) (%)	0.03 (0.05)	0.03 (0.04)	0 (0.05)	0.04 (0.05)	0.06 (0.08)	0.02 (0.07)	0.21
P-Myristic acid (C14:0) (%)	1.04 (0.31)	0.96 (0.25)	−0.08 (0.24)	1.09 (0.38)	1.2 (0.29)	0.12 (0.34)	0.01
P-Palmitic acid (C16:0) (%)	23.3 (1.25)	22.8 (1.91)	−0.51 (1.35)	24.1 (1.88)	24.2 (1.81)	0.14 (1.23)	0.03
P-Palmitoleic acid (C16:1) (%)	2.45 (0.68)	2.18 (0.65)	−0.26 (0.38)	2.74 (0.93)	2.81 (0.97)	0.07 (0.47)	0.001
P-Stearic acid (C18:0) (%)	7.8 (0.92)	7.76 (0.89)	−0.03 (0.76)	7.52 (0.73)	7.7 (0.63)	0.13 (0.53)	0.32
P-Oleic acid (C18:1n-9cis) (%)	25.9 (3.58)	25.8 (3.89)	−0.12 (3.12)	27.0 (3.82)	26.4 (3.33)	−0.49 (2.06)	0.55
P-Linoleic acid (C18:2n-6cis) (%)	24.4 (3.42)	26.1 (3.54)	1.64 (2.19)	24.0 (3.54)	23.9 (3.45)	−0.07 (2.59)	0.003
P-Arachidonic acid (C20:4n-6) (%)	6.42 (2.51)	6.32 (2.62)	−0.06 (0.85)	5.46 (2.35)	5.34 (2.29)	−0.08 (0.84)	0.92
P-Total SFA (%)	34.7 (1.86)	33.9 (2.74)	−0.77 (1.97)	35.1 (2.33)	35.6 (2.02)	0.44 (1.54)	0.005
P-Total PUFA (%)	36.7 (4.4)	37.9 (4.61)	1.19 (2.82)	34.9 (4.63)	34.9 (4.6)	−0.04 (3.17)	0.08
P-Total MUFA (%)	28.5 (3.73)	28.2 (4.07)	−0.42 (3.24)	30.0 (4.14)	29.5 (3.82)	−0.4 (2.45)	0.98

%E, percent of total energy consumed, kJ kilojoule; MUFA, mono-unsaturated fatty acids; P, plasma; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
All values are means (SD) calculated from raw data.
[a] Comparisons between treatment groups were performed using independent samples *t*-test on change values.

indicating these findings should be treated with caution. Moreover, while some previous studies have shown that regular almond consumption reduces traditional markers of glycaemic control including fasting blood glucose and insulin, and HbA1c [19,39,43], similar to the present study, others have not [44–46], or have demonstrated differential effects across patient sub-groups with differing levels of glucose control [46]. It is therefore possible that discrepancies between studies could be related to differences in study design including populations, background diet and diet plans [19,43].

Similar to the present study, previous research has shown that consumption of various nut varieties including almonds, at doses ranging from ~15 to 150 g/day had no influence on markers of general adiposity including body weight, BMI or visceral adiposity (inferred by waist circumference) [47]. Conversely, an almond enriched (dose individualized to 15% of daily energy) energy-restricted diet, compared to a nut-free energy-restricted diet led to statistical and clinically relevant reductions in several anthropometric and body composition outcomes [4]. Similarly, Berryman et al. reported that daily consumption of 42 g of almonds as part of a

Table 3
Glycaemic variability, fasting glucose, insulin and glycated haemoglobin during the study and comparisons between groups.

Variable	Almond Snack (n = 39)			Biscuit Snack (n = 37)			*P*-value[a]
	Baseline	8 weeks	Change	Baseline	8 weeks	Change	
Overall Minimum Glucose (mmol/L)	4.36 (1.11)	4.51 (0.85)	0.15 (0.92)	4.30 (0.59)	4.25 (0.82)	−0.06 (0.91)	0.34
Overall Maximum Glucose (mmol/L)[b]	11.17 (2.69)	11.18 (2.80)	0.01 (1.41)	10.91 (2.55)	10.31 (2.08)	−0.60 (2.19)	0.14
Overall Mean Glucose (mmol/L)[b]	6.78 (1.04)	6.87 (1.01)	0.09 (0.40)	6.73 (0.81)	6.76 (0.90)	0.03 (0.45)	0.50
Glucose Range (mmol/L)	6.81 (2.23)	6.67 (2.72)	−0.14 (1.72)	6.26 (1.56)	5.99 (1.98)	−0.26 (1.97)	0.77
Overall SD of Glucose[b]	1.19 (0.46)	1.13 (1.00)	0.01 (0.23)	1.09 (0.42)	1.00 (0.33)	−0.09 (0.36)	0.18
SD of daily glucose means ($SD_{intraday}$)[b]	0.49 (0.23)	0.51 (0.26)	0.02 (0.23)	0.46 (0.23)	0.47 (0.21)	0.01 (0.30)	0.94
CONGA-1[b]	1.05 (0.40)	1.03 (0.41)	−0.02 (0.22)	1.05 (0.41)	0.92 (0.34)	−0.13 (0.34)	0.11
CONGA-3[b]	1.44 (0.65)	1.49 (0.68)	0.05 (0.31)	1.41 (0.61)	1.25 (0.47)	−0.16 (0.46)	0.04
MAGE[b]	2.75 (1.23)	2.78 (1.18)	0.04 (0.60)	2.68 (1.15)	2.32 (0.83)	−0.36 (0.87)	0.02
MODD[b]	1.11 (0.43)	1.12 (0.46)	0.01 (0.25)	1.08 (0.35)	1.00 (0.31)	−0.07 (0.33)	0.27
Plasma glucose (mmol/L)[b]	6.79 (1.40)	6.97 (1.49)	0.18 (0.43)	6.79 (0.77)	6.90 (0.87)	0.14 (0.42)	0.66
Serum insulin (mU/L)[b]	15.6 (7.87)	16.9 (7.43)	1.30 (4.82)	16.9 (10.6)	18.7 (16.4)	1.77 (9.67)	0.50
HbA1c (%)[b]	5.74 (0.76)	5.91 (0.91)	0.17 (0.30)	5.60 (0.49)	5.75 (0.53)	0.19 (0.32)	0.63
HbA1c (mmol/mol)[b]	38.7 (8.36)	40.6 (9.95)	1.82 (3.21)	37.2 (5.35)	38.9 (5.81)	2.06 (3.51)	0.57

SD, standards deviation; CONGA, continuous overall net glycaemic action; CONGA-1 and CONGA-3, SD of differences between observations 1 and 3 h apart, respectively; MAGE, mean amplitude of glycaemic excursions; MODD, mean of daily blood glucose differences; HbA1c, glycated haemoglobin.
All values are means (SD) calculated from raw data.
Differences in CONGA-3 and MAGE no longer significant after adding covariates (BMI, sex and age) to the statistical model ($P \geq 0.21$).
[a] Comparisons between treatment groups were performed using mixed effects longitudinal models. *P*-value represents the Time × Treatment interaction effect.
[b] Specified analyses were performed on log transformed data.

Table 4
Changes in anthropometric outcomes, hepatic triglyceride content and liver enzymes during the study and comparisons between groups.

Variable	Almond Snack (n = 39)			Biscuit Snack (n = 37)			*P*-value[a,b]
	Baseline	8 weeks	Change	Baseline	8 weeks	Change	
Weight (kg)	102 (18.5)	103 (18.7)	0.78 (1.82)	95.8 (18.4)	97.4 (18.6)	0.68 (1.33)	0.88
BIA weight (kg)	102 (18.5)	102 (18.6)	0.78 (2.10)	95.2 (18.1)	96.9 (18.6)	0.79 (1.66)	0.89
BIA FFM (kg)	62.0 (13.1)	62.9 (13.6)	0.91 (1.73)	58.6 (12.9)	59.5 (13.1)	0.40 (1.25)	1.00
BIA body fat mass (kg)	39.6 (12.1)	39.4 (12.1)	−0.14 (1.61)	36.5 (11.5)	37.4 (11.8)	0.39 (1.34)	0.77
BIA muscle mass (kg)	34.9 (7.74)	35.1 (8.11)	0.18 (2.30)	32.9 (7.80)	33.4 (7.90)	0.25 (0.73)	0.89
Waist circumference (cm)	112 (14.0)	111 (13.7)	−0.25 (2.66)	108 (12.8)	108 (12.8)	0.22 (2.44)	0.91
SCAT (cm[b])	349 (111)	350 (106)	0.46 (16.1)	352 (109)	361 (105)	4.04 (16.1)	0.78
VAT (cm[b])	231 (97.6)	227 (99.1)	−3.18 (15.9)	209 (116)	202 (113)	−0.99 (19.4)	0.97
VAT/SCAT (%)	76.0 (53.7)	75.0 (54.2)	−0.95 (7.21)	68.3 (55.7)	59.0 (44.1)	−9.26 (39.7)	0.87
Liver Fat (%)	8.23 (1.21)	9.76 (8.74)	1.53 (4.64)	10.2 (9.25)	12.3 (10.8)	1.88 (3.51)	0.84
Liver Fat Saturation (arbitrary units)	0.97 (0.03)	0.98 (0.03)	0.004 (0.02)	0.963 (0.02)	0.971 (0.02)	0.007 (0.02)	0.63
Serum ALT (IU/L)	26.2 (12.5)	26.0 (12.2)	−0.17 (9.70)	34.2 (23.1)	39.7 (33.9)	5.42 (33.6)	0.19
Serum AST (IU/L)	22.5 (5.82)	24.1 (6.97)	1.62 (4.65)	28.6 (13.1)	30.7 (19.7)	1.89 (21.0)	0.73

BIA, bioelectrical impedance analysis; FFM, fat free mass; SCAT, (abdominal) subcutaneous adipose tissue; VAT, visceral adipose tissue; ALT, alanine transaminase; AST, aspartate transaminase.
All values are means (SD) calculated from raw data.
There were no significant differences between treatments in the changes from baseline to week 8 for any of the outcomes.
[a] Comparisons between treatment groups were performed using mixed effects longitudinal models. *P*-value represents the Time × Treatment interaction effect.
[b] All analyses were performed on log transformed data.

6-week cholesterol lowering diet reduced abdominal fat and leg fat, despite no total body weight differences [3]. Cross-sectional [48] and prospective epidemiologic [49] studies also showed that nut (including almond) consumers gained less weight gain over time compared to non-nut consumers. Additionally, this is the first known study to examine the effects of almond consumption on VAT and LF assessed using MRI/^{1}H-MRS. However, no effects between the groups on VAT and liver fat (both % and level of saturation) was evident.

Cholesterol lowering properties of almonds and nuts are well documented, particularly in hypercholesterolemic, non-medicated individuals [3,5,31,43,50–54]. However, in the present study no such effects were evident, despite the use of an almond dose that has been previously shown to elicit a hypocholesterolemic response. This is possibly due to the relatively modest baseline LDL-C concentrations [50], the use of lipid lowering medication by 25% of participants; and/or high BMI levels that is known to reduce LDL-C responses to diet interventions [50,55]. Other almond or nut interventions conducted in overweight/obese populations have also shown no hypocholesterolemic effects [46,56,57]. Berryman et al. [58] showed that substituting almonds for a carbohydrate-rich snack favourably affected HDL-C efflux in a normal-weight group with elevated LDL-C, but not in overweight individuals.

The 8% (−0.22 mmol/L) reduction in serum TC/HDL-C ratio in women in the AS group compared to BS group, was slightly greater than the average 5.6% reduction shown in previous studies [50]. Group differences in TC/HDL-C were driven by both a decrease in TC and an increase HDL-C in the AS group, whereas, in the BS group the HDL-C increase was accompanied by increases in TC. HDL-C is generally not affected by almond consumption [5]. Changes in background diet promoted by inclusion of almonds may in part explain the effects on TC/HDL-C. It is well known that TC and LDL-C are reduced when dietary PUFA replace SFA and HDL-C is favourably affected by reduced carbohydrate and increased fat intake [59]. Berryman et al. showed that substituting almonds for an isocaloric high-carbohydrate snack attenuated a reduction in HDL-C associated with a low SFA diet and maintained a favourable circulating HDL sub-population distribution and function [58].

The reason why the effect was only present in females, and not in males, is unclear. Sabate et al. [50] concluded that lipid responses

Table 5
Changes in lipid profiles and inflammatory markers during the study and comparisons between treatments.

Variable	Almond Snack (n = 39)			Biscuit Snack (n = 37)			*P*-value[a]
	Baseline	8 weeks	Change	Baseline	8 weeks	Change	
Serum TC (mmol/L)	5.41 (0.98)	5.43 (1.00)	0.02 (0.41)	5.38 (0.83)	5.62 (0.84)	0.19 (0.43)	0.08
Serum LDL-C (mmol/L)	3.33 (0.76)	3.30 (0.78)	−0.03 (0.39)	3.24 (0.79)	3.43 (0.76)	0.13 (0.37)	0.07
Serum HDL-C (mmol/L)[b]	1.30 (0.30)	1.35 (0.29)	0.06 (0.14)	1.24 (0.30)	1.32 (0.34)	0.07 (0.13)	0.87
Serum TAG (mmol/L)[b]	1.71 (1.18)	1.70 (1.27)	−0.01 (0.48)	1.96 (0.95)	1.92 (0.96)	−0.03 (0.70)	0.86
Serum TC/HDL-C (mmol/L)[b, c]	4.36 (1.20)	4.18 (1.19)	−0.18 (0.39)	4.53 (1.15)	4.48 (1.14)	−0.07 (0.34)	0.15
Female[b, d]	4.03 (0.89)	3.67 (0.85)	−0.36 (0.26)	4.04 (0.98)	3.93 (0.99)	−0.14 (0.32)	0.05
Men[b, e]	4.54 (1.33)	4.46 (1.27)	−0.08 (0.42)	4.93 (1.15)	4.94 (1.06)	−0.00 (0.36)	0.46
Serum hCRP[b] (mg/dL)	5.58 (10.3)	4.31 (7.07)	−1.27 (8.11)	3.01 (2.54)	4.17 (5.72)	1.07 (5.18)	0.12
Serum IL-6 (pg/mL)[b]	7.66 (5.64)	8.49 (7.26)	0.84 (3.92)	7.29 (6.44)	6.67 (4.69)	−0.82 (5.72)	0.39
Serum TNFα (pg/mL)[b]	12.2 (4.26)	12.2 (4.77)	0.00 (1.85)	12.7 (4.42)	12.8 (4.71)	0.16 (2.79)	0.99

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TAG, triacylglycerol; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNFα, tumour necrosis factor alpha.
All values are means (SD) calculated from raw data.
[a] Comparisons between treatment groups were performed using mixed effects longitudinal models. *P*-value represents the Time × Treatment interaction effect.
[b] Specified analyses were performed on log transformed data.
[c] Significant interaction effects were shown with gender (gender x treatment x time, $P < 0.01$) resulting in a significant treatment x time effect ($P = 0.04$). Subsequent post-hoc analysis was performed stratified for gender.
[d] n = 14 for almond snack; n = 17 for biscuit snack.
[e] n = 25 for almond snack; n = 20 for biscuit snack.

were not affected by gender in their pooled analysis. Relative to body weight women consumed more almonds than men, however, the percent of energy intake from almonds were similar in men (16% of total energy) and women (17% of total energy). Women also had significantly lower TC/HDL-C levels at baseline compared to men, but this difference is more likely to attenuate the reduction in women rather than enhancing the response. Irrespective of the reason for the differential gender response these results should be interpreted with caution as the sub-analysis was not an *a priori* objective and the study was not powered to investigate gender-by-treatment effects. Furthermore, after applying Bonferroni correction for multiple comparisons, statistically significance was not maintained. Nevertheless, further exploration of the effects of almonds on HDL-C while considering the potential modulating effects of the background diet is warranted.

The present data showed a significantly higher desire to consume the almonds with a higher rating of likeness compared to the biscuit snack, consistent with previous research [31]. These data suggest almonds, at least when compared to biscuits, has a superior level of acceptance and would be considered a more appealing option in the free-living setting.

A key study strength is that this is the first known study to assess the effects of a simple dietary change (consuming almonds as mid-meal snack) in a free-living setting on a comprehensive array of novel and related metabolic health markers. The study was adequately powered and retention and compliance were high. The 8-week intervention duration has previously been sufficient to detect metabolic changes [50]; nevertheless, longer-term studies should be conducted to elucidate the chronic effects. The almond dose (56 g/day, ~16% of total energy intake) used was also at the upper feasible limit in the free-living environment that maintained a high liking rating. A study limitation is that the experiment was designed to examine the primary outcomes of GV and LF concentration, and it is possible the individuals examined were not the most appropriate to observe the well-known lipid lowering properties of almonds. Participants were also predominantly Caucasian and future studies should investigate the glycaemic effects of almonds in diverse ethnic populations, particularly those with greater risk of poor glycaemic control and T2D such as Asian and African American populations.

In conclusion, compared with BS, twice daily consumption of AS was well accepted but did not differentially alter diurnal GV, liver fat or function, systemic inflammation, adiposity or cardiometabolic health markers, although modest improvements in serum TC/HDL-C ratio in women only with AS was observed. Further research should examine the longer-term health effect of regular almond consumption and gender differential effects on lipid responses with consideration of the modulating effects of background diet.

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The authors' responsibilities were as follows—GDB: was the guarantor of this work and, as such, had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; All authors: obtainment of funding, study concept and design, analysis and interpretation data, drafting and approval of the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2018.12.088.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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