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Acute whole apple consumption did not influence postprandial lipaemia: a randomised crossover trial

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Abstract

Whole apples are a source of pectin and polyphenols, both of which show potential to modulate postprandial lipaemia (PPL). The present study aimed to explore the effects of whole apple consumption on PPL, as a risk factor for CVD, in generally healthy but overweight and obese adults. A randomised, crossover acute meal trial was conducted with seventeen women and nine men (mean BMI of 34·1 (SEM 0·2) kg/m^2). Blood samples were collected for 6 h after participants consumed an oral fat tolerance test meal that provided 1 g fat/kg body weight and 1500 mg acetaminophen per meal for estimating gastric emptying, with and without three whole raw Gala apples (approximately 200 g). Plasma TAG (with peak postprandial concentration as the primary outcome), apoB48, chylomicron-rich fraction particle size and fatty acid composition, glucose, insulin and acetaminophen were analysed. Differences between with and without apples were identified by ANCOVA. Apple consumption did not alter postprandial TAG response, chylomicron properties, glucose or acetaminophen ($P > 0·05$), but did lead to a higher apoB48 peak concentration and exaggerated insulin between 20 and 180 min ($P < 0·05$). Overall, as a complex food matrix, apples did not modulate postprandial TAG when consumed with a high-fat meal in overweight and obese adults, but did stimulate insulin secretion, potentially contributing to an increased TAG-rich lipoprotein production.

Key words: **Apples: Cardiovascular risk: Gastric emptying: Overweight: Pectin: Polyphenols: Postprandial lipaemia**

Postprandial lipaemia (PPL) is characterised by the dynamic changes in TAG and TAG-rich lipoproteins (i.e. chylomicrons, VLDL and their remnants) that occur following ingestion of dietary lipid[1]. Chylomicrons, specifically, transport reassembled TAG from the enterocytes to the liver and their number (characterised by apoB48 which is constitutive) and/or size increase when a fat-containing meal is ingested, then return to baseline over several hours as TAG are cleared from circulation[2]. The nature of the PPL response is recognised as an important risk factor for CVD[1]. In fact, non-fasting TAG concentrations are a more significant risk factor for CHD events than fasting levels[3]. Oral fat tolerance tests (OFTT) in which an individual consumes a standard high amount of fat with serial blood sampling for analysis of blood lipids can be used to study PPL and identify individuals with impaired lipid metabolism[4]. For example, chylomicron overproduction is a characteristic of individuals with insulin resistance, type 2 diabetes[5] and obesity[6]; smaller and more numerous chylomicrons have been reported in older *v.* younger populations[7] and in hypertriacylglycerolaemic abdominally obese *v.* lean women[8]. Smaller chylomicrons are also postulated to clear more slowly from circulating plasma[9], which correlates with their potential atherogenicity as they are able to penetrate the arterial wall and induce inflammation and oxidative stress in the subendothelial space[10]. OFTT can also be used to study the influence of dietary strategies on the extent and/or duration of PPL. Such investigations are especially warranted in overweight and obese individuals as they tend to have impaired lipid metabolism, involving increased production and/or insufficient clearance of TAG-rich lipoproteins, resulting in elevated plasma TAG levels and prolonged PPL[5,11].

Fruit and vegetable consumption, which is generally recommended to reduce CVD risk, may impact PPL. This is partly related to their contents of dietary fibre, which can alter various parameters that contribute to lipid digestion and absorption[12]. For example, apple skin contains the soluble fibre pectin. Apple pectin is a polysaccharide consisting of a homogalacturonan backbone esterified with methoxyl groups[13]. It has a relatively high molecular weight (10^4–10^6 Da) and degree of esterification (>70 %)[14] and gels at a pH of 3·6 in the presence of a co-solute (e.g. sucrose), inducing viscosity[13]. Apple pectin consumption has been shown to delay gastric emptying[15,16] and to lower fasting blood cholesterol[17,18] in humans. In another instance, purified pectin (unknown source but highly esterified) also reduced fasting plasma TAG in a hamster model[19], and pectin-rich dried

Abbreviations: CMRF, chylomicron-rich fraction; FA, fatty acid; HOMA-IR, homoeostatic model assessment of insulin resistance; iAUC, incremental AUC; OFTT, oral fat tolerance test; PPL, postprandial lipaemia; UFA, unsaturated fatty acids.

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beet pulp powder lowered the postprandial TAG response in pigs[20]. *In vitro*, pectins have also been shown to alter the rate and/or extent of lipid digestion[21,22] and to bind bile salts[23,24], which otherwise promote the solubilisation of hydrolysed lipids in the digestate. Apples are also rich in polyphenols, mainly flavanols (e.g. catechin and proanthocyanidins) and hydroxycinnamic acid derivatives[25] that can play a role in PPL. For example, apple procyanidins inhibited pancreatic lipase activity *in vitro* and reduced postprandial TAG elevation in mice[26]. Apple procyanidins also reduced the secretion of TAG-rich lipoproteins in human Caco-2/TC7 enterocytes *in vitro,* pointing to a possible hypolipidaemic effect *in vivo*[27]. Another study with rats found that apple pectin improved large intestinal fermentation and reduced fasting plasma TAG and cholesterol more efficiently in the presence of apple polyphenols, suggesting synergy between these molecules[28] with respect to PPL. That said, human studies related to apples and lipaemia have largely focused on fasting blood lipids and have yielded mixed results. In hyperlipidaemic overweight men, 8-week consumption of Golden Delicious apples (300 g/d) increased fasting serum TAG and VLDL[29], whereas another study found that 4-week consumption of whole apples (unknown variety) (550 g/d) or apple pomace (22 g/d), compared with fibre-free apple juice (500 ml/d), decreased fasting LDL-cholesterol concentrations in healthy participants, highlighting the role of apple fibres in lowering LDL[30]. Whole apples and apple-derived products also contain sugars, especially fructose, which can also impact PPL[31].

In summary, apples as a complex food matrix show potential to alter fasting lipids, but their role in PPL has not been investigated. Therefore, the present study aimed to use an OFTT to explore the effects of whole Gala apple (a common variety produced in Ontario, Canada) consumption on PPL (plasma TAG, apoB48, chylomicrons and remnants particle sizes and fatty acid (FA) composition), as well as on gastric emptying, glycaemia and insulinaemia. Overweight and obese individuals were selected given their greater propensity for impaired PPL metabolism and because fruit and vegetable consumption is recommended to these individuals as a strategy both for weight management and metabolic disease risk reduction[32]. It was hypothesised that apple consumption would reduce the magnitude of PPL by slowing down gastric emptying of the OFTT meal.

Materials and methods

Participants

Participants were recruited through flyer, Internet, newspaper and radio advertisements in Guelph, Ontario, Canada, and the surrounding communities. Inclusion criteria were: 18–75 years; BMI ≥ 25·0 kg/m^2; generally healthy; non-alcoholic; non-smoker; non-diabetic; free of digestive, cardiovascular or inflammatory diseases/disorders; stable body weight (<5 % fluctuation) for the previous 3 months and no intention to gain or lose weight. Individuals were excluded if they were: hospitalised due to serious medical conditions within the last year; taking medications that could interfere with the study outcomes; allergic or intolerant to any ingredients in the test meals; pregnant, breast-feeding or post-menopausal. A fasting TAG concentration ≥ 1·69 mmol/l was initially required, but it was not feasible to find hyperlipidaemic individuals who were otherwise eligible, so this criteria was removed. Of the 179 persons screened, fifty-one were invited for further screening, among which twenty-eight met the criteria and were enrolled in the study, all of which took place from January 2017 to September 2018.

Study design and protocols

This was a randomised, cross-over acute meal study conducted at the Human Nutraceutical Research Unit at the University of Guelph. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the University of Guelph Human Research Ethics Board (REB no. 16JA013) and registered at clinicaltrials.gov (NCT03523403, The Apple Study: Investigating the Effects of Whole Apple Consumption on Risk Factors for Chronic Metabolic Diseases in Overweight and Obese Adults, https://clinicaltrials.gov/ct2/show/NCT03523403). All participants provided written informed consent. Each participant attended two study visits separated by a 1-week wash-out period. They were randomised based on their order of study enrolment using a random number table (generated by Excel, Microsoft Ltd, organised by an external party) to consume a dairy-based OFTT meal with or without apples at each visit. Due to the nature of the treatments, participants and researchers were not blinded at each study visit, although samples and data were coded based only on study visit days in order to conceal treatment allocation during their handling and analyses. For 2 weeks before the first study visit, participants were counselled to avoid foods from a three-page long list of foods rich in polyphenols and/or dietary fibres (e.g. berries, purple and red potatoes, chocolate and coffee), but to otherwise maintain their dietary and exercise habits which were recorded in a study diary. For 48 h before each study visit, participants abstained from alcohol, exercise and over-the-counter medications (including acetaminophen-containing products). Participants consumed a standardised low-fat (12·0–12·5 g fat per serving) dinner the night before each study visit. This consisted of one serving of vegetarian lasagna (President's Choice Blue Menu), a cranberry almond granola bar (President's Choice Chewy Trail Mix), a pudding (Hunt's, chocolate or vanilla flavoured), and a juice box (President's Choice, apple or fruit punch). On the morning of each study visit, participants arrived at the research centre having fasted for 10–12 h and completed a brief questionnaire for compliance to protocol. Water consumption was encouraged for up to 1 h before a phlebotomist inserted an intravenous catheter for fasting and periodic blood sampling over 6 h. Participants consumed the test meals within 15 min, and baseline (i.e. 0 min) was from when they started to consume the meal. Throughout the day, participants remained seated with only short washroom breaks.

Test meal treatments

During each study visit, participants consumed the OFTT meal, which was a 500 ml mixture of 35 % fat whipping cream and skimmed milk, standardised with additional skimmed milk powder such that each meal provided 1 g fat/kg body weight and

14·6 g protein. Extra Strength Tylenol® (00559407) containing 1500 mg acetaminophen was ground and added to each meal shortly before consumption by participants, for indirect assessment of gastric emptying[33]. Fat consumption was based on body weight to take into account the larger blood volume in overweight and obese participants[4], but the same approach did not apply for acetaminophen to avoid potential overdose in extremely obese participants (total amount taken in a day should not exceed 4000 mg[34]). Regarding protein, its content in the cream-skimmed milk mixture decreased with an increasing fat content; therefore, skimmed milk powder was added to eliminate any potential PPL-lowering effects based on differences in protein consumption[35]. The OFTT meal was well tolerated by most participants, although one participant vomited 20 min after its consumption and discontinued participation. Three Gala apples (Martin's Family Fruit Farm and Norfolk Fruit Growers Association) were cored and sliced without removing the skin (approximately 200 g total) and then consumed along with the OFTT meal at one of the study visits. The pectin and total polyphenol contents of the Gala apples were measured using colorimetric assays. Whole apples were cored and sliced and then freeze-dried (VirTis Genesis 35 L pilot lyophilizer; Stone Ridge). For pectin analysis, alcohol insoluble solids were extracted from the dried apples and then hydrolysed by sulphuric acid to galacturonic acid, which was reacted with m-hydroxydiphenyl. Absorption was measured at 525 nm using a UV–vis spectrophotometer (Hewlett Packard 8451 A Diode Array Spectrophotometer), and concentration was determined using a standard curve based on D-galacturonic acid (0–0·5 mg/ml)[36]. For the total phenolic analysis, the dried apples were homogenised with acidified methanol to remove vitamin C and the extract was mixed with the Folin–Ciocalteu reagent and sodium carbonate solution, followed by absorbance measurements at 750 nm and using a standard curve based on gallic acid (0–0·156 mg/ml)[37]. The meals' nutrient compositions are provided in Table 1.

Table 1. Nutrient composition of the test meals*

Nutrients (per meal)	OFTT meal (500 ml)	OFTT meal (500 ml) + apples (200 g)
Energy content (kcal†)‡§	792·7–1524·1	906·7–1638·1
Fat (g)§	71·6–154·0	71·6–154·0
Sugar (g)§	20·3–21·5	41·1–42·3
Fructose	–	11·9
Sucrose	–	5·6
Glucose	–	3·3
Protein (g)	14·6	15·8
Pectin (g)‖	–	2·2
Total polyphenols (g)¶	–	0·2
Acetaminophen (g)	1·5	1·5

OFTT, oral fat tolerance test.
* Values calculated based on nutrient reports in Health Canada Nutrients Database[46], unless otherwise stated.
† To convert kcal to kJ, multiply by 4·184.
‡ Calculated based on product labels (for the OFTT meal) and nutrient reports in Health Canada Nutrients Database (for apples).
§ Depends on participants' body weight, aiming to provide 1 g fat/kg body weight.
‖ Presented as galacturonic acid equivalents.
¶ Presented as gallic acid equivalents.

Blood sampling and analysis

Blood samples were taken at baseline, every 20 min within the first 3 h, and at 4, 5 and 6 h. All samples were drawn from the antecubital vein into EDTA vacutainer tubes via an intravenous line (Vacutainer®; K2EDTA, BD). After centrifugation at 625 ***g*** (Allegra TM X-22 R Centrifuge; Beckman Coulter Incorporated) at 4°C for 10 min, plasma was collected, aliquoted into cryovials in small volumes and immediately stored at −80°C until analysis by commercial assays. Plasma concentrations of the primary outcome, that is, plasma TAG (Wako Diagnostics), and secondary outcomes, that is, apoB48 (Cloud Clone), acetaminophen (Neogen), glucose (Wako Diagnostics) and insulin (Mercodia AB) were determined according to the manufacturers' instructions. The intra-assay variabilities were 10·0, 7·3, 5·3, 8·8 and 5·9 % for TAG, apoB48, acetaminophen, glucose and insulin, respectively. Fasting cholesterol concentrations were determined immediately from a drop of whole venous blood using the Cholestech LDX Lipid Analyzer (Cholestech).

Chylomicron-rich fraction analysis

The chylomicron-rich fraction (CMRF) was separated from plasma based on Vors *et al.*[38] with slight modifications for determination of the secondary outcomes CMRF particle size and FA composition[37]. Briefly, 3·0 ml plasma was overlaid with 2·5 ml saline solution ($d = 1{\cdot}006$ g/ml) and centrifuged at $3{\cdot}9 \times 10^5$ ***g*** (at r_{max}; Beckman Coulter) at room temperature for 23 min (Sorvall WX Ultra 80; ThermoFisher Scientific). The top layer, which was white and cloudy, was aspirated for immediate particle size analysis by dynamic light scattering (NanoZetasizer S, Malvern Instruments), using a refractive index of 1·450 for human plasma protein and absorption of 0·001 as input parameters for calculations[39]. A portion was also stored in an Eppendorf tube at −80°C and analysed for FA composition by GC within 1 month of sample collection. Lipid extractions for GC analysis were performed based on the Folch method with slight modifications[40]. Briefly, 0·03 ml of CMRF was vortexed with 0·97 ml of 0·1M KCl and 4 ml of chloroform–methanol (2:1) until well mixed. The mixture was flushed with N_2 and set at 4°C overnight before centrifugation at 300 ***g*** for 10 min. The lower chloroform layer was collected and dried under N_2 and then saponified by 2 ml of 0·5M KOH in methanol at 100°C for 1 h. After cooling to room temperature, methylation was carried out with 2 ml hexane and 2 ml 14 % BF_3-methanol at 100°C for 1 h. After the mixture was cooled, 2 ml water was added to stop the methylation and the mixture vortexed and centrifuged at 300 ***g*** for 10 min. The top hexane layer containing the FA methyl esters was collected and dried down under N_2 and then reconstituted in 1 ml of hexane for analysis. FA methyl esters were separated by GC (Agilent 6890 Network GC System; Agilent Technologies) with a Supelco SP 2560 fused-silica capillary column (100 m × 0·25 mm internal diameter, 0·2 µm film thickness; Sigma-Aldrich). The FA composition is presented as % total area count, calculated by dividing the peak area of the target FA by the total area. SFA and unsaturated fatty acids (UFA) from C10 : 0 to C24 : 0 were grouped separately for calculating their % total area counts and the ratio between them (SFA:UFA ratio). The same methods were used to determine FA profiles of the OFTT meal.

Table 2. Characteristics and fasting blood measurements for male (*n* 9) and female (*n* 17) participants*
(Mean values with their standard errors; ranges)

Characteristics	Mean	SEM	Range
Age (years)	45·5	3·1	19–69
Weight (kg)	100·0	4·5	
Male	118·0	7·5	91·6–154·0
Female	90·5	4·1	71·6–131·6
BMI (kg/m^2)	34·1	1·2	
Male	36·6	2·3	27·2–48·2
Female	32·7	1·3	25·4–41·5
Waist circumference (cm)	112·8	2·0	
Male	122·1	4·6	106·0–149·0
Female	107·9	3·0	87·0–133·0
Total cholesterol (mmol/l)	4·88	0·12	2·59–6·70
LDL-cholesterol (mmol/l)	3·14	0·09	1·75–4·27
HDL-cholesterol (mmol/l)	1·11	0·05	0·57–2·06
Glucose (mmol/l)†	4·98	0·18	3·49–6·76
Insulin (pmol/l)	61·15	9·54	14·50–183·50
HOMA-IR	1·14	0·17	0·30–3·30
TAG (mmol/l)‡	1·38	0·08	
Male	1·30	0·11	0·75–1·89
Female§	1·42	0·10	0·66–2·31
ApoB48 (mg/l)	23·09	1·90	
Male	27·02	3·23	15·62–40·74
Female	21·01	2·25	1·29–38·53
CMRF particle size (nm)	55·25	1·82	45·56–85·01
CMRF SFA:UFA ratio	0·50	0·01	0·40–0·59

HOMA-IR, homoeostatic model assessment of insulin resistance; CMRF, chylomicron-rich fraction; UFA, unsaturated fatty acids.
* Values are the average of the fasting measurements on both study visit days.
† *n* 25 (one male participant outlying value eliminated from the data sets).
‡ *n* 25.
§ *n* 16 (one female participant outlying value eliminated from the data sets).

Data and statistical analyses

The required number of participants was 34, based on an *a priori* calculation using a two-tailed model for a matched pairs mean comparison in TAG C_{max}, with a medium effect size (Cohen's *d* coefficient = 0·5[41]), a probability of a type I error of 0·05 and a power of 0·8 (G*Power Software, version 3.1.9.4., Universität Düsseldorf). All measurements were completed with replicates (duplicates for plasma endpoint analyses by kits and triplicates for GC and particle size analyses), and the data were then averaged and analysed using Statistical Package for the Social Sciences version 21 (SPSS; IBM Corporation). Two outlying values (one for TAG and another one for glucose, based on *z*-scores method, Table 2) were excluded from the statistical analysis. Data normality was assessed using Shapiro–Wilk testing. All normally distributed data are presented as the arithmetic means with their standard errors. When not normally distributed, data were log-transformed and presented as geometric means with average transformed standard errors, as indicated. All fasting endpoints were compared between study visits 1 and 2 using paired-sample *t* tests. Fasting glucose and insulin concentrations were used to calculate the homoeostatic model assessment of insulin resistance (HOMA-IR), using the HOMA2 Calculator (version 2.2.3, Diabetes Trials Unit, University of Oxford). For the postprandial time-wise data, repeated-measures ANCOVA were performed with centred baseline value (i.e. individual baseline value minus mean baseline value) as a covariate, as suggested in Schneider *et al.*[42], and Bonferroni *post hoc* testing with a significance level of $P < 0·05$. For each endpoint, the effects of treatment (OFTT meal with or without apples), time (postprandial time points) and treatment × time interactions were assessed. Participant characteristics, including sex, age, body weight and BMI, were added into the ANCOVA models as between-subject factors or covariates (for nominal or continuous values, respectively) to assess their effects on the endpoints, but none of them was significant in the models. Therefore, the ANCOVA for each endpoint included only the centred baseline value as a covariate. Incremental AUC (iAUC) was calculated using the linear trapezoidal method (GraphPad Prism version 7.04 for Windows, www.graphpad.com) and compared using ANCOVA using centred baseline values as a covariate. Maximum concentrations (C_{max}) of each endpoint were identified as the greatest value in an individual's data set and maximum peak times (T_{max}) identified as the corresponding nominal sampling time. Mean C_{max} and median T_{max} are reported. Values of C_{max} were compared using paired-sample *t* testing, while T_{max} values were compared using median test for two independent samples *t* test. Statistical analyses were also performed on change from baseline values, but no differences in results were observed from those based on absolute values (data not shown). Effect size was estimated as Cohen's *d*, calculated as the difference between pairwise means divided by pooled standard deviations[41].

Results

Participant characteristics

Of the twenty-eight participants enrolled, twenty-six completed the study (Fig. 1). Participant characteristics and fasting measurements are shown in Table 2. There were no differences in participant characteristics or fasting blood measurements between the two study visits ($P > 0·05$ for all measurements, data not shown).

Postprandial TAG and apoB48

Plasma TAG concentrations increased rapidly in the first 3 h ($P < 0·05$) after the consumption of both meals and began to decrease after 5 h (Fig. 2(a)). There was no effect of apple ingestion on postprandial TAG concentration ($P_{treatment} = 0·731$, Fig. 2(a)). Similarly, peak TAG concentration (C_{max}), TAG iAUC and time to peak concentration (T_{max}) were not different when the OFTT meal was consumed alone *v.* with three apples ($P = 0·921$, 0·385 and 0·266, respectively, Table 3). There were significant effects of time ($P_{time} = 0·007$), but not treatment, in terms of apoB48 concentration ($P_{treatment} = 0·088$, Fig. 2(b)). ApoB48 iAUC and median T_{max} values also did not differ between treatments ($P = 0·257$ and 0·375, respectively, Table 3), although apoB48 C_{max} was higher with apples ($P = 0·007$, Table 3).

Postprandial size and fatty acid composition of the chylomicron-rich fraction

CMRF particle size increased rapidly within 2 h of meal ingestion ($P < 0·05$) and reached a plateau, not changing between 4 and 6 h ($P = 0·420$, Fig. 3(a)). There were no differences in

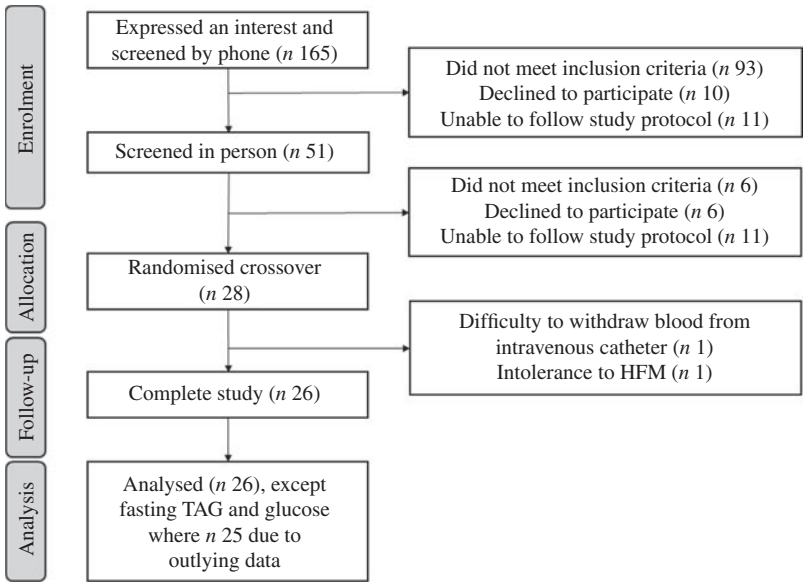

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study. HFM, high-fat meal.

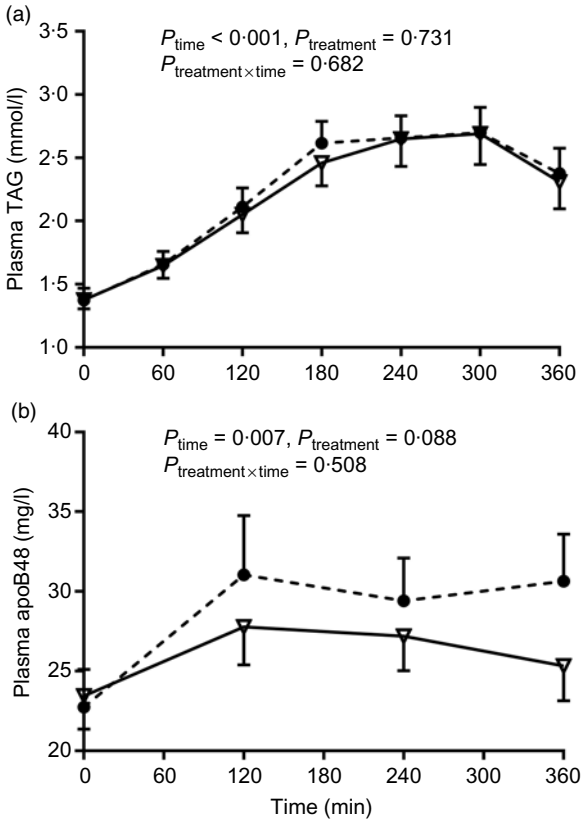

Fig. 2. Postprandial plasma TAG (a) and apoB48 (b) concentrations following an oral fat tolerance test (OFTT) meal with and without apples. Data are means with their standard errors, (a) *n* 25 per treatment (one female participant outlying data removed from database) and (b) *n* 26. P_{time}, $P_{treatment}$ and $P_{treatment \times time}$ refer to results from repeated-measures ANCOVA, using centred fasting TAG (a) and apoB48 (b) values as covariates, followed by Bonferroni *post hoc* testing. –●–, With apple; –▽–, without apple.

postprandial CMRF size between treatments over 6 h ($P_{treatment}$ = 0·935, Fig. 3(a)). The main FA identified in the CMRF were myristic (C14 : 0), palmitic (C16 : 0), palmitoleic (C16 : 1), stearic (C18 : 0), oleic (C18 : 1c9), vaccenic (C18 : 1c11) and linoleic (C18 : 2). The OFTT meal (FA composition shown in Table 4) also contained decanoic (C10 : 0) and lauric (C12 : 0) acids, but these species were not present in appreciable amounts in the CMRF (% total area count < 2 % at peak value, data not shown). For both study visits, the baseline fasting CMRF SFA:UFA ratio was approximately 0·5 (P = 0·110). This ratio increased following OFTT meal ingestion, peaking at 6 h (0·90 (SEM 0·02) and 0·84 (SEM 0·02) with and without apples, respectively), but there was no difference between treatments at any time point except for a small but significant decrease without apples at 6 h (P < 0·05, Fig. 3(b)). The proportion of individual SFA, including C14 : 0, C16 : 0 and C18 : 0, did increase substantially with time (P_{time} < 0·001, Table 4), as the UFA C16 : 1, C18 : 1c9 and c11, and C18 : 2 decreased (P_{time} < 0·01, Table 4). However, apple consumption was not associated with any differences in CMRF FA composition ($P_{treatment}$ > 0·05, Table 4).

Postprandial glucose and insulin

Glucose concentration fluctuated during the postprandial period (P_{time} < 0·05) but did not differ between treatments ($P_{treatment}$ = 0·749, Fig. 4(a)). There was an expected, but statistically insignificant, early rise with the apple treatment. Apple consumption also did not influence postprandial glucose iAUC, C_{max} and T_{max} values (P = 0·446, 0·923 and 1·000, respectively, Table 3). Insulin concentration did increase quickly (P < 0·05) with and without apples and gradually returned to fasting levels by 360 min (Fig. 4(b)). Higher insulin concentrations were observed from 20 to 180 min when apples were ingested (P < 0·05, Fig. 4(b)). iAUC and C_{max} values were also both higher with apples (P < 0·001, Table 4),

Table 3. Postprandial TAG, apoB48, glucose, insulin and acetaminophen iAUC, C_{max} and T_{max} values following ingestion of oral fat tolerance test meals with and without apples*
(Mean values with their standard errors)

		Without apples		With apples		
		Mean	SEM	Mean	SEM	*P*
TAG†	iAUC (mmol/l×h)	5·1	0·7	5·4	0·5	0·921
	C_{max} (mmol/l)	3·0	0·3	3·0	0·2	0·385
	T_{max} (min)	300	–	240	–	0·266
ApoB48	iAUC (mg/l×h)	30·9	6·4	44·8	10·0	0·257
	C_{max} (mg/l)	32·7	1·9	40·8	3·5	0·007
	T_{max} (min)	180	–	240	–	0·375
Glucose‡	iAUC (mmol/l×h)	2·3	0·4	2·8	0·5	0·446
	C_{max} (mmol/l)	6·3	0·1	6·3	0·2	0·923
	T_{max} (min)	160	–	120	–	1·000
Insulin	iAUC (pmol/l×h)	243·6	38·0	434·6	47·3	< 0·001
	C_{max} (pmol/l)	250·8	31·6	356·0	30·2	< 0·001
	T_{max} (min)	60	–	60	–	0·760
Acetaminophen	iAUC (μmol/l×h)	448·2	53·6	476·8	47·5	0·440
	C_{max} (μmol/l)	146·8	16·2	140·1	13·0	0·661
	T_{max} (min)	120	–	120	–	0·577

iAUC, incremental AUC; C_{max}, maximum concentration; T_{max}, time to reach the maximum concentration.
* iAUC and C_{max} of different treatment groups were compared using ANCOVA with fasting values as covariates, while T_{max} were compared using the median test. iAUC and C_{max} values are presented as means with their standard errors and T_{max} are medians; *n* 26 per treatment.
† *n* 25, as one female participant outlying data removed from the database.
‡ *n* 25, as one male participant outlying data removed from the database.

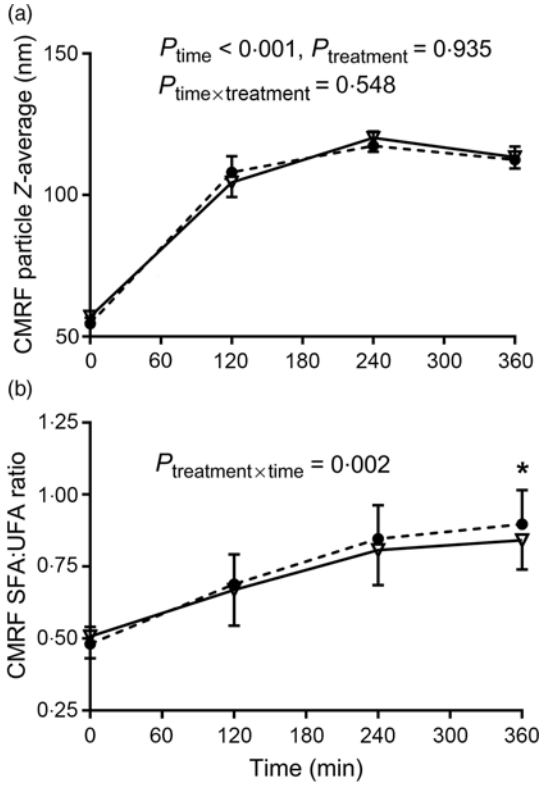

Fig. 3. Postprandial chylomicron-rich fraction (CMRF) particle size (a) and ratio between percentage total area count of SFA and unsaturated fatty acids (UFA) (SFA:UFA ratio) (b) after consumption of an oral fat tolerance test (OFTT) meal with or without apple. Data are means with their standard errors, *n* 26 per treatment. P_{time}, $P_{treatment}$ and $P_{treatment×time}$ refer to results from repeated-measures ANCOVA using centred fasting CMRF particle size (a) and SFA:UFA ratio (b) values as covariates, followed by Bonferroni *post hoc* testing. * $P < 0·01$ between with and without apples. *Z*-average, intensity weighted harmonic mean size measured by dynamic light scattering. –●–, With apple; –▽–, without apple.

although T_{max} was 60 min, regardless of treatment ($P = 0·760$, Table 3).

Postprandial acetaminophen as an indicator of gastric emptying

Acetaminophen concentration increased after ingestion of the meals and slowly decreased towards baseline after 3 h (Fig. 5, $P_{time} < 0·001$). However, treatment did not have an overall effect on plasma acetaminophen concentration ($P_{treatment} = 0·065$, Fig. 5), nor did apples affect acetaminophen iAUC, C_{max} or T_{max} ($P = 0·440$, 0·661 and 0·577, respectively, Table 3).

Discussion

While a few human studies have examined the effects of apples on fasting lipid profiles[29,30,43–45], their influence on PPL, as a risk factor for CVD, is understudied and remains equivocal. Therefore, the present study aimed to evaluate the influence of consuming whole raw Gala apple with a dairy-based OFTT on PPL in otherwise healthy overweight and obese participants. The three small Gala apples (approximately 200 g edible parts) were equivalent to the weight of one large apple to mimic a fruit serving consumed as part of a meal[46]. Postprandial glycaemia and insulinaemia were investigated, as was gastric emptying, to elucidate the mechanisms involved. The main findings were that apple consumption did not influence PPL and that gastric emptying was not implicated.

Study participants' BMI ranged from 25·4 to 48·2 kg/m^2 and with nine and seventeen participants classified as overweight and obese, respectively. The study also included both normolipidaemic (<1·69 mmol/l, *n* 20) and hyperlipidaemic (≥1·69 mmol/l, *n* 6, 2 of whom were obese) individuals. Although the average fasting plasma TAG level was 1·42 (SEM 0·10) mmol/l, this ranged from 0·66 to 2·31 mmol/l. Interestingly, of the nine overweight participants, four were

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Table 4. Fatty acid composition of the oral fat tolerance test (OFTT) meal and chylomicron-rich fraction at fasting and throughout the 6-h postprandial period* (Mean values with their standard errors)

	OFTT meal			0 h		2 h		4 h		6 h		ANCOVA results†	
Fatty acid	Mean	SEM	After meal	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P_{time}	$P_{treatment}$
C14 : 0	12·0	0·1	With apple	1·7	0·1	4·4	0·2	5·6	0·2	5·5	0·2	<0·001	0·429
			Without apple	2·0	0·1	4·3	0·2	5·4	0·2	5·1	0·4		
C16 : 0	36·7	0·1	With apple	24·1	0·4	26·6	0·4	29·2	0·5	30·3	0·4	<0·001	0·815
			Without apple	24·7	0·4	26·9	0·4	28·9	0·4	30·0	0·4		
C16 : 1c9	0·2	0·0	With apple	3·4	0·2	3·0	0·1	2·9	0·1	2·9	0·1	<0·001	0·467
			Without apple	3·6	0·2	3·1	0·1	3·0	0·1	2·9	0·1		
C18 : 0	11·6	0·1	With apple	5·0	0·1	6·3	0·2	7·4	0·2	7·7	0·2	<0·001	0·052
			Without apple	5·4	0·3	6·4	0·2	7·4	0·2	8·1	0·4		
C18 : 1c9	22·1	0·1	With apple	32·6	0·4	29·8	0·4	27·7	0·3	26·9	0·4	<0·001	0·900
			Without apple	31·9	0·5	29·8	0·4	27·8	0·3	27·3	0·4		
C18 : 1c11	2·4	0·0	With apple	2·7	0·1	2·6	0·1	2·5	0·0	2·5	0·1	0·006	0·471
			Without apple	2·6	0·1	2·6	0·1	2·6	0·1	2·6	0·1		
C18 : 2	2·9	0·0	With apple	19·5	0·5	15·9	0·5	13·2	0·4	12·5	0·3	<0·001	0·109
			Without apple	18·3	0·4	15·2	0·4	13·1	0·4	12·5	0·3		

C14 : 0, myristic acid; C16 : 0, palmitic acid; C16 : 1, palmitoleic acid; C18 : 0, stearic acid; C18 : 1c9, oleic acid; C18 : 1c11, vaccenic acid; C18 : 2, linoleic acid.
* Values are expressed as the percentage of total area counts (%) in GC, *n* 3 for the OFTT meal and *n* 26 for postprandial measurements.
† P_{time} and $P_{treatment}$ refer to main effects of time and treatment in ANCOVA after OFTT meal consumption, while $P_{time \times treatment}$ was not reported because it was larger than 0·05 for each fatty acid.

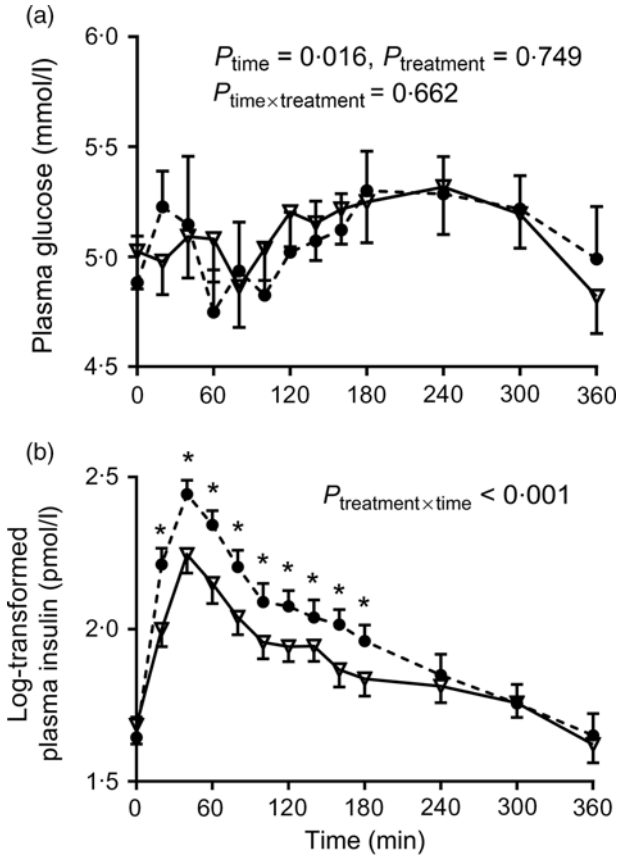

Fig. 4. Postprandial plasma glucose (a) and log-transformed plasma insulin (b) concentrations. (a) Data are means and their standard errors, *n* 25 per treatment (one male participant outlying data removed from database). (b) Data are geometric means and their average transformed standard errors, *n* 26 per treatment. P_{time}, $P_{treatment}$ and $P_{treatment \times time}$ refer to results from repeated-measures ANCOVA with Bonferroni post hoc testing. * $P < 0·05$ between with and without apples. -●-, With apple; -▽-, without apple.

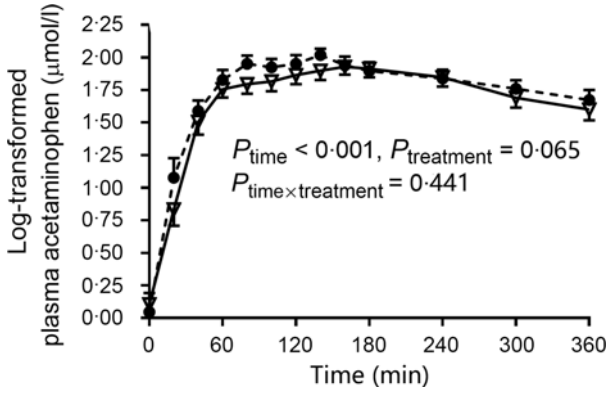

Fig. 5. Log-transformed postprandial plasma acetaminophen concentrations. Data are geometric means and their average transformed standard errors; *n* 26 per treatment. P_{time}, $P_{treatment}$ and $P_{time \times treatment}$ refer to results from repeated-measures ANCOVA with Bonferroni *post hoc* testing. * $P < 0·05$ between with and without apples. -●-, With apple; -▽-, without apple.

hyperlipidaemic and, of the seventeen obese participants, only two were hyperlipidaemic. This may have contributed to the absence of expected correlations between postprandial TAG and BMI that has been reported in other instances[47]. Overall, the observed PPL response (i.e. the extent and length of TAG elevations) was comparable to previous OFTT studies wherein the same 1 g fat/kg body weight was provided to healthy and overweight[48–50] and obese[50] participants.

Apple consumption did not alter postprandial TAG, even though animal studies have shown attenuations when purified pectin and polyphenols were ingested[20,26]. This discrepancy could be due to the relatively lower levels of these bioactives present in the test meal. For example, Leclere *et al.*[20] fed 30 g dried sugar beet pulp fibre containing 4·11–7·32 g soluble fibre (mainly pectin)[51] to pigs weighing approximately 30 kg. In the

present study, participants weighed between 71·6 and 154·0 kg and consumed only 2·17 g pectin. Sugiyama *et al.*[26] observed that apple polyphenol extract at 1000 mg/kg body weight completely inhibited postprandial TAG elevations in mice. This is also much higher than the levels of apple polyphenols consumed in the present study (i.e. 1·49–3·21 mg/kg body weight). In addition, procyanidins are the most active apple polyphenols in terms of lipase inhibition[26]. The content of procyanidins in the Gala apples consumed by participants is unknown and may have been insufficient to alter lipid digestion. Moreover, compared with the OFTT meal alone, the apple meal contained higher and different amounts of digestible carbohydrates, especially fructose and sucrose (Table 1), which can promote hypertriacylglycerolaemia[52–54]. The proposed mechanisms for fructose-induced hypertriacylglycerolaemia include a lower activation of adipose tissue lipoprotein lipase and reduced plasma TAG clearance[53,55], improved *de novo* lipogenesis[55,56] and greater enterocyte chylomicron secretion[57]. Therefore, apple consumption may have led to fructose-induced postprandial hypertriacylglycerolaemia, counteracting any lipid-lowering potential from the pectin and polyphenols present. Sample size may also have precluded observing statistical significance, as the study was underpowered. Although twenty-six participants are similar to the number of participants in studies where treatment differences in plasma TAG were observed[50,58,59], participants' postprandial TAG responses were highly variable in the present study (the mean difference of TAG C_{max} between with and without apples was −0·04 (SEM 1·11) mmol/l), making the effect size very low (=0·04).

The changes observed in CMRF FA composition reflected the composition of the OFTT meal, as expected[7,60]. Specifically, the proportions of SFA (C16 : 0, in particular) increased in the CMRF as the UFA (C18 : 1c9 and C18 : 2) decreased, postprandially. The OFTT had an SFA:UFA ratio of 1·5 (data not shown), and the OFTT consumption significantly increased this ratio in the CMRF (i.e. from approximately 0·5 at fasting to 0·9 and 0·8 at 6 h with and without apples, respectively). The CMRF FA composition did not exactly match the meal, probably because of contributions from FA in the enterocyte storage pools[60]. Research investigating the contribution of dietary fibre to postprandial chylomicron FA composition, in relation to CVD risk, is limited. However, there is demonstrated potential for chylomicron FA composition to influence various metabolic events associated with CVD risk, including the conversion rate from chylomicrons to remnants in rats[61], interactions between chylomicron remnants with isolated rat hepatocytes[62], and chylomicron remnant-like particle uptake by macrophages *in vitro*[63]. Nevertheless, in the present study, apple consumption had no effect on FA CMRF incorporation within the 6-h postprandial period.

A wide range of fasting apoB48 concentrations (1·29–40·74 mg/l) were observed, likely related to the variability in participants' metabolic and health status, including fasting TAG[64]. The levels observed are consistent with Sakai *et al.*[64], which reported fasting apoB48 levels of 5·2 *v.* 6·4–92·4 mg/l in normolipidaemic *v.* hyperlipidaemic populations. Postprandial apoB48 increased from baseline (5–10 mg/l) to similar extents as reported by Sakai *et al.*[64] where participants consumed 30 g fat/m^2 body surface of 35 % cream. Because obesity has been associated with exaggerated postprandial apoB48 elevations after a fat load[6], differences in postprandial apoB48 increases were explored between the obese and overweight participants, but no differences were found ($P = 0·649$, data not shown). Changes in apoB48 point to increase in the number of circulating chylomicron and remnant particles, which were modest in the present study. In contrast, CMRF particle size increased more significantly during the postprandial period, reflecting the loading that occurs as dietary TAG are reassembled[65], and agreeing with previous observations that postprandial increases in chylomicron size are much greater than particle number[65,66]. Postprandial changes in chylomicron size and number are relevant since smaller chylomicrons (and their remnants) in higher numbers tend to increase atherosclerotic risk, due to the ability of these particles to penetrate the arterial intima[7] coupled with their slower clearance from plasma[66]. However, in the present study, apples did not influence chylomicron number or size, hence did not show potential to impact atherosclerotic risk related to postprandial chylomicron metabolism.

Dietary carbohydrates contribute to postprandial glycaemia and insulinaemia and can therefore influence lipid metabolism, including PPL, through complex processes[31]. Participants in the present study generally had fasting glucose and HOMA-IR values consistent with non-elevated risk for diabetes, although oral glucose tolerance tests were not performed. All twenty-six participants had a fasting plasma glucose below 7·0 mmol/l, that is, the cut-off for a diagnosis of diabetes[67]. Three participants did have HOMA-IR values above 2·6, that is, the cut-off for a diagnosis of insulin resistance in a population with normal glucose metabolism[68], although factors that can influence the HOMA-IR cut-off value, including ethnicity, sex and age[69], were not accounted for in the present study. The dairy-based OFTT meal in the present study contained 20·3–21·5 g sugars comprised mainly of lactose. Relative to other dietary sugars, lactose induces a low glycaemic response[70]. This explains the minimal changes observed in postprandial glucose for the OFTT meal alone. Although the 200 g apples provided an additional 20·8 g digestible carbohydrates (i.e. 11·9 g fructose, 5·6 g sucrose and 3·3 g glucose, Table 1), this did not lead to a significant rise in postprandial glucose. This may be because much of apples' available carbohydrate is the disaccharide fructose which induces a lower postprandial plasma glucose rise compared with other common carbohydrates, for example, glucose, dextrose and sucrose[71]. Also, potential rises in plasma glucose owing to the apple carbohydrates could have been offset by contributions from the soluble fibres present, for example, increased gut content viscosity[72,73].

Despite the non-significant rises in postprandial glucose, insulin increased substantially both with and without apples. This may be related to the whey protein in the OFTT meal since it is known to promote insulin release[74]. Postprandial increases in insulin with the OFTT without apples were similar, although slightly delayed, compared with that observed for whole milk containing similar amounts of protein and carbohydrates, but much less fat[75]. The differences are potentially related to the high fat in the present study, which could lead to a slower gastric emptying comparing with lower fat meals[76]. Significant rises in plasma insulin were observed with apples between 20 and

180 min. This is similar to observations by Theytaz *et al.*[77], where fructose consumption led to higher plasma insulin, despite no effects on plasma glucose. Insulin concentration is a recognised modulator of TAG-rich lipoprotein synthesis and secretion[31]. Although there was no correlation between insulin and apoB48 concentration in the present study (r^2 0·018, $P = 0{\cdot}169$, data not shown), the higher peak insulin concentration may partly explain the higher peak apoB48 concentration observed with apple consumption. These results highlight the interactions between lipid and carbohydrate metabolism and the complexity of food matrix effects on postprandial changes, which warrants further investigation.

Gastric emptying controls the rate of digesta delivery to the upper small intestine and is affected by food composition and structure through various mechanisms[78]. For example, as related to glucose, there is a bi-directional relationship since gastric emptying influences peak postprandial glucose concentrations, but the rate of emptying itself is modulated by acute hyperglycaemia after a meal[79]. Pectin, like other soluble dietary fibres, has shown potential to delay gastric emptying in human studies in purified form and at relatively high doses (10–20 g per meal)[15,16,80]. In the present study, the apples contained 2·17 g pectin, which may have been too low to affect gastric emptying. Moreover, the OFTT meal alone was completely liquid and the presence of apples introduced solids, potentially altering gastric emptying dynamics[79]. Meal volumes also differed. The OFTT meal was 500 ml which is comparable with other OFTT studies[81,82]. Total meal size increased substantially with the apples (500 ml OFTT + 200 g apple) and, while similar to other mixed meal studies[11,83], would potentially lead to differences in acetaminophen distribution, emptying and absorption. Also, for comparison with other studies, it is worth noting that the OFTT beverage is an emulsion of milk fat stabilised by dairy protein and therefore susceptible to pepsinolysis and phase separation in the stomach[84]. The effects of whole apples on gastric emptying and lipid digestion may be different based on whether lipids are bulk or dispersed and based on the nature of any emulsifier present.

The present study is the first to examine the effects of consuming whole apples on postprandial responses in humans and has the strengths of reflecting a mixed meal scenario using a crossover study design. Nevertheless, the relatively small sample size and high inter-individual variability are limitations, as is the lack of apple compositional data, in particular the polyphenol profiles, since different polyphenols may influence lipid digestion differently[26]. Given that procyanidins are identified as the main effective polyphenols for lipase inhibition, it would be interesting to investigate the lipid-lowering effects of apple varieties containing higher levels of procyanidins (e.g. Red Delicious and Granny Smith apples[85]). Lastly, although plasma acetaminophen correlates well with liquid emptying, more direct measurements (e.g. scintigraphy) should be considered for a better representation of gastric emptying of meals containing solids[33].

In summary, the present study examined the influence of consuming 200 g Gala apples, with skin, on PPL, glycaemia, insulinaemia, chylomicron size and number, and gastric emptying using a dairy-based OFTT in overweight and obese adults. In this mixed meal scenario, only postprandial insulin was altered by the presence of apples, potentially owing to the relatively low levels of pectin and/or polyphenols and/or interactions between carbohydrate and lipid metabolism from the mixed meal. The most important observation is that Gala apples, as a commonly consumed food, did not attenuate the PPL response induced by a high-fat meal when consumed by overweight and obese adults. Differences in gastric emptying that might have mediated the rate of nutrient absorption were not observed using the indirect acetaminophen method. Future research should explore the amount and type of apples or apple products that could realise any benefits of apple's functional ingredients on metabolism and CVD risk in the postprandial period and with larger numbers of participants. Also, close attention should be paid to interactions between food ingredients, for example, lipids and carbohydrates in mixed meals.

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X. L., D. M. L., L. E. R. and A. J. W. designed the research; X. L., D. M. L., H. R. N., L. E. R. and A. J. W. conducted the research; X. L., D. M. L., H. R. N. and A. J. W. analysed the data; X. L. and A. J. W. performed the statistical analysis; X. L. and A. J. W. wrote the paper; and X. L., D. M. L., L. E. R. and A. J. W. had primary responsibility for the final content. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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