Manipulation of lipid bioaccessibility of almond seeds influences postprandial lipemia in healthy human subjects

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ABSTRACT

Background: Plant cell walls are known to influence the rate and extent of lipid release from plant food tissues during digestion; however, the effect of cell wall structure on postprandial lipemia is unknown.

Objective: The objective was to investigate the effects of lipid release (bioaccessibility) on postprandial lipemia by comparing lipid encapsulated by cell walls with lipid present as free oil.

Design: A randomized crossover trial (n = 20 men) compared the effects of 3 meals containing 54 g fat provided as whole almond seed macroparticles (WA), almond oil and defatted almond flour (AO), or a sunflower oil blend as control (CO) on postprandial changes in oxidative stress (8-isoprostane F2α concentrations), vascular tone (peripheral augmentation index), and plasma triacylglycerol, glucose, and insulin concentrations.

Results: The postprandial increase in plasma triacylglycerol was lower [74% and 58% lower incremental area under curve (iAUC)] after the WA meal than after the AO and CO meals (P < 0.001). Increases in plasma glucose concentrations (0–180 min) were significantly higher after the WA meal (iAUC: 95% CI: 76, 153) than after the AO meal (iAUC: 74; 95% CI: 48, 99) (P < 0.05), but no significant differences from the CO meal were observed (iAUC: 88; 95% CI: 66, 109). The peak reductions in peripheral augmentation index after the WA, AO, and CO meals (−9.5%, −10.1%, and −12.6%, respectively, at 2 h) were not significantly different between meals. Plasma 8-isoprostane F2α and insulin concentrations did not differ significantly between meals.

Conclusions: The bioaccessibility of lipid in almond seeds, which is regulated by the structure and properties of cell walls, plays a primary role in determining postprandial lipemia. Am J Clin Nutr 2008;88:922–9.

INTRODUCTION

An elevated and prolonged postprandial lipemic response is associated with an increased risk of coronary heart disease (1) via effects on lipoprotein remodeling (2), vascular function (3), oxidative stress (4), and thrombotic activity (5). The magnitude and duration of the postprandial response are influenced by physiologic, genetic, and dietary factors that in turn affect rates of gastric emptying, intestinal digestion and absorption, chylothron synthesis, lipolysis, and receptor-mediated removal (6). Previous research into the effects of diet on postprandial lipemia has focused on the quantity, composition, and properties of dietary fat, whereas the role of food structure and properties has received less attention.

There is increasing evidence indicating that plant cell walls (dietary fiber) play a key role in the rate and extent of nutrient release (ie, bioaccessibility) during digestion, because cell walls are largely resistant to degradation in the upper gut and provide a physical barrier to the release of nutrients (7–9). Bioaccessibility is defined as the proportion of nutrients released from a food matrix and therefore potentially available for absorption in the gastrointestinal (GI) tract (10). The processing of plant foods (eg, milling, homogenization, and extrusion cooking), can influence the bioaccessibility of starch, lipids, and other nutrients, mainly as a result of changes in cell wall structure and properties. The importance of starch bioaccessibility in regulating the kinetics of starch digestion and postprandial glycemia is well documented (8, 9, 11–13). However, there is a paucity of information on the effects of lipid bioaccessibility on postprandial lipemia. Moreover, previous studies on dietary fiber and lipemia have focused only on fiber supplements (14, 15) rather than on the physical state of the cell walls.

Fecal excretion and ileostomy studies have shown that many of the cell walls of masticated almond seeds remain intact in the GI tract and inhibit the release of intracellular lipids (7). Also, in vitro digestion experiments have shown that lipid bioaccessibility is dependent on many factors, notably the size of almond particles, which determines the number of almond cells that are ruptured and therefore the amount of intracellular lipid available for the early stages of digestion (7, 16). Because bioaccessibility plays a key role in influencing lipid digestion in almonds, further work is required to investigate the effects of lipid bioaccessibility on postprandial lipemia and associated postprandial events. Recent studies have also shown that the addition of almonds to starch-rich meals decreases postprandial glycemia and insulinemia (17, 18); such effects may be influenced by the bioaccessibility of nutrients (eg, lipids) and phytochemicals present in almond seeds.

In the present study, it was hypothesized that whole almond seeds consumed as macroparticles, in which much of the lipid is encapsulated by cell walls, will produce a lower postprandial lipemic response than the macroparticles in which much of the lipid is released from the cell walls.
lipemic response than will an almond sample comprising separated fractions of oil and defatted seed flour. The almond macroparticles and almond oil and flour samples represent low- and high-bioaccessible lipid meals, respectively. It is also hypothesized that manipulating lipid bioaccessibility will alter the postprandial rise in blood glucose and insulin and oxidative stress and may influence vascular tone.

SUBJECTS AND METHODS

Subjects
Twenty-two male subjects were recruited from among staff and students of King's College London, University of London. The subjects were healthy, and the exclusion criteria included a history of cardiovascular disease, diabetes, body mass index (in kg/m$^2$) <20 or >35, plasma cholesterol >7.8 mmol/L, plasma triacylglycerol >3 mmol/L, plasma glucose <6.0 mmol/L, current use of antihypertensive or lipid-lowering medication, allergy to nuts or seeds, and a self reported intake of > 28 units alcohol/wk (1 unit = 10 mL ethanol). Fasting plasma lipoprotein lipid concentrations, body weight, blood pressure, blood cell count, and liver function were confirmed to be within the prescribed limits before entry into the study. Habitual nutrient intake was assessed from a 3-d food intake diary, and the Microdiet program (Downlee Systems, United Kingdom) was used to estimate nutrients. Subject details are shown in Table 1. Dietary intakes were close to recommended dietary guidelines (19).

Study design
A randomized crossover study design was used. Each subject received 3 experimental meals with ≥1 wk between treatments. On the day preceding each postprandial test, subjects were advised to avoid consuming foods high in fat and were provided with a standardized low-fat dinner (containing <10 g fat) to consume as their evening meal. To control for physical activity levels, subjects were asked to refrain from strenuous exercise, including cycling and sporting activities, and from the use of alcohol and caffeine-containing drinks on the day before and on the day of the test meal. Subjects fasted overnight from 2200, and the following morning a cannula was inserted into the forearm (antecubital vein) of each subject between 0800 and 1000 and fasting venous blood samples were obtained. After a 15-min rest, vascular function measurements (pulse wave analysis; PWA) were conducted, after which the test meal was consumed within 15 min. Additional venous blood samples were obtained at 20, 30, 45, 60, 90, 120, and 150 min and thereafter at 3, 4, 5, 6, 7, and 8 h. Additional PWA measurements were made at 2, 4, 6, and 8 h. After the 3-h blood sample was collected, subjects received a standardized lunch (1.7 MJ) consisting of low-fat yogurt (<1 g fat). Yogurt was previously shown not to influence the postprandial lipemic response (20). The study protocol was reviewed and approved by King’s College Research Ethics Committee (reference no. 06-07/142), and all participants gave their written informed consent.

Formulation of test meals and chemical and physical analyses of lipid and almond seeds
The test meals consisted of 3 muffins (each containing 17 g test fat) and custard (Bird’s Instant Custard; Premier Ambient Products, Lincolnshire, United Kingdom) and were formulated to provide 4.5 MJ, 31 g protein, 116 g carbohydrate (mostly starch), and 54 g fat (50 g fat from control or almond oil or whole almond seeds, the remainder from custard powder (3 g), and trace amounts from other meal ingredients (1 g)). Details of the test meal composition and ingredients of the muffins are shown in Table 2. The fat was provided in the form of whole almond seeds cut into 2–3-mm particles (WA), almond oil plus defatted almond flour (AO), and a sunflower oil blend as control oil (CO). High–oleic acid sunflower oil was mixed with standard sunflower oil at a ratio of 73% to 27%, respectively (both supplied by Archer Daniels Midland Pura Limited, Erith, United Kingdom) to match the control oil to the almond oil fatty acid composition. The sunflower oil blend was used as a control oil, as in previous studies (21), to study the effect of free oil alone compared with free oil (almond oil) plus defatted almond flour (containing nonlipid nutrients and phytochemicals). Whole seeds (kernels) of the almond tree plant (Amygdalus communis L., nonpareil variety), supplied by the Almond Board of California, were selected for this study and were not blanched before use. The muffins were made in 2 batches using a simple recipe (as outlined in Table 2); all the ingredients were weighed into a mixing bowl to which the almond oil or control oil were added and were then homogenized with a commercial blender. The muffins were baked in a standard domestic gas oven at a temperature of 190–200 °C for 20 min and subsequently stored at −20 °C.

The almond samples were prepared to maximize differences in lipid bioaccessibility (7, 16). Thus, macroparticles of whole almond seeds were prepared with a defined particle size range with a food processor (type588; Moulinex Multichef, Alençon, France). The particle size distribution, estimated using laboratory test sieves (Endecotts Ltd, London, United Kingdom), was between 1.7 and 3.4 mm. Preliminary experiments showed that this was the maximum particle size possible to ensure minimal churning without loss in palatability while allowing for minimal lipid release during digestion. Lipid bioaccessibility of these particles was estimated to be 10% (range: 8–12%), compared with 100% for AO and CO samples, which was predicted from

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.8 ± 4.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.0 ± 10.6</td>
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<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.8 ± 3.1</td>
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<tr>
<td>Serum cholesterol (mmol/L)</td>
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</tr>
<tr>
<td>Serum HDL cholesterol (mmol/L)</td>
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<tr>
<td>Serum LDL cholesterol (mmol/L)</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Serum triacylglycerol (mmol/L)</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>Dietary intake$^2$</td>
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<tr>
<td>Energy (MJ)</td>
<td>9.6 ± 3.4</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>15.4 ± 4.3</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>51.5 ± 7.3</td>
</tr>
<tr>
<td>Total fat (% of energy)</td>
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<td>Saturated fat (% of energy)</td>
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<tr>
<td>Monounsaturated fat (% of energy)</td>
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<tr>
<td>Polyunsaturated fat (% of energy)</td>
<td>5.0 ± 2.9</td>
</tr>
</tbody>
</table>

1 All values are ± SD.
2 Analyzed with the Microdiet program (Downlee Systems, Chapel-en-le Frith, United Kingdom).
TABLE 2
Test meal composition of the whole almond seed macroparticles (WA), almond oil and flour (AO), and control oil (CO) meals

<table>
<thead>
<tr>
<th></th>
<th>WA</th>
<th>AO</th>
<th>CO</th>
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<tbody>
<tr>
<td>Muffin²</td>
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<td>—</td>
<td>—</td>
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<tr>
<td>Control oil</td>
<td>—</td>
<td>—</td>
<td>50.0</td>
</tr>
<tr>
<td>Almond oil</td>
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<td>—</td>
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<tr>
<td>Whole almonds</td>
<td>96.5</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Defatted almond flour</td>
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<td>47.0</td>
<td>—</td>
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<td>Plain wheat flour³</td>
<td>37.0</td>
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<td>44.5</td>
</tr>
<tr>
<td>White sugar⁴</td>
<td>37.0</td>
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<td>37.0</td>
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<td>Cornflour⁴</td>
<td>12.0</td>
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<tr>
<td>Egg white powder⁴</td>
<td>6.5</td>
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<td>Baking powder⁴</td>
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<td>2.6</td>
<td>2.6</td>
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<td>Skim milk⁴</td>
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<td>62.0</td>
<td>62.0</td>
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<tr>
<td>Vanilla flavoring⁴</td>
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<td>Custard</td>
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<td>50.0</td>
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<td>Custard powder⁴</td>
<td>37.5</td>
<td>37.5</td>
<td>37.5</td>
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<tr>
<td>Egg white⁴</td>
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<td>14.0</td>
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<tr>
<td>Energy (MJ)</td>
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<tr>
<td>Fiber (g)</td>
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<td>1.6</td>
</tr>
<tr>
<td>Fat (g)⁵</td>
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<td>54</td>
<td>54</td>
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<tr>
<td>16:0 (g)</td>
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<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>18:0 (g)</td>
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<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>18:1n-9 (g)</td>
<td>36.3</td>
<td>36.3</td>
<td>36.3</td>
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<tr>
<td>18:2n-6 (g)</td>
<td>11.2</td>
<td>11.2</td>
<td>11.3</td>
</tr>
</tbody>
</table>

¹ Data from proximate analysis of ingredients as previously described by Ellis et al (7) and determined with the use of British food-composition tables.
² Total for 3 muffins as given per test meal (g).
³ Sainsbury’s own brand; Sainsbury’s Plc, London, United Kingdom.
⁴ Bird’s Instant Custard; Premier Ambient Products, Lincolnshire, United Kingdom.
⁵ 50 g fat was from the test oil or whole almonds; the remainder was from custard powder (3 g) and trace amounts of other meal ingredients (1 g).

our recently developed mathematical model based on the average particle size of almond particles and the diameter of the lipid-rich almond cells (22). The percentage of fractured cells, relative to the total number of cells, was estimated to be ≈15% and 7%, corresponding to the size limits of 1.7 and 3.4 mm, respectively.

Solvent extraction with commercial hexane was used to prepare the almond oil and defatted almond flour from the same batch of almond seeds (POS Pilot Plant Corporation, Saskatoon, Canada). The oil was provided as crude filtered oil with minimal refining (not bleached or deodorized), to ensure that there were no compositional differences between the WA and the AO meals.

After the control and test muffins were baked and frozen, a microstructural analysis of the original almond seeds and the almond seed particles (WA) contained in the muffins was carried out to assess the morphology of the lipid-rich cells of the almond cotyledon and the integrity of the cell walls. All the almond samples were infiltrated with Spurr resin, embedded in molds, and polymerized at 60 °C. Sections (1 μm for light microscopy) were cut with a Reichert Ultracut ultramicrotome (Leica Microsystems Ltd, Milton Keynes, United Kingdom), mounted on glass slides, and stained with 1% (wt:vol) Toluidine Blue. Sections were viewed with a Zeiss Axioskop 2 mot plus microscope (Carl Zeiss, Göttingen, Germany) using image acquisition and analysis software (AxioVision 3.5; Carl Zeiss Vision GmbH). Micrographs showed that the cellular structure of almond seed tissue remained physically intact after being baked and frozen (online supplementary information). Thus, most of the cells showed the presence of intact intracellular lipid encapsulated by unbroken cell walls.

Collection and handling of blood samples
Venous blood samples were collected from a cannula, previously inserted into the forearm vein of each subject, into a syringe and dispensed into evacuated tubes. Blood samples were processed within 15 min of blood collection. Blood for lipid analysis [nonesterified fatty acid (NEFA) and triacylglycerol concentrations and plasma fatty acid composition] was collected at hourly intervals (0–8 h postprandially) into 4-mL EDTA-containing Vacutainers (Becton Dickinson, Oxford, United Kingdom), and plasma was separated by centrifugation at 1500 × g for 15 min at 4 °C.

Biochemical assays
Enzymatic assays were used to analyze plasma glucose, insulin, and triacylglycerol concentrations as described elsewhere (21). Plasma NEFA concentrations were analyzed with an enzymatic colorimetric method assay (WAKO NEFA-HR; WAKO Diagnostics, Neuss, Germany) on an ILab 650 Clinical Chemistry System (ILab, Milan, Italy). Plasma concentrations of oleic and linoleic acid were determined by gas-liquid chromatography with pentadecanoic acid as an internal standard (23) on a BP75 column (25 m × 220 μm × 0.25 μm; SGE Analytical Science, Victoria, Australia) on an Agilent 6890 (Agilent Technologies, Cheshire, United Kingdom).

Vascular function measurements
All vascular measurements were performed while the subjects were supine in a quiet, darkened, and temperature-controlled room (23 °C). Radial PWA was measured after a 5-min rest, in triplicate, 5 min apart. An automatic oscillometric digital blood pressure monitor (OMRON, Tokyo, Japan) was used to measure blood pressure before each pulse wave measurement, according to British Hypertension Society guidelines (25). Applanation tonometry of the radial artery was performed with a sensitive transducer (SyphgomoCor, AtCor Medical, Sydney, Australia)
FIGURE 1. Geometric mean plasma triacylglycerol (TAG) concentrations and 95% CIs in healthy men (n = 20) after test meals containing 50 g test fat from whole almond seed macroparticles (A), almond oil and flour (C), or sunflower oil (control, ■). Deviations from fasting values were analyzed by ANOVA, with the 3 diets and time (0–8 h) as factors: diet effect (P = 0.002), time effect (P < 0.001), and diet × time interaction (P < 0.001). Inset: incremental area under the curve (0–8 h) values are inset and presented as geometric means and 95% CIs (n = 20) for whole almond seed macroparticles (■), almond oil and flour (□), and control oil (□). Significantly different from whole almond seed macroparticles, P < 0.01 (Bonferroni multiple comparison test).

FIGURE 2. Mean (± SEM) plasma oleic acid and linoleic acid concentrations in healthy men (n = 20) after test meals containing 50 g test fat from whole almond seed macroparticles (A), almond oil and flour (C), or sunflower oil (control, ■). Deviations from fasting values were analyzed by ANOVA, with the 3 diets and time (0–8 h) as factors. Oleic acid: time effect (P < 0.001), diet effect (P < 0.001), and diet × time interaction (P < 0.001). Mean incremental area under the curve (iAUC; 0–8 h) values and 95% CIs (n = 20) were as follows: whole almond seed macroparticles (3.4; 2.0, 4.9)1,2, almond oil or flour (12.8; 9.1, 16.5)2, and control oil (8.6; 5.3, 14.1).1 Significantly different from whole almond seed macroparticles, P < 0.01 (Bonferroni multiple comparison test). Linoleic acid: time effect (P < 0.001), diet effect (P < 0.001), and diet × time interaction (P < 0.001). iAUC (0–8 h) values and 95% CIs (n = 20) were as follows: whole almond seed macroparticles (1.5; 0.7, 2.3)1,2, almond oil or flour (4.7; 3.3, 6.2)2, and control oil (3.2; 2.3, 4.0).1 Significantly different from whole almond seed macroparticles, P < 0.05 (Bonferroni multiple comparison test).

RESULTS

Postprandial plasma lipids

A total of 20 subjects completed the study. Plasma triacylglycerol concentrations (Figure 1) were significantly lower after the WA meal than after the AO and CO meals (diet effect: P = 0.002; diet × time interaction: P < 0.001). The iAUC values for plasma triacylglycerol were 74% and 58% lower after the WA meal than after the AO and CO meals, respectively (P < 0.01 for both) and 34% lower after the CO meal than after the AO meal (NS). Concentrations reached peak values 5 h after all test meals, increasing from mean fasting values (% of 40.6 (95% CI: 24.9, 58.4), 115.3 (95% CI: 84.4, 151.5), and 103.0 (95% CI: 77.1, 132.7) after the WA, AO, and CO meals, respectively. Plasma fatty acid concentrations of the major fatty acid constituents of the test meals (oleic and linoleic acids) had a similar pattern of response to the plasma triacylglycerol concentrations (Figure 2). The iAUC for oleic and linoleic acids were significantly lower after the WA meal than after the AO and CO meals (P < 0.01 for oleic acid and P < 0.05 for linoleic acid) and significantly lower after the CO meal than after the AO meal (P < 0.01 for oleic acid and P < 0.05 for linoleic acid). NEFA concentrations elicited a different pattern of response after the test meals (diet × time interaction; P < 0.001), but there was no overall effect of diet (Figure 3).

Postprandial plasma glucose and insulin concentrations

The changes in plasma glucose and insulin concentrations, which had a different pattern of response after the test meals (diet

Statistical analysis

Data that were not normally distributed were log transformed before analysis. Incremental area under the curves (iAUCs) were calculated with GraphPad Prism (version 3.0; GraphPad, San Diego, CA) using the trapezoid rule. Statistical analysis of the data was carried with repeated-measures analysis of variance (ANOVA), with diet and time as within-subject factors, using SPSS PC version 10 (SPSS Inc, Chicago, IL). iAUC values were analyzed by ANOVA, and Bonferroni post hoc analysis was carried out using GraphPad Prism when significant differences between diets were found. Results are presented as means ± SDs, geometric means with 95% CIs, or mean changes from baseline (with 95% CIs).
× time interaction: \( P < 0.001 \) for both) but had no overall effect of diet, are shown in Figure 4. However, the iAUC (0–180 min) for plasma glucose was significantly lower after the AO meal than after the WA meal (\( P < 0.05 \)), but was not different from the CO meal. Moreover, the mean iAUC for the most dynamic part of the glucose curve (0–60 min) was significantly lower after the AO meal (40.3; 95% CI: 29.3, 51.3) than after both the WA (70.7; 95% CI: 65.2, 76.0) and CO (63.7; 95% CI: 50.0, 77.4) meals (\( P < 0.01 \) for both). No differences in iAUC plasma insulin values were observed between the diets.

Vascular function and oxidative stress

Postprandial PAIx is shown in Figure 5. PAIx reduced from baseline values after all 3 test meals, reaching mean peak reductions 2 h after the WA (−9.5%; 95% CI: −12.8, −6.1), AO (−10.1%; 95% CI: −13.3, −6.8), and CO (−12.6%; 95% CI: −15.5, −9.8) meals (time effect: \( P < 0.001 \), but were not significantly different between meals. There was a significant time effect for postprandial peripheral systolic blood pressure, diastolic blood pressure, and heart rate after the 3 test meals (\( P < 0.01 \) for all), but no significant differences between meals (data not shown). 8-Isoprostanate-F2α concentrations did not change significantly from fasting values 4 or 8 h postprandially and were not different between the test meals (data not shown).
LIPID BIOACCESSIBILITY AND POSTPRANDIAL LIPEMIA

DISCUSSION

The primary aim of this study was to investigate the effect of manipulating lipid bioaccessibility on postprandial lipemia. Unlike previous studies on postprandial lipemia, the test meals were designed to have an identical composition, but with physical structures that had been manipulated to maximize differences in lipid bioaccessibility. This was achieved by preparing test meals containing structurally intact almond seed (with encapsulated lipid) or extracted almond oil plus defatted almond seed flour, thus producing meals with a predicted low- and high-lipid bioaccessibility, respectively.

As predicted, the low-lipid-bioaccessibility meal (WA) resulted in a significantly attenuated lipemic response compared with the high-lipid-bioaccessibility meals (AO and CO). The substantially lower iAUC value for triacylglycerol after the WA meal (ie, a 74% reduction relative to AO; see inset in Figure 1) was consistent with the predicted low level of lipid release for WA, which was estimated to be about 8–12% (22). We attributed this effect to the structurally intact cell walls of almonds, which act as a physical barrier and thereby encapsulate the intracellular lipid and hinder the lipid release during digestion. Indeed, direct evidence for a cell wall barrier mechanism comes from in vitro digestion experiments of almond lipid (16) and from a human study showing that the ingestion of almond seeds increases the fecal excretion of encapsulated lipid (7).

However, the postprandial phase is highly dynamic, and plasma triacylglycerol measurements do not provide an absolute measure of the extent of lipid digestion and absorption, but instead are an integrated measurement of the rate of lipolysis, absorption, chylomicron remnant clearance and endogenous triacylglycerol production. Indeed, in a similar recent study, Burton-Freeman et al (26) reported a delayed peak in plasma triacylglycerol concentrations (indicating differences in the rates of absorption and/or clearance) after a meal containing masticated almond seeds (300 min) compared with a meal containing almond oil (180 min), but no difference was observed in the magnitude of lipemia (indicating a similar extent of lipid absorption). In the current study however, plasma triacylglycerol concentrations peaked at the same time (300 min) after all 3 meals, but to a different extent. One likely explanation for the apparent lack of effect of lipid availability on the degree of postprandial lipemia reported by Burton-Freeman et al (26) is the relatively small differences in the amount and bioaccessibility of lipid in the test meals. Thus, only 17 g lipid was derived from almond seeds, out of a total of 29 g lipid in the meal (26). Additionally, using published values for the particle size distribution of masticated almond seeds (between <0.4 and 2 mm) (27) and our predictive model (22), we estimated that not more than about 9 g of the 17 g of lipid was in the encapsulated form, which is unlikely to result in detectable differences in lipemia. In contrast, in the present study, the WA and AO test meals contained 50 g lipid derived from almond seeds, with large differences in predicted bioaccessibility.

Because of the previously reported effects of lipid on postprandial glycemia and lipid release in the upper GI tract on gastric emptying and the release of gut hormones (28), the role of lipid bioaccessibility on postprandial glycemic and insulineic responses was determined. Previously, Josse et al (17) reported that the addition of almond seeds to a wheat-bread meal resulted in a dose-response reduction in the glycemic response, consistent with the findings of Jenkins et al (18), who reported that the addition of almond seeds to a similar meal reduced the postprandial rise in blood glucose and insulin concentrations. The dose-response effect shown by Josse et al (17) may be a consequence of the different amounts of lipid (from the almond seeds) influencing gastric emptying rates and gut hormone responses. However, these authors also suggested that other components of almond seeds, in addition to fat, may be responsible for the attenuated response, including protein, phytic acid, and phenolic compounds (29–31). In the current study, the overall glycemic effect (iAUC: 0–180 min) was significantly higher after the WA meal than after the AO meal, but not when compared with the CO meal, whereas the 0–60-min glucose iAUC (the most dynamic part of the glycemic response) was significantly higher after both the CO and WA meals than after the AO meal. This may be related in part to the different postprandial lipemic response after the test meals and/or to an enhanced gut hormone release and delayed gastric emptying induced by high-bioaccessibility lipid. However, the pattern of the insulin response after the test meals (a greater early peak in insulin after the WA meal than after the AO and CO meals) does not explain these differences. One other possible explanation for the additional glucose-lowering effect of the AO meal relative to the CO meal, is the likely higher bioaccessibility of nonlipid components in almond flour (16), including phenolic compounds, which are derived mainly from the almond seed coat (testa) (32). Certain phenolic compounds are reported to exert a blood glucose-lowering effect through a variety of mechanisms, possibly through the inhibition of amylysis and glucose absorption via sodium-glucose cotransporter-1 and/or enhanced insulin secretion (30, 31, 33).

Plasma NEFA concentrations were measured in the current study because of the influence of postprandial lipemia, glycemia, and insulinaemia on NEFA concentrations. Acute changes in insulin reduce plasma triacylglycerol and NEFA concentrations; therefore, the greater reduction in NEFAs after the WA meal than after the AO and CO meals is consistent with the pattern of response observed for plasma insulin.

A secondary aim of this study was to investigate the influence of lipid bioaccessibility on postprandial changes in oxidative stress. It was hypothesized that attenuated postprandial lipemia after the WA meal would result in attenuation of oxidative stress compared with the corresponding responses to the AO and CO meals; however, there were no postprandial changes in oxidative stress from fasting values and no significant differences between meals. It is plausible that this may have been due in part to the high concentration of antioxidant compounds, such as vitamin E, found in almond seeds and in the CO. Indeed, studies have shown that coadministration of antioxidants with a high-fat meal prevents the postprandial increase in oxidative stress (34–36). Studies of lipid bioaccessibility in whole almond seeds during in vitro digestion and also in ileostomy patients found that vitamin E release correlated with the release of lipid (16). Therefore, the expected postprandial increase in oxidative stress may have been prevented via different mechanisms, depending on the test meals; by the higher antioxidant bioavailability from the AO and CO meals; and by the lower postprandial lipemia after the WA meal.

After all 3 meals, there was a significant reduction in PAIx, which is a measure of peripheral arterial tone and reflects...
vasodilatation caused by endothelial-dependent and endothelial-independent mechanisms. Previous studies of the effects of high-fat and high-carbohydrate meals have also reported postprandial peripheral vasodilatation (37–39). It has been proposed that this may have been due to increased plasma insulin concentrations (40), which may induce systemic vasodilatation via nonendothelial-dependent mechanisms. Therefore, the similar reductions in PAI-1 observed after all 3 meals, despite differences in lipemia and glycemia (which influence endothelial-dependent mechanisms via reductions in nitric oxide bioavailability), may be explained by the similar overall increases in postprandial insulin concentrations.

In conclusion, the consumption of lipid in the form of whole almond seeds has a beneficial effect on postprandial lipemia compared with the equivalent free lipid. Although previous studies have shown that food structure is an important influence on postprandial metabolism, few studies have linked this effect directly to the integrity of cell walls and the process of bioaccessibility. We have shown that the degree of lipid encapsulation in almond seeds, which is determined by the structure and properties of the cell walls, plays a crucial role in determining postprandial lipemia.

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REFERENCES