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Rapid Communication

Impact of cell wall encapsulation of almonds on *in vitro* duodenal lipolysis

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ABSTRACT

Although almonds have a high lipid content, their consumption is associated with reduced risk of cardiovascular disease. One explanation for this paradox could be limited bioaccessibility of almond lipids due to the cell wall matrix acting as a physical barrier to digestion in the upper gastrointestinal tract. We aimed to measure the rate and extent of lipolysis in an *in vitro* duodenum digestion model, using raw and roasted almond materials with potentially different degrees of bioaccessibility. The results revealed that a decrease in particle size led to an increased rate and extent of lipolysis. Particle size had a crucial impact on lipid bioaccessibility, since it is an indicator of the proportion of ruptured cells in the almond tissue. Separated almond cells with intact cell walls showed the lowest levels of digestibility. This study underlines the importance of the cell wall for modulating lipid uptake and hence the positive health benefits underlying almond consumption.

1. Introduction

Plant foods, such as lipid-rich almond seeds are complex matrices with structures ranging in scale, from the cm dimensions of plant tissue to the nm scale of nutrient molecules inside plant cells. In order to be digested in the human stomach and small intestine, intracellular lipids have to be released from the tissue and emulsified to increase susceptibility of the lipid to lipase action. Previously, it was assumed, by many researchers, that most of the lipid and other macronutrients are released during mastication and available for digestion and absorption in the upper gastrointestinal (GI) tract (Bauer, Jakob, & Mosenthin, 2005). However, it has been known for some time that the digestion of nutrients from various edible plants can vary substantially and that food structure and properties are important factors in explaining this variation (Parada & Aguilera, 2007). For instance, the cell walls (i.e. the main source of dietary fibre) of almonds and other plant foods can behave as physical barriers that hinder the bioaccessibility and hence digestion of entrapped lipid (Ellis et al., 2004). Indeed the role of cell walls, in regulating the bioaccessibility (release) of lipid and other nutrients in edible plants, has received considerable attention (Ellis et al., 2004; Tydeman et al., 2010; Grundy et al., 2015). Bioaccessibility is defined, in this paper, as the proportion of a nutrient "released" from a complex food matrix and, therefore, potentially available for digestion and/or absorption in the GI tract.

The composition of almond seeds varies according to a number of factors, including the variety and the harvest; but, typically, the seeds contain approximately 50% of lipids and 12% of dietary fibre, which is mainly derived from cell wall polysaccharides (Yada, Lapsley, & Huang, 2011). The lipid components of the seeds are mainly located in intracellular oil-bodies in the form of triacylglycerol (TAG) (Ellis et al., 2004), the predominant fatty acids of which are oleic, linoleic and palmitic. The oil-bodies have an average diameter of 2–3 μm, approximately, and are surrounded by a single layer of phospholipids in which proteins, mainly oleosins, are embedded (Beisson, Ferte, Voultoury, & Arondel, 2001).

Recent studies have provided evidence to show why a cell wall barrier mechanism impairs the bioaccessibility and extent of digestion of lipid in almonds, despite their status as a high energy food (Novotny, Gebauer, & Baer, 2012; Grassby et al., 2014; Mandalari et al., 2014; Grundy et al., 2015). The presence of intact cell walls from almond tissue during digestion also has a significant influence on postprandial lipaemia, as shown by Berry et al. (2008). They reported that muffins made with large almond particles,

Abbreviations: DAG, diacylglycerol; FFA, free fatty acids; GI, gastrointestinal; L, linoleic acid; MAG, monoacylglycerol; NaGDC, sodium glycodeoxycholate; NaTC, sodium taurocholate hydrate; O, oleic acid; P, palmitic acid; SEM, standard error of the mean; TAG, triacylglycerol.

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comprising mainly intact cells with encapsulated lipid, elicited a lower lipaemic response in healthy human subjects than did the muffins containing defatted almond flour and free almond oil. Furthermore, the results of our recent mastication study showed that 35–40% of the almond bolus was composed of particles with a size superior to ~500 μm (Grundy et al., 2015), suggesting that the particles reaching the stomach probably contain a significant proportion of intact cells with encapsulated lipid. Also, negligible changes in structure and particle size seem to occur during *in vitro* gastric digestion (Mandalari et al., 2014). Thus we concluded that lipid present on the surface of the almond particles is available for hydrolysis by lipase, whereas the intracellular lipid located in intact cells is largely unavailable and remains undigested in the early stages of digestion (Mandalari et al., 2008, 2014; Grundy et al., 2015).

The purpose of the present study was to determine the effect of varying the proportion of ruptured/intact cells on the rate and extent of *in vitro* lipid digestion (expressed as the amount of free fatty acids released) in raw and roasted almond materials. The samples were manipulated such that they exhibited marked differences in lipid bioaccessibility, and included ground and chewed particles of almond and also intact almond cells. To address this main objective, two methods were employed to measure the free fatty acids release: the pH-stat titrimetric method and gas chromatography (GC) analysis. As far as we are aware, however, this is the first time that the pH-stat method has been used to study the effects of lipid bioaccessibility on lipolysis. This study focussed on simulating the digestion in the duodenal compartment only, because the majority of lipid hydrolysis is considered to take place in the duodenum (Bauer et al., 2005).

2. Materials and methods

2.1. Materials

Natural raw and roasted almonds (*Amygdalus communis* L.; variety Nonpareil) were produced by Hughson Nut and supplied by the Almond Board of California. The roasted almonds were produced by using a standardised method of hot air (dry) roasting (150 °C for 15 min). Powdered beta-lactoglobulin (β-Lg) was donated by Davisco Foods International (JE 002-8-415, Le Sueur, USA). Almond oil, glyceryl tributyrate (99%), glyceryl trioleate (65%), sodium dihydrogen phosphate (99%), disodium hydrogen phosphate (99%), trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA, 98.5%), sodium metabisulphite (⩾99%), sodium chloride (99.5%), calcium chloride (93%), sodium glycodeoxycholate (NaGDC, ⩾97% TLC) and pancreatin from porcine pancreas (L3126, activity 100–400 units/mg protein, where 1 unit corresponds to 1 micro-equivalent of fatty acid released from olive oil in 1 h at pH 7.7, 37 °C) were purchased from Sigma–Aldrich Chemical Co. (Poole, UK). The oil of roasted almond was obtained from Huilerie Croix Verte (Neuillé, France) and sodium taurocholate hydrate (NaTC, ⩾97% TLC) was obtained from Alpha Aesar (Ward Hill, USA). Internal standards for gas chromatography analysis, C15:0 (pentadecanoic acid, monopentadecanoin, 1,3-dipentadecanoin, tripentadecanoin), were purchased from Nu-Chek- Prep, Inc (Elysian, USA).

2.2. Emulsion preparation and characterisation

Almond oil emulsion was included as a reference sample with a high lipid bioaccessibility (100%). β-Lg solution was prepared by dissolving 1 wt% of powdered β-Lg in 10 mM phosphate buffer (pH 7.0 at 37 °C) and stirring for at least 2 h. Emulsions were made from either synthetic lipids commonly used to determine lipase activity, namely, tributyrin and triolein, or almond oil. Raw and roasted almond oils contained approximately 64.1% and 63.1% of oleic acid (O), 26.1% and 25.9% of linoleic acid (L), and 6.8% and 7.2% of palmitic acid (P), respectively (analysis performed by gas liquid chromatography as described below). The emulsions were obtained by pre-emulsifying 1.6 wt% of oil in β-Lg solution using a homogeniser (Ultra-Turrax T25, IKA® Werke, from Fisher Scientific Ltd.) for 1 min at 1100 rpm. The pre-emulsion was then sonicated with an ultrasonic processor (Sonics & Materials Inc, Newtown, USA) at 70% amplitude for 2 min. The lipolysis of unemulsified oils, for raw and roasted almond samples, as shown in Section 3, was performed by dispersing the oils directly into the reaction vessel without preliminary preparation.

The droplet size distributions of the emulsions were measured with a laser light scattering instrument (Beckman Coulter LS13320®, Beckman Coulter Ltd., High Wycombe, UK). Water was used as a dispersant (refractive index of 1.330), and the absorbance value of the oil droplets was 0.001. Almond oil has a refractive index of 1.471, tributyrin 1.435 and triolein 1.470, as measured using a refractometer (Rhino Brix90 Handheld Refractometer, Reichert, Inc., New York, USA). The particle size measurements were reported as the average volume diameter: $d_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of droplets of diameter d_i. Values are presented as the means ± SEM of at least three replicates.

2.3. Preparation of almond samples

Almond cells were separated by soaking 2–3 mm almond particles for 4 weeks, with rotation, in a solution containing a chelating agent (50 mM CDTA) and a preservative (5 mM $Na_2S_2O_5$) at pH 7.0 (Mandalari et al., 2014). The samples were briefly rinsed and then mashed using a mortar and pestle to a paste consistency. The almond pieces were loaded onto a sieve of 53 μm and a 20 μm nylon mesh, as well as a sieve base to collect the liquid. After elimination of most of the water, the material present on the nylon mesh was then transferred into a dialysis membrane (Float-A-Lyzer G2 10 ml, 3.5–5 Kd, Sigma). The membrane was placed in phosphate buffer (10 mM, pH 7.0) for about 4 h; the operation was repeated 4 times as recommended by the manufacturer. The dialysis permitted the removal of CDTA from the separated cells, which is important since CDTA is known to inhibit lipase activity (Weaber, Freedman, & Eudy, 1971).

Almond particles of four different size ranges (1000–2000, 500–1000, 250–500, and <250 μm) were obtained by grinding raw and roasted almonds in a blender (E825bk, Lloytron PLC, Leigh, UK) before sieving and collecting the particles from sieves of 1000, 500 and 250 μm aperture, as well as a sieve base (size < 250 μm).

Masticated samples (triplicate boluses) were obtained, following a protocol described in detail elsewhere (Grundy et al., 2015). This study was approved by the Research Ethic Committee of the North London's National Research Ethics Service (Reference No. 10/H0717/096) and registered at isrctn.org as ISRCTN58438021. Predicted values of lipid (mainly TAG) bioaccessibility of the almond samples were obtained from our theoretical model, using average particle size data (Grassby et al., 2014; Grundy et al., 2015).

2.4. In vitro duodenal digestion (pH-stat)

The pH-stat method is a rapid and convenient tool to study lipolysis occurring in the duodenal compartment and on synthetic lipids (e.g. tributyrin and triolein) and olive oil (Beisson, Tiss, Riviere, & Verger, 2000). This method has been widely used and previous investigations have not been restricted to pancreatic