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Salivary concentration of *N*-acylethanolamines upon food mastication and after meal consumption: Influence of food dietary fiber

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ABSTRACT

The primary objective of this study was to evaluate the impact of the amount and type of food dietary fiber on salivary concentrations of *N*-acylethanolamines (NAEs) and glucose upon food mastication and in the post-prandial phase.

Three types of biscuits enriched with 3% barley β-glucan (βGB) or whole-wheat bran (WWBB) or without dietary fiber (control, CB) were developed. A crossover randomized human study was carried out by collecting saliva samples from eighteen healthy and fasting participants in a resting condition, upon mastication of parafilm and one of the three biscuits. Subsequently, the amount of biscuits consumed in an *ad-libitum* breakfast was measured and post-prandial saliva samples, blood glucose, appetite, and food liking were collected over the following 2 h.

Salivary concentration of oleoylethanolamide (OEA) and linoleoylethanolamide (LEA) significantly increased during all biscuits mastication compared to food-free conditions, with OEA increasing more (~138 folds) than LEA (~7 folds). Subjects consumed always 75 g of biscuits at breakfast. Salivary OEA peaked at 15 min and returned to baseline concentration at 60 min after consumption of all types of biscuits whereas LEA peaked only after WWBB. Fifteen minutes after βGB consumption all NAE levels were significantly lower than those after WWBB. No difference of biscuit type on post prandial blood glucose was recorded.

Results demonstrated that NAEs were released in saliva during biscuit mastication, independently from dietary fiber composition. The type of dietary fiber could influence the persistence of NAEs in saliva over 30 min after consumption. Future studies will clarify the mechanisms behind these findings and the role of salivary NAEs in food liking and appetite cues after food consumption.

1. Introduction

Satiation is the satisfaction of appetite developing during eating and eventually results in the termination of eating (Slavin & Green, 2007; Suzuki, Jayasena, & Bloom, 2012). Satiation is influenced by a series of fine physiological factors comprised in the cephalic phase response to eating and includes the action of homeostatic and tonic signals from the gastro-intestinal tract and adipose tissue as well as the reward signals (Hansen, 2014).

Food composition and structure can modulate satiation by influencing chewing time and individual hedonic value of the food (Blundell & Halford, 1994; De Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004). Among food constituents, fats and sugars are positively associated with food palatability and overeating (D'Addario et al., 2014; Ifland et al., 2009) whereas dietary fibers can increase satiation through their bulking and textural properties thus possibly beneficing the control of energy balance (Howarth, Saltzman, & Roberts, 2001; Slavin & Green, 2007). This is well documented in short-term studies where reduced appetite feelings and energy intake at the meal containing dietary fiber and to that following dietary fiber consumption were associated with the amount of fiber consumed and, sometimes, to its viscosity (Slavin & Green, 2007).

However, the reduced palatability of dietary fiber rich foods is often an issue for long-term consumption of these products thus failing the possibility to work for weight management (Alfieri, Pomerleau, Grace, & Anderson, 1995; Hess, Birkett, Thomas, & Slavin, 2011; Howarth et al., 2001). A better understanding of the physiological factors underpinning sensory mechanisms may help to develop new foods being both satiating and palatable.

Abbreviations: βGB, β-glucan biscuits; WWBB, whole-wheat bran biscuits; CB, control biscuits; NAEs, *N*-acylethanolamines; OEA, oleoylethanolamide; LEA, linoleoylethanolamide; PEA, palmitoylethanolamide; ECs, endocannabinoids; 2-AG, 2-acylglycerol; AEA, arachidonylethanolamide; TFEQ, Three Factor Eating Questionnaire; VAS, visual analogue scales; HPLC, high performance liquid chromatography; LC/MS/MS, liquid chromatography tandem mass spectrometry; SEM, standard error of means; AUC, area under the curve; ANOVA, analysis of variance; NAPEs, *N*-acylphosphatidylethanolamines; NAPE-PLD, *N*-acylphosphatidylethanolamine-phospholipase D.

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In this context we have recently demonstrated that tasting of a food (before swallowing) influences plasma levels of the endocannabinoids (ECs) - 2-acylglycerol (2-AG) and arachidonylethanolamide (AEA) -, of the congeners NAEs - OEA, linoleoylethanolamide (LEA) and palmitoylethanolamide (PEA) – and of gut peptides in a manner that is dependent from the individual liking of the tasted food (Mennella, Ferracane, Zucco, Fogliano, & Vitaglione, 2015). This finding was in line with the well-known role of gustatory system on the cephalic phase of eating and suggested that some mediators could act during mastication and elicit the plasma response.

Post-prandial variation of plasma ECs and NAEs were also reported in the literature with different effect on appetite: ECs being mainly associated with an increase and NAEs with a decrease of appetite (Hansen, 2014; Nielsen, Petersen, Astrup, & Hansen, 2004). In addition, intestinal levels of NAEs and AEA were correlated with the levels of the constituting fatty acids (Hansen, 2014).

Matias et al. (Matias et al., 2012) demonstrated that ECs and NAEs are present in human saliva at a concentration dependent from people nutritional status: higher is individual body mass index, higher is the salivary ECs and NAEs level. Moreover they showed no variation of salivary ECs and NAEs concentration 1 h after meal consumption.

In this study the hypothesis that NAEs could be formed in the mouth during food mastication and might influence satiation upon eating biscuits with different content and types of dietary fiber was tested. Moreover, the appetite sensations and liking of the different biscuits over 2 h after food consumption was monitored together with salivary NAEs and blood glucose.

To this purpose, biscuits enriched with 3% gel-forming barley β-glucan (βGB), 3% insoluble whole wheat bran (WWBB) and without dietary fiber (CB) were developed and a crossover randomized design protocol was performed in healthy normal weight subjects.

2. Materials and methods

2.1. Foods

Three types of biscuits containing 3% barley β-glucan (βGB), 3% whole wheat bran (WWBB), or without dietary fiber (CB) were developed using a traditional recipe for biscuits. All the biscuits were prepared with the following ingredients purchased from local supermarket: flour (Divella, Bari, Italy), sugar (Eridania, Bologna, Italy), margarine (Vallé, Milan, Italy), yeast (PaneAngeli, Brescia, Italy). To produce βGB, 3% wheat flour were replaced by a barley β-glucan concentrate (Glucagel™, containing >77.5% dietary fiber) purchased from DKSH (Miribel Cedex, France); whereas for WWBB, a whole-wheat bran concentrate (VITACEL, containing 97% dietary fiber) purchased from ITALI (Reggio Emilia, Italy) was used.

The dough was prepared and after layering, circular biscuits with a diameter of 3.5 cm were formed and baked at 190 °C for 15 min.

2.2. Subjects selection

The recruitment was performed among the students of the Department of Agricultural Sciences of University of Naples, who were interviewed about their medical status, subjective eating habits and food preferences (100 recipes, scores from 1 to 9). The selected subjects were healthy, they were not undergoing any medication or drug therapy, they usually had breakfast, they were not on a restrictive diet and had a normal eating behaviour as assessed by the Three Factor Eating Questionnaire (TFEQ) (Stunkard & Messick, 1985).

Eligible subjects signed an informed written consent before entering this study. They were advised not to vary their physical activity during all the period of the study, always avoiding it the day before the experimental days.

2.3. Study design

The study design and protocol were approved by the Ethics Committee of University of Naples.

The protocol had a crossover, single blind, randomized design. It was characterized by three treatments per each subject that were conducted on separate days with a 1-week washout period from each other (Fig. 1). Each subject participated in three tests and to a training session which was performed two days before the first test and was finalized to train subjects on detailed procedures to collect saliva samples, to rate their appetite feelings and food liking on visual analogue scales (VAS) questionnaires and to set chewing time and rate for the mastication protocol. The subjects were instructed to consume a standardized dinner in the evening before the experimental days within the 22:00 h. On the experimental days fasting subjects reached the nutritional laboratory of the Department of Agricultural sciences at 08:30 h and after 10 min of rest, baseline blood glucose was measured and they were asked to collect baseline non-stimulated and mechanically stimulated (by parafilm and by food) saliva samples. Immediately after, participants were offered a breakfast comprising 150 g of the same type of biscuit they had just masticated and were asked to eat biscuits until they felt satisfied within 15 min. A glass of water (125 mL) was also offered. The remaining biscuits in subject plates were weighted and energy intake consumed was calculated. After breakfast and 15, 30, 60 and 120 min after breakfast, subjects were asked to collect saliva for 5 min, and to rate appetite feelings and actual liking of the biscuit on VAS questionnaires. Blood glucose at the same time points was also measured.

2.4. Saliva sample collection and preparation for analysis

Resting drooling was used to collect non-stimulated saliva from the oral cavity. Participants were asked to sit comfortably in an upright position, having their heads down slightly to pool saliva in the mouth and letting saliva fall into a pre-labeled sterile container for 5 min.

To collect mechanically stimulated saliva samples, the participants were asked to chew onto a piece of inert and tasteless paraffin film (0.29 g; PARAFILM purchased by Sigma-Aldrich, St Louis, USA) for 160 s at a speed of one mastication per second (every 40s saliva were collected as performed with real biscuits, rhythm was given by a metronome) and then expectorate only the saliva into a pre-labeled sterile container.

Once collected both types of sample were immediately placed on ice to minimize degradation of components until further processing. Saliva samples were aliquoted in pre-labeled Eppendorf tubes (2 mL) and frozen (−40 °C) for storage until analysis. Saliva was separated from the bolus immediately after collection by centrifuging samples at 4000 rpm/min per 10 min at 4 °C. Then the supernatant saliva was collected and treated as above.

2.5. Salivary N-acylethanolamines measurement

NAEs (OEA, LEA, and PEA) were simultaneously quantified in saliva samples prepared as aforementioned by liquid chromatography tandem mass spectrometry (LC/MS/MS). Extractions were performed using the solid-phase method. Saliva samples (1 mL) were centrifuged at 16,000 ×*g* for 5 min at 4 °C. The supernatants were collected and 1 mL were separated in another tube and spiked with 200 μg/mL of AEA-d8 internal standards. Oasis HLB 1 cm^3, 30 mg cartridges (Waters) were preconditioned using 1 mL methanol and 1 mL H_2O under a vacuum manifold. Samples were introduced onto the cartridges and drawn under gentle vacuum at a flow rate of approximately 1 mL/min. The cartridges were washed with 1 mL 40% aqueous methanol and NAEs were eluted in 1 mL acetonitrile. The eluents were dried under nitrogen stream before reconstitution in acetonitrile/water (50:50 v/v) (100 μL) for HPLC/MS/MS analysis. To estimate the extraction efficiency, peak