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Testing the Food Experience in Healthy Human Volunteers: a Proof-of-Concept Study

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ABSTRACT

Background & Aims: For a healthy food to be introduced to the consumer's diet, it has to be attractive, yet testing for food acceptance and the sensory postprandial responses is still not standardized. The main objective of this study was to demonstrate that healthier foods can be obtained without impact on the responses to ingestion.
Methods: A randomized, cross-over, double-blind, pilot study in non-obese, healthy men (n=8) comparing the responses to a standard sausage rich in animal fat (mortadella) versus a modified product based on a plant-derived fat analogue and an aroma. Palatability and postprandial sensations were measured on 10 cm scales and brain activity was evaluated by functional magnetic resonance imaging before and after each meal on separate days.
Results: Both meals were rated equally palatable and induced the same degree of homeostatic sensations (satiety, fullness) with a similar hedonic dimension (improved mood and digestive well-being). Both meals induced similar changes in brain connectivity: decreased activity in the frontal-parietal, basal ganglia and thalamus, visual occipital, sensory-motor, temporal superior and in the "default-mode" networks, while increased activity was detected in the network associated with white matter.
Conclusion: A substantial improvement in the nutritional profile of food can be achieved without affecting the responses to ingestion.

Key words: healthy foods – meal ingestion – postprandial sensations – brain imaging

Abbreviations: fMRI: functional magnetic resonance imaging.

INTRODUCTION

The relation between food and health, and the interest in developing healthy foods have been well established. However, for a healthy food to be introduced in the consumer's diet, it has to be attractive [1]. One of the main factors that determine food acceptance is how likable it is; likability is formed by the initial tasting experience [2]. A second, and probably more important factor, is how the consumer feels after eating the food, which is determined by the postprandial experience [3-5]. Both factors, palatability during ingestion and postprandial sensations, have to be taken into consideration when evaluating the potential acceptability of foods [1].

The main objective of this study was to demonstrate that healthier foods can be obtained without an impact on the responses to ingestion. We designed a pilot study to compare the responses to a standard sausage rich in animal fat (mortadella) versus a modified product with animal fat replaced by a fat analogue. To test the responses to foods, we applied a methodological paradigm that combines measurements of subjective sensations, using perception scales, and of brain activity in different regions, by means of functional magnetic resonance imaging (fMRI) [6].

METHODS

Participants

Eight healthy men (age range 25-32 years) with a body mass index between 18 and 25 kg/m^2 were recruited by public advertising to participate in the study during February-March 2017. Inclusion criteria were age range between 18 and 70 years, male gender and right-handedness; right-handed men were recruited to include a homogeneous population for brain imaging. Handedness was determined using the Edinburgh test (laterality index). Exclusion criteria were obesity, organic disorders, history of digestive symptoms and psychological or eating disorders. Absence of current digestive symptoms was verified using a standard abdominal symptoms questionnaire (no symptom ≤2 on a 0-10 scale). Psychological and eating disorders were excluded using the following tests: Hospital Anxiety and Depression scale (HAD), Dutch Eating Behaviour Questionnaire (DEBQ- Emotional eating, External eating, Restrained eating), and Physical Anhedonia Scale (PAS).

The protocol for the study was approved by the Institutional Review Board of the University Hospital Vall d'Hebron, and all participants gave written informed consent.

Experimental design and procedure

This was a randomized double-blind, cross-over study comparing the responses to a sausage rich in animal fat (mortadella) and a modified low-fat product. The responses were studied after a 4 h fast on 2 consecutive days in random order. Brain imaging and perception measurements were obtained before and after the test meals. The test meals were eaten in a quiet room next to the brain imaging room. For this pilot study, no formal sample size calculations were performed.

Test meals

Each test meal consisted of a sandwich (19 g bread, 50 g mortadella) and 50 mL water. Two different types of mortadella were used: a standard product with 16.3% total fat, 6.6% saturated fat, and a modified low-fat product with 4.1% total fat, 0.9 saturated fat; fat content was determined using food composition tables. In the modified low-fat product, animal fat was replaced by a plant-derived fat analogue (15%) with an additive (0.015%) that provided an animal fat aroma (Carinaroma Grasa SL104330, Carinsa, Sant Quirze del Valles, Spain). The fat analogue was an emulsion of sunflower oil and water stabilized with sodium alginate (E401), microcrystalline cellulose (E-460i), and carboxymethyl cellulose (E-466); the emulsion turned into a gel after 4 h at 4°C. The sandwich was ingested at room temperature and at the rate of choice.

Outcomes

Ten centimeter scales graded from -5 to + 5 were used to measure perception regarding: a) palatability (very bad/disagreeable to very good/delicious), b) hunger/satiety, c) digestive well-being (unpleasant sensation/dissatisfaction to pleasant sensation/satisfaction), and d) mood (negative to positive). Abdominal bloating/fullness was measured on a scale graded from 0 (not at all) to 10 (very much). The palatability scale was scored only once, immediately after ingestion of the meal. All other scales were scored before and after brain imaging, both pre- and post-ingestion; mean fasting and postprandial scores were obtained by averaging the measures obtained before and after brain imaging.

Brain imaging was performed using a 3.0T magnet (Skyra, Siemens, Erlangen, Germany) with a 32-channel head coil including a structural image (MPRAGE) before and resting-state fMRI (BOLD) before and after meal ingestion (see Supplemental Material).

Data analysis

Mean values (±SE) were calculated. Normality of data distribution was evaluated by the Kolmogorov-Smirnov test. Comparisons of parametric, normally-distributed data were made by the paired Student's t-test; otherwise, the Wilcoxon signed rank test for paired data was used.

RESULTS

Perception

Before the meals (baseline fasting period), subjects reported hunger sensation and a positive mood (Fig. 1). Test meals were ingested within 203±12 s (at the rate of choice). The palatability score was reported as positive for the two meals (3.3±0.3 and 3.1±0.3 for the standard and low-fat meal, respectively; p=0.516). Meal ingestion suppressed hunger, improved mood, induced mild fullness sensation and postprandial satisfaction (digestive well-being) (Fig 1). Compared to the pre-ingestion levels, these effects were significant for both meals (p≤0.040 vs for all), and no differences were detected between the standard and the low-fat meal; indeed, the responses were remarkably similar to both meals (Fig 1).

Brain imaging

The overall effect of meal ingestion on "resting-state" brain activity was evaluated pooling the results of all subjects together. Meal ingestion induced significant increases and decreases in brain connectivity; specifically, decreased activity was detected in the following networks: frontal-parietal, basal

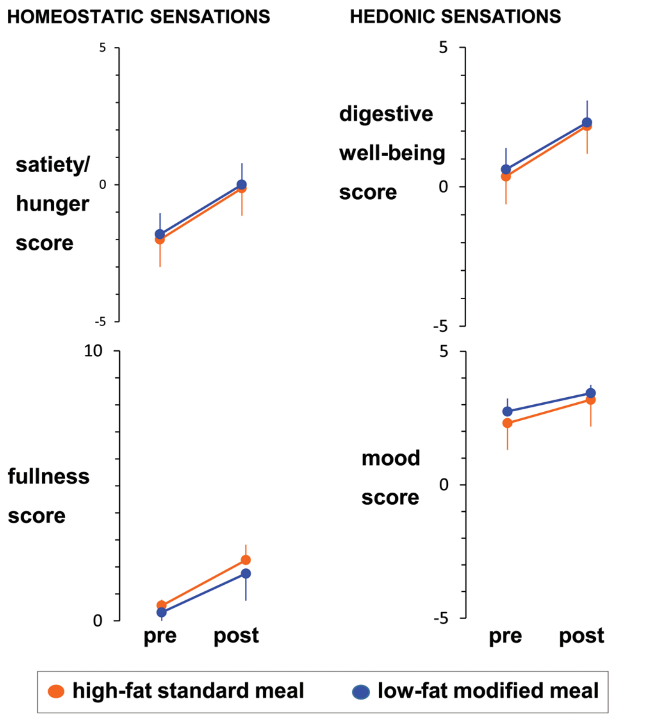

Fig. 1. Sensory responses to meal ingestion. Homeostatic and hedonic sensations pre- and post-ingestion of standard and modified meals (n=8). Note similar responses to both meals.

ganglia and thalamus, visual occipital, sensory-motor, temporal superior and in the "default-mode"; increased activity was detected in the network associated to white matter (Table I, Fig. 2). No differences were detected between meals.

DISCUSSION

Ingestion of a meal activates the digestive system to initiate the digestive process which is modulated by a complex set of reflexes [7]. The digestive response is associated with subjective sensations, including homeostatic sensations, such as hunger/satiety and fullness, as well as with hedonic sensations, such as meal liking, digestive well-being and changes in mood [1, 8]. Meal ingestion has been shown to induce changes in the activity of the central nervous system that can be detected by fMRI [5]. In a previous study, we demonstrated correlations between conscious sensations and changes in the activity of specific brain regions [6]. These data suggested that brain responses to meal ingestion can be taken as objective surrogate markers of perception.

The sensations induced by meal ingestion depend on the characteristics of the meal and on a series of conditioning factors that influence the receptivity of the eater [1]. To test the effect of a specific characteristic of the meal, it is essential to keep other factors under control. Meal palatability is a major determinant of the postprandial response [9]. In a previous study, we compared the responses to a palatable potato and cheese cream dish and a vanilla cream dessert, both with the same color, texture and temperature, either as a two-course meal or mixed together [9]. The mixed meal induced more fullness and less satisfaction than the standard two-course meal. Meal composition also influences the postprandial response: two meals with identical taste and flavor, but with a different composition, induced different degrees of postprandial satisfaction [10]. Fat, particularly animal fat, is a key component of food in terms of health implications, and a diet low in animal fat is advisable [11-13]. However, fat is the key component in comfort foods and has a major effect on postprandial satisfaction and mood [5]. Hence, reducing fat without affecting the hedonic responses is a crucial and challenging aspect in the production of healthy foods. Palatability depends on several factors, including food appearance, taste, smell and texture; a decrease in fat may alter these characteristics [1, 10, 14, 15]. Our paradigm showed that the addition of a plant-derived fat analogue and an aroma allowed a substantial reduction of animal fat content in a sausage without affecting either palatability or the hedonic response after ingestion.

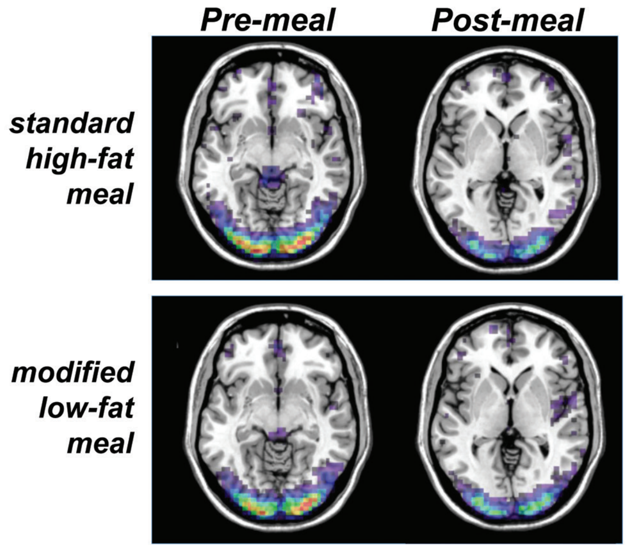

Fig. 2. Brain activity responses to meal ingestion. Example of changes in the synchronization of the visual occipital network (pre- and post-ingestion images) in response to standard and modified meals in a healthy subject. Note similar response (decrease) to both meals

Table I. Resting-state changes in response to meal ingestion

Resting-state network	Change	Location	MNI coordinate	Voxel extension	p
dorsal attention	D	L temporal inf	(-46, -46, -28)	45	0.015
BG & thalamus	D	R caudate	(-6, 6, 0)	105	0.022
visual occipital	D	R occipital inf	(30, -98, -16)	29	0.022
		L occipital inf	(-30, -102, -12)	25	0.024
		L lingual	(-14, -86, -12)	12	0.035
sensory-motor	D	R postcentral	(18, -26, 60)	52	0.015
		L parietal inf	(-38, -46, 52)	11	0.027
		L postcentral	(-26, -34, 48)	11	0.035
		L precentral	(-14, -26, 56)	11	0.031
temporal sup	D	R temporal sup	(58, -34, 8)	92	0.018
		L temporal sup	(-58, -34, 16)	69	0.011
default-mode	D	L precuneus	(-10, -62, 52)	126	0.002
white matter	I	brainstem	(14, -38, -28)	62	0.022
		L lingual	(-22, -70, -8)	69	0.024

Abbreviations: D=decrease; I=increase; BG=basal ganglia; MNI=Montreal Neurological Institute; L=left; R=right; inf=inferior; sup=superior.

It is important to note that our study had several limitations. In the first place, this is a pilot study with a small sample size. Furthermore, the paradigm has to be validated in a dose-response study with a graded reduction in fat.

CONCLUSION

Our pilot, proof-of-concept study indicates that a substantial improvement in the nutritional profile of food might be achieved without affecting responses to ingestion. Testing for food acceptance and the sensory postprandial experience is not standardized and perception measurements are subjective. The data derived from our study may be relevant to the development of attractive, healthy foods guided by biological markers.

Conflicts of interest: Quiro Lopez and Eduardo Lucas are employees at Carinsa. No competing interests were declared by the other authors

Authors' contributions: T.P.: study management, conduction of experiments, data analysis. Q.L., E.L.: study design and development of tests meals. D.M.L.: manuscript revision. A.R.: supervision of brain imaging studies. A.A.: supervision of clinical studies. F.A.: study design, data interpretation, manuscript preparation and guarantor of the article. D.P.: study design, brain imaging and interpretation, manuscript revision.

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SUPPLEMENTAL MATERIAL: BRAIN IMAGING AND ANALYSIS

Image acquisition. Images were acquired in a 3.0T magnet (Skyra, Siemens, Erlangen, Germany). Acquisitions were performed with a 32-channel head coil. The MRI protocol included a structural image (MPRAGE) and a resting-state fMRI (BOLD) before and a resting-state after the meal ingestion. Acquisition parameters were TR= 2300 ms,TE= 2.98ms, 176 sections, voxel size=1x1x1mm3 for the MPRAGE; and TR=2700ms, TE=35ms, voxel size=3.6x3.6.x3.0mm3, 180 volumes for the resting-state fMRI.

Image analysis. Resting-state fMRI data was analyzed by using the MELODIC toolbox implemented in FSL (1). The first 5 volumes were discarded due to signal inhomogeneity. After image realignment, a temporal concatenated approach was chosen and it was applied to all the studies. In order to estimate the changes, a predefined set of 20 resting state networks (RSNs) was chosen as template. This set included the following RSNs: visual medial, occipital, lateral, default-mode, cerebellum, sensory-motor, auditory, frontal-executive, right and left executive among other RSN. This set was used to generate subject-specific versions of these spatial maps and associated time series, using dual regression. Briefly, for each subject, the set of 20 RSN was regressed (as spatial regressors in a multiple regression) into the subject's 4D space-time dataset. This results in a set of subject-specific time series, one per group-level spatial map. Next, those time series are regressed (as temporal regressors, again in a multiple regression) into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per group-level spatial map. We then tested for differences, using FSL's randomize permutation-testing tool (number of permutations was set at 10000). Finally, the cluster tool was applied to the threshold-free cluster enhancement corrected p-images; significance level was set at $p<0.05$ and an extended threshold of 10 voxels. The main effect of meal ingestion was assessed by pooling all the studies together (as paired t-tests). Interactions between the two meals were computed as the difference of differences between the two meal paradigms.

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