Melanoidins from coffee and bread differently influence energy intake: A randomized controlled trial of food intake and gut-brain axis response

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1. Introduction

Heat-treated and highly-processed foods including bakery products, coffee, milk, beer, cocoa, soy sauce and vinegar are dietary sources of high molecular weight compounds named melanoidins (Borrelli et al., 2003; Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002; Fogliano & Morales, 2011; Moreira, Nunes, Domingues, & Coimbra, 2012). They are the final heterogeneous polymers formed by the Maillard reaction between amino acids and reducing carbohydrates in food. Maillard reaction products have been attributed a nutritional quality lowering effect due to reduced protein digestibility and, particularly, the advanced glycation end-products, which have been linked with health conditions and diseases such as aging, diabetes and atherosclerosis (Aljahdali & Carbonero, 2019). In contrast, Maillard reaction products can provide around 10 g/day of melanoidins (Fogliano & Morales, 2011; Morales, Sozoza, & Fogliano, 2012). In addition, melanoidins possess antioxidative (Borrelli et al., 2002) and anti-inflammatory properties (De Marco, Fischer, & Henle, 2011) shown in vitro and in mice (Choi, Jung, & Ko, 2018) as well as a hepatoprotective effect shown in a rat model of liver disease (Vitaglione et al., 2010). Since these molecules are formed in food products upon heating, their structure and composition vary depending on the availability of precursors and the presence of other food components that may be involved in the reaction. Therefore, melanoidins from coffee and bread, which are the most abundant melanoidins in the Western diet (Fogliano & Morales, 2011), are chemically different. In coffee, melanoidins are formed mainly by digestible dietary elements and the gut microbiota. Since a common diet can provide around 10 g/day of melanoidins (Fogliano & Morales, 2011; Pastoriza & Rufian-Henares, 2014), melanoidins are good contributors to the digestion-resistant polymers that reach the colon and are fermented by the local microbiota (Fogliano & Morales, 2011; Morales, Sozoza, & Fogliano, 2012). Therefore, melanoidins from coffee and bread, which are the most abundant melanoidins in the Western diet (Fogliano & Morales, 2011), are chemically different. In coffee, melanoidins are formed mainly by...
polysaccharides and polyphenols due to the presence of arabinogalactans and chlorogenic acids in green coffee beans (Morales et al., 2012) and have a limited ability to form gel structures in the stomach and the intestine (Nunes & Coimbra, 2007). In contrast, bread melanoidins derive mainly from gluten proteins and starch (Morales et al., 2012). Bread melanoidins are only partially digested by digestive enzymes in the stomach and remain insoluble throughout the gastrointestinal tract (Helou et al., 2015, 2017). Although melanoidins share physicochemical, biochemical and biological properties with dietary fibers (Fogliano & Morales, 2011), the effect of melanoidins on satiety and biochemical pathways underpinning appetite sensations in humans is unknown.

Humans have an innate tendency to overeat, which is an important component of the current obesity epidemic. An elegant study recently demonstrated that ultra-processed diets cause excess calorie intake by affecting appetite cues (Hall et al., 2019). Food intake results in a systemic modulation of hormones, peptides, signaling molecules, and hedonic feelings leading to satiety (Berthoud, 2011; Keesey & Powley, 2008) through feedback mechanisms that enable the gut-brain axis to monitor changes in the nutrient status within the body (Camilleri, 2015). The body’s response to food ingestion results in an increase of plasma glucose, insulin, PYY, and GLP-1, which elicits satiety and limits food intake through the down-regulation of activity in the hypothalamus (Zanchi et al., 2017), while the orexigenic peptides ghrelin and orexin-A (So et al., 2018) along with the endocannabinoids (ECS), anandamide (AEA) and 2-arachidonoylglycerol (2-AG), can stimulate hypothalamic activity (Di Marzo, Ligresti, & Cristina, 2009; Zanchi et al., 2017). In addition, endocannabinoid-like molecules, including N-acylthanolamines (NAEs) such as oleoylethanolamide (OEA), linoleoyethanolamide (LEA), and palmitoylethanolamide (PEA), have a pleiotropic activity in the body and affect energy metabolism leading to appetite reduction through a wide range of receptors located in the central nervous system and along the gastrointestinal tract (Mennella et al., 2016; Mennella, Savarese, Ferracane, Sacchi, & Vitaglione, 2015; Witkamp, 2018).

The primary aim of this study was to evaluate whether coffee and bread melanoidins influence appetite sensations and energy intake in humans. Secondarily, we aimed to assess the postprandial responses of blood glucose and 21 biomarkers of the gut-brain axis involved in appetite cues and to display relationships between appetite sensations, metabolic biomarkers and energy intake.

2. Subjects and methods

2.1. Isolation of coffee melanoidins

Prepackaged Lavazza Suerte ground coffee (Lavazza), a medium roast coffee, was purchased from a local market. The coffee powder was diluted with water (1:4 wt:vol) and cooked at 90 °C with a stirring rate of 2,000 rpm for 30 min in a BIMBY cooker (Vorwerk). The coffee mix was allowed to settle for 10 min and was poured into a French press (Brazil model, Bodum). The French press coffee was filtered through unbleached filter paper (Melitta), collected, and transferred to dialysis tubing with a molecular weight cut-off of 12–14 kDa (Spectra/Por, Spectrum Laboratories). The filtered coffee was dialyzed against tap water at 4 °C until the water remained clear. The dialyzed coffee was collected and the weight of the coffee melanoidins was determined by freeze-drying the dialyzed coffee melanoidins solution. The freeze-dried powder was stored at −20 °C until use in the preparation of the coffee melanoidins-enriched bread.

2.2. Isolation of bread melanoidins

Bread melanoidins were prepared following the protocol of Fogliano and Morales with a few modifications (Fogliano & Morales, 2011). Bread crisps dough was prepared with wheat flour (tipo 00) and water at a ratio of 1.8:1 (wt:vol). The dough was spread to a thickness of 0.3 cm, cut into circular disks with a diameter of 6 cm, and holes were made using a fork to minimize the formation of air pockets during baking. Crisps were baked for 60 min at 200 °C in a convective oven (Electrolux Rex). The baked crisps were finely ground at 4,000 rpm for 30 sec followed by 10,000 rpm for 5 sec using the BIMBY mixer. The ground crisps were sifted through a 300 µm sieve and stored at −20 °C until needed.

The melanoidins content in the bread crisps powder were determined (Fogliano & Morales, 2011). A 500 mg sample of bread crisp powder was incubated in 6 mL of 20 mM Tris pH 8.0 along with 0.1 mg/mL protease Pronase E from Streptomyces griseus (Sigma-Aldrich) for 40 h at 37 °C. The supernatant was cleared at 2,800 g for 10 min. Three mL of supernatant were incubated with 3 mL of 40% trichloroacetic acid for two h at room temperature. The precipitated bread crust was concentrated in a Centricon YM-10 (Merck-Millipore). The retentate was freeze-dried and the melanoidins present in the bread crisps were calculated.

2.3. Preparation of the foods for the randomized, controlled, crossover study

Three breads were formulated and prepared. They were a conventional 100% refined wheat flour bread (control bread, CT) and two breads enriched with 3% coffee-melanoidins (CM) or bread melanoidins (BM). To design the melanoidins-enriched breads we took into account the reported daily intakes of coffee and bread melanoidins (which are 0.5–2 g/d and 1.8–15 g/d, respectively) (Fogliano & Morales, 2011) and we estimated that the addition of an extra 3 g of coffee or bread melanoidins would represent a large intake for both types of melanoidins at one meal. Coffee melanoidins or bread melanoidins were incorporated into the dough by replacing wheat flour in the recipe of the conventional bread in order to have a final amount of 3 g melanoidins per 100 g bread. The doughs were colored with a 0.3% (w:w) brown food coloring (Wilton Industries) to mask the color differences arising from melanoidins enrichment. The composition of the three breads is summarized in Table S1. The doughs were baked for 25 min at 200 °C in a convective oven (Electrolux Rex). The prepared breads were all weighed, placed in freezer bags, and frozen until the study session.

A study day breakfast consisted of 100 g of one of the breads and 125 mL of 1% lactose-free milk (Parmalat). The nutritional composition of the three breakfasts is reported in Table S2. The experimental day ad libitum lunch provided pre-weighed portions of typical Italian dishes in a buffet style including pasta with tomato sauce, meat, fish, green salad, French fries, bread, and fruit. The food was prepared by a local restaurant following fixed recipes on each experimental day. The selected dishes were based on the subjects’ preferences as determined from their written responses in a food preference questionnaire that each subject completed during the enrollment visit. After lunch, the dishes were weighed again and the food consumed was measured to the nearest 1 g. The energy intake and nutrient intake were then calculated based on the food analysis from the Italian research center for food and nutrition (CREA - Centro di ricerca per gli alimenti e la nutrizione, Italy).

2.4. Subjects

Recruitment was conducted at the University of Naples among people working or studying in the Department of Agricultural Sciences and informed of the study by public announcements on social media. The participant flow and their general characteristics were reported in Fig. 1 and Table 1, respectively. Twenty subjects were screened and fourteen subjects were selected based on the data obtained from a questionnaire of their medical status, their food preferences and their subjective eating habits. The selected subjects were healthy. They were not taking any medications (birth control pill was allowed) or drugs. The subjects had to be habitual consumers of breakfast and could not be...
Each study day, which minimized the consumption of dietary fats, was separated by a 1-week washout period. The experimental design for each study day is illustrated in Fig. S1. The order of treatments was randomized for each subject via computer-generated randomization tables (Microsoft Excel) and the values calculated with a logistic 5-parameter regression line equation using the Bio-Plex Manager 5.0 software (Bio-Rad).

Table 1: Characteristics of the study participants. Data shown as means ± SE.

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<th>Unit</th>
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<tr>
<td>Body mass index</td>
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<td>19–25</td>
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in a restrictive diet regimen. The subjects signed an informed consent form to be enrolled in the study.

2.5. Study design

This study was registered at clinicaltrials.gov as NCT01851304. It was conducted following the guidelines laid down by the Code of Ethics of the World Medical Association (Declaration of Helsinki) and all procedures involving human subjects were approved by the Ethics Committee of the University of Naples (Protocol number: 235/13). Written informed consent was obtained from all subjects and the privacy rights of all subjects were observed. The study started in September 2014 and finished in July 2015.

A randomized, controlled, crossover, and single blind designed study was performed. The sample size was calculated on the study primary outcome: appetite feelings and energy intake at the subsequent lunch. It was calculated that an α = 0.05 with a power of 80%, 12 subjects would be needed to detect a 19% difference in energy intake at the ad libitum lunch. Considering the postprandial hormonal responses as secondary outcomes, it was also calculated that 8 subjects would allow for the detection of a 23% and 16% difference in AUCO-180 of ghrelin and PYY, respectively. Each blindered subject participated in three study days that were separated by a 1-week washout period. The order of treatments was randomized for each subject via computer-generated randomization tables (Microsoft Excel) that were provided by an experimenter to the nutritionist in charge of the meal planning system.

The consumption of either the CM or the BM was well tolerated and avoided the second-meal effect (Wolever, Jenkins, Ocana, Rao, & Collier, 1988). They were also instructed to consume white rice and animal protein-containing foods; to avoid alcoholic and soft drinks; to always consume the same foods at dinner before each study day; to finish eating by 22:00 h; and to fast overnight with ad libitum access to water. Before beginning a study day, subjects completed a questionnaire about their current health. Subjects experiencing any physiological or psychological discomfort were excluded from the current visit and asked to come to the next experimental day.

The fasted subjects arrived at 08:30 h at the laboratory of Food and Health of the Department of Agricultural Sciences of the University of Naples. The appetite sensations of hunger, desire to eat, fullness, and satiety were measured at time points 0 (just prior to breakfast), 30 min, 60 min, 120 min and 180 min after breakfast using a 100 mm Visual Analog Scale (VAS) anchored by specific questions (Green, Delargy, Joanes, & Blundell, 1997). The questions were 'How hungry do you feel?' 'How much could you eat at this moment?' 'How full do you feel?' 'How satisfied do you feel?' and the recorded mark indicated the strength of each sensation (Green et al., 1997).

Immediately after the recording of baseline appetite sensations, blood glucose was measured with a skin prick test using a One Touch meter (LifeScan). Then a cannula was placed into an arm vein of each subject and blood was collected at that time and at 30 min, 60 min, 120 min and 180 min after breakfast. Fifteen milliliters of blood were drawn into three EDTA-coated vacutainer tubes (Becton Dickinson). The protease inhibitors dipeptidyl peptidase IV (DPPIV, EMD Millipore) and phenylmethylsulfonyl fluoride (PMSF, Sigma-Aldrich) were added immediately after blood collection to inhibit the degradation of acylated ghrelin and PYY. The tubes were centrifuged at 2,400 g for 10 min at 4 °C. The supernatant was collected and stored at −40 °C until sample measurement.

After the collection of the last blood sample (180 min) subjects were offered an ad libitum lunch as described above (Par. 2.3.). Before leaving the laboratory, subjects were instructed on how to compile a weighted food diary throughout the rest of the day until the following morning. They received a food diary entry scheme with four columns: one to report the time, one for the food or the beverage consumed, one for the weight/volume of the food, and one for the notes. Subjects were instructed to write everything they consumed in the food diary. When subjects consumed a commercial food, they were required to report the commercial product and brand into the notes. For food prepared at home, they were required to report in the notes the weight added fats or sugars, as well as indicate the percentage of ingredients in the dish in order to allow a proper calculation of the nutritional composition of the related food item. On the day after an experimental day, the weighed food diary was sent by e-mail from each subject to the nutritionist collaborating with the study. The nutritionist called each subject by phone to confirm the contents of their food diary and to avoid any mistakes possibly occurring due to the subject’s forgetfulness. The weighed food diaries were then analysed by the nutritionist to calculate the energy intake using the same food nutritional composition database that was used for assessing the energy intake at the ad libitum lunch (Par. 2.3.).

2.6. Gastrointestinal hormones

Insulin, ghrelin, leptin, peptide YY (PYY), glucagon peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), pancreatic polypeptide (PP) and glucagon were measured in plasma samples prepared with protease inhibitors using the EMD Millipore human metabolic hormone magnetic bead panel following the manufacturer’s instructions. The plate was read in a Bio-Plex 200 System with Luminex Technology (Bio-Rad) and the values calculated with a logistic 5-parameter or 4-parameter regression line equation using the Bio-Plex Manager 5.0 software (Bio-Rad).
2.7. Postprandial insulin sensitivity

Postprandial oral glucose insulin sensitivity (OGIS) was calculated by the meal glucose tolerance test (MGTT) over 3 h according to Mari et al. using an Excel spreadsheet for 3 h OGTT (Mari et al., 2002; Mari, Tura, Gastaldelli, & Ferrannini, 2002).

2.8. Neuropeptides

Cortisol, β-endorphin, α-melanocyte stimulating hormone (α-MSH), oxytocin, orexin-A, neotensin, and substance P were simultaneously measured using the EMD Millipore human neuropeptide magnetic bead panel; read with a Bio-Plex 200 system using Luminex Technologies; and the measurements calculated with the Bio-Plex Manager 5.0 software. Melatonin was measured by ELISA following the manufacturer’s protocol (IBL International). Each plate was scanned on a dual-wavelength plate reader (Thermo Scientific Multiscan FC, Thermo Scientific) at 405 nm with reference wavelength of 620 nm and data collected with the ScanIt Software version 2.5.1 (Thermo Scientific). The melatonin concentration was determined using a logistic 4-parameter regression line.

2.9. Plasma endocannabinoids (ECs) and N-acylethanolamines (NAEs)

Plasma samples were spiked with an internal standard and two ECs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), along with three NAEs, linoleoyl ethanolamide (LEA), palmitylolethanolamide (PEA) and oleoylolethanolamide (OEA) were extracted from the plasma samples and analyzed by LC/MS/MS (Mennella, Ferracane, Zucco, Fogliano, & Vitaglione, 2015).

2.10. Statistical analysis

The statistical differences between time points and bread treatments were calculated using SPSS 16.0 and SigmaPlot 11.0. For all the measured markers, the mean ± standard error mean (SE) for each time point was determined. The area under the curve (AUC) for each marker was calculated using the linear trapezoidal rule. All measured markers including the energy intake data and the area under the curves were normalized using LN transformation. The transformed data were subjected to repeated measures analysis of variance (ANOVA) over time for each bread and to one-way ANOVA between breads at each time point and AUC interval. Statistically significant differences (p < 0.05) were further investigated by the Bonferroni post-hoc test. Paired t-tests with a significant difference of p < 0.05 were done between CM and BM at all time points and AUC intervals for each marker.

The AUC of each variable calculated over all treatments by all participants was subjected to a pairwise Pearson’s correlation test. The heatmap correlation matrix was visualized using the Hmisc package and the function heatmap.2 of R software, version 3.6.2 (https://www.r-project.org).

3. Results

3.1. Short-term appetite sensations

Participants perceived similar hunger, fullness, satiety and prospective food consumption over the three hours after breakfast with all three types of bread (Table S3). Compared to baseline, however, both CM and CT consumption reduced hunger after 30 min and it remained low at 60 min after CT (Fig. 2A). That profile paralleled a decreased prospective consumption at 30 min (Fig. 2B) and an increased satiety and fullness at 30, 60 and 120 min vs baseline only after CT (p < 0.05) (Fig. 2C-D). Participant sensations did not vary over time when BM was consumed.

3.2. Daily energy intake

In accordance with the similar appetite sensations recorded over the three hours after the three breakfasts, participants did not vary their energy intake at the subsequent lunch offered 180 min after breakfast (Fig. 3). However, energy intake over the period following lunch was significantly reduced by 26% when participants consumed CM bread at breakfast compared with CT bread (901 ± 102 kcal (3,770 ± 427 kJ) vs 1,216 ± 97.1 kcal (5,088 ± 406 kJ); p = 0.04). A similar trend was observed with BM but the 18% decrease was not enough to achieve a statistically significant difference (967 ± 114 kcal (4,046 ± 477 kJ) vs 1,216 ± 97.1 kcal (5,088 ± 406 kJ); p = 0.08).

3.3. Influence on glucose and gastrointestinal markers

The blood glucose response was similar over the three hours after all of the breakfasts (Table S3). Participants experienced blood glucose responses with a peak between 30 min and 60 min followed by a rebound to baseline values at 180 min (Fig. 4A). Interestingly, the blood glucose peak at 60 min after breakfast was significantly lower with CM than after BM (1.31 ± 0.24 mmol/L vs 1.85 ± 0.17 mmol/L, p = 0.04). Moreover, CM elicited a lower late postprandial response of insulin than CT (Fig. 4B and Table S4). After CM ingestion, plasma insulin concentration peaked at 30 min, and at 120 min it had returned to a value that was similar to baseline but significantly lower than after CT (0.877 ± 0.178 µg/L vs 1.50 ± 0.220 µg/L, p = 0.024); while at 180 min insulin concentration after CM was lower than after BM (0.456 ± 0.062 µg/L vs. 0.619 ± 0.134 µg/L, p = 0.017). The OGIS index did not show any difference in the individual insulin sensitivity over 180 min after breakfasts (Fig. S2). On the other hand, data showed that ghrelin concentration within 60 min after breakfast with CM decreased faster and deeper than after either BM or CT (Fig. 4C). This fall was followed by a rapid increase within 180 min after CM consumption that resulted in a significantly higher late response of ghrelin after CM than BM (Table S4).

The counteraction of anorexigenic peptides explained the lack of effect on energy intake at lunch despite the stronger response of the orexigenic ghrelin following CM consumption (Fig. 4D–I). Although ghrelin, leptin, PP, PYY, glucagon, GIP, and GLP-1 showed similar responses after all bread consumption, we found differences in time-concentration profiles of some of them between breads. Particularly, plasma GIP strongly increased in the first hour after both CM and BM bread ingestion followed by a decrease towards baseline at 180 min after breakfast. Pancreatic polypeptide showed a strong response to CM bread ingestion 180 min after breakfast while BM and CT displayed a broader peak over the entire 180 min after breakfast. Plasma glucagon and leptin levels were affected only after CT ingestion.

3.4. Influence on endocannabinoids and N-acylethanolamines

We did not find any difference between the entire 180 min postprandial ECs and NAEs response with any of the breads (Table S3; Fig. 5). In general, 2-AG concentration did not change postprandially after bread ingestion. AEA and the NAEs, on the other hand, decreased significantly lower than baseline. All three breads maintained a significant reduction in plasma GIP and ghrelin at 180 min. Lower concentrations of all of the NAEs at 180 min were always observed after CM vs BM (LEA was 4.96 ± 0.527 µg/L vs 6.20 ± 0.785 µg/L, OEA was 10.6 ± 1.10 µg/L vs 12.8 ± 1.65 µg/L, PEA was 6.08 ± 0.570 µg/L vs 7.45 ± 0.846 µg/L, p < 0.05, respectively).
3.5. Influence on neuropeptides

For 5 out of 8 neuropeptides monitored, we did not observe any difference in the postprandial plasma response between breads (Fig. 6A–D and Fig. S3A-D) while three neuropeptides showed significant variations. Compared to BM, CM showed a significantly lower plasma response of \( \alpha \)-MSH over 180 min (Table S3), orexin-A over 30–180 min (AUC\(_{30-180\text{min}}\) were 44.9 ± 5.82 µg*min/L vs 52.0 ± 5.72 µg*min/L, \( p = 0.042 \), respectively) and \( \beta \)-endorphin over 60–180 min (Table S4). Moreover, we found a decrease in cortisol concentration 120 min after CM bread consumption compared to baseline and a lower plasma \( \beta \)-endorphin concentration 120 min after CM than BM.

3.6. Postprandial interplay between gut and brain biomarkers

In order to evaluate the postprandial interplay between responses of gut and brain biomarkers with evolution of appetite sensations and the subsequent energy intake, we performed a correlation analysis between variables monitored within 180 min postprandially. Fig. 7 illustrates the strength of the Pearson’s correlations between the postprandial responses for each pair of biomarkers. We confirmed some relationships between specific appetite sensations and the response of GLP-1, PP, insulin, leptin, \( \beta \)-endorphin, substance-P and orexin-A. Moreover, we unraveled novel relationships between \( \alpha \)-MSH and neurotensin with hunger, all NAEs and AEA with satiety neurohormonal response, and 2-AG with energy intake.

4. Discussion

In this study, we aimed to assess whether coffee and bread melanoidins modulate energy intake and how they affect postprandial dynamics of 21 gut-brain modulators of appetite, including 8 gastrointestinal peptides, 2 endocannabinoids, 3 \( N \)-acylethanolamines, and 8 neuropeptides in humans. Participants reported similar appetite sensations over 180 min after the three breakfasts and energy intake at the subsequent lunch. However, CM significantly decreased energy intake by 26% over the post lunch period compared to CT, while the 18% reduction recorded with BM indicated a clear trend although it did not reach statistical significance.
The reduction of energy intake over the day after lunch suggested that melanoidin-enriched breads mainly targeted physiological mechanisms taking place in the distal gastrointestinal tract. This is a typical effect of low processed and whole food because of a delayed digestion compared with highly processed and finely grounded food, as previously demonstrated with a rye-based product (Isaksson et al., 2011). A similar effect was also observed with microencapsulated bitter compounds, which were specifically designed to release the bioactive compounds into the duodenum in order to target the chemosensory receptors on intestinal mucosa (Mennella et al., 2016). Recalling the evidence that coffee melanoids lead to intestinal bacterial growth (Morales et al., 2012), we can hypothesize in this study that coffee melanoids are metabolized by the gut microbiota in the colon, thus releasing metabolites that can elicit the secretion of anorexigenic hormones by enteroendocrine cells (Fetissov, 2017). Therefore, a concomitant effect of the food structure delaying digestion of macronutrients and delivering polyphenols from CM or proteins from BM, as well as a satiety response induced by microbial metabolites likely contributed to the delayed satiety observed in our study.

Looking at postprandial metabolic responses, results showed a generally lower glucose and insulin concentration following CM consumption. This effect was independent from postprandial insulin sensitivity as assessed by OGIS and a delayed gastric emptying. Indeed, we found a stronger response of ghrelin that might indicate an even faster gastric emptying following CM (Levin et al., 2006). A similar effect on postprandial insulin response in humans was also observed in previous studies with a lupin-based bread and a wholegrain bread (Keogh, Atkinson, Eisenhauer, Inamdar, & Brand-Miller, 2011) as well as with a β-glucan-enriched bread (Vitaglione, Lumaga, Stanzione, Scalfi, & Fogliano, 2009). Interestingly, rye products also displayed an improved postprandial glycemic response when served at breakfast (Rosen, Ostman, & Bjorck, 2011) that was likely due to their content of bioactive phenolic compounds inhibiting activity of carbohydrate digestive enzymes (Rosen et al., 2011). We hypothesize that high molecular weight coffee melanoids having chlorogenic acids incorporated into their structures (Moreira et al., 2012) could elicit a metabolic effect similar to polyphenol-bound dietary fiber present in cereals (Vitaglione et al., 2015). This idea that the polyphenol-based and antioxidant structure of CM (Borrelli et al., 2002) might have affected the postprandial metabolic response was also supported by the observation of a lower α-MSH response after CM vs BM. That result suggested a lower impact of CM on postprandial oxidative and inflammatory phenomena along the intestine because α-MSH has been indicated as an anti-inflammatory peptide secreted by many tissues, including intestinal cells, to counteract inflammation (Hoggard, Hunter, Duncan, & Rayner, 2004; Singh & Mukhopadhyay, 2014).

Regarding the response of ECs, that 2-AG did not respond to food intake was already found in previous studies in normal weight subjects (Gatta-Cherifi et al., 2012; Mennella, Savarese et al., 2015) while it was in disagreement with one study reporting an increase of 2-AG 60 min
after a meal in overweight subjects (Tischmann et al., 2019).

A previous study demonstrated that plasma OEA could reflect the fatty acid intake (Fu et al., 2011). It could then be hypothesized that the lower concentration of NAEs at 180 min after CM vs BM could be caused by a slower digestion of the breakfast containing CM that provided fewer precursors for LEA, OEA, and PEA synthesis in the intestine and/or a slower spill-over of NAEs from the gut into the bloodstream (DiPatrizio, Astarita, Schwartz, Li, & Piomelli, 2011; Naughton, Mathai, Hryciw, & McAinch, 2013). Moreover, since plasma NAEs concentration is dependent on the formation and degradation pathways operated by specific enzymes such as the NAPE-PLD and FAAH (Ahn, McKinney, & Cravatt, 2008; Hansen, 2014), it is also likely that the two types of melanoidins differently influenced, by direct or indirect mechanisms, the activity of these enzymes. Further studies should test these hypotheses.

The lack of a breakfast effect on individual appetite sensations and behavior at lunch was observed despite different responses after breakfast from several biomarkers including insulin, β-endorphin, LEA, OEA and PEA. This supported the evidence that eating episodes result from the cumulative effects of a number of submaximal hormone responses that may have additive or opposing effects. In order to shed light on the biochemical modulation of appetite cues and eating behavior, we displayed the pattern of relationships between the post-prandial responses of the monitored biomarkers.

Our data confirmed the results from previous studies that fullness and satiety increase is associated with increased responses of the anorectic hormones GLP-1, leptin, and insulin; hunger reduction is associated with increased insulin and the anorectic hormones PP, PYY, leptin, and GLP-1 (Murphy & Bloom, 2006; Suzuki, Simpson, Minnion, Shillito, & Bloom, 2010); and hunger increase is associated with an increased orexigenic β-endorphin (Veening & Barendregt, 2015), substance-P (Karagiannides et al., 2008) and orexin-A (Edwards et al., 1999). In disagreement with the previous literature, we provided evidence for possible involvement of the neuropeptides α-MSH and

Fig. 5. Endocannabinoids and N-acyl ethanolamines response (A–E). Measurement of the endocannabinoids and N-acyl ethanolamines are shown as means ± SE, n = 14. *p < 0.05 for the measured time point compared with baseline within each bread treatment (repeated-measures ANOVA, Bonferroni post-hoc test).

^p < 0.05 between CM and BM at this time point (paired t-test).
neurotensin in the evolution of hunger (Izaguirre, Catalan, & Frühbeck, 2016; Sohn, 2015).

In parallel with expected correlations between the responses of gut peptides and neuropeptides, new correlations between those responses and the dynamics of NAEs and ECs were displayed. Three previous studies postprandially evaluated only ECs and gut peptides: one study had normal weight subjects (Monteleone et al., 2012), one study had obese subjects (Rigamonti et al., 2015), and one study had patients with Prader-Willi syndrome (Rigamonti et al., 2017). No previous study focused on NAEs, gut peptides and neuropeptides in humans.

While it is known that circulating OEA elicits satiety (Witkamp, 2018), our data showed that all three NAEs may contribute to the anorexic effect of gut peptides and neuropeptides. In contrast with the previous evidence that AEA increases appetite and energy intake (Di Marzo & Matias, 2005), we found that this endocannabinoid correlates with some gut peptides and neuropeptides likely eliciting an anorexic effect. We hypothesized that the longer postprandial period evaluated by us (180 min) compared to the study by Gatta-Cherifi et al. (60 min) might account for this difference (Gatta-Cherifi et al., 2012). Nonetheless, the positive and strong correlation we found between 2-AG and energy intake is in agreement with all previous literature indicating the implication of this endocannabinoid in the regulation of food intake (Witkamp, 2018).

One study limitation involves the consumption of milk at breakfast, as we cannot exclude the possibility that milk proteins influenced CM and BM effects. However, providing milk at breakfast aligned the experimental meal with the usual dietary habits of the participants thus avoiding a possible bias of laboratory experimental procedures.

5. Conclusions

For the first time, we show in this study that melanoidins, particularly from coffee, affect daily energy intake in humans by targeting physiological mechanisms taking place in the distal intestinal tract. Although both types of melanoids similarly influence appetite and subsequent energy intake, coffee melanoidins lower the blood glucose peak and insulin response enhancing ghrelin, and blunt the response of all NAEs, α-MSH, orexin-A and β-endorphin. These effects are likely due to the chemical structure of coffee melanoidins that incorporate chlorogenic acids and possibly elicit metabolic effects similar to polyphenol-bound dietary fiber of cereals, such as modulation of digestive enzyme activity, control of oxidative and inflammatory processes in the gastrointestinal tract and modulation of the gut microbiota composition.

Additionally, we shed light on the interplay between postprandial appetite sensations and neurohormonal responses in humans. We confirm some relationships between specific appetite sensations and the response of GLP-1, PP, insulin, leptin, β-endorphin, substance-P and orexin-A. We unravel novel relationships between α-MSH and neurotensin with hunger, all NAEs and anandamide with satiety neurohormonal response, and 2-AG with energy intake.

Overall, our findings have impact in both food science and physiology. Thermal treatment of food can lead to the formation of often disregarded molecules, such as melanoidins, possessing metabolic properties that can support their isolation and utilization as novel functional ingredients to control daily energy intake. The validation of food physiological effects by a multi-system approach integrating human sensations and behaviors with homeostatic and non-homeostatic postprandial responses, despite being costly and time consuming, is mandatory to explain the mechanisms underpinning appetite cues and dietary behavior. This approach can provide a proper scientific foundation for the design of personalized food, which is the stepping stone to open the era of personalized nutrition.

Clinical Trial Registry

This study was registered at ClinicalTrials.gov as NCT01851304.
Data sharing statement

The generated and analyzed datasets after deidentification in the current study are available from the corresponding author on reasonable request.

Ethics statements

The study was conducted following the guidelines laid down by the Code of Ethics of the World Medical Association (Declaration of Helsinki) and all procedures involving human subjects were approved by the Ethics Committee of the University of Naples (Protocol number: 235/13).

Written informed consent was obtained from all subjects and the privacy rights of all subjects were observed.

CRediT authorship contribution statement

Joel M. Walker: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Supervision, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing.
Ilario Mennella: Methodology, Investigation, Supervision.
Rosalia Ferracane: Investigation.
Silvia Tagliamonte: Investigation, Software, Formal analysis.
Ann-Katrin Holik: Formal analysis.
Mark M. Somoza: Conceptualization, Writing - review & editing.
Vincenzo Fogliano: Conceptualization, Methodology, Resources, Writing - review & editing.
Paola Vitaglione: Conceptualization, Methodology, Resources, Supervision, Project administration, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References


