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Subjective satiety and plasma PYY concentration after wholemeal pasta

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A B S T R A C T

Dietary fiber and whole grain foods may contribute to the regulation of appetite; however, evidence has produced inconclusive findings. The objective was to evaluate the effects of an experimental wholemeal pasta on appetite ratings, plasma concentrations of gastrointestinal hormones involved in appetite control, and postprandial glucose/insulin responses in healthy adults. Fourteen healthy adults (7M/7F), mean age 30±2 yrs (mean±SEM), participated in a randomized, controlled, crossover trial. Participants consumed on two different days, at one week interval, 117g of wholemeal pasta or 100g of refined wheat pasta (control pasta), similar in energy and macronutrient composition except for fiber amount, which was higher in wholemeal pasta (11 vs 3 g). Appetite ratings, glucose/insulin/lipid and gastrointestinal hormone responses were measured at fasting and for 4-h after the ingestion of the pasta tests, after which *self-reported* energy intake for 8-h was evaluated. After the wholemeal pasta, the desire to eat and the sensation of hunger were lower (-16%, p=0.04 and -23%, p=0.004, respectively) and satiety was higher (+13%; p=0.08) compared with the control pasta; no effect on self-reported energy intake at subsequent meal was observed. After wholemeal pasta, glucose, triglyceride increased and GLP-1 responses were not different compared to control pasta but insulin response at 30 min (p<0.05) and ghrelin at 60 min (p=0.03) were lower and PYY levels higher (AUC=+44%, p=0.001). The appetite rating changes correlated with PYY plasma levels (p<0.03). In conclusion, consumption of whole grain instead of refined wheat pasta contributed to appetite control but did not seem to influence acute energy balance. Appetite ratings were associated with modifications in PYY hormone concentrations.

1. Introduction

Obesity represents one of the most important public health issues worldwide (Ng et al., 2014). This condition is a well-known risk factor for metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease (Burke et al., 2008; Janghorbani, Salamat, Amini, & Aminorroaya, 2017; Kivimäki et al., 2017); therefore, it is necessary to find strategies to prevent weight gain and, thus, the incidence of metabolic diseases. Regulation of energy intake and appetite represents a way to prevent obesity; however, the mechanisms underlying the regulation of energy intake and appetite are complex and interrelated. Indeed, physiological as well as psychological, social and cultural factors influence appetite, and the sensory properties of foods determine the way individuals select their food and how much they eat (Sørensen, Møller, Flint, Martens, & Raben, 2003).

Epidemiological evidence suggests that higher dietary fiber and whole grain intake is associated with improved body mass index (BMI), body weight and abdominal adiposity (Liu et al., 2003; McKeown et al., 2009) and with a lower risk of type 2 diabetes and cardiovascular disease (Buil-Cosiales et al., 2016; Chanson-Rolle et al., 2015). The main characteristics of whole grain products responsible for potentially beneficial effects on body weight include reduced energy density, a high volume of products and a high fiber content (Karl & Saltzman, 2012).

Several studies have shown that dietary fiber and whole grain

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foods may promote feelings of satiety and reduce hunger in acute condition (Clark & Slavin, 2013). Some studies on this topic used foods rich in fermentable and viscous fibers, given their known beneficial effects on appetite and energy intake (Beck, Tosh, Batterham, Tapsell, & Huang, 2009; Vitaglione, Lumaga, Stanzione, Scalfi, & Fogliano, 2009; Wanders et al., 2011). In particular, viscous fibers can delay gastric emptying and slow down nutrient digestion and absorption, resulting in reduced postprandial blood glucose response; they can also modulate the secretion of appetite regulating hormones from the small intestine (Flint et al., 2007; Jenkins, Kacinik, Lyon, & Wolever, 2010; Kristensen et al., 2010; Wanders et al., 2014). Although viscous fibers result consistently effective on appetite modulation and energy intake, the evidence of this effect is based on a very few studies. On the contrary, the studies which used non viscous fibers (Cioffi et al., 2016; Isaksson, Fredriksson, Andersson, Olsson, & Aman, 2009; Juvonen et al., 2011; Kristensen et al., 2010; Samra & Anderson, 2007; Schroeder, Gallaher, Arndt, & Marquart, 2009; Weickert et al., 2007) have provided conflicting results. Therefore, at present no definitive conclusions can be drawn on the effects of fiber – mainly of non-viscous fibers – on appetite modulation. This inconsistency can be ascribed to specific features of the different studies, such as the amount of dietary fibers used, dietary fiber properties (gel-forming, fermentability, molecular weight and size), different preparation and food matrix (rye or whole wheat bread or pasta) and different nutrient composition of the food. In the light of this, a recent review has also highlighted a remarkable variability of the effects of fiber on appetite, failing to show any clear relation between their properties and efficacy (Poutanen et al., 2017). Moreover, very few studies have evaluated the effects of non-viscous fiber consumption on appetite ratings and, at the same time on gastrointestinal hormones related to appetite regulation and the metabolic glucose/insulin response. Therefore, the aims of the present study were to evaluate the effects of an experimental wholemeal pasta with a high cereal fiber content on appetite ratings and determine its effects on the plasma concentrations of a subset of gastrointestinal hormones involved in appetite control and postprandial glucose, insulin and lipid responses in healthy adults.

2. Material and methods

2.1. Participants

Thirty-five individuals were recruited through advertisings posted at the Department of Clinical Medicine and Surgery of the Federico II University in Naples. Following assessment of their eligibility for the study, eight candidates were excluded because they did not meet the inclusion criteria. Exclusion criteria were known chronic illness, diabetes, regular intensive physical activity, renal failure (serum creatinine >1.7 mg/dL), liver disease, anemia, any other chronic diseases, or use of drugs able to influence glucose and lipid metabolism. Thirteen of those selected declined to participate; overall, fourteen individuals of both sexes (7 male and 7 female), aged 20–50 years, BMI 18–25 kg/m^2, were recruited (Table 1). All participants provided written informed consent, and the study was approved by the Ethics Committee of the "Federico II" University. This trial was registered at Clinicaltrials.gov as NCT02842606.

2.2. Study design

The study was based on a randomized, controlled, cross-over design (Fig. 1). The participants consumed on two different days, at one-week interval, two isoenergetic standardized meals, one based on wholemeal pasta and other on refined wheat pasta (control pasta). Participants were randomly assigned to consume wholemeal or refined wheat pasta using a web-based program. The randomization was carried out with stratification for sex.

At screening, the health status and medical history of the subjects were examined by interview; body weight, height were measured according to standardized procedures (Lohman, Roche, & Martorell, 1988) and habitual fiber intake was evaluated by a 7-day food record. During the previous 24-h before each test day, participants were instructed to consume a low fiber diet (≤8g/4180 kJ) avoiding to consume whole grain cereal products, legumes, some vegetables (i.e. artichokes) and no more than one portion/day of fruit. Moreover, participants were instructed to abstain from performing vigorous physical activities 24 h before each test day.

On the two experimental days, volunteers were invited to the research centre 12-h after an overnight fast. They were interviewed by a dietary 24 h-recall to assess fiber intake on the day before; thereafter, an i.v. cannula (BD Saf-T-intima, Becton Dickinson) was inserted into an antecubital vein to be used for blood sampling at fasting and over 240 min after consuming the test meals. A Visual Analogue Scale (VAS) (Flint, Raben, Blundell, & Astrup, 2000) was performed to assess subjective appetite sensation before and at same time points immediately after blood sampling over 240 min after the test meal. The test meal was served at 9.00 a.m. Participants were allowed to drink only 150 ml of water with the meal and were not allowed to eat or drink anything else over the 240 min after the test meal. On the morning of the test day, participants were asked to abstain from drinking water for at least 2 h before the study.

2.3. Test meal composition

The two isoenergetic standardized meals were composed of 117g of experimental wholemeal pasta (spaghetti) and of 100g of refined wheat pasta (spaghetti, control pasta) seasoned with 150g of tomato sauce. The two pasta tests, based on durum wheat, were similar in energy and macronutrient composition but different in fiber content – which was higher in wholemeal pasta (11 vs 3 g) (Table 2). The portion of wholemeal pasta was larger so as to achieve the same amount of available carbohydrates in the two test meals. Consequently, also the volume of wholemeal pasta meal was slightly larger than that of control pasta meal.

Both wholemeal and control pasta were prepared respectively with whole and refined wheat flour obtained from an Italian cultivar of Triticum durum. However, whole wheat flour was achieved by grinding the wheat kernels decorticated with a dehulling process, to preserve the aleurone layer intact. This process limits to 6% by weight the loss of kernels, preserving most of the dietary fiber and micronutrients in the flour. This way, the fiber content of the final pasta product was about 9,4%. Both whole wheat and refined wheat flour were then obtained according to the standard procedures adopted for grain milling. Participants were instructed to consume pasta (whether test or control) within 10–15 min, according to their randomly assigned sequence.

The two test meals were prepared in the metabolic ward by a dietitian utilizing standardized amounts of wholemeal and refined wheat pasta and tomato sauce. Both wholemeal and control pasta were cooked in 1 L of water containing 4 g of salt for 10 min, drained and seasoned with 150 g of tomato sauce. Tomato sauce was prepared with 250 g of raw tomato puree, oil, garlic, salt and allowed to cook for 30 min. Energy and nutrient composition of each portion of tomato sauce (150g), calculated by a computer program using the food database of the Italian National Institute for Food and Nutrition, provided 1705 kJ, 2 g of protein, 7.5 g of carbohydrate, 15 g of fat and 1.5 g of fiber. The meals were consumed in 15–20 min. Energy and nutrient composition of both the wholemeal and control pasta were provided by manufacturer (Baronia De

Table 1
Characteristics of participants in the study.[a]

Sex (M/F)	7/7
Age (y)	30±2
Weight (kg)	64±2
Height (cm)	169±2
BMI (kg/m^2)	22±1
Systolic blood Pressure (mmHg)	101±2
Diastolic blood Pressure (mmHg)	67±2
Fasting plasma triglycerides (mmol/L)	0.8±0.1
Fasting plasma cholesterol (mmol/L)	4.2±0.2
HDL-cholesterol (mmol/L)	1.4±0.1
Fasting plasma glucose (mmol/L)	5.8±0.1
Fasting plasma insulin (pmol/L)	64.6±7.2
Homa Index	2.3±0.3

[a] Mean±SEM (all such values).

Table 2
Energy content and macronutrient composition of the two types of pasta.

	Wholemeal Pasta (117g)	Control Pasta (100g)
Energy (kJ)	1431	1322
Protein (g)	13.8	11.0
Carbohydrates (g)	69.0	69.2
Starch (g)	66.7	66.6
Sugars (g)	2.3	2.6
Fiber (g)	11.0	3.0
Total Fat (g)	2.6	1.3

Matteis Agroalimentare Spa, Flumeri, Avellino, Italy).

2.4. Appetite sensation and self-reported energy intake after the meals

Subjective appetite sensation (desire to eat, hunger and satiety) was assessed using the Visual Anologue Scales (VAS). Measurements were carried out 30 min and 0 min before the two pasta meals and 30, 60, 90, 120, 150, 180 and 240 min after meals, immediately after blood sampling. These measures were obtained using a 100-mm scale ranging from 0 ("not at all") to 100 ("extremely") (Flint et al., 2000). After the last blood sample drawing (240 min after the test meal was served), self-reported energy intake at subsequent meals was evaluated for 8-h by food diary record in which the participants filled in all foods and drinks consumed, indicating accurately the weight of the portion and the brand of industrial products consumed. Food records were analyzed by a computer program using the food database of the Italian National Institute for Food and Nutrition.

2.5. Plasma metabolite and hormone measurements

Blood samples were collected from an antecubital arm vein in the morning, after a 12-h overnight fast, before the two pasta meals and at 30, 60, 90, 120, 180 and 240 min after the meal, for the measurement of plasma glucose, insulin, triglycerides and gut hormones. Blood drawn in EDTA- or EDTA and aprotinin (for GLP-1 assay) tubes were centrifuged and plasma stored at −80 C until measurement.

2.6. Analytic methods

Plasma glucose and triglyceride concentrations were assayed by enzymatic colorimetric methods (ABX Diagnostics, Montpellier, France) on an ABX Pentra 400 Autoanalyzer (ABX Diagnostics, Montpellier, France). Plasma insulin, ghrelin and PYY concentrations were measured by sandwich enzyme-linked immunosorbent assay methods (ELISA; DIAsource ImmunoAssays S.A., Nivelles, Belgium; Merck-Millipore, Darmstadt, Germany) on Triturus Analyzer (Diagnostics Grifols, S.A., Barcelona, Spain). Plasma active GLP-1 was also assayed by a nonradioactive, highly specific sandwich enzyme-linked immunosorbant assay method (Merck-Millipore, Darmstadt, Germany), which has 100% cross-reactivity with active isoforms of GLP-1 (7–36 amide and 7–37 glycine extended) but no reactivity with inactive isoforms (9–36 amide and 9–37 glycine extended), GLP-2, or glucagon (Di Marino, Griffo, Maione, & Mirabella, 2011).

3. Sample size calculation and statistical analysis

The primary outcome of the present study was the differences in

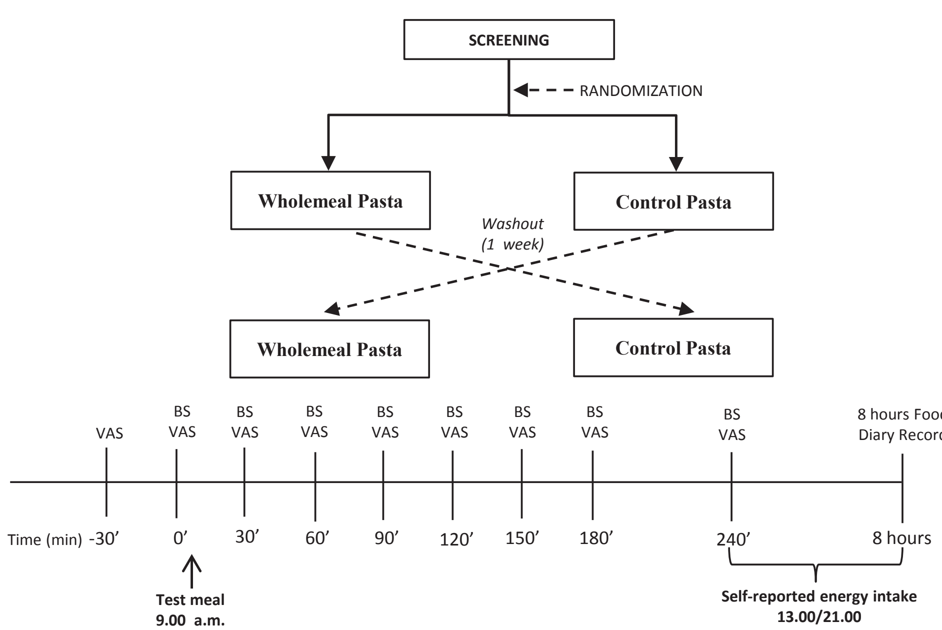

Fig. 1. Study design. BS: Blood sample collection; VAS: Visual Analogue Scale.

appetite ratings. The number of participants needed to detect a 10% difference in appetite ratings between the wholemeal and control pasta, based on a crossover study, was estimated to 13–18 participants, with a type I error $\alpha=0.05$, a type II error $\beta=0.1$ (power 80%) (Flint et al., 2000). All data are expressed as mean±SEM unless otherwise stated. The area under the curve (tAUC) was calculated as the total area above zero for glucose, insulin, triglycerides, desire to eat, hunger, satiety and gut hormones using the trapezoidal rule.

A repeated measures ANCOVA was performed to examine the effects of meal and time and meal × time interaction effect on postprandial response of appetite ratings, gastrointestinal hormones and glucose, insulin and triglyceride responses. In this analysis, postprandial values measured every 30 min over an interval of 240 min were included as levels of the within-subject "time" factor, and wholemeal pasta and control pasta were included as levels of the within-subject "meal" factors; BMI and sex were included as covariates. Differences between time-points of the postprandial curve, were tested by post-hoc analysis (Bonferroni Test). Bivariate associations were assessed by Pearson's correlation. For all analyses, the level of statistical significance was set at $p=0.05$ (two tails). Statistical analysis was performed according to standard methods using the SPSS software 20.0 (SPSS/PC; IBM Armonk, NY, USA).

4. Results

Fourteen individuals, 7 men and 7 women, mean age 30±2 years (mean±SEM), BMI 22.3 kg/m^2, with normal fasting blood glucose, insulin and lipid levels participated in the study (Table 1). Habitual dietary fiber intake obtained from 7-day food records was of 11±3 and 10±3g/4184 kJ per day in men and women, respectively. Dietary fiber intake during the 24-h before each test day was 6.5±0.5 and 6.1±0.3 g/4184 kJ in men and women, respectively. All participants stated that they found the meals appetizing on both test days.

4.1. Appetite sensation and 8-h energy intake after the meals

Responses in ratings of desire to eat, hunger and satiety with corresponding tAUCs are reported in Fig. 2. The desire to eat was significantly lower after the wholemeal pasta compared to the control pasta ($F=3.611$, $p=0.002$; for time × meal interaction; repeated measures ANCOVA). In particular, it was significantly lower at 120, 150 and 180 min after eating the wholemeal pasta compared to the control meal ($p<0.05$); consequently, the corresponding tAUCs were different [9761±1386 vs 11683±1066 mm*240 min (M±SEM), respectively; $F=4.940$, $p=0.046$] (Fig. 2A).

Hunger sensation was significantly lower after the wholemeal pasta compared to the control pasta ($F=3.319$, $p=0.004$ for time × meal interaction; repeated measures ANCOVA). In particular, it was significantly lower at 120, 150, 180 and 240 min after eating the wholemeal pasta compared to the control pasta ($p<0.05$) and consequently the corresponding tAUCs were different [8850±1237 vs 11463±1095 mm*240 min (M±SEM), respectively; $F=13.003$, $p=0.004$] (Fig. 2B). Satiety sensation was not different after the wholemeal pasta compared to the control pasta ($F=1.905$, $p=0.082$ for time × meal interaction; repeated measures ANCOVA). However, it was significantly higher from 90 to 120 min ($p<0.05$) after eating the wholemeal pasta compared to the control pasta while no difference in the corresponding tAUCs were found [14527±1196 vs 12860±962 mm*240 min (M±SEM), respectively; $F=3.446$, $p=0.088$] (Fig. 2C).

Subsequent *self-reported* 8-h energy intake was about 218 kJ lower after the wholemeal pasta than after the control pasta [6004±653 vs 6222±435 kJ (M±SEM), respectively], but this difference was not statistically significant ($F=3.446$, $p=0.693$). Moreover, there was no difference in *self-reported* 8-h energy intake between men and women.

4.2. Gastrointestinal hormone responses

Ghrelin, GLP-1 and PYY responses after the wholemeal and control pasta are reported in Fig. 3.

Postprandial plasma ghrelin levels were not different after the wholemeal pasta compared to the control pasta ($F=0.203$, $p=0.960$ for time × meal interaction; repeated measures ANCOVA). However, ghrelin concentrations were significantly lower at 60 min after eating wholemeal pasta compared to control pasta ($p<0.05$), with no differences in the corresponding tAUCs [110042±8602 vs 119743±10962 pg/ml*240 min, (M±SEM), respectively; $F=1.548$, $p=0.237$] (Fig. 3A). Postprandial plasma GLP-1 levels were not different after eating wholemeal pasta compared to control pasta ($F=0.812$, $p=0.580$ for time × meal interaction; repeated measures ANCOVA), either at each time point of the curve or as tAUCs [978±66 vs 1022±85 pmol/L*240 min, (M±SEM), respectively; $F=1.512$, $p=0.211$)] (Fig. 3B). Postprandial plasma PYY levels were significantly higher after wholemeal pasta than after control pasta ($F=12.817$, $p=0.005$ for time × meal interaction; repeated measures ANCOVA). In particular, they were significantly higher from 60 to 240 min after eating wholemeal pasta than after the control pasta ($p<0.05$) and consequently the corresponding tAUCs were different [14582±735 vs 11671±556 pg/ml*240 min (M±SEM), respectively; $F=11.055$, $p=0.011$] (Fig. 3C). Postprandial plasma PYY levels were inversely associated with hunger sensation ($r=-0.409$, $p=0.038$), and positively with satiety sensation ($r=0.414$, $p=0.035$) (Fig. 4). No significant correlation was found between postprandial plasma insulin concentrations, desire to eat, hunger, and satiety sensations.

Plasma glucose, insulin and triglyceride responses and corresponding tAUC are reported in Fig. 5. Postprandial plasma glucose levels were not different after eating wholemeal pasta compared to control pasta ($F=0.216$, $p=0.981$ for time × meal interaction; repeated measures ANCOVA), either at any time point of the curve or as tAUCs [1599±36 vs 1589±31 mmol/L*240 min, (M±SEM), respectively; $F=0.062$, $p=0.810$] (Fig. 5A). Postprandial plasma insulin levels were not different after eating wholemeal pasta compared to control pasta ($F=0.813$, $p=0.579$ for time × meal interaction; repeated measures ANCOVA). However, insulin concentrations were significantly lower at 30 min after eating wholemel pasta compared to control pasta ($p<0.05$) with no differences in the corresponding tAUCs [41299±3932 vs 46759±5359 pmol/L*240 min, (M±SEM), respectively; $F=1.466$, $p=0.254$] (Fig. 5B). Postprandial plasma triglyceride levels were not different after eating wholemeal pasta compared to control pasta ($F=1.352$, $p=0.628$ for time × meal interaction; repeated measures ANCOVA), either at any time point of the curve or as tAUCs [175±17 vs 187±18 mmol/L*240 min, (M±SEM), respectively; $F=0.238$, $p=0.636$] (Fig. 5C).

5. Discussion

The present study investigated whether an experimental wholemeal pasta compared to a refined wheat pasta affected appetite ratings and self-reported energy intake at subsequent meals for an 8-h period; at the same time, it evaluated the effect on postprandial response of a subset of gastrointestinal hormones involved in appetite control and glucose, insulin and lipid responses in healthy adults. The results indicated that acute consumption of a wholemeal pasta reduced the desire to eat and the hunger sensation and increased satiety. In fact, compared with refined wheat pasta, wholemeal pasta significantly reduced by 16%

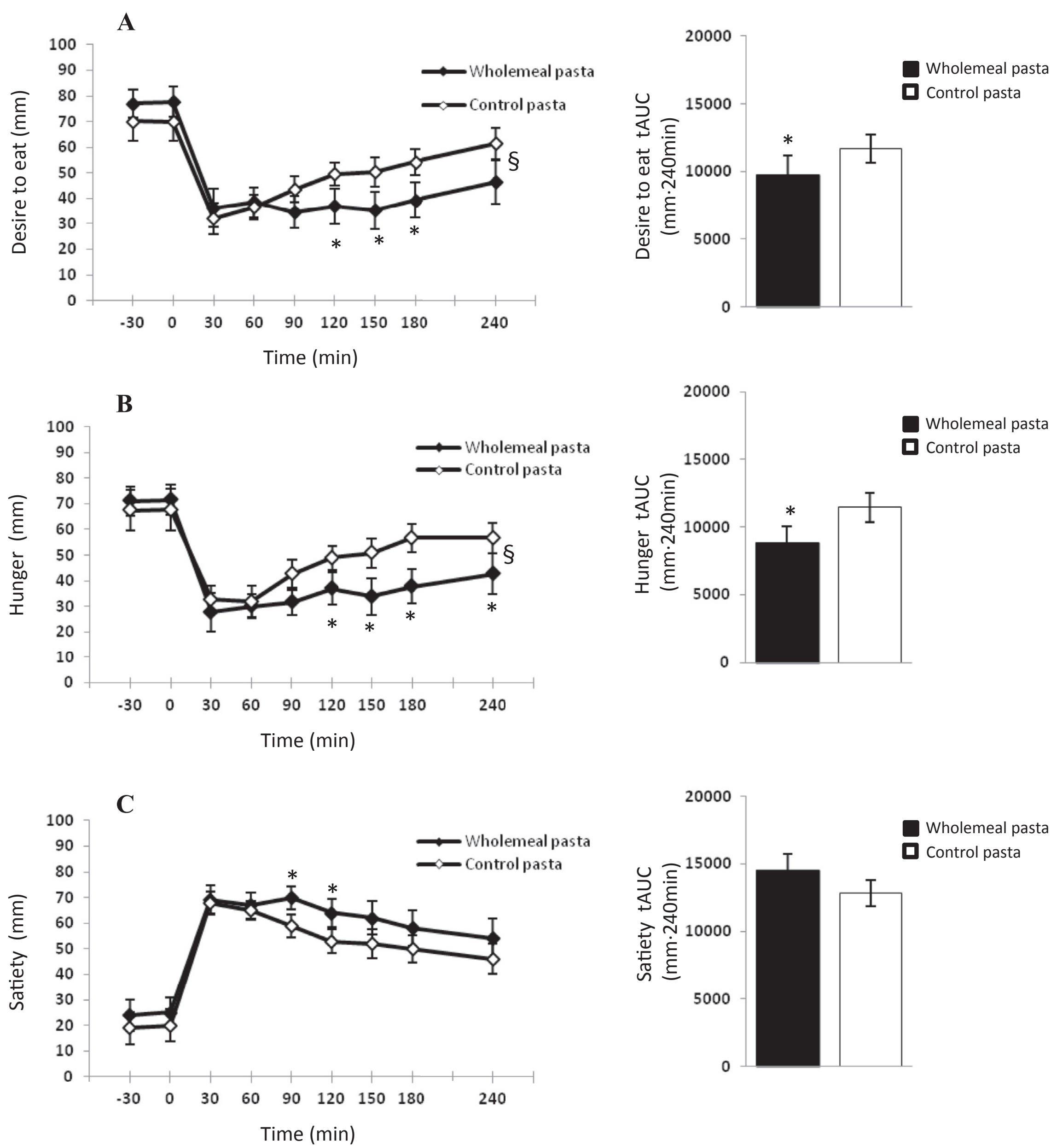

Fig. 2. Postprandial mean rating of Desire to eat (A) Hunger (B) and Satiety (C) and corresponding total area under the curve (tAUC) (means±SEM) after the intake of the wholemeal and control pasta in healthy young volunteers. *p<0.05; Different from Control (Bonferroni-corrected post-hoc *t*-test); §p<0.005 for time × meal interaction by repeated measures ANCOVA.

the desire to eat and by 23% the feeling of hunger, and the satiety sensation was higher by 13%, although this increase did not achieve statistical significance. Our data are in line with the results of Kristensen's study (Kristensen et al., 2010) who reported a greater reduction in hunger sensation and prospective food consumption and a more marked increase in the feelings of satiety and fullness after the consumption of a whole grain wheat bread compared to white wheat bread and with those reported by Cioffi et al. (2016), who compared wholemeal pasta vs refined wheat pasta. Furthemore, Rosén, Ostman, and Björck, (2011) reported similar effects on appetite ratings for different whole rye bread consumed at breakfast compared to refined wheat bread, in addition to a significant reduction in energy intake at a subsequent meal. Samra's study (Samra & Anderson, 2007) also observed a significant reduction in appetite sensation after a meal rich in insoluble fibers from grains; however, in this study, the suppression of appetite parameters was observed with an amount of fiber equal to 33 g, a dose significantly higher than that used in our study (11g), and in Kristensen's (11.9g) and Cioffi's (8.2g) studies (Cioffi et al., 2016; Kristensen et al., 2010).

The changes in appetite ratings observed in our study were associated with a change in gastrointestinal orexigenic (ghrelin) and anorexigenic (PYY) hormones. In particular, wholemeal pasta suppressed the early phase of ghrelin response (at 60 min) more than control pasta and increased postprandial plasma PYY response by 44% (p=0.001). In addition, the wholemeal pasta compared with

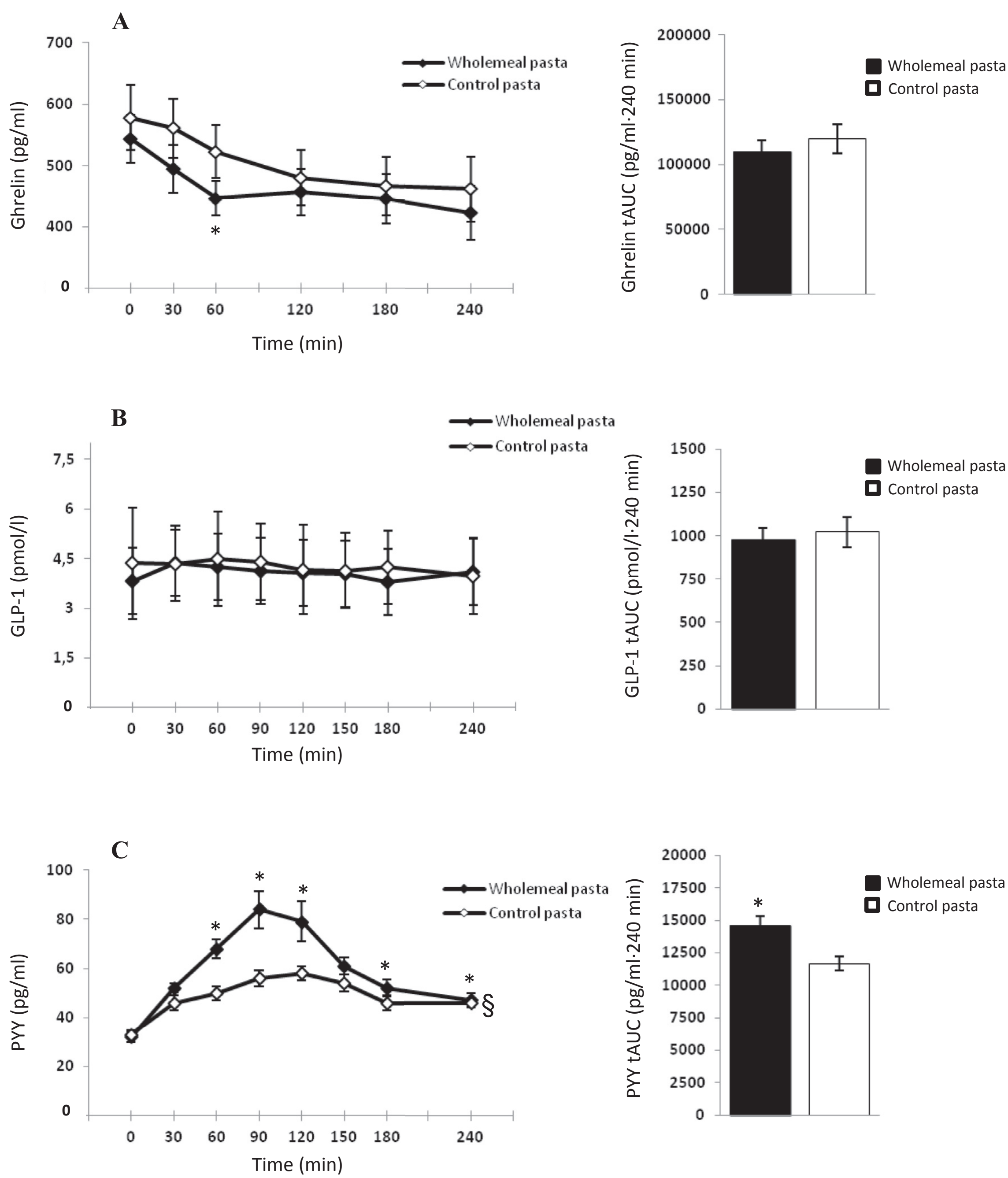

Fig. 3. Postprandial plasma Ghrelin (A) GLP-1 (B) and PYY (C) concentrations (means) and corresponding total area under the curve (tAUC) (means±SEM) after the intake of wholemeal and control pasta in healthy young volunteers. *p<0.05; Different from Control (Bonferroni-corrected post-hoc *t*-test);[§]p=0.005 for time × meal interaction by repeated measures ANCOVA.

control pasta induced the largest increase in postprandial PYY levels between 60 and 120 min with a peak at 90 min – a time point when PYY plasma concentrations were about three fold higher than fasting levels. These results represent the most noteworthy data of our study. In turn, postprandial PYY response significantly correlated with lower hunger and higher satiety ratings, thus suggesting a possible relationship. The association of PYY levels with appetite ratings reduces the possibility that the perception of hunger and satiety might be affected by the general perception of whole grain foods being healthier. Nevertheless, the physiological role PYY in the regulation of satiety in humans has not yet been defined. In fact, some studies investigating the eating-inhibitory effect of peripheral PYY have shown that only a PYY intravenous infusion at a pharmacological dose (~0.8 pmol * kg^{-1}* min^{-1}) resulted in a dose-dependent reduction in food intake mediated by higher plasma PYY levels than those produced by the ingestion of a meal (Batterham et al., 2002). Other studies have shown that the intravenous infusion of supraphysiological dose of PYY inhibited eating

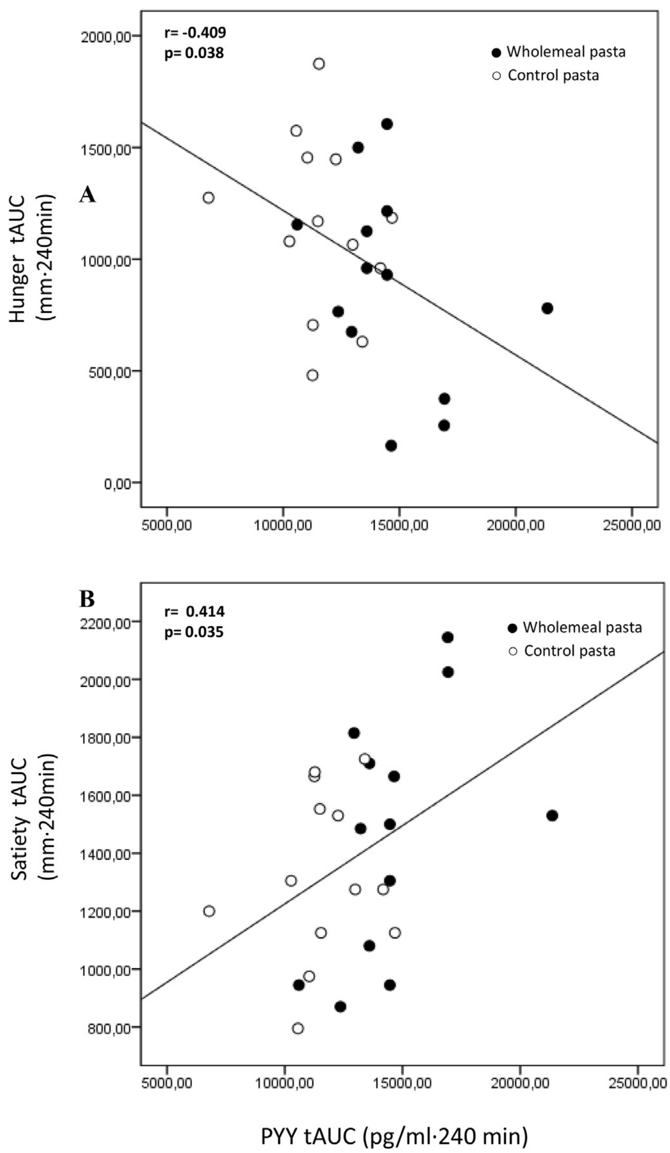

Fig. 4. Correlation between postprandial PYY concentration (tAUC) and Hunger (A) and Satiety (B).

and increased fullness with PYY plasma concentrations similar to that produced by ingestion of meals (Steinert et al., 2017). However, these studies did not rule out an alternative physiological pathway of PYY as satiety hormone; indeed, the molecule could act locally within the gut affecting vagal afferents that mediate the satiating effect. In any case, whether locally released PYY is sufficient to stimulate neuronal pathways that mediate the satiating effects should be investigated.

It is worth stressing that the eating-inhibitory effect in humans and rats of peripheral PYY is due to PYY (3–36) (active form) rather than to PYY (1–36). In our study, plasma PYY (1–36) levels were measured and found increased. Of course, it is likely that the increase in total PYY is accompanied also by an increase in the active form, which actually affects the feeling of satiety induced by wholemeal pasta.

In contrast to the PYY effects, wholemeal pasta did not affect GLP-1 response. It may be due, at least in part, to the same composition of the two test meals in terms of energy and macronutrient intake, except for fiber content. Thereafter, it can be hypothesized that wheat fiber is unable to influence GLP-1 secretion. This hypothesis is supported by results of previous studies where no effects on GLP-1 response were observed after the intake of a wheat bread rich in arabinoxylan (Hartvigsen et al., 2014) or after a whole grain rich diet based mainly on whole wheat products (Giacco et al., 2014). The GLP-1 response after both pasta meals was rather flat, which may be attributed also to the rapid inactivation of GLP-1 by the DPP-4 enzymes (Steinert et al., 2017). As for the satiating effect, previous studies in humans and animal models have reported slower gastric emptying after pharmacological intervention with GLP-1, which is likely to contribute to the satiating effect of GLP-1 (Steinert, Beglinger, & Langhans, 2015), whereas the effect of endogenous GLP-1 is presumably smaller and does not occur in all experimental conditions, as observed in our study.

In our study, the appetite ratings did not result in a significant reduction of self-reported 8-h energy intake at subsequent meals, which was only 3.6% lower after wholemeal pasta in comparison with control pasta. However, a large intra-individual variability was observed after both the experimental and control meals, suggesting that a larger sample size may be necessary to detect a significant change in *self-reported* 8-h energy intake after wholemeal pasta compared to control pasta. However, we cannot rule out that, in our study, the absence of any effect of wholemeal pasta on this parameter could be due to a less accurate method of measurement.

Most of the studies cited above evaluated *ad libitum* energy intake at subsequent meals using a standard method, the buffet approach, namely by providing participants with a standard meal *ad libitum* in order to accurately quantify energy intake. Of course, this method has the advantage of being very sensitive and accurate but it has also the disadvantage that the food or meal to be consumed *ad libitum* may not be what the participant would choose to eat, thus affecting intake. In our opinion, the measurement in free living conditions of *self-reported* energy intake after the pasta tests by 8-h food diary record may provide a result closer to real life situations. The lower sensitivity of this method and the small sample size may have led to some error in our results. Thus, these are limitations of our study. Nevertheless, it remains possible that the reduction of 218 kJ observed after wholemeal pasta – if maintained over time – would have a positive impact in counteracting weight gain, since a higher intake of whole grains is associated with lower BMI in epidemiological studies (Giacco, Della Pepa, Luongo, & Riccardi, 2011) and has shown beneficial effects on body fat in clinical trials (Pol et al., 2013). In addition, a higher fiber intake leads to modest weight-loss effect in diets not intended to be calorically restricted (Kim et al., 2016). Moreover, even in the absence of effects on *self-reported* energy intake at subsequent meal, the improvement of subjective appetite ratings might influence pleasure, hunger reduction as well as mood, possibly contributing, in the long term, to weight loss and adherence to a healthy diet (Hetherington et al., 2013).

The effect of wholemeal pasta on appetite was observed in the absence of changes in glycemic response. Postprandial insulin response was not different between the two test meals, although, 30 min after wholemeal pasta, insulin levels were slightly lower. In addition, insulin response did not correlate with the improved appetite rating changes. In our study, the findings may be partially related to a higher amount of fiber and to different components of whole grains, including specific protein fractions or other fiber-associated molecules. However, we could not rule out that the particle size of fiber and its high amount as well as the slightly higher volume of the wholemeal pasta could have acutely slowed down gastric emptying respect to control pasta, thus resulting in a lower early insulin response after ingestion. In our study, the consumption of wholemeal pasta did not affect postprandial

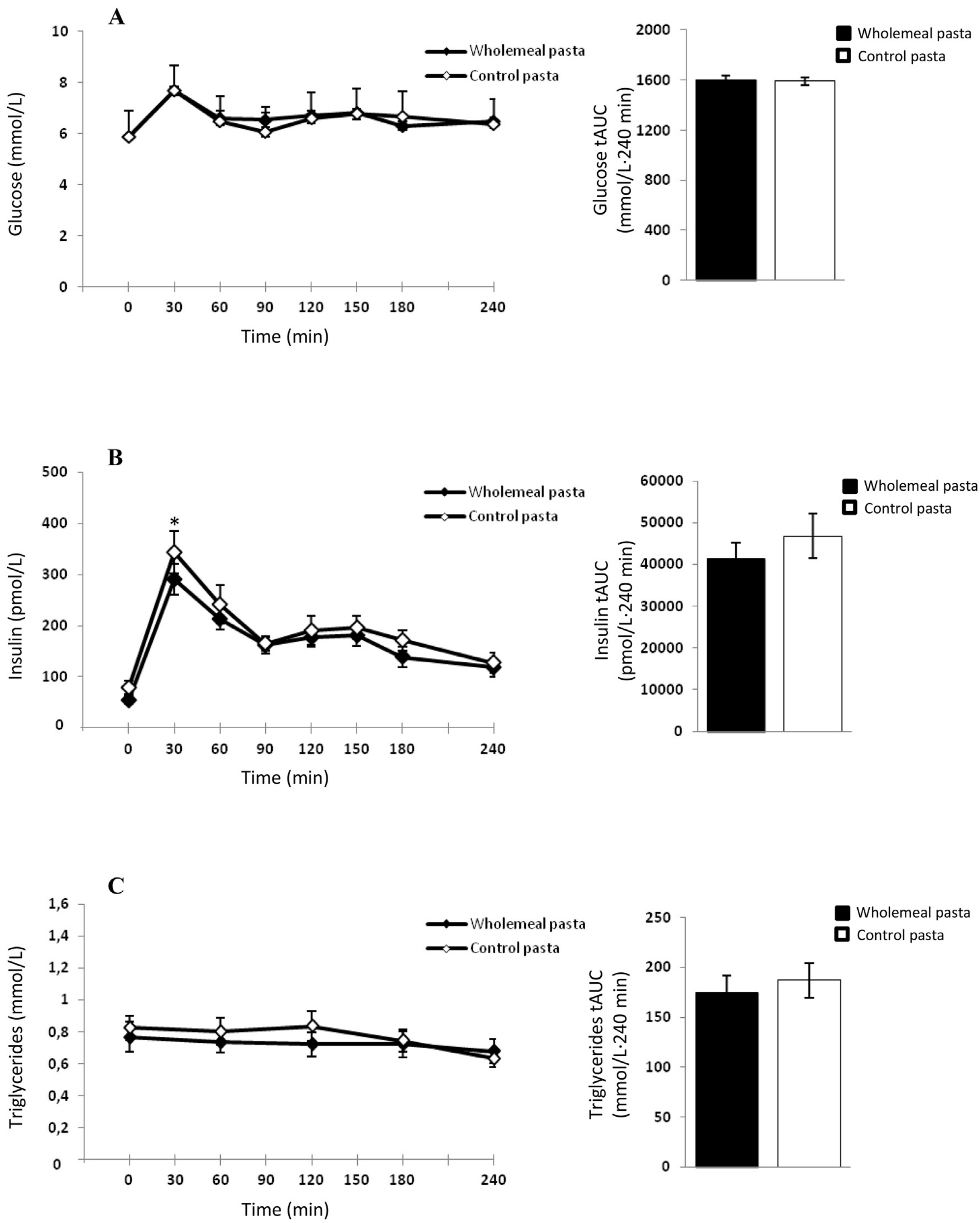

Fig. 5. Postprandial plasma glucose (A) insulin (B) and triglycerides (C) concentrations (means) and corresponding total area under the curve (tAUC) (means±SEM) after the intake of the wholemeal and control pasta in healthy young volunteers. *p<0.05; Different from Control (Bonferroni-corrected post-hoc *t*-test).

triglyceride response. This lack of effect is probably due to the acute nature of the study since the consumption for 3 months of a diet based on whole grains products was able to improve postprandial lipid response (Giacco et al., 2014).

Our study had several strengths. It was one of the first to concurrently evaluate subjective parameters related to the sense of hunger-satiety, gastrointestinal hormones involved in appetite control and metabolic parameters after a wholemeal pasta consumption. Another point of strength is the amount of fiber contained in the wholemeal pasta, which is close to a portion realistically consumed daily by the general population. Pasta is a traditional food of the Italian diet and thus represents a valuable and feasible tool to reach the recommended intake of dietary fiber. The two types of pasta used (wholemeal and control) had equivalent amounts of available carbohydrates. This allowed us to evaluate the effect of fiber without the potential influence of carbohydrate-mediated increase in blood glucose, a parameter that may affect the sense of hunger-satiety. The amount of fiber in wholemeal pasta consumed in our experiment is slightly higher than that of the commercial wholemeal pasta available on the market (6.5g/100g of spaghetti) but this did not affect the sensorial properties of the product, as appreciated by the participants. For these reasons, the consumption of wholemeal pasta should be recommended to the general population to increase their daily fiber intake.

Our study also had some limitations: 1) the small sample size for the evaluation of energy intake at subsequent meals; 2) the slightly larger volume of the wholemeal pasta meal, which might affect the perception of appetite; 3) the absence of any information on particle size of wheat fiber which may influence the physicochemical properties and gel morphology of wheat starches (Sun, Wu, Bu, & Xiong, 2015); 4) the acute design did not permit us to assess whether the effect on appetite was maintained over time or affected body weight; 5) we were unable to evaluate other possible mechanisms through which dietary whole grain could modulate appetite in the long term, including effects of fiber on microbiota composition and short-chain fatty acids production (SCFAs), which may affect appetite or gene expression of an anorexigen hormones (Tremaroli & Bäckhed, 2012).

In conclusion, the results of our study indicate that the consumption of wholemeal pasta reduced the desire to eat and the feeling of hunger, and tended to increase the feeling of satiety. The appetite rating changes seemed to be mediated by gastrointestinal hormones. However, further intervention studies with a larger sample size could help clarify the possible effects on *ad libitum* energy intake at the subsequent meals. Long-term interventions would also help to evaluate whether the effect of whole grain products on appetite suppression is maintained over time and has beneficial effects on body weight. The habitual consumption of wholemeal pasta together with other whole grain products could be a useful way to counteract obesity and chronic-degenerative diseases therewith associated.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.appet.2018.02.004.

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