



Food and health

# Metabolic response to amylose-rich wheat-based rusks in overweight individuals

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## Abstract

**Background/objectives** The amylose-amylopectin ratio influences starch properties. A higher amylose content is associated with slower starch digestion thus reducing the postprandial plasma glucose response and improving the overall postprandial metabolism. So far, limited evidence is available on the metabolic effect of wheat-based foods rich in amylose. This randomised controlled study investigated the acute metabolic effects of amylose-rich wheat-based rusks in overweight subjects focusing on potential mechanisms.

**Subjects/methods** Ten overweight subjects consumed in random order two test meals differing only in the carbohydrate source: rusks prepared with amylose-rich wheat flour (ARR) or conventional wheat flour (control). Blood samples were taken at fasting and over 4 h after the meal. Satiety and intestinal fermentation were evaluated by VAS and H<sub>2</sub>-breath test, respectively.

**Results** ARR reduced plasma glucose response during the first two hours after the meal and the desire to eat, and increased breath hydrogen concentration at 4 h ( $p < 0.05$  for all). Moreover, according to computational models, the ARR slightly reduced intestinal glucose absorption in the first hour after the meal and increased the overall postprandial insulin sensitivity.

**Conclusions** Rusks made with amylose-rich flour could be useful for improving postprandial glucose metabolism and reduce the desire to eat, thus possibly contributing to the prevention and treatment of overweight/obesity, impaired glucose tolerance or diabetes.

## Introduction

Carbohydrates content of the meal is considered the main dietary factor influencing postprandial glucose homeostasis [1–4]. The available evidence indicates that

increased plasma glucose and insulin levels in the postprandial period are risk factors for adverse cardiovascular events and type 2 diabetes also in non-diabetic individuals [5–7]. However, a large reduction of carbohydrates in the habitual diet is neither easy to be accomplished nor recommended, since in a normocaloric diet it would require an increase of protein and/or fat intake with potential untoward consequences on health.

Against this background, it is important to take into account that not only the amount but also the type of carbohydrate foods in the habitual diet may influence postprandial glucose metabolism [8–10]. According to the International Scientific Consensus Summit from the International Carbohydrate Quality Consortium [7], different carbohydrate sources may modulate their impact on postprandial metabolism depending on their characteristics (i.e. carbohydrates accessibility, type and amount of fibre, rate of digestion).

Undoubtedly, starch is the largest source of carbohydrates in the diet worldwide, and it is mainly derived by

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**Table 1** Composition of the test meals

	Rusks		Wheat-bran powder		Fatless ham		Tomato salad		EVO oil		Lactose-free skimmed milk		Orange juice		Water		Sum	
	CRR	ARR	CRR	ARR	CRR	ARR	CRR	ARR	CRR	ARR	CRR	ARR	CRR	ARR	CRR	ARR	CRR	ARR
Amount (g/ml)	117	130	20	—	40	40	250	250	27	28	100	100	90	100	150	150	—	—
Energy (kcal)	438	459	45	—	62	62	49	49	243	252	32	32	28	36	—	—	897	890
Available CHO <sup>a</sup> (g)	81	81	5	—	—	—	7	7	—	—	5	5	7	8	—	—	105	101
Sugars (g)	2	3	1	—	—	—	7	7	—	—	5	5	7	8	—	—	23	23
Resistant starch <sup>b</sup> (g)	—	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7
Dietary fibre (g)	9	18	9	—	—	—	2	2	—	—	—	—	—	—	—	—	20	20
Protein (g)	15	18	4	—	11	11	3	3	—	—	3	3	0	1	—	—	36	36
Fat (g)	6	7	1	—	2	2	1	1	27	28	0	0	—	—	—	—	37	38
SFA (g)	1	1	0	—	1	1	0	0	4	4	0	0	—	—	—	—	6	6

ARR test meal with amylose-rich wheat rusks, CHO carbohydrates, CRR test meal with conventional refined-wheat rusks (control meal); EVO extra-virgin olive, SFA saturated fatty acids

<sup>a</sup>Available CHO = sugar plus digestible starch

<sup>b</sup>Resistant starch was assessed in the flour by AOAC 2002.02

cereals and cereal products [11]. Starch is composed by two different types of glucose polymers: amylopectin and amylose, present in a 3:1 ratio in most cereals foods. The relative amounts of amylose and amylopectin influence the properties of starch and its functionality, due to their different chemical structure—i.e. amylose is essentially linear, whereas amylopectin is highly branched. In particular, starches with higher amount of amylopectin are rapidly digested by  $\alpha$ -amylases present in the human duodenum because the branched structure provides multiple sites for enzymatic hydrolysis; on the contrary, high-amylose starches undergo a slower digestion for the tendency of amylose chains to re-associate (during retrogradation) in double helices structures that are less accessible to the  $\alpha$ -amylases [12]. For these reasons amylose-rich starch is expected to lower plasma glucose concentrations in the postprandial glucose.

Indeed, an accumulating body of evidence shows that amylose-rich cereals consumption may reduce postprandial glucose and insulin concentrations [13–20]. However, the mechanisms underlying these effects have been poorly explored.

In addition, to the best of our knowledge, only one study [20] has been carried out with wheat-based products that represent a relevant source of starch in the human diet.

During the last decade, several technical approaches have been applied to produce high-amylose wheat varieties [21, 22]. In particular, mutant wheat lines that present an increased amylose content have been developed. These lines could be used for the preparation of foodstuffs that might have a lower impact on postprandial glucose response [21–23].

Therefore, the aim of this study was to evaluate with a pilot experiment whether the consumption of amylose-rich wheat-based rusks could induce a better postprandial metabolic response than a conventional wheat-based rusks in a group of non-diabetic subjects with a slightly increased cardiometabolic risk. In addition, potential mechanisms underlying the metabolic effect of amylose-rich starch have been explored.

## Methods

### Participants

Ten non-diabetic volunteers (three men and seven women), aged  $46 \pm 12$  years, with overweight or moderate obesity (BMI  $30 \pm 5$  kg/m<sup>2</sup>) were included in the study. Their main fasting biochemical parameters were: plasma glucose  $100 \pm 9$  mg/dl, total cholesterol  $172 \pm 30$  mg/dl, HDL-cholesterol  $50 \pm 14$  mg/dl and triglycerides  $77 \pm 22$  mg/dl. Exclusion criteria included: established diabetes, dyslipidemia (fasting triglycerides  $\geq 400$  mg/dl; fasting cholesterol  $>270$  mg/dl), history of cardiovascular or peripheral vascular diseases, renal or liver diseases, anaemia (Hb  $< 12$  g /dl), any other chronic disease, use of drugs that influence glucose and lipid metabolism.

The study protocol was approved by the “Federico II” University Ethics Committee and registered at ClinicalTrials.gov, number NCT02702934. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. All participants gave their written informed consent to participate in the study.

## Study design

The study had a randomised crossover design with each subject studied on 2 occasions at least 1 week apart. The randomisation was performed by coin toss. The participants were asked to standardise their dinner and avoid foods rich in dietary fibre (i.e. legumes and wholegrain foods) the day prior to each experiment. Furthermore, they were asked to avoid alcohol or probiotic-containing foods and to refrain from intense physical exercise in the previous day.

On the two experimental days, the volunteers were invited to the research centre after an overnight fast (10–12 h). 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 4 h after the test meals consumption. All participants were interviewed for a dietary 24h-recall and a Visual Analogue Scale (VAS) [24] to assess subjective appetite sensations (hunger, satiety, desire to eat and prospective of food intake) at fasting and 30, 60, 90, 120, 150, 180, 240 min after the test meal consumption. Each subjective appetite sensation was measured using 10 cm VAS with the most positive and the most negative rating at each end of the line. At each time point the participants were given a small notebook containing VAS for each sensation. Participants were instructed to mark on the line the corresponding grade of the sensation they felt.

Moreover, hydrogen was measured in exhaled breath as a marker of colonic fermentation using a handheld Gastro + Gastrolyzer® (Bedfont Scientific Ltd.) [25] at fasting and 60, 120, 150, 180, 240 min after the test meal consumption. Participants were instructed to breathe in deeply, hold their breath for 10 s and then exhale at a steady pace into the cardboard mouthpiece of the device until their lungs felt empty.

## Test meal composition

The two test meals were designed to have the same energy and macronutrient composition; in particular, they had the same amount of available carbohydrate and dietary fibre (Table 1). They differed only for the carbohydrate source: rusks prepared with amylose-rich wheat flour or conventional wheat flour. The conventional refined-wheat rusks (CRR) were commercially available rusks (Barilla G&R F.lli. SpA, Parma, Italy) and were used in the control meal. The amylose-rich wheat rusks (ARR) were prepared using a flour from a mutant wheat genotype with an elevated amylose content (>50%; Botticella et al. in preparation). In this genotype the three homoeoalleles coding the Starch Branching Enzymes IIa (SBEIIa) have been silenced by a Targeting Induced Local Lesions IN Genomes (TILLING) approach [26]. The flour was provided by the University of Tuscia, Italy, and the wheat rusks were kindly manufactured by Barilla (Barilla G&R F.lli. SpA, Parma, Italy) for the

study (more details can be found in supplementary table 1 and supplementary table 2, and supplementary figure 4).

Since the ARR had a higher amount of dietary fibre (18 g vs. 9 g, ARR and control, respectively; Table 1), 20 g of pure wheat-bran powder (Albios Giuliani, Italy) was added to the control meal in order to achieve the same amount of dietary fibre in the two test meals, thus avoiding any possible interference of dietary fibre [27]. The other foodstuffs included in the test meals are reported in Table 1.

The two test meals were prepared in the metabolic kitchen by a dietitian and were consumed in 15–20 min.

## Blood sampling and laboratory methods

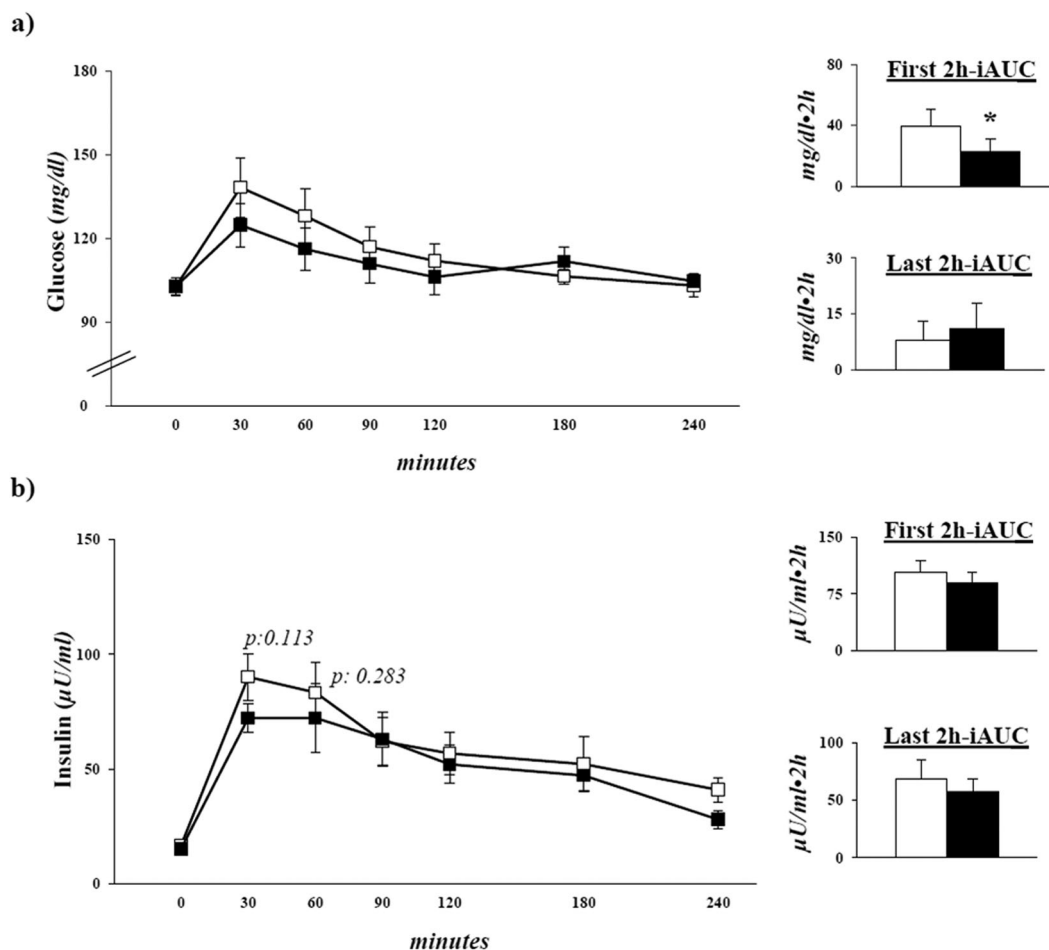
Blood was sampled at fasting and at different time points in the postprandial period according to the specific parameter to be measured: glucose and insulin (30, 60, 90, 120, 180 and 240 min), triglyceride and FFA (60, 120, 180 and 240 min) and GLP-1 and ghrelin (15, 30, 60, 90, 120, 150 and 180 min).

Blood drawn in EDTA- or EDTA and aprotinin tubes was centrifuged, and plasma was stored at  $-80^{\circ}\text{C}$  until the analyses. Glucose, FFA and triglyceride concentrations were assayed by enzymatic colorimetric methods (ABX Diagnostics, Montpellier, France; Roche Diagnostics, Milan, Italy) on a ABX Pentra 400 (HORIBA Medical, Montpellier, France). Insulin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA; DIA-source ImmunoAssays S.A., Nivelles, Belgium) on Triturus Analyzer (Diagnostics Grifols, S.A., Barcelona, Spain). Active GLP-1 was assayed by a nonradioactive, highly specific sandwich ELISA method (Merck-Millipore, Darmstadt, Germany) that had 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 [28].

Human total ghrelin (both intact and des-octanoyl forms) was assayed by a highly specific sandwich ELISA method (Merck-Millipore, Darmstadt, Germany) with 100% cross-reactivity with des-octanoyl human ghrelin, 80% human ghrelin (active) and 70% canine ghrelin (active). The intra- and interassay coefficient of variation for the GLP-1 assay was <5% and for ghrelin assays was <10%. All analyses were performed by personnel blinded to the sequence of the test meals.

## Calculations and statistical analyses

Results are presented as means  $\pm$  SEM, unless otherwise stated. Variables not normally distributed were analysed after logarithmic transformation or expressed as absolute increment/decrement, calculated by subtracting the fasting value from that of each time point of the curve. Postprandial



**Fig. 1** Plasma glucose (a) and insulin (b) response after the two test meals. White square: conventional refined-wheat rusks (CRR; control); black square: amylose-rich wheat rusks (ARR). First 2h-iAUC and last 2h-iAUC represent the incremental area under the curve above the

baseline value for plasma glucose and insulin concentrations evaluated during the first two hours or the last two hours after the meal. Mean  $\pm$  SEM. \*  $p < 0.05$  vs. control (paired sample *t*-test)

responses were evaluated as the area under the curve above/below the fasting value (incremental/decremental) area under the curve (iAUC) calculated with the trapezoidal method.

Insulin sensitivity index (ISI) was calculated as the ISI-comp parameter, according to the method by Matsuda et al. [29].

Intestinal glucose absorption during the meal test was calculated through a mathematical model validated in a non-diabetic population [30]. In brief, the increase of postprandial circulating glucose (dglu) over time (dt) is the result of gain from gut glucose absorption (ABS) and endogenous glucose production (EGP), and loss because of glucose uptake (Rd), predominantly by skeletal muscle. Thus, changes in glucose concentration over time can be expressed as:

$$dglu/dt = 1/VG \times (BW \times (EGP - Rd) + ABS) \quad (a)$$

where BW is the body weight and VG is the oral glucose

distribution volume, and with initial conditions  $glu(0) =$  fasting glucose concentration,  $ABS(0) = 0$ ,  $EGP(0) = Rd(0)$ . Since EGP and Rd are calculated through specific formulas [30], and glucose concentration changes during the meal test are measured, by equation (a) ABS is finally estimated.

Another mathematical model was exploited to assess the FFA kinetics, and calculate some specific parameters [31]. In our representation of FFA kinetics, it is assumed that insulin enters a compartment remote from plasma, where it exerts an inhibitory effect on FFA production. The corresponding compartmental variable is a delayed profile of insulin. Starting from basal production at basal insulin, FFA production decreases with slope  $S_{FFA}$  for suprabasal increases in remote insulin, up to a value beyond which FFA production becomes constant. Thus,  $S_{FFA}$  provides a measure of the lipolysis inhibition due to suprabasal insulin variations, which represents the sensitivity of FFA inhibition to increasing insulin.

**Table 2** Dynamic indices of glucose absorption rate, insulin sensitivity index and FFA inhibition during the two test meals

Parameters	Test meal with conventional rusks	Test meal with amylose-rich rusks
Glucose absorption (g/4 h)	86 ± 5	89 ± 5
ISI <sub>comp</sub> ((mg/dL) <sup>2</sup> /(μU/mL) <sup>2</sup> ) <sup>-1/2</sup>	3.4 ± 0.4	4.0 ± 0.6*
FFA inhibition (ml/microU/4 h)	3.7 ± 1.3	0.7 ± 0.5*

Data are expressed as mean ± SEM

\*  $p < 0.05$  vs. control (paired sample  $t$ -test)

Differences between the two test meals were evaluated by paired sample  $t$ -test. Statistical analysis was performed according to standard methods using the Statistical Package for Social Sciences software version 21.0 (SPSS, Chicago, IL, USA).

## Results

### Fasting parameters

No differences were observed between the two experimental days in relation to fasting plasma concentrations of glucose (ARR: 101 ± 9 mg/dl vs. control: 101 ± 8,  $p$ : 0.931), insulin (ARR: 15 ± 2 μl/ml vs. control: 17 ± 1 μl/ml,  $p$ : 0.246), triglycerides (ARR: 73 ± 7 mg/dl vs. control: 81 ± 7 mg/dl,  $p$ : 0.085), FFA (ARR: 0.49 ± 0.2 mEq/L vs. control: 0.51 ± 0.2 mEq/L,  $p$ : 0.723), GLP-1 (ARR: 5.22 ± 1 pmol/L vs. control: 5.01 ± 2 pmol/L,  $p$ : 0.737), ghrelin (ARR: 494 ± 74 pg/ml vs. control: 419 ± 113 pg/ml,  $p$ : 0.196) and for breath hydrogen excretion (ARR: 16 ± 5 ppm vs. control: 19 ± 4 ppm,  $p$ : 0.481).

### Metabolic response to the test meal

After the ARR-test meal, plasma glucose response was significantly lower (−43%) during the first 2 h as compared with the control meal: first 2h-iAUC were 23 ± 8 mg/dl/2 h vs. 40 ± 11 mg/dl/2 h, ARR and control, respectively;  $p$ : 0.045 (Fig. 1a). Conversely, in the late period after the meal, plasma glucose concentrations were similar for the ARR and the control meal: last 2h-iAUC were 11 ± 7 mg/dl•2 h vs. 8 ± 5 mg/dl•2 h, ARR and control, respectively;  $p$ : 0.588 (Fig. 1a).

Plasma insulin concentrations were constantly lower after the ARR-test meal than after the control meal; however, differences between the two test meals did not reach the conventional level of statistical significance both for the early (first 2h-iAUC, ARR: 90 ± 14 μl/ml/2 h vs. CRR: 103 ± 16 μl/ml/2 h;  $p$ : 0.189) and the late response (first 2h-iAUC, ARR: 57 ± 11 μl/ml/2 h vs. CRR: 68 ± 17 μl/ml/2 h;  $p$ : 0.236) (Fig. 1b).

In order to evaluate the mechanisms underlying the metabolic effects, we utilised computational models that allow to calculate both the intestinal glucose absorption rate and the insulin sensitivity, calculated as insulin sensitivity index (ISI) after the meal.

Results from the computational models are shown in Table 2.

The overall postprandial glucose absorption, calculated as 4h-iAUC, was not different between the two test meals; however, the iAUC of glucose absorption was significantly reduced during the first hour after the ARR meal as compared with the control meal (23 ± 1 g/h vs. 27 ± 2 g/h, respectively;  $p$ : 0.048).

As for insulin sensitivity index (ISI), it was significantly higher (+18%) after the ARR meal than after the control meal (4.0 ± 0.6 vs. 3.4 ± 0.4, respectively;  $p$ : 0.041).

Plasma FFA concentrations fell after both meals; however, the ARR-test meal induced a significantly smaller reduction of plasma FFA concentrations at 120, 180 and 240 min (Fig. 2a). This finding was further substantiated by the FFA inhibition index ( $S_{\text{FFA}}$ ) that was significantly lower after the ARR meal than after the control meal (Table 2).

No significant differences were found for plasma triglyceride and GLP-1 concentrations after the two test meals (Supplementary figure 1 and 2, respectively).

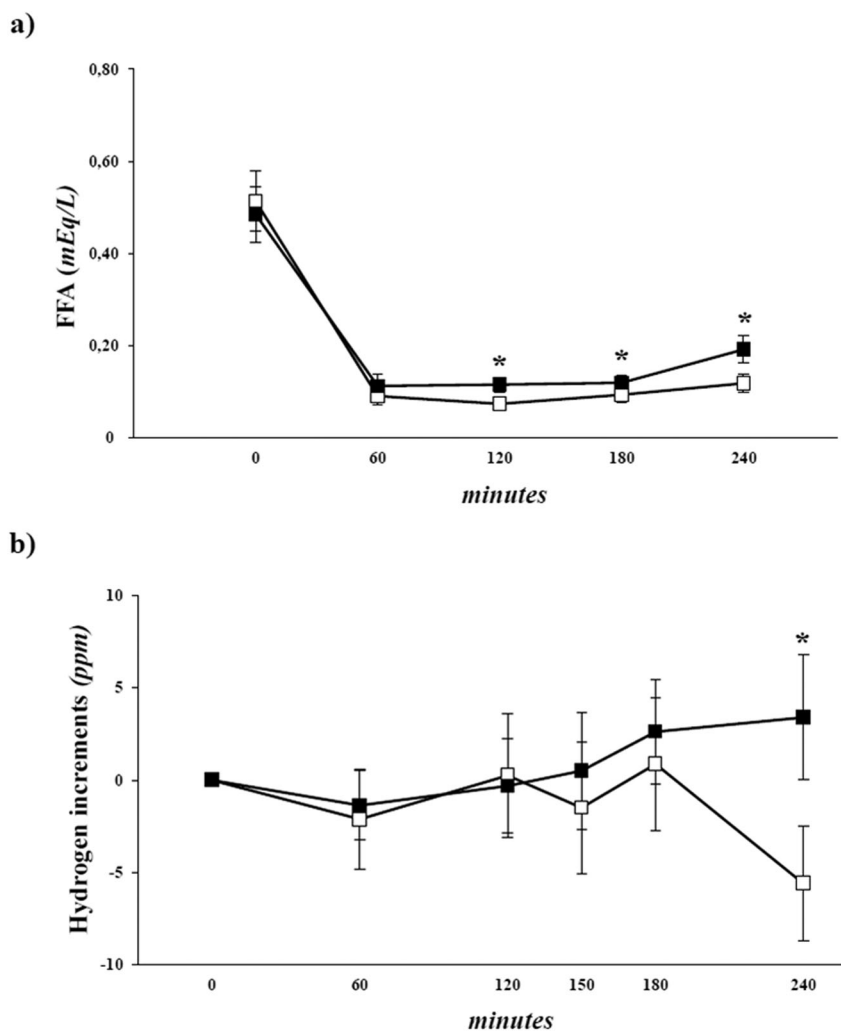
### Intestinal fermentation

The intestinal fermentation was evaluated by the hydrogen excretion during the breath test. No significant differences were observed between the two test meals during the early phase of the postprandial period; however, hydrogen excretion was significantly higher at 240 min after the ARR-test meal as compared with the control meal (Fig. 2b).

### Appetite

After the ARR-test meal, the participants reported a significant reduction of the desire to eat that persisted during the 4 h (Fig. 3). No difference were observed for the other subjective appetite sensations (hunger, satiety and prospective of food intake; data not shown).

**Fig. 2** Plasma FFA response (a) and breath hydrogen excretion (b) after the two test meals. White square: conventional refined-wheat rusks (CRR; control); black square: amylose-rich wheat rusks (ARR). Plasma FFA concentrations are presented as Mean  $\pm$  SEM. Breath hydrogen excretion is presented as mean increment/decrement from fasting value for the single time point  $\pm$  SEM. \*  $p < 0.05$  vs. control (paired sample t-test)



In addition, no statistically significant differences were detected between the two test meals in relation to ghrelin concentrations (Supplementary figure 3).

## Discussion

The main and most relevant finding of this study is that the acute consumption of amylose-rich rusks (ARR; amylose >50%), within the context of a standard meal, significantly reduces glucose response in the first two hours after the meal compared to conventional refined rusks.

It is noteworthy that the composition of the two test meals was similar in terms of the amount of available carbohydrates and dietary fibre. This allows to interpret all differences in postprandial metabolic parameters as the consequence of the proportion of amylose and amylopectin in the wheat flour utilised for the preparation of the two types of rusks tested in this study, since it was the only nutritional parameter that was different between the two meal.

A lower postprandial plasma glucose response has been reported previously in studies carried out with food prepared with barley [13, 14], rice [15, 16], corn [16–19], and, in a preliminary aspect, also with amylose-rich wheat [20], supporting the potential role of high-amylose cereals in plasma glucose control.

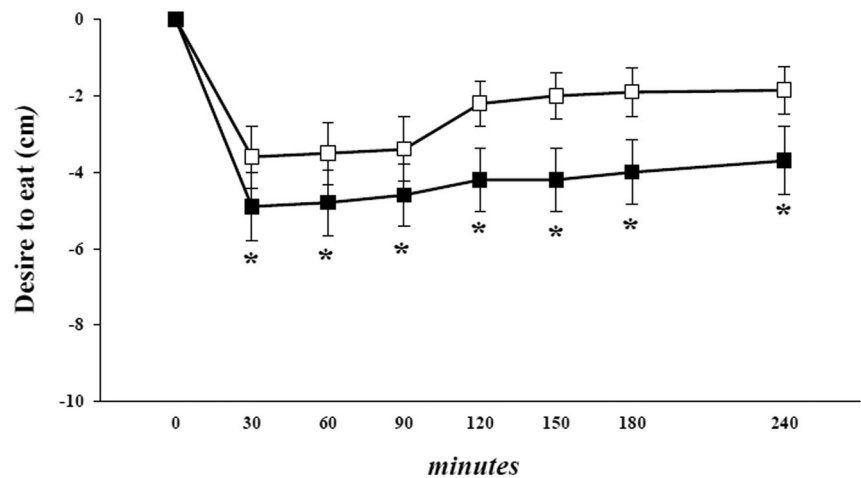
Our study confirms and extends the results of previous studies providing relevant information on the mechanisms underlying the improvement of postprandial plasma glucose response to foods based on amylose-rich starch.

The results of our study indicate that ARR may beneficially influence the postprandial plasma glucose response likely through a slower digestion rate of the starch molecules that may limit also insulin requirement. As a matter of fact, although 4 h-glucose absorption was not different between the two meals, the significant reduction of the iAUC of glucose absorption during the first hour after the meal confirms this hypothesis.

In this study the insulin response was constantly lower after the ARR meal, but differences did not reach the



**Fig. 3** Desire to eat after the two test meals. White square: conventional refined-wheat rusks (CRR; control); black square: amylose-rich wheat rusks (ARR). Data are presented as mean decrement from fasting value for the single time point  $\pm$  SEM. \*  $p < 0.05$  vs. control (paired sample *t*-test)



conventional level of statistical significance. This finding is in line with previous studies where no significant effect of amylose-rich products was observed on postprandial insulin concentrations. However, some studies carried out with foods containing higher amounts of amylose (60–70%) were able to show a significant reduction of insulin concentrations [17–19]. Lower plasma insulin concentrations after the ARR meal may explain the impaired suppression of FFA response after the ARR meal. In fact, it is known that insulin is the most potent hormonal inhibitor of lipolysis; thus, even a small insulin reduction may have triggered the lower FFA inhibition and the smaller postprandial FFA reduction observed in our study after the consumption of ARR meal.

This mechanism may have counteracted the FFA-reducing effect of an increased intestinal fermentation; however, given the acute nature of this study, it is plausible that the observation time was too short to allow a major metabolic effect of SCFA production to become apparent.

Computational models based on the ratio between plasma insulin and glucose concentrations indicate that in our study also postprandial insulin sensitivity was increased after the ARR meal.

In this study, insulin sensitivity was evaluated as ISI index that has not been validated for mixed meal tests. However, although the results may not be completely accurate on this aspect, ISI index is widely used to assess insulin sensitivity in clinical trials, also after mixed meals [32–35].

The increase in insulin sensitivity, calculated as ISI index over 4 h, might be due to the reduction of glucotoxicity as a consequence of the lower postprandial glucose concentrations after the ARR meal. Moreover, it may also be due to the intestinal fermentation, at least in the later period after the meal consumption, as showed by the increment of hydrogen excretion during the breath test at 4 h after the meal. In fact, an increased amylose content in starch

promotes the production of resistant starch that skips intestinal digestion and absorption, and undergoes fermentation by colonic microbiota. The fermentation products of resistant starch include not only gases (methane, hydrogen, carbon dioxide), but also short chain fatty acids (SCFAs: acetate, propionate, butyrate) that can beneficially influence insulin sensitivity as well as glucose and lipid metabolism [36, 37].

Finally, we observed a significant effect of the ARR on the desire to eat; this suggests that ARR may influence appetite and reduce the energy intake, thus representing a potential useful tool for body weight reduction/maintenance. However, this finding was not confirmed by the other subjective parameters relevant for the satiety process (hunger, satiety and prospective of food intake); moreover, it was not corroborated by the results on ghrelin and GLP-1 since their postprandial concentrations did not differ between the two test meals.

One limitation of our study is represented by the small sample size; in fact, it was calculated to detect a mean reduction of about 30% of the glucose response, but it could be not sufficient to observe changes in other parameters evaluated in this study and, in particular, differences in plasma hormonal responses (insulin, GLP-1 and ghrelin).

The strengths of our study are the well-controlled study design, the balanced composition of the test meal, and the optimal compliance of the participants.

In conclusion, the results of the present study show that the consumption of amylose-rich rusks improves postprandial glucose metabolism, increases insulin sensitivity and intestinal fermentation. Therefore, products made with amylose-rich flour could be useful for the prevention and treatment of overweight/obesity, impaired glucose tolerance or diabetes.

Further studies are needed to clarify the effects of amylose-rich products and their mechanisms of action in the long term. Meanwhile, this study adds new information on

the potential benefits of the amylose/amylopectin ratio modification.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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