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ORIGINAL CONTRIBUTION

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In vitro starch digestibility and *in vivo* glucose response of gluten-free foods and their gluten counterparts

Summary *Background* Recently there has been increasing interest in the production of gluten-free (GF) foods and studies on minor cereals and pseudocereals without celiac activity in order to fulfill the specific needs of people affected by celiac disease. GF bread, pasta, biscuits are usually manufactured using different combinations of thickenings and particular food processing procedures that could affect starch digestibility. Carbohydrates, mainly starch from cereals, play an important part in a balanced diet, and dietary guidelines suggest a diet with low glycemic index foods, that is to say rich in slowly digested carbohydrates. *Aim* The present study was aimed at evaluating: 1) the importance of some GF food characteristics in relation to their effects on *in vitro* starch accessibility to digestion, in comparison with traditional gluten products; 2) the *in vivo* metabolic responses to GF foods. *Methods* Firstly, starch digestibility of several products was evaluated *in vitro*. Then, an *in vivo* study was performed on a group of healthy volunteers. Postprandial glucose and insulin responses were evaluated after administration of three GF foods and traditional bread. Triglycerides and free fatty acids (FFA) were also evaluated. Attempts were also made to explore differences in metabolic responses to GF foods in healthy subjects with respect to celiac subjects. *Results* The area under the curve (AUC) of digested starch of GF bread was slightly higher than that of the traditional counterpart. No significant difference was observed in AUCs of digested starch between GF pasta and the traditional pasta. The AUCs of digested starch of quinoa and the two samples of pasta were not statistically different. Significant differences were observed between GF bread and bread-like products. Statistic differences in glucose responses to GF pasta were observed between healthy and celiac subjects. In healthy subjects, the AUCs of glucose response after GF bread were higher than those after bread with gluten. No significant differences were observed between the AUCs of insulin responses to all products tested. Glycemic index (GI) for GF pasta was similar to GI for GF bread while GI for quinoa was slightly lower than that of GF pasta and bread. Two-way ANOVA revealed that quinoa induced lower FFA levels with respect to GF pasta. In addition, triglyceride concentrations were significantly reduced for quinoa with respect to GF bread and bread. *Conclusions* Our results indicate that the different formulations and the food processing procedures used in the manufacturing of GF products may affect the rate of starch digestion both *in vitro* and *in vivo*. It may be worthwhile improving the formulation of these products. Furthermore, quinoa seems to represent a potential alternative to traditional foods, even if further and larger studies are required to demonstrate its hypoglycemic effects.

Key words gluten-free foods – starch digestibility – metabolic responses – *Chenopodium quinoa*

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Introduction

Recently, great interest has been centred on the production of gluten-free foods which could fulfill the specific needs of people affected by celiac disease (CD), the only treatment for which is a lifelong strict diet of foods devoid of wheat, barley and rye [1, 2]. Gluten-free starchy materials, such as maize, rice and potato, are usually used in the manufacturing of bread, pasta, biscuits, and textured using different combinations of thickenings (guar gums, carboxymethyl cellulose, carob flour), and particular food processing procedures different from the conventional ones. In order to produce foods with textural and nutritive characteristics suitable for replacing, at least partially, traditional cereal-based products, food scientists and biochemists have been studying minor cereals and pseudocereals without celiac activity, such as buckwheat, amaranth, and quinoa. The use of these pseudocereals is of great nutritional interest in view of their peculiar composition and of the qualities of some of the minor components in these grains [3].

Carbohydrates, mainly starch from cereals, play an important part in a balanced diet. The degree of digestion and absorption of starches is affected by a number of factors [4, 5]. The glycemic response and consequently the insulin demand appear to be closely related to the enzymic susceptibility of starch [6]. In particular, food composition and processing affect carbohydrates availability and cause different glycemic response [7–9].

Reduction of postprandial blood glucose and insulin response has been shown to improve overall blood glucose [10] and lipid concentrations [11], so dietary guidelines suggest a diet with low glycemic index foods, in other words a diet rich in slowly digested carbohydrates [12].

Since celiac disease is associated with a high incidence of type I diabetes [13], an important task for these patients is to maintain good glycemic control whilst adhering to a strict gluten-free diet. In fact, scanty reports exist on the blood glucose response to GF foods, although this information could be useful to optimise diet planning for celiacs. Thus, the aim of the present study was to evaluate the nutritional properties of carbohydrates of some gluten-free foods.

Firstly, starch digestibility was studied *in vitro* in order to evaluate the importance of some GF food characteristics in relation to their effects on starch accessibility to digestion. Then, an *in vivo* study was performed on a group of healthy volunteers. Postprandial glucose and insulin responses were evaluated after administration of gluten-free pasta and bread, quinoa and bread. Triglycerides and free fatty acids were also evaluated, along with satiety and palatability scores. Attempts were also made to explore differences in metabolic responses to gluten-free foods in healthy subjects with respect to celiac subjects with at least 2 years treated celiac sprue.

Materials and methods

Sample preparation and analysis

Pasta with gluten ("Fusilli" by Barilla Alimentari, Parma, Italy), gluten-free (GF) pasta ("Fusilli" by Bi-Aglut, Plasmon Dietetici Alimentari, Latina, Italy), bread sliced containing gluten ("White Bread" by Mulino Bianco, Barilla Alimentari, Parma, Italy), white GF bread (Bi-Aglut, Plasmon Dietetici Alimentari, Latina, Italy), GF "Crackerbread" (Bi-Aglut, Plasmon Dietetici Alimentari, Latina, Italy), GF "Grissini" – bread sticks – (Bi-Aglut, Plasmon Dietetici Alimentari, Latina, Italy), GF "Crispbread" (Dr. Schär, Bolzano, Italy), GF "Crackers toast" (Glutafin, Nutricia Dietary, Lainate, Italy), quinoa grains (*Chenopodium quinoa*) (Anapqui, Asociacion Nacional de Productores de Quinua, Bolivia) were studied. Products directly edible, i. e. breads, were minced in a blender. Pasta and quinoa were boiled in salted water (10 g NaCl/L) following cooking instructions on the packages, before mincing.

"In vitro" digestibility

In vitro digestibility was evaluated by a multi-enzymic digestion confined within a dialysis tube followed by analysis of the reducing sugars released to permeate [14]. Aliquots of foods containing 2 g starch were placed in a dialysis tube to undergo sequential attacks with pepsin and pancreatic α-amylase to simulate digestion.

Each food underwent three different treatments, the first with active enzymes (digestion), the second with deactivated enzymes (blank), and the third with deactivated enzymes plus a known amount of maltose to allow measurement of sugar diffusibility from the dialysis tube in the presence of food (diffusion). The amount of reducing sugars in the permeate was measured at time 0 and every 30 min for 5 h by colorimetric analysis. A standard curve was prepared by using maltose. The extent of hydrolysis was calculated as 100 × mg maltose equivalents × 0.95/mg starch in sample.

"In vivo" glycemic response

Quinoa, bread with and without gluten and GF pasta were studied *in vivo*, prepared as described in "sample preparation" and consumed whole and dressed with the same amount of seasoning. Each portion contained 50 g available carbohydrates. Nutrient composition of samples was evaluated by standard chemical analysis of the food "as eaten" (Table 1).

Bread and pasta were served after adding 50 g of tomato sauce (by Barilla Alimentari, Parma, Italy). The addition of tomato sauce to foods does not seem to af-

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Table 1 Weight of meal and nutrient composition of 50 g available carbohydrate portions of the foods studied as served

Test foods	Weight of meal (g)	Protein (g)	Water (g)	Carbohydrate (g)	Fat (g)	Total dietary fibre (g)
Bread	100	9.4	31.4	50	3.6	2.8
GF Bread	125	5.7	38.5	50	9.7	3.9
GF Pasta	156	3.2	61.2	50	0.6	2.1
Quinoa	320	3.4	75.9	50	2.0	2.8

GF gluten-free

fect the glycemic response [15]. Boiled quinoa grains were seasoned with 100 g tomatoes, 4.2 g olive oil and 2.5 g basil the day before the test and kept at 4 °C until the test. The samples were cooked in individual portions. White bread sliced was considered as the standard food for normal healthy people.

Seven healthy women aged 20–45 years with BMI 18.5 ± 0.8 kg/m^2 and six celiac women with treated celiac sprue aged 20–45 years with BMI 20.8 ± 3.1 kg/m^2 participated in the study. Informed consent was obtained from each patient before beginning the study, and formal approval of the study protocol was given by the Local Ethical Committee.

Glucose, insulin, free fatty acids (FFA) and triglyceride responses to gluten-free products were evaluated in both groups. Traditional bread was eaten only by the normal group.

The subjects were asked to consume a standard dinner to avoid the "second meal effect" [16]. Furthermore, the volunteers fasted from 10:00 pm the evening preceding the test until the start of the experiment at 8:00 am the next day. An intravenous catheter was inserted into an antecubital vein for blood sampling. A fasting blood sample was obtained immediately before the test meal was eaten. Subjects were required to eat the foods within 15 minutes and to avoid undue physical exertion for the 3 h of the test. Additional blood samples were taken at 15, 30, 45, 60, 90, 120, 150, 180 min after the start of the meal.

Plasma glucose (mg/dL), FFA (mmol/L) and triglyceride (mg/dL) concentrations were assayed by fluorimetric methods using a Cobas Fara II centrifugal analyser (Roche, Basel, Switzerland). Serum insulin concentration (μU/mL) was evaluated by a MEIA (Microparticle Enzyme Immunoassay, Abbott Laboratories, IL, USA).

Satiety and palatability score

Sensations related to satiety were estimated using visual-analogue scales (VAS; mm) [17], which are 100 mm segments anchored at the ends with the terms weak and strong and preceded by the question regarding to the sensation [18]. Sensations indagated were fullness, desire to eat and satiety. Oral instructions were provided about the meaning of these sensations. The volunteers were requested to rank their sensations before and after the consumption of the test meal. Satiety, fullness and desire to eat sensation ratings were expressed as difference between the scores obtained after and before the consumption of the meal. After the consumption of the meal, a score of palatability was ranked, too.

Data analysis

Results are expressed as means ± SD. The areas under the curves (AUCs) of glucose and insulin response over 90 min and *in vitro* starch digested over 5 h were calculated geometrically, according to the method described by [19]. The effect of different foods on *in vitro* starch digestibility incremental areas was evaluated by means of one-way ANOVA for repeated measures. Shapiro-Wilks' W test was used to verify normal distribution of variables. The effect of different foods on glucose and insulin incremental areas in healthy or celiac subjects was analysed by means of one-way ANOVA when variables were normally distributed; differently, Friedman's two-way analysis of variance was applied. Glycemic index (GI) was expressed as per cent of the response of the test food to the standard food, in this case bread with gluten. Differences between the GI of GF foods were evaluated by means of one-way ANOVA.

A two-way ANOVA for repeated measures design was used to verify the effect of the type of food and time (dependent factors) on blood levels of metabolic variables.

A one-way ANOVA was used to evaluate the palatability ratings and the effects of products on satiety sensation of the two groups of subjects.

Differences between the two groups of subjects were analysed by two-way ANOVA using subjects as independent factor.

Results

In vitro digestion study

Table 2 shows the AUCs of digested starch over 5 h for the test meals, expressed as mg dL^{-1}. The AUCs were signifi-

Table 2 Areas under the curves (AUC) (mg min dL^{-1}) of digested starch for the tested foods over 5 h. Data are expressed as mean ± standard deviation

Foods	AUC (mg min dL^{-1})
Pasta	1018 ± 185[a]
GF Pasta	1133 ± 174[a]
Quinoa	1255 ± 275[a]
Bread	3914 ± 191[b]
GF Crispbread	4292 ± 218[b]
GF Bread	4426 ± 656[b]
GF Cracker toast	4468 ± 208[b]
GF Grissini	5502 ± 791[c]
GF Crackerbread	5701 ± 483[c, d]

Values in columns without a common letter are significantly different.
[b] significantly different from [a] ($p < 0.01$)
[c] significantly different from [b] ($p < 0.05$)
[d] significantly different from [b] ($p < 0.01$)
GF gluten-free

cantly lower for the two samples of pasta than for breads ($p < 0.01$), the AUC of GF bread being slightly higher than that of the traditional counterpart. No significant difference was observed in AUCs of digested starch between gluten-free pasta and the traditional pasta.

The AUCs of digested starch of quinoa and the two samples of pasta were not statistically different.

Significant differences were observed between GF bread and like-bread products. In particular, the AUCs of digested starch for GF bread were significantly lower than for Grissini ($p < 0.05$) and Crackerbread ($p < 0.01$). Crackerbread and Grissini were similar and significantly higher than the other like-bread products.

In vivo digestion study

The AUCs of blood-glucose response and insulin response over 90 min for healthy and celiac subjects are presented in Table 3. AUCs of glucose responses to GF foods in celiacs appeared higher than in healthy subjects. In particular, statistic analysis showed significant differences in glucose responses to GF pasta ($p < 0.05$) between healthy and celiac subjects.

Comparison of AUC of glucose response in healthy subjects demonstrated a significant difference between GF bread and the traditional gluten product ($p < 0.01$), the glucose responses after GF bread being higher than those after bread with gluten. No significant differences were observed between GF pasta and GF bread. AUC for quinoa was slightly lower than AUC for GF pasta and GF bread.

Although no significant differences were observed between the AUCs of insulin responses to all products tested, insulin response to bread was the highest while the response to quinoa was the lowest.

Table 3 Areas under the blood glucose curve (AUC) and insuline curves over 90 min after test foods in healthy and celiac subjects. Data are expressed as mean ± standard deviation

Food	AUC of glucose response (mg min dL^{-1})	AUC of insulin response (mU min mL^{-1})
Healthy subjects		
Bread	594 ± 370[a]	2202 ± 862
Quinoa	980 ± 852[a, b]	1194 ± 756
Gluten-free Pasta	1127 ± 758[b]	1656 ± 589
Gluten-free Bread	1266 ± 855[b, c]	1581 ± 851
Celiacs subjects		
Gluten-free Bread	2094 ± 1030	1442 ± 353
Gluten-free Pasta	2261 ± 2473[1]	1569 ± 899

Values in columns without a common letter are significantly different.
[b] significantly different from [a] ($p < 0.05$)
[c] significantly different from [a] ($p < 0.01$)
[1] significantly different from healthy subjects ($p < 0.05$)

The glycemic indices (GI) for the gluten-free foods in healthy subjects are shown in Table 4. GI for quinoa was the lowest but was not statistically significant.

FFA and triglyceride blood responses to the intake of the test meals are reported in Fig. 1. No differences were observed in FFA and triglyceride responses to the different products between healthy and celiac subjects.

Two-way ANOVA used to verify the effect of the type of food and time on blood FFA and triglyceride concentrations revealed that in healthy subjects quinoa induced lower FFA levels with respect to GF pasta ($p < 0.05$). In addition, triglyceride concentrations were significantly reduced for quinoa with respect to GF bread ($p < 0.01$) and bread ($p < 0.001$).

In celiac subjects GF bread induced a higher triglyceride response than GF pasta ($p < 0.01$).

Satiety and palatability score

The palatability scores expressed for test foods and the variation, before and after the consumption of the meal, in satiety, fullness and desire to eat sensation ratings are represented in Table 5.

Palatability for GF bread expressed by the healthy subjects was the lowest; palatability for quinoa was significantly lower than for GF pasta. Comparison of the palatability ratings expressed for GF pasta and bread be-

Table 4 Glycemic index (GI) of gluten-free foods evaluated in healthy subjects. Bread with gluten is the standard food, GI = 100

Food	GI
Gluten-free bread	230
Gluten-free pasta	255
Quinoa	186

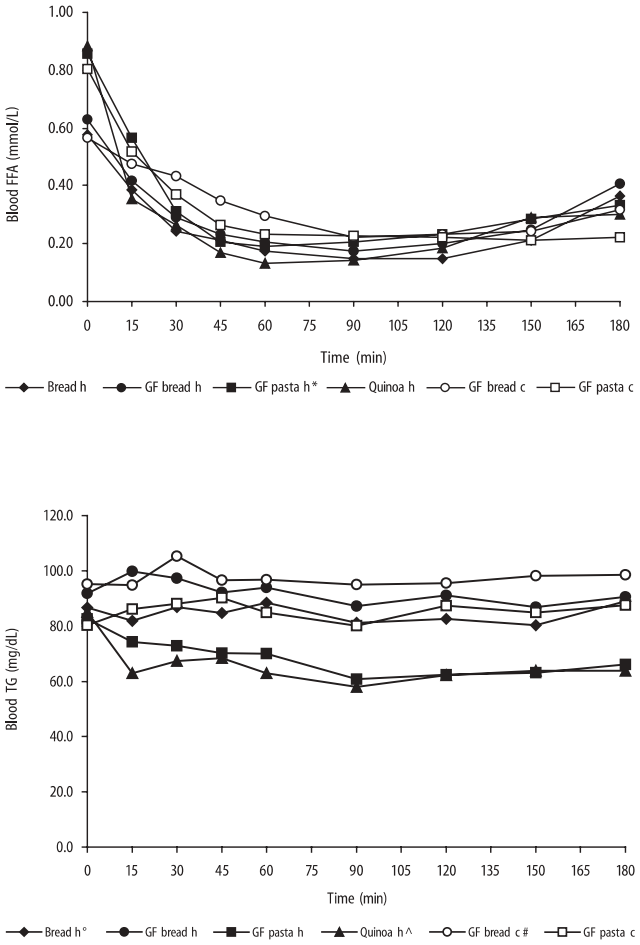

Fig. 1 Mean blood free fatty acid (FFA) and triglyceride (TG) concentrations after the consumption of tested foods in healthy (h) and celiac (c) subjects (*GF* gluten-free; * Significantly different from Quinoa ($p < 0.05$) and Bread ($p < 0.01$) in healthy subjects; ° Significantly different from the other products ($p < 0.01$) in healthy subjects; ^ Significantly different from Bread ($p < 0.001$) in healthy subjects; # Significantly different from GF Pasta ($p < 0.01$) in celiac subjects)

tween healthy and celiac subjects showed a significant difference for GF bread ($p < 0.01$), being higher for celiacs.

ANOVA on satiety sensations (reported as the difference of the rating expressed before and after the consumption of the meal) showed no significant differences among the different products in healthy subjects.

The comparison between gluten-free products showed that, although not significant, the highest decrease in the desire to eat was induced by quinoa consumption while the lowest by GF bread.

In celiacs, GF bread was more effective in modifying sensations than GF pasta, although not significantly.

No significant differences were observed in satiety sensations between the two groups of subjects.

Table 5 Palatability scores and differences in the scores of the satiety sensations expressed after and before the consumption of the test foods for healthy and celiac subjects. Data are expressed as mean ± standard deviation

Foods	Palatability scores	Satiety	Fullness	Desire to eat
Healthy subjects				
Bread	6.9 ± 1.6^{c}	3.7 ± 4.4	3.5 ± 3.9	-3.8 ± 3.8
Gluten-free Bread	1.9 ± 2.0^{a}	5.6 ± 3.5	6.1 ± 1.1	-4.2 ± 2.5
Gluten-free Pasta	7.8 ± 1.7^{c}	5.8 ± 1.2	6.2 ± 2.0	-4.9 ± 2.5
Quinoa	4.7 ± 3.2^{b}	5.6 ± 3.1	6.5 ± 2.0	-6.0 ± 4.1
Celiac subjects				
Gluten-free Bread	8.1 ± 2.0^{1}	4.2 ± 1.6	5.3 ± 0.6	-2.8 ± 2.0
Gluten-free Pasta	7.0 ± 2.6	5.2 ± 2.4	5.8 ± 3.9	-4.0 ± 3.9

[a] significantly different from [b] ($p < 0.05$)
[a] significantly different from [c] ($p < 0.001$)
[b] significantly different from [c] ($p < 0.05$)
[1] significantly different from Gluten-free Bread scores in healthy subjects ($p < 0.01$)

Discussion

Starch digestibility and glycemic response for the majority of gluten-free foods is unknown, although in the "International Tables of Glycemic Index", published in 2002 [20], the glycemic indexes (GIs) of some gluten-free products are shown. Jenkins [8] reported that the GI of gluten-free bread was significantly higher than that of traditional bread, while Packer et al. [21] found that the GIs of gluten-free digestive biscuits, pasta, sliced and unsliced white bread, sliced and unsliced fibre bread were comparable to the published GIs of their gluten replete counterparts.

Theoretically the glycemic response of carbohydrates may be increased following the removal of gluten [8], as the gluten protein network surrounds the starch granules so not allowing amylase to easily access the granule and inhibiting the rate of starch hydrolysis in the lumen of the small intestine.

Since celiac disease is associated with a high incidence of type I diabetes [13], an important task for these patients is to select foods with both beneficial effects on lipid metabolism and minimal hyperglycemic activity whilst adhering to a strict gluten-free diet.

Among the possible mechanisms governing the glycemic response, the rate of starch digestion is likely to play the principal role. Measuring the rate at which carbohydrates in foods are digested *in vitro* has been suggested [8] as a cheaper and less time-consuming method for predicting *in vivo* features. Thus, in the present work the first step was to evaluate starch digestibility of several products *in vitro*. Our results indicate that the different formulations and the food processing procedures used in the manufacturing of gluten-free products may affect the rate of starch digestion. Starch digestibility of

GF bread and bread-like products was in fact generally higher than that of traditional bread, supporting the observation [8] that removal of gluten from flours resulted in an increased rate of amylolytic digestion.

A particularly high starch digestibility was obtained for GF Grissini and Crackerbread. These results support the view that the more processed a food is, the higher the digestibility of starch. Many authors showed that modern technologies of food processing, such as extrusion cooking, explosion puffing etc, increase the availability of starches to amylase because of the higher temperatures and pressures involved which enhance the degree of gelatinisation or result in expansion of the product [4, 9, 22].

As expected, *in vitro* starch digestibility of pasta samples was significantly lower than that of breads [23]. Furthermore, the similar digestibility observed for pasta and GF pasta seems to highlight that the technological process applied facilitates the transformation of starches and flours into textured foods independently from the presence of gluten. Interestingly, the digestibility of quinoa starch was similar to that of pasta.

Despite the fact that *in vitro* approaches could allow the rapid screening of foods for diet planning purposes, some authors [20, 23] have found that the *in vitro* procedure did not always offer a reliable indication of the metabolic behaviour of starchy foods. It is possible that some factors that significantly affect glycemia *in vivo*, such as the rate of gastric emptying, gut hormone profiles, glucose absorption, can determine significant differences with *in vitro* procedures, where all of the human digestive processes cannot be mimicked. Within limits determined by methodologic variables and by the day-to-day variation of glycemic response, the glycemic index (GI) predicts the ranking of the glycemic potential of different meals in individual subjects [24]. Despite controversial beginnings, the GI is now recognised as a reliable, physiologically based classification of foods according to their postprandial glycemic effects [20]. In view of these considerations and of the interesting results we obtained *in vitro*, we turned our attention to those foods that may be considered particularly interesting for celiac individuals such as bread and pasta, which are typical of the traditional eating habits of the Italian population, and quinoa. This crop is very distantly related to cereals that are toxic for celiacs and is nutritionally interesting because of the content of high-quality lysine-rich proteins [25], of polyunsaturated fatty acids, and micronutrients [3, 26, 27]. This means that mixing cereal grains with quinoa can enhance the nutritional value of the resultant product [25].

Even if the number of subjects involved in the study was small, because of problems in their recruitment, some suggestion for future studies could be drawn. In particular, results could give a contribution to the understanding of the potential impact of GF foods on celiac subjects. Our results showed that the AUCs of glucose response to gluten-free products are higher in celiacs than in healthy subjects, suggesting a higher insulin resistance in celiacs. Furthermore, the AUCs of glucose response to gluten-free products are higher than those of bread and quinoa, but statistic differences were observed only between bread and GF bread. Contrary to that expected, insulin response to GF bread was lower than that of bread. These results could be explained by the higher bread protein content which seems to increase insulin secretion. In fact, some authors [28] observed that high protein meals stimulate a higher insulin response which results in lower blood glucose concentration.

To calculate the glycemic index we chose white bread with gluten as the standard food because the low palatability score (1.9%) for the gluten-free bread could have affected the results. It is in fact known that cephalic phase influences the insulin release lowering blood-glucose level [29].

Results obtained *in vivo* do not completely confirm *in vitro* results, as only glycemic responses to GF bread correlated positively with the starch digestibility *in vitro*. Once again, the higher starch digestibility of bread *in vitro* compared with its low glycemic response may be explained by its high protein content. Consequently, in agreement with previous investigations [19, 20, 23], our results suggest that *in vitro* procedures do not have the potential of predicting the metabolic behaviour of starchy foods.

In light of the knowledge that insulin resistance and a raised postprandial triglyceride concentration are risk factors for cardiovascular disease [11], another aim of the present study was to determine the impact of test foods on lipaemia. No differences were observed between the two groups of subjects. Bread stimulated a lower FFA response but a higher triglyceride response than GF pasta, while quinoa induced lower FFA and triglyceride responses than GF pasta. The lowering of the serum triglyceride concentration might be a consequence of the slow release of starch in the small intestine which suppresses the FFA level in blood [11]. FFAs have been shown to impair insulin-mediated glucose disposal and enhance hepatic glucose output and triglyceride production. Thus, the prolonged FFA suppression could result in improved insulin sensitivity and lower blood glucose and triglyceride concentrations.

As concerns the effect of the products tested on food intake, our data suggest that the GF products seem to influence appetite in the same way as foods containing gluten. Only quinoa induced a lower desire to eat and a higher fullness and satiety sensations than the other gluten-free products. The high satiating properties of quinoa may be explained by the weight of 50 g available carbohydrate per portion served. Furthermore, considering that its GI was the lowest we support a study from

Lodwing [30] which, examining the short-term satiating effects of foods, showed that low-GI foods are relatively more satiating than are their high-GI counterparts.

In summary, our results underline how the absence of gluten may induce different metabolic responses eliciting a high glucose response. Considering that there is a strong association between type 1 diabetes and celiac disease, and that celiac subjects participating in our study seemed to have a higher insulin resistance to GF foods, benefits of low GI diets seem to be difficult to achieve in a GF diet because of the limited choice of foods. Thus, it may be worthwhile improving the formulation of these products. Furthermore, our results concerning quinoa suggest that this *Chenopodiacea* should be considered as a viable alternative to traditional foods.

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