Eur J Nutr (2012) 51:947–954
DOI 10.1007/s00394-011-0272-6

ORIGINAL CONTRIBUTION

In vitro and in vivo assessment of the glycemic index of bakery products: influence of the reformulation of ingredients

A. Ferrer-Mairal · C. Peñalva-Lapuente · I. Iglesia · L. Urtasun · P. De Miguel-Etayo · S. Remón · E. Cortés · L. A. Moreno

Received: 12 July 2011 / Accepted: 26 October 2011 / Published online: 10 November 2011

Abstract
Purpose To evaluate whether the modification of ingredients of two bakery products, muffins and bread, reduces their glycemic index, by means of in vitro and in vivo procedures.
Methods In vitro and in vivo glycemic index were evaluated for two types of bread and two types of muffins including one standard product for each category. For the in vitro determination, kinetics of starch digestion method was used. For the in vivo procedure, postprandial glucose measured as IAUC was obtained in a group of eighteen healthy volunteers (ten did the test with muffins and eight with breads).
Results In in vitro, a reduction in the expected glycemic index regarding the control muffin was achieved with the partial substitution of wheat flour by a mixture of resistant starch, dextrin and lentil flour. In breads, with the partial substitution of wheat flour by a mixture of resistant starch and dextrins, a decrease in the expected glycemic index was also observed. In in vivo, a reduction in GI was also achieved both in muffin and in bread. All the obtained GI was higher in in vitro method.
Conclusions Despite the fact that in vitro overestimate in vivo method, the trend in the reduction in GI seems to be similar in both methods. With the substitution assayed, a reduction in the expected glycemic index and the glycemic index were obtained both in muffins and in breads.

Keywords Glycemic index · In vitro · In vivo · Bakery products · Starch hydrolysis · Blood glucose response

A. Ferrer-Mairal · C. Peñalva-Lapuente · L. Urtasun
Tecnología de los Alimentos, Universidad de Zaragoza, Zaragoza, Spain

I. Iglesia · P. De Miguel-Etayo · L. A. Moreno (✉)
GENUD "Growth, Exercise, NUtrition and Development" Research Group, University of Zaragoza, Zaragoza, Spain
e-mail: lmoreno@unizar.es

I. Iglesia · P. De Miguel-Etayo · L. A. Moreno
School of Health Sciences, University of Zaragoza, Zaragoza, Spain

S. Remón
I+D+I, Fundación Parque Científico Tecnológico Aula Dei, Zaragoza, Spain

E. Cortés
Novapan, S.L., Rebola-Panishop Group, Zaragoza, Spain

Introduction

Type 2 diabetes is one of the commonest lifestyle-related diseases [1]. Clinical management of type 2 diabetes requires an adequate selection of carbohydrates. The glycemic index (GI) concept was introduced by Jenkins et al. [2] for diabetic patients in 1981 to quantify the glycemic response to carbohydrates in different foods. It is defined as the incremental blood glucose area following the test food, expressed as the percentage of the corresponding area following a carbohydrate-equivalent load of a reference product [3]. A high-GI food induces a larger area under the glucose curve over the postprandial period when comparing with an equivalent carbohydrate contained in a low-GI food. It was also suggested that reducing the rate of carbohydrate absorption by lowering the GI of the diet could benefit the general population by preventing or delaying the development of diseases that are linked to insulin resistance [4, 5]. In large-scale observational studies, diets with the highest average GI were associated with a greater risk of type 2 diabetes [6], coronary heart disease [7] and

certain cancers [8, 9]. Nowadays, there is significant debate about the role of GI for disease prevention or treatment.

It has been argued that the GI values of food intended to form the basis for glycemic control choices should be based on clinical measurements of the human response to foods. However, since clinical determination is impractical for routine use [10], a promising alternative is in vitro digestion. In vitro models mimic the physicochemical processes involved in carbohydrate digestion that occur in the upper gastrointestinal tract of humans and essentially measure carbohydrate hydrolysis as glucose equivalents. They are economical, non-invasive and applicable to large numbers of samples. A universal standardized method has, as yet, not been adopted from the many available options currently in use [11] and there are large discrepancies about the correlations between in vitro and in vivo determinations [12, 13]. During food product development, in vitro measurements of the glycemic potency are recommended [11].

Reducing the glycemic index is of particular interest in frequently consumed cereal products such as bread and muffins. In general, due to their richness in sugar and white flour, bakery products belong to medium-to-high glycemic index (GI) categories. Both raw materials and baking processes can therefore influence glycemic response [14]. However, the technological role of ingredients in this food group hampers the development of lower GI products. It has been previously showed that it is possible to reduce the GI of bakery products by mixing wheat flour with other types of flours or grains or by adding fiber [15, 16]. Resistant starch can also have potential health benefits and functional properties [17]. The addition of resistant starch has been studied both in bread and cakes and in muffins [18, 19], showing positive effects on texture and slight modifications on sensory characteristics. Legume flours are also interesting candidates to replace wheat flour in bakery products. Legume flours are rich in soluble dietary fiber, with strong interactions between amylose chains [20], leading to lower glycemic indices than cereal or tuber starches. Lentil flour has one of the lowest glycemic indices among pulse flours [21].

The objective of the present study is to evaluate the effects of ingredients reformulation, on reducing the glycemic index of two bakery products. As the GI was determined through in vitro and in vivo procedures, the agreement of both methods was also assessed.

Materials and methods

Materials and sample preparation

Two formulae for each product, muffins and breads, were prepared. The most remarkable modifications of ingredients to reduce glycemic index for each sample are shown in Tables 1 (muffins) and 2 (breads).

The control muffin (MC) was based on a commercial recipe. Sample M1 was prepared with a partial replacement of wheat flour by a mixture of 5% resistant starch, 3% dextrins and 7% lentil flour (Los Pisones, Spain). Ingredients were whipped in a mixer (BM11 SAMMIC, Spain). Muffins were baked at 240 °C for 7 min.

The control bread (BC) was based on a commercial recipe for long shelf-life bread. B1 was formulated by a mixture of 6% resistant starch (ActiStar 11700, Cargill, France) and 3% dextrins (Nutriose FB06, Roquette, France).

Bread was fermented for 90 min at 30 °C and a relative humidity of 80% in a proofer (IVERPAN FC-18/00, SALVA, Spain) and baked for 20 min at 180 °C in a conventional industrial oven (LT-4 + H Oven, SALVA, Spain).

All the samples were cooled at room temperature for an hour and then wrapped in a plastic film until in vitro and in vivo analysis sampling.

The in vitro study

In vitro starch hydrolysis

A previously reported in vitro method [22] with slight modifications was used. The aim of the in vitro starch

Table 1 Muffins formulations showing major ingredients components and type of substitution (% on flour basis)

Muffins		
Ingredients	MC	M1
Sucrose	23	23
Soft wheat flour	29	14
Resistant starch	–	5
Dextrins	–	3
Lentil flour	–	7

MC, control muffin; M1, muffin 1

Table 2 Breads formulations showing major ingredients components and type of substitution (% on flour basis)

Bread		
Ingredients	BC	B1
Forced wheat flour	36	30
Soft wheat flour	24	20
Rye flour	–	–
Resistant starch	–	6
Dextrins	–	3

BC, control bread; B1, bread 1

hydrolysis was to simulate the in vivo procedure. Samples were formerly dehydrated and those with more than 5% of fat (muffins) were defatted using Soxtec extraction with petroleum ether (SOXTEC System 2055, Foss, Denmark) [23]. The oral phase was simulated by means of mechanical disaggregation through an 8-mm plate (Model MG450, Kenwood, North Ryde, NSW) of 50 mg food portions. The gastric phase was developed for 1 h at 40 °C with 10 ml of HCl-KCl buffer pH = l.5 and pepsin (Sigma P-7000). The intestinal phase was carried out in sodium potassium phosphate buffer 0.05 M pH 6.9 containing pancreatic amylase (Sigma A3176). Samples were then incubated at 37 °C in a shaking water bath. 0.2 mL aliquot samples were taken from each tube at 0, 30, 60, 90, 120 and 180 min and then immediately analyzed for reducing sugars. This was done using 3, 5-dinitrosalicylic acid method using a glucose standard curve patron. The glucose was converted into starch by multiplying by 0.9. Samples were analyzed in triplicate on two different days, obtaining 6 replicates for each sample. Commercial white bread (WB) was also analyzed as reference product.

A non-linear model established by Goñi et al. [22] was applied to describe the kinetics of starch hydrolysis. The area under the hydrolysis curve (AUC) was calculated using the following equation:

$$\mathrm{AUC} = C_{\infty}(t_{\mathrm{f}} - t_0) - \left(\frac{C_{\infty}}{k}\right)\left[1 - \mathrm{e}^{-k(t_{\mathrm{f}} - t_0)}\right]$$

C_{∞} corresponds to the concentration at equilibrium (t_{180}), t_{f} is the final time (180 min), t_0 is the initial time (0 min) and k is the kinetic constant. The calculated hydrolysis index (calcHI) was obtained by dividing the area under the hydrolysis curve of the sample by the area obtained for white bread (WB). The expected glycemic index (eGI) was calculated using the equation described by Granfeldt et al. [24].

$$\mathrm{eGI_{HI}} = 0.862 \times \mathrm{calcHI} + 8.198$$

The in vivo study

The procedures for the determination of the glycemic index (GI) in food products were based on the ISO/DIS 26642 provided by the Spanish Association of Standardisation and Certification (AENOR) [25] and also from the reviewed literature [13, 26, 27].

Subjects

Between April and June 2009, twenty-two subjects were recruited as volunteers for the in vivo glycemic index study in the city of Zaragoza (Spain). The inclusion criteria were that subjects should be healthy males or females, aged between 18 and 40 years old. The exclusion criteria were the use of medications affecting glucose tolerance (excluding oral contraceptives), the presence of diseases or drugs influencing digestion or absorption of nutrients, major medical or surgical events requiring hospitalization within the past 3 months, known diabetes mellitus and related conditions, and food allergy or intolerance. Each subject gave written informed consent for the study, and approval was obtained from the Ethical Committee of Clinical Research of Aragón (CEICA). Subject identification numbers were assigned sequentially in the order in which participants were enrolled.

The weights and heights of subjects were obtained using standardized procedures.

Due to the abnormal results obtained in the first oral glucose tolerance test (50 g of anhydrous glucose), three subjects were excluded and the final number was nineteen. Ten subjects participated in the test of muffins and nine in the testing of bread. One of the subjects who participated in bread testing was excluded as a result of the analysis of GI of the modified bread 1 because the value obtained for the GI was unusually high (>2SD) [26]. Finally, eighteen young healthy subjects (4 males and 14 females) were included for further analysis (27.0 ± 4.63 years; 22.68 ± 2.93 kg/m^2 of BMI; 1.74 ± 0.19 m^2 of body surface using the Mosteller formula).

Test meals

Each subject completed the set of five visits: 2 days to obtain the IAUC (Incremental Area under the Curve) for the reference food (50 g of anhydrous glucose) and three more for testing the products. The test meals consisted of two types of muffins and two types of bread containing 50 g of available glycemic carbohydrate, defined as total carbohydrate minus dietary fiber. The final edible amount of each test product was 110 g of MC, 133 g of M1, 113 g of BC and 129 g of B1. Test products were consumed with a maximum of 500 mL of water. Each product was compared with the results obtained from the reference food. Incremental area under the curve was calculated for each test product using the trapezoidal method and compared with the average AUC ($n = 2$) for the reference food in each subject. Any area under the baseline (fasting point) was ignored. Participants were asked to fast 12 h before the analysis. The GI was determined as the mean ± SE in 10–8 different subjects and was calculated by dividing the AUC obtained after the tested products by the AUC obtained after glucose intake as previously described**.** Therefore, the GI of any one food was based on between 160 and 128 separate glucose determinations (10–8 subjects, 8 time points, 2 reference food tests and 1 food test). Blood glucose points were determined in a Clinical

Analysis Laboratory by electrochemical detection coupled enzyme system [25].

Data analysis

Data were analysed using SPSS v.16 for the in vivo method. Results of GI are expressed as means, SD (standard deviation) and SEM (standard error of the mean). The coefficients of variation (CV = 100 × mean/SD) for the AUC (Area under the curve) and for the GI were calculated. The CV of AUC of the two tests with the reference food (anhydrous glucose) to check the intra-individual variation, and the CV of the GI for each product to check the inter-individual variability. A paired *t* test was applied to look for differences between the first and the second test with the reference food (anhydrous glucose) in all the subjects and also to know whether obtained differences in GI between products were significant. A *t* test was applied to search for differences between weight status regarding the obtained GI. Correlations were performed to search for relations between BMI or body surface area and obtained GI. To assess the agreement between in vitro and in vivo methods, an adaptation of Bland–Altman scatter plot was used. Instead of using the average value of in vitro and in vivo measurements, only the in vivo values were used, as this is the reference method. Analysis was conducted at the 0.05α level.

Results

The in vitro study

Table 3 shows the equilibrium constant (C_∞), the kinetic constant (k), the calculated hydrolysis index (calcHI) and the estimated glycemic index (eGI) for muffins and breads. In muffins, partial replacement of wheat flour by a mixture of resistant starch, dextrins and lentil flour (M1) slightly reduced the eGI (68.1 ± 1.6 vs. 64.5 ± 1.8). In breads, partial replacement of wheat flour by a mixture of resistant starch and dextrin (B1) slightly reduced the eGI (82.2 ± 2.9 vs.76.4 ± 2.3). Calculated HI values for muffins and bread samples showed similar trends to estimated glycemic index.

Figure 1 shows the AUCs of digested starch over 3 h for the test products. The AUCs were significantly lower for the bread and muffins than for white bread. Muffins showed lower AUCs than breads.

The in vivo study

Obtained GI and AUC were normal distributed. Mean AUC and subsequent GI values for food products with their standard deviations, standard errors and coefficients of variation (CV) are shown in Table 4. The mean within-subject CV (±SEM) for the two tests of the reference food were 40.33 ± 9.56% for the subjects who made this study with muffins and 26.76 ± 6.47% for the subjects who tested bread. There were no significant differences between the first and the second measures.

A reduction in GI was obtained for muffins and breads regarding the control products. Control muffin showed a GI of 62.70. With the substitution assayed, a GI of 39.14 was measured. For breads, it was also observed a reduction in GI from 64.39 (BC) to 59.91 (B1).

The CV of GI for muffins was in the range from 69.70% for M1 to 79.01% for the MC; and for bread, from 31.33% for B1 to 45.77% for BC.

Table 3 Concentration at equilibrium (t_{180}), kinetic constant (k), calculated hydrolysis index (calcHI) and expected glycemic index (eGI) for all types of muffins and breads

Samples	C_∞	k	calcHI	eGI
Mean MC	29.8	0.25	69.5	68.1
SEM	0.84	0.03	1.8	1.6
Mean M1	28.5	0.13	65.3	64.5
SEM	0.98	0.01	2.1	1.8
Mean BC	36.4	0.35	85.8	82.2
SEM	1.44	0.01	3.4	2.9
Mean B1	33.6	0.32	79.1	76.4
SEM	1.13	0.01	2.6	2.3

MC, control muffin; M1, muffin 1; BC, control bread; B1, bread 1

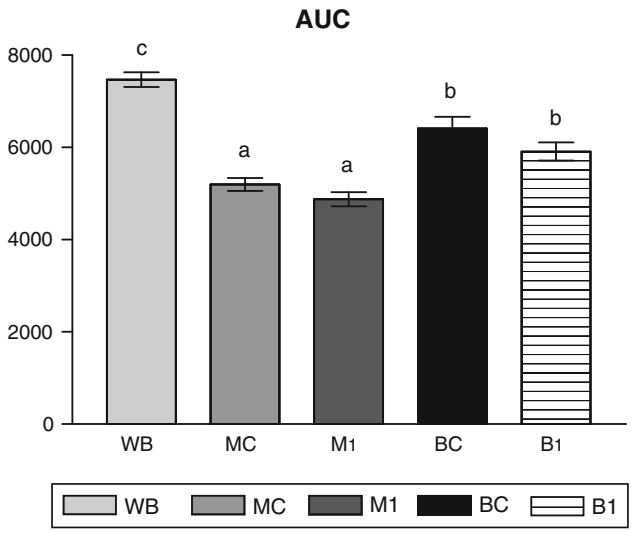

Fig. 1 Area under the curve for the in vitro starch hydrolysis at 180 min for all types of muffins and breads. WB, white bread reference product; MC, control muffin; M1, muffin 1; BC, control bread; B1, bread 1. Data with the same *letter* are not significantly different at the $P < 0.05$ level

Table 4 Glycemic index values and AUC for all types of muffins and breads and for the reference food (based on the results of eight subjects in case of muffins tests and in ten subjects in case of breads tests)

	Mean	Standard error	Standard deviation	Coefficient of variation (%)
Muffins				
Glucose 1 (AUC)	170.92	27.56	87.14	50.98
Glucose 2 (AUC)	167.74	28.70	90.76	54.11
MC (AUC)	89.86	14.11	44.63	49.67
M1 (AUC)	61.88	9.73	30.78	49.74
GI MC	62.70	15.67	49.54	79.01
GI M1	39.14	5.76	18.22	46.55
Bread				
Glucose 1 (AUC)	122.86	25.03	75.08	61.11
Glucose 2 (AUC)	155.11	31.58	94.75	61.09
BC (AUC)	89.22	22.16	66.48	74.51
B1 (AUC)	85.12	13.34	37.74	44.34
GI BC	64.39	9.82	29.47	45.77
GI B1	59.91	6.64	18.77	31.33

AUC, area under curve; MC, control muffin; M1, muffin 1; GI, glycemic index; BC, control bread; B1, bread 1

When the *t* test was applied, no significant differences were noted in the obtained GI values for any of the products, whether muffin or bread, between overweight or non-overweight and male or female subjects (results not shown). There was no correlation between the body mass index or body surface area and the obtained GI for any of the products, regardless of the product.

Bland–Altman figures were used to determine the difference in the obtained GI between the two methods (Fig. 2). It showed a disagreement between them: glycemic indices obtained through in vitro analysis were higher than those obtained through in vivo analysis for breads and muffins.

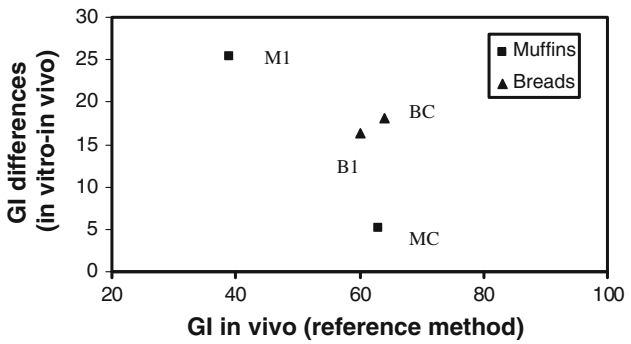

Fig. 2 Difference in GI values obtained from in vitro and in vivo analysis. Adaptation of Bland–Altman method. MC, control muffin; M1, muffin 1; BC, control bread; B1, bread 1

Discussion

Our results show the lack of a strong correlation between the results obtained by means of in vitro and in vivo methods, probably explained by both the characteristics and limitations of both methods and the complex matrix characteristics of the bakery products.

Matrix could influence the starch hydrolysis index. The glycemic effect of foods depends on the food texture and particle size [28], type of starch (the amylose/amylopectin ratio) [29], the physical entrapment of starch molecules within food, food processing and other ingredients, such as sugars, fat, protein, dietary fiber and anti-nutrient content [30–32].

For the determination of in vitro GI, procedural analysis was based on starch hydrolysis and the analysis of reducing sugars. In vitro determination to evaluate the effect of ingredients substitution on the glycemic index has been previously described by other researchers but there is no standardized methodology for the determination of carbohydrate digestion rate [22, 24, 33]. Sample preparation is different in each particular method. Hydrolysis may be performed under unrestricted or restricted (dialysis) conditions. The sample may be broken down by milling or by using subjects to chew the samples. Recent studies concluded that a non-restricted (test tube) mincing method showed good potential as a new in vitro starch digestibility method for predicting GI in grain foods [33].

Other source of variation regarding in vitro methods is the use of proteolytic enzymes in combination with amylases in different combinations and concentrations [22, 24, 33]. Also, the time point from the hydrolysis curve to establish the relationship with in vivo data was different from one method to another being 90 min the time of preference in most studies. However, all methods measured the starch hydrolysis from 0 to 180 min.

Besides, the hydrolysis index measured using in vitro methods is then transformed into estimated GI by using different empiric equations.

The values of eGI obtained for control bread (82.2) were similar to the values obtained by other authors (70, 65.31) [33, 34]. Muffins have been less studied, although there are studies on bakery products like conventional cakes with similar eGI values [23]. Moreover, eGI values were in the range of in vivo data [35] for both bread (62–95) and muffins (44–102).

When a mixture of resistant starch and dextrins was added both to bread and muffins at low percentage, a little reduction in the calcHI and consequently eGI was achieved. Resistant starch is known to exert a similar effect to some dietary fibers by increasing the indigestible carbohydrate portion in small and large intestines [36], thus reducing the rate of dietary carbohydrate absorption. Some

authors [15, 29, 36–39] observed a diminution of the starch hydrolysis rate in vitro when high contents of resistant starch were added. In summary, according to the results obtained by means of the in vitro method, the joint addition of several ingredients with reducing GI properties, added in low percentages, shows a decreasing tendency.

Regarding in vivo determination, results suggested a GI reduction achieved through the reformulation of every product. In an in vivo study in which white kidney bean extract was added in bread in different amounts and formats, significant reductions were not observed in all the studied formulae [40]. However, the GI of all the bread in our study is included in the range of values obtained in other studies for white bread as well as their CV [41]. Sample size characteristics could be another important explanation. According to FAO/WHO/ISO standards [25], at least 10 subjects are necessary to develop these tests; in the case of the bread test, only eight subjects were finally used. However, published material highlights other studies, which reported a similar sample size [42]. Another possible limitation could be gender imbalance, as the difference in our sample is quite considerable (14 females against 4 males); however, material from other studies shows that there are no significant differences between genders [13, 43]. Additional subject characteristics did not appear to have a significant effect on mean GI values, which have already been described [13] as they were also observed in our study.

Another important issue was the intra- and inter-subject variability obtained in the assessment of the GI of the products in vivo, similar to those previously reported [26, 41]. This variability has been largely discussed in existing published material. One of the points mentioned is the use of venous blood instead of capillary blood. Procedures recommended by FAO/WHO (1998) allow variation in blood sampling. However, current recommendations are that capillary blood is preferred in determining GI because the oscillations in venous blood are higher than in capillary blood. Factors like stress, recent exercise, alcohol consumption and smoking habits have been shown to impair glucose tolerance and insulin sensitivity [44]; length of fasting time, and previous meal consumption can also influence this variability [45] and that simply advising subjects to avoid certain types of foods is cost-effective [46]. In our study, these factors were not controlled. However, there are some studies which concluded that in practice, there is no need for rigorous control of exercise, smoking or diet the day before the test [13, 44].

The fact that we only performed twice the tests with the reference foods (anhydrous glucose) could be considered as a limitation as well. Nevertheless, a recent study suggested that there is no evidence to justify repeating it three times rather than two, because the differences were small and not significant [13, 43]. However, it is probable that if we had performed the test three times with the reference food, we would have obtained shorter within-person coefficients of variation.

Concerning between-person coefficients of variation, muffins had higher mean CV. One explanation could be that muffins have more proportion of other macronutrients which could potentially reduced the postprandial glycemic responses [47], although the measurement of GI of the carbohydrate has been shown to be reliable in mixed meals [40, 43].

As an indication of the veracity of this study, standardized strategies were employed to minimize inter- and intra-subject variability, including the restriction of the inclusion criteria to certain age and health status. The glycemic index value determinations were assessed following a protocol that was as consistent as possible among volunteers.

The obtained GI values for the modified muffin and bread were lower than the control products using both in vitro and in vivo methods.

Despite the fact that the trend toward GI reduction seems to be similar for both in vitro and in vivo methods, the Bland–Altman plot shows important differences between both approaches. The in vitro method overestimated the GI obtained with the in vivo method (reference test). This overestimation is lower if the measured GI measured in in vivo is higher. Some researchers have found correlations between the rate of in vitro glucose release from starchy foods using pancreatic and brush-border enzymes and the glycemic response in vivo [13]. However, associations between in vivo and in vitro results have not been consistently found [48]. Physiological factors (gastrointestinal function, glucose tolerance, the rate of food consumption) and meal factors (physical form, other nutrients) can confound the relationship (Glycemic index and health, 2001). Given the large variation between subjects and between methods in values of glycemic index for bakery products seems adequate to suggest the need of standardization regarding in vitro methods and the combined use of both in vivo and in vitro analysis. In vitro methodology could be used in a previous screening phase in the development of a product, although in vivo method would be necessary to determinate the GI value for the labeling.

Low-GI diets may have implications in the prevention and management of chronic diseases like type 2 diabetes, coronary heart disease or some cancers. Published material suggests that the recommendations of low-fat and high-carbohydrate diets could be implemented with low-GI food choices [4]. For this reason, information on glycemic responses of foods can be used in fine-tuning glycemic control.

Acknowledgments The authors wish to thank all participants for their collaboration, some of whom were colleagues in the GENUD and in the Veterinary research groups. We would like to sincerely thank the *Laboratorio de Análisis Clínicos. Dra. M[a] Jesús Vicente Lobera* for their assistance in obtaining biological samples throughout the study. Bread and Muffins were supplied by NOVAPAN S. L. Zaragoza. Spain. This study was supported by a grant from CDTI (Centro de Desarrollo Tecnológico Industrial) (Project CENIT HIGEA CEN-20072003).

Conflict of interest The authors declare no conflict of interest in the carrying out of the study and the writing of the manuscript.

References

1. Probst-Hensch NM (2010) Chronic age-related diseases share risk factors: do they share pathophysiological mechanisms and why does that matter? Swiss Med Wkly 140:w13072
2. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM et al (1981) Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 34(3):362–366
3. Bjorck I, Liljeberg H, Ostman E (2000) Low glycaemic-index foods. Br J Nutr 83(Suppl 1):S149–S155
4. Augustin LS, Franceschi S, Jenkins DJ, Kendall CW, La Vecchia C (2002) Glycemic index in chronic disease: a review. Eur J Clin Nutr 56(11):1049–1071
5. Brand-Miller JC (2004) Postprandial glycemia, glycemic index, and the prevention of type 2 diabetes. Am J Clin Nutr 80(2):243–244
6. Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB (2004) Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. Am J Clin Nutr 80(2):348–356
7. Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L et al (2000) A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. Am J Clin Nutr 71(6):1455–1461
8. Augustin LS, Dal Maso L, La Vecchia C, Parpinel M, Negri E, Vaccarella S et al (2001) Dietary glycemic index and glycemic load, and breast cancer risk: a case-control study. Ann Oncol 12(11):1533–1538
9. Franceschi S, Dal Maso L, Augustin L, Negri E, Parpinel M, Boyle P et al (2001) Dietary glycemic load and colorectal cancer risk. Ann Oncol 12(2):173–178
10. Monro JA, Mishra S (2010) Database values for food-based dietary control of glycaemia. J Food Compos Anal 23:406–410
11. Woolnough J, Monro J, Brennan C, Bird A (2008) Simulating human carbohydrate digestion in vitro: a review of methods and the need for standardization. Int J Food Sci Technol 43:2245–2256
12. Brand-Miller J, Holt S (2004) Testing the glycaemic index of foods: in vivo, not in vitro. Eur J Clin Nutr 58(4):700–701
13. Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G et al (2005) Glycaemic index methodology. Nutr Res Rev 18(1):145–171
14. Fardet A, Leenhardt F, Lioger D, Scalbert A, Remesy C (2006) Parameters controlling the glycaemic response to breads. Nutr Res Rev 19(1):18–25
15. Akerberg A, Liljeberg H, Bjorck I (1998) Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. J Cereal Sci 28(1):71–80
16. Angioloni A, Collar C (2010) Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. LWT-Food Sci Technol 44(3):747–758
17. Fuentes-Zaragoza E, Riquelme-Navarrete MJ, Sánchez-Zapata E, Pérez-Álvarez JA (2010) Resistant starch as functional ingredient: a review. Food Res Int 43(4):931–942
18. Baixauli R, Salvador A, Martínez-Cervera S, Fiszman SM (2008) Distinctive sensory features introduced by resistant starch in baked products. LWT-Food Sci Technol 41(10):1927–1933
19. Sanz T, Salvador A, Baixauli R, Fiszman SM (2009) Evaluation of four types of resistant starch in muffins. II. Effects in texture, colour and consumer response. Eur Food Res Technol 229(2):197–204
20. Tharanathan RN, Mahadevamma S (2003) Grain legumes—a boon to human nutrition. Trends Food Sci Technol 14(12):507–518
21. Chung HJ, Liu Q, Hoover R, Warkentin TD, Vandenberg B (2008) In vitro starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. Food Chem 111(2):316–321
22. Goñi I, Garcia-Alonso A, Saura-Calixto F (1997) A starch hydrolysis procedure to estimate glycemic index. Nutr Res 17(3):427–437
23. Sanchez-Pardo ME, Ortiz-Moreno A, Mora-Escobedo R, Necoechea-Mondragon H (2007) In vitro starch digestibility and predicted glycemic index of microwaved and conventionally baked pound cake. Plant Foods Hum Nutr 62(3):99–105
24. Granfeldt Y (1994) Foods factors affecting metabolic responses to cereal products. University of Lund, Lund
25. Standardization IOf (2008) Food products-Determination of the glycemic index (GI) and relevant classification. International Organization for Standardization, Geneva
26. Brand-Miller JC, Stockmann K, Atkinson F, Petocz P, Denyer G (2009) Glycemic index, postprandial glycemia, and the shape of the curve in healthy subjects: analysis of a database of more than 1,000 foods. Am J Clin Nutr 89(1):97–105
27. Wolever TM, Vorster HH, Bjorck I, Brand-Miller J, Brighenti F, Mann JI et al (2003) Determination of the glycaemic index of foods: interlaboratory study. Eur J Clin Nutr 57(3):475–482
28. Tovar J, Sayago-Ayerdi SG, Penalver C, Paredes-Lopez O, Bello-Perez LA (2003) In vitro starch hydrolysis index and predicted glycemic index of corn tortilla, black beans (Phaseolus vulgaris L.), and Mexican "taco". Cereal Chem 80(5):533–535
29. Behall KM, Scholfield DJ (2005) Food amylose content affects postprandial glucose and insulin responses. Cereal Chem 82(6):654–659
30. Cavallero A, Empilli S, Brighenti F, Stanca AM (2002) High (1 -> 3, 1 -> 4)-beta-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci 36(1):59–66
31. El SN (1999) Determination of glycemic index for some breads. Food Chem 67:67–69
32. Marques C, D'Auria L, Cani PD, Baccelli C, Rozenberg R, Ruibal-Mendieta NL et al (2007) Comparison of glycemic index of spelt and wheat bread in human volunteers. Food Chem 100(3):1265–1271
33. Germaine KA, Samman S, Fryirs CG, Griffiths PJ, Johnson SK, Quail KJ (2008) Comparison of in vitro starch digestibility methods for predicting the glycemic index of grain foods. J Sci Food Agric 88(4):652–658
34. Utrilla-Coello RG, Osorio-Díaz P, Bello-Pérez LA (2007) Alternative use of chickpea flour in breadmaking: chemical composition and starch digestibility of bread. Food Sci Technol Int 13(4):323–327
35. Foster-Powell K, Holt SH, Brand-Miller JC (2002) International table of glycemic index, glycemic load values. Am J Clin Nutr 76(1):5–56
36. Yamada Y, Hosoya S, Nishimura S, Tanaka T, Kajimoto Y, Nishimura A et al (2005) Effect of bread containing resistant

starch on postprandial blood glucose levels in humans. Biosci Biotechnol Biochem 69(3):559–566
37. Goni I, Valentin-Gamazo C (2003) Chickpea flour ingredient slows glycemic response to pasta in healthy volunteers. Food Chem 81(4):511–515
38. Rosin PM, Lajolo FM, Menezes EW (2002) Measurement and characterization of dietary starch. J Food Compos Anal 15:367–377
39. Zabidi MA, Aziz NAA (2009)) In vitro starch hydrolysis and estimated glycaemic index of bread substituted with different percentage of chempedak (Artocarpus integer) seed flour. Food Chem 117(1):64–68
40. Udani JK, Singh BB, Barrett ML, Preuss HG (2009) Lowering the glycemic index of white bread using a white bean extract. Nutr J 8:52
41. Vega-Lopez S, Ausman LM, Griffith JL, Lichtenstein AH (2007) Interindividual variability and intra-individual reproducibility of glycemic index values for commercial white bread. Diabetes Care 30(6):1412–1417
42. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH (1999) Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. Am J Clin Nutr 69(3):448–454
43. Chen YJ, Sun FH, Wong SH, Huang YJ (2010) Glycemic index and glycemic load of selected Chinese traditional foods. World J Gastroenterol 16(12):1512–1517
44. Campbell JE, Glowczewski T, Wolever TM (2003) Controlling subjects′prior diet and activities does not reduce within-subject variation of postprandial glycemic responses to foods. Nutr Res 23:621–629
45. Granfeldt Y, Wu X, Bjorck I (2006) Determination of glycaemic index; some methodological aspects related to the analysis of carbohydrate load and characteristics of the previous evening meal. Eur J Clin Nutr 60(1):104–112
46. Wolever TM, Brand-Miller JC, Abernethy J, Astrup A, Atkinson F, Axelsen M et al (2008) Measuring the glycemic index of foods: interlaboratory study. Am J Clin Nutr 87(1):247S–257S
47. Wolever TM (2006) The glycaemic index: a physiological classification of dietary carbohydrate. Cabi Pub, Oxford
48. Urooj A, Puttaraj S (2000) Glycaemic responses to cereal-based Indian food preparations in patients with non-insulin-dependent diabetes mellitus and normal subjects. Br J Nutr 83(5):483–488