European Journal of Nutrition
https://doi.org/10.1007/s00394-018-1813-z

ORIGINAL CONTRIBUTION

Predicting mixed-meal measured glycaemic index in healthy subjects

Simon Ballance[1] · Svein Halvor Knutsen[1] · Øivind Winther Fosvold[2] · Aida Sainz Fernandez[3] · John Monro[4]

Received: 26 April 2018 / Accepted: 6 September 2018

Abstract

Purpose To determine the influence of meal composition on the glycaemic impact of different carbohydrate staples, and the accuracy of "adjusted calculated meal GI" compared with "measured mixed-meal GI".

Methods In a non-blind randomized crossover trial fasted healthy subjects consumed four dinner-type mixed meals of realistic serving size comprising a carbohydrate staple of either mashed potato, pasta, rice or a glucose drink, combined with fixed portions of boiled carrots, poached salmon and herb sauce. Blood samples collected between 0 and 180 min were analysed for glucose and insulin concentrations. Adjusted calculated meal GI values were determined against a 50 g reference glucose drink, and compared to corresponding measured mixed-meal GIs, supplemented with data from four previous mixed-meal postprandial glycaemic response studies.

Results The common carbohydrate staples, and the glucose drink, ingested as part of the salmon mixed meal induced a significantly lower post-prandial relative glycaemic response (RGR) and concurrent higher relative insulin response than the same amount of staple eaten alone. Adjusted calculated mixed-meal GI closely predicted measured mixed-meal GI in healthy subjects for 15 out of 17 mixed meals examined, showing the need to account for effects of fat and protein when predicting measured mixed-meal GI. Further, we showed the validity of using customarily consumed food amounts in mixed-meal postprandial RGR study design.

Conclusions Adjusted calculated mixed-meal GI appears a useful model to predict measured mixed-meal GI in healthy subjects and with further development and validation could aid nutrition research and rational design of healthy meals for personalized nutrition and particular consumer groups.

Keywords Blood sugar · Insulin · Potato · Rice · Pasta · Starch · Meal

Introduction

Glycaemic response (GR) is the postprandial change in blood glucose elicited by a food or meal. Glycaemic index (GI) is both a standardized and relative GR to a food containing a fixed amount (usually 50 g) of available carbohydrate expressed as a percentage of the GR to an equivalent amount of reference carbohydrate (usually glucose) [1, 2]. An ability to predict postprandial GR in mixed meals would be a valuable tool in nutrition research as many carbohydrate-rich staple foods such as rice, pasta and potatoes are most often eaten together with other foods, where at least one contains predominantly fat or protein. Such a combination of foods may be defined as a mixed meal [3]. Another valuable application would be in formulation of foods and meals for specific end-user groups and for different eating occasions. For example, the general stimulating effect of protein on insulin might be beneficial in subjects with insulin resistance while in the long term it could be harmful for healthy subjects (often also referred to as normal subjects), where hyperinsulinemia may ultimately cause a decrease in insulin sensitivity, increasing the risk of developing type 2 diabetes [4].

For nearly 30 years it has been generally accepted that the GRs to mixed meals of equivalent nutrient content are proportional to their scores on a parameter known as the 'calculated meal glycaemic index (CMGI)' [5]. This is calculated

✉ Simon Ballance
simon.ballance@nofima.no

1 Nofima AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Osloveien 1, 1433 Ås, Norway

2 Fjordland AS, Brynsengveien, Oslo, Norway

3 Leatherhead Food Research, Epsom, UK

4 The New Zealand Institute for Plant and Food Research Limited, Palmerston North, New Zealand

Published online: 14 September 2018

as the weighted average of the GI of each food comprising the mixed meal with the weighting based on the proportion each food's carbohydrate contributes to total carbohydrate in the mixed meal [6]. However, CMGI only takes into account the source and amount of available carbohydrate in a mixed meal. It does not take account of effects of non-carbohydrate components on GR. Therefore, alone this model cannot predict relative GRs of mixed meals that are not equivalent in nutrient content, as is the case in most meals, which contain substantial and different amounts of particular types of protein, fat or fibre.

A recent extension of the CMGI model to take protein and fat into account in healthy subjects has been proposed by Wolever [5]. Using data from two dose–response studies [7, 8] the effect on GR of adding fat (corn or canola oil) and protein (soy or whey) to 50 g glucose was estimated. This information was then used together with knowledge of the macronutrient content of the meal to calculate an 'adjusted calculated meal GI' (adjusted-CMGI) [5]. It was shown, using data re-evaluated from two previously published postprandial clinical studies on typical dinner-type mixed meals in healthy subjects [9, 10], that this new model could predict clinically measured relative GR of mixed meals. This new adjusted-CMGI model can thus be a potential predictive measure of mixed-meal GR. A prerequisite is that the protein, available carbohydrate and fat amount in the meal are known together with an accurate GI of the individual meal components (foods). In addition, one needs knowledge of the dose–response effect on GR for specific sources of protein and fat in the mixed meal. Further direct testing and validation of the current format of the 'adjusted-CMGI' is required because at present this is lacking.

Many studies [9, 11, 12] have also determined another 'standardized' GR parameter; due to the way in which it is measured and what it represents, has become to be known as 'measured meal GI' (MMGI) [10]. Here the incremental area under the curve (iAUC) of the GR to available carbohydrate in a mixed meal is expressed as a percentage of the response to an equivalent amount of available carbohydrate reference, usually 50 g in the form of glucose. This is essentially the same approach and methodology as for the conventional GI determination of carbohydrate-rich foods. However, and to our knowledge, there have been no comparisons made between MMGI and adjusted-CMGI for healthy subjects consuming mixed meals.

A limitation of current postprandial clinical GR studies involving complex mixed meals is the conventional practice that it should contain 50 g of available carbohydrate. However, for many mixed-meal types, this is way beyond realistic serving sizes. For example, for cooked potato as the main source of staple carbohydrate in a mixed meal, 50 g available carbohydrate is equivalent to roughly 2–3 servings or about 350–475 g potato depending on its moisture content [13]. Another limitation with trying to have absolute fixed amounts of available carbohydrate in a study is that it severely restricts the composition of the mixed meals, especially if more than one food component comprising the meal also contains available carbohydrate. For a whole host of practical reasons during meal preparation for crossover studies it can also be difficult to make a set of matched meals with a fixed and identical available carbohydrate content, especially if the major source of available carbohydrate is starch and there are other sugars present. A further adaption of the adjusted-CMGI model would be to see if it is possible to widen its scope and increase the flexibility of postprandial GI studies for any type of mixed-meal dominated by large contributions of fat and protein in addition to a large amount of available carbohydrate.

The aim of the work reported in this paper was to determine the differences between MMGI, CMGI, and adjusted-GMGI with the aim of validating the calculated adjusted-GMGI values. The comparison was extended using supplementary data from previous mixed-meal postprandial glycaemic response studies in healthy subjects [5, 9, 10, 14].

Materials and methods

Study meals

Foods to make eight different test meal/food combinations for the study subjects were prepared (Table 1). Of these, four were dinner mixed meals. These comprised 140 g poached, minced, bone and skin free, farmed Atlantic salmon; 100 g cooked minced carrots; and 100 g herb sauce. A carbohydrate staple of either 160 g boiled mashed potato, 84.2 g cooked rice, or 82.3 g cooked pasta was added to three of the mixed meals. For the fourth mixed meal instead of a staple, a supplementary 250-ml 23.21 g glucose drink was gradually consumed along with the remaining meal components. The remaining meals comprised potato, pasta or rice alone or salmon with carrot and herb sauce. All consumed carbohydrate staples and glucose had an equal total available carbohydrate content. A 50 g dose of glucose in 250 ml water was used as the reference food consumed by each subject on three separate occasions.

Fresh vacuum-packed salmon fillets were from Lerøy AS, Bergen, Norway. Peeled and quartered frozen raw carrots were from Findus Norge AS, Tønsberg, Norway. Peeled, salted, blanched and vacuum-packed potatoes were of the variety Folva (Superior Potet, Hoff SA, Gjøvik, Norway). These were all pre-cooked and packaged at Fjordkjøkken AS, Varhaug, Norway. Herb sauce containing 86% water, 6% double-cream, 3.4% milk powder, 2.9% modified maize starch (Cargill C-TEX 06205, acetylated distarch adipate) with the remaining 1.3% comprising a mixture of salt,

Table 1 Nutrient composition of the test foods and mixed meals in grams per portion

	Serving size[a]	ACHO	Digestible starch	Resistant starch	Sugars	Total fibre	Fat	Protein	Ash	Water[a]	Unaccounted	Energy (kcal)
Potato	160	24.6	21.1	1.8	1.4	2.1	ND	1.9	1.4	127.8	4.3	102
Rice	84.2	23.4	21.1	0.8	0.2	1.3	0.3	2.1	0.3	56.3	2.6	99
Pasta	82.3	23.3	21.1	0.8	0.1	2.1	0.5	4.9	0.2	51.1	2.3	113
Salmon (S)	140	0.1	ND	ND	0.1	ND	13.4	30.9	2.2	94.1	0.0	245
Carrot (C)	100	6.6	1.1	ND	5.5	2.3	0.2	0.5	0.5	90.2	0.0	35
Herb sauce (H)	100	2.5	2.4	0.1	0.1	N.D	4.0	1.2	1.8	88.3	2.2	51
S + C + H with glucose	363	32.9	3.5	0.1	28.9	2.3	17.6	32.6	4.5	273.0	0.6	423
S + C + H with potato	500	34.4	24.6	1.9	7.4	4.4	17.6	34.6	5.9	400.8	4.7	433
S + C + H with pasta	424	33.0	24.6	0.9	5.8	4.4	18.1	37.5	4.7	324.1	4.8	443
S + C + H with rice	422	33.2	24.6	0.9	5.9	3.6	17.9	34.8	4.8	329.3	1.1	430
S + C + H alone	340	9.7	3.5	0.1	5.7	2.3	17.6	32.6	4.5	273.0	0.8	330

ND below detection limit

[a]Not including 250 ml glass of water (or glucose drink) consumed with the meal

pepper, aroma and dried herbs was also prepared and packaged in portions at Fjordkjøkken AS. Macaroni short pasta (ANCO professional) was from Soubry N.V., Roeselare, Belgium. Parboiled long-grain rice was sourced from Harlem Foods AS, Oslo, Norway.

In a professional kitchen at Fjordland AS, Oslo, the pasta was cooked for 8 min in boiling water and the rice was cooked for 20 min in one part rice two parts water. Salmon was minced to homogeneity through an 8-mm mesh plate and then mashed by hand. Potatoes were boiled until soft to the centre, drained and pressed through a potato ricer into a large bowl before mashing by hand. Carrots were drained and minced to homogeneity through a 3-mm mesh plate. The salmon, carrot and potato, therefore, had a semi-solid paste-like consistency. The pasta and rice were considered to be solid.

All these foods were immediately and separately vacuum packed in ready-to-eat meal portions. All packed meals received heat-treatment in a Convotherm combi-steamer for 30 min at 98 °C. They were then cooled in running cold water for 20 min, frozen, and then transported chilled to Leatherhead, UK. Prior to consumption each food item was thawed overnight in the fridge and re-heated in its vacuum bag for 7–8 min in boiling water. A measured glass of water (250 ml) was supplied for consumption with the test meals/foods except in the cases where glucose was consumed as a drink.

The nutrient composition of the meals was analysed as follows. Protein was estimated (N × 6.25) from the analysis of N by the method of Kjeldahl. Fat was determined gravimetrically following acid hydrolysis, extraction into diethyl ether and petroleum ether and evaporation. Total dietary fibre was determined gravimetrically according to AOAC 985.28. Sugars were determined as the sum of sucrose, glucose and fructose after extraction in 50% water:methanol followed by analysis with anion-exchange chromatography with pulsed amperometric detection. Total and resistant starch 'as eaten' was determined by AOAC 2002.02 within 1 h of re-heating the foods. Available CHO was subsequently calculated as described by [15]. Moisture content was determined gravimetrically following drying at 103 °C to constant weight. Ash was determined as the inorganic residue remaining after removal of all water and organic matter by heating at 550 °C. Total energy content was calculated according to EU Council Directive 1169/2011. The nutrient composition of the test foods is shown in Table 1.

Subjects

Volunteers were pre-screened and asked initial recruitment questions to determine their suitability to take part in the study. The nature of the study and their involvement and responsibilities were described to them. Eligible volunteers

who were willing to participate were presented with an information sheet, containing study details, along with a written consent form at least 3 days before starting the study. The inclusion criteria were age 18–65 years, gender: male or female, BMI 18–27 kg/m^2, self-diagnosis as healthy at the time of recruitment confirmed by medical questionnaire and fasting blood glucose 4–6.1 mmol/l. Subjects were excluded from the study if they had any history of diabetes or had consumed anything apart from water 12 h prior to starting the test.

Fifteen healthy subjects were recruited for one single cohort. Fourteen subjects (12 female, 2 male) completed the study. The mean age of these subjects was 47.3 (SEM 3.5) years with a mean BMI of 23.7 (SEM 0.6) kg/m^2. Nine subjects completed all 11 visits. Five subjects missed one visit, while one subject missed three visits. At least 13 subjects attended each visit. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and the study design was approved by the West Kent Research Ethics Committee, Aylesford, UK. Written informed consent was obtained from all subjects. All clinical testing was conducted at Leatherhead Food Research, UK, within a 3-month period between February and April 2016.

Study protocol

The night before the test the subjects were instructed to avoid strenuous physical activity, and refrain from consuming alcohol the day before a test and smoking during the day of the test. The subjects were instructed to consume a similar carbohydrate-based evening meal before each test session. Subjects were also instructed to fast from 20:00 the night before a test. Water consumption was not restricted until 1 h before the start of the test. Subjects should not have had a similar test for the last 48 h (wash-out time). On each test day, the volunteers arrived at the Human Nutrition Unit, having fasted for at least 12 h prior to commencement, and they were seated and asked to remain so for the duration of the test. Upon arrival, their blood glucose levels were checked using a hand-held glucometer to ensure they had fasted correctly and were suitable to take part. Once each subject was relaxed and comfortable, they were asked to provide a baseline glucose and insulin measurement for that day, against which all of that day's subsequent assessments were measured. The subjects were given the different meals in a non-blind randomized order on separate days (crossover) with at least 48-h wash-out between testing. Meals for testing were randomized in blocks of up to four meals with consumption of the reference food (glucose) before and after each block. Each subject presented with a study meal/food including a glass of water was instructed to consume the whole amount within a 15-min period. The first blood sample was collected exactly 15 min after the first bite of the sample food. After this point blood samples were taken at 15-min intervals for the first hour, 30-min intervals for the second hour and then after a 1-h interval for the third hour. Samples were collected at 0, 15, 30, 45, 60, 90, 120, 180 min.

Capillary blood samples were collected into small tubes containing lithium–heparin following a finger prick, and centrifuged at 3000 rpm for 10 min to separate the plasma. The plasma samples were then analysed for glucose by an YSI 2300 Stat Plus Glucose and Lactate analyzer. The sensitivity of the analyser is 0–50 mmol/l and the margin of error is ± 2% or 0.2 mmol/l. Insulin was analysed in plasma using a sandwich-ELISA (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions. Prior to insulin analyses, all plasma samples were stored at − 80 °C.

Calculations, power and statistical analysis

The incremental area under the glucose response curves ($iAUC_{120min}$) above baseline was calculated for 0–120 min using the standard trapezoid geometric method [3]. This was programmed into, validated and performed in a standardized way in R-Studio version 0.99.491. The mean and CV (coefficient of variation) (CV = 100 × SD/mean) of within-individual $iAUC_{120min}$ values for repeated ($n = 3$) measures of the reference food (50 g glucose) was calculated for each subject. The mean CV for the subject group was 17.7 and, therefore, inside the upper recommended threshold of 30 [16]. The one-phase exponential association dose–response equation: RGR (iAUC relative to that elicited by 50 g glucose) = $GI \times 1.49 \times (1 - e^{-0.0222g \text{ available carbohydrate}})$ according to [3] was used to calculate $iAUC_{120min}$ for the reference food corrected for an equivalent available carbohydrate content in the test food/mixed meal. Measured GI values were calculated for foods and mixed meals, respectively, by expressing the $iAUC_{120min}$ for the test food/mixed meal in each subject as a percentage of the same subjects' corrected mean reference $iAUC_{120min}$. The mean of the resulting values was the measured GI for the food/mixed meal. Measured GI values for a food/mixed meal for individual subjects greater than the mean plus 2 SDs were considered outliers and excluded [16]. iAUCs and other responses (fasting, peak and incremental peak) for identified outlier subjects for a specific food/mixed meal were also excluded from any further statistical comparisons.

For mixed meals, CMGI was calculated according to [6] using GI values determined for the meal components (potato, rice, pasta, etc.) measured in this study (see Table 2). Adjustment factors for the combined effect of fat, protein and available carbohydrate dose in calculating adjusted-CMGI were made according to [5]. Using the potato mixed-meal as an example the individual and overall adjustment factors are calculated as follows. Adjustment

Table 2 Adjustment factors, calculated mixed-meal GI, adjusted calculated mixed-meal GI and mean measured mixed-meal GI ± SD of variation of estimates in individual subjects

	Avail. CHO	Adj. factor		Overall Adj.	CMGI	Adjusted-CMGI	MMGI (mean ± SD)
		Fat	Protein				
Potato	–	–	–	–	–	–	81 ± 14.6
Rice	–	–	–	–	–	–	57 ± 17.8
Pasta	–	–	–	–	–	–	63 ± 11.0
S + C + H with glucose	1.29	0.95	0.53	0.65	79	51	52 ± 11.9
S + C + H with potato	1.27	0.95	0.53	0.64	66	42	35 ± 18.2
S + C + H with pasta	1.29	0.95	0.53	0.65	53	34	33 ± 14.4
S + C + H with rice	1.29	0.95	0.53	0.65	49	32	28 ± 7.9
S + C + H alone	–	–	–	–	–	–	29 ± 23.5

For mean % reductions in AUC when calculating adjusted mixed-meal GI, a value of 0.29%/g fat and 1.45%/g protein was used

S salmon, *C* carrots, *H* herb sauce, *Adj*. adjustment

for available carbohydrate = $1.49 \times (1 - e^{-0.0222g})$, where g = grams of available carbohydrate. This dose–response equation describing the effect of available carbohydrate on glycaemic response predicts that the effect of an increase in available carbohydrate from 24.6 g for the potato-only test food to 34.4 g in the potato meal is a difference of a decimal percent of 0.27. Given

$$\text{RGR} = 1.49 \times (1 - e^{-0.0222\times24.6}) = 0.627 \text{ and } 1.49 \times (1 - e^{-0.0222\times34.4}) = 0.796, \quad (1)$$

and where

$$\text{Adj. factor avail. CHO potato meal} = (0.796/0.627) = 1.27. \quad (2)$$

An adjustment factor of 1 (i.e. no adjustment) represents the potato eaten on its own.

For fat in the potato meal:

$$\text{Adj. factor fat potato meal} = 1 - ((0.29 \times (M - F)/100)) = 0.95, \quad (3)$$

where *M* represents meal fat content (17.6 g) and *F* represents potato fat content (0 g). The value of 0.29 is the mean % reduction in AUC/g fat taken from [5].

For protein in the potato meal:

$$\text{Adj. factor protein potato meal} = 1 - ((1.45 \times (M - P)/100)) = 0.53, \quad (4)$$

where *M* represents meal protein content (34.6 g) and *P* represents potato fat content (1.9 g). The value of 1.45 is the mean % reduction in AUC/g protein taken from [5].

The overall adjustment factor is the product of the individual three adjustment factors. For the potato meal: overall adj. = $1.27 \times 0.95 \times 0.53 = 0.64$. Adjusted-CMGI is the CMGI (for the potato meal = 66) × overall adj. which gives an adjusted-GMGI of 42 for the potato meal. To calculate GMGI we used the method of [6]. A worked example is found in [3]. For the potato meal CMGI calculation, we used GI values determined in this study (Table 2) for potato alone and the salmon, carrots and herb sauce eaten on their own without a carbohydrate staple. The available carbohydrate content of these is found in Table 2.

Minitab version 17 was used for all statistical analysis and power calculations. The primary endpoint was $iAUC_{120min}$. To calculate sample size the within-heathy subject standard deviation of 25 was used [17]. Using a sample size of $n = 12$ subjects provided 80% power to detect a difference in $iAUC_{120min}$ of 30% (two-tailed *t* test) with α set at 0.05. To allow for a 20% dropout 15 persons were recruited to the study. Statistical differences between fasting, peak, incremental peak and $iAUC_{0–120min}$ for glucose and natural logarithm transformed insulin responses for mixed meal/food (fixed factor) were assessed for subjects (random factor) by repeated measures ANOVA using a general linear model. The criterion for significance was a two-tailed $P < 0.05$. Comparison between foods/mixed meals was made with the post hoc Bonferroni pairwise test at a confidence interval of 95%.

Simple linear regression was utilized to assess how well adjusted-CMGI predicts MMGI in healthy subjects. To increase the power of the regression model, additional data were evaluated from clinical postprandial GR studies of mixed meals from the literature. Criteria for study selection included the existence of data on MMGI, mixed-meal macronutrient composition, GI of carbohydrate-rich food that make up the meal are measured in the same study, and mixed-meal GI is calculated. Where it was measured, data on specific adjustment factors for a particular studied protein source, or previously calculated values for adjusted-CMGI were used. In total, three published clinical mixed-meal GR studies [9, 10, 14], supplemented by one review [5] satisfied these criteria.

Results

The four mixed-meal dinners contained similar amounts of available carbohydrate (32.9–34.4 g) and protein (32.6–37.5 g) with a significant but smaller (17.9–18.1 g) contribution of fat (Table 1). Consequently, the mixed meals all had a very similar energy content (423–443 kcal). They also contained, including the 250-ml glass of water co-consumed with the mixed-meal, a large (525–650 g) but variable, amount of water (Table 1). The vast majority of all the fat and protein originated from the salmon. The herb sauce contained a small amount (4 g) of milk-derived fat (Table 1). For a near equivalent available carbohydrate content, the potato contained more than double the amount of water than in the pasta and rice (Table 1). Apart from the meal with the glucose drink nearly three quarters of the available carbohydrate was in the form of digestible starch while the rest were as free sugars (Table 1). All mixed meals were medium to low (< 5 g) in their dietary fibre content. The mixed meals contained 10 g more available carbohydrate load than the meals containing carbohydrate staple alone (Tables 1, 2; Fig. 1), mostly arising from the carrot and herb sauce.

Blood glucose responses to the staple carbohydrate foods ingested alone compared with their ingestion as part of the mixed meal showed a number of significant differences ($p < 0.001$; Figs. 2a, b, 3a, b). Potato ingested alone induced a significantly greater RGR (incremental peak height and $iAUC_{120min}$) than rice or pasta alone, which were similar, and not significantly different to one another. When eaten with the mixed meal, all corresponding RGR parameters were significantly reduced for all three staples (except for incremental RGR peak for pasta), and the RGR to potato was no longer significantly greater than for rice and pasta. The RGR ($iAUC_{120min}$) for the glucose reference was significantly higher than for the carbohydrate staple foods and

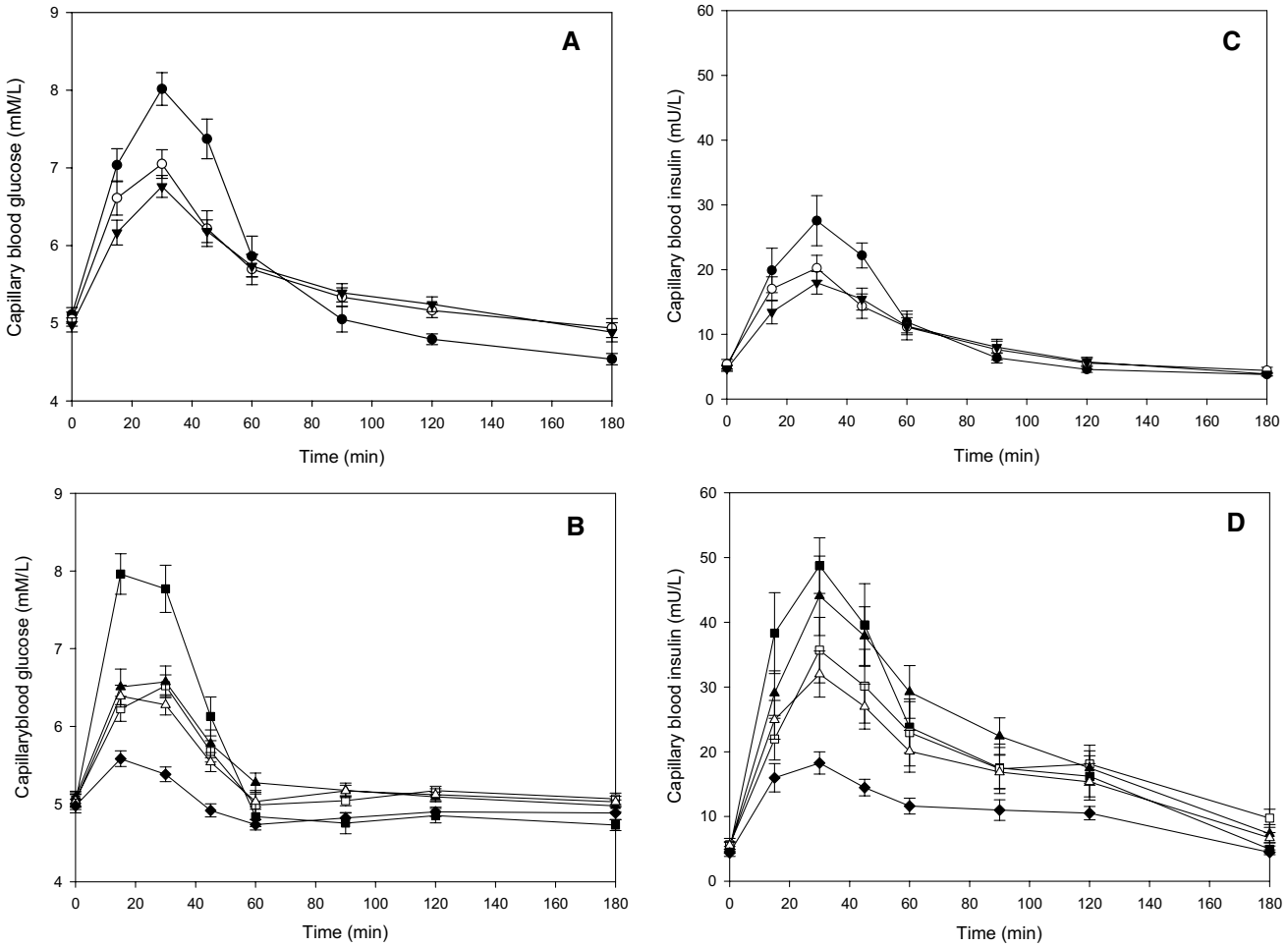

Fig. 1 Mean (±SEM) changes in capillary blood glucose (**a, b**) and insulin (**c, d**) in healthy subjects after the postprandial consumption of the test foods (**a, c**) or mixed meals (**b, d**). Mashed potato (filled circle), rice (open circle), pasta (upside-down filled triangle), salmon, carrot, herb sauce (S + C + H) with glucose drink (filled squares); S + C + H with pasta (open squares), S + C + H with potato (filled triangle), S + C + H with rice (open triangle), S + C + H alone (filled diamond)

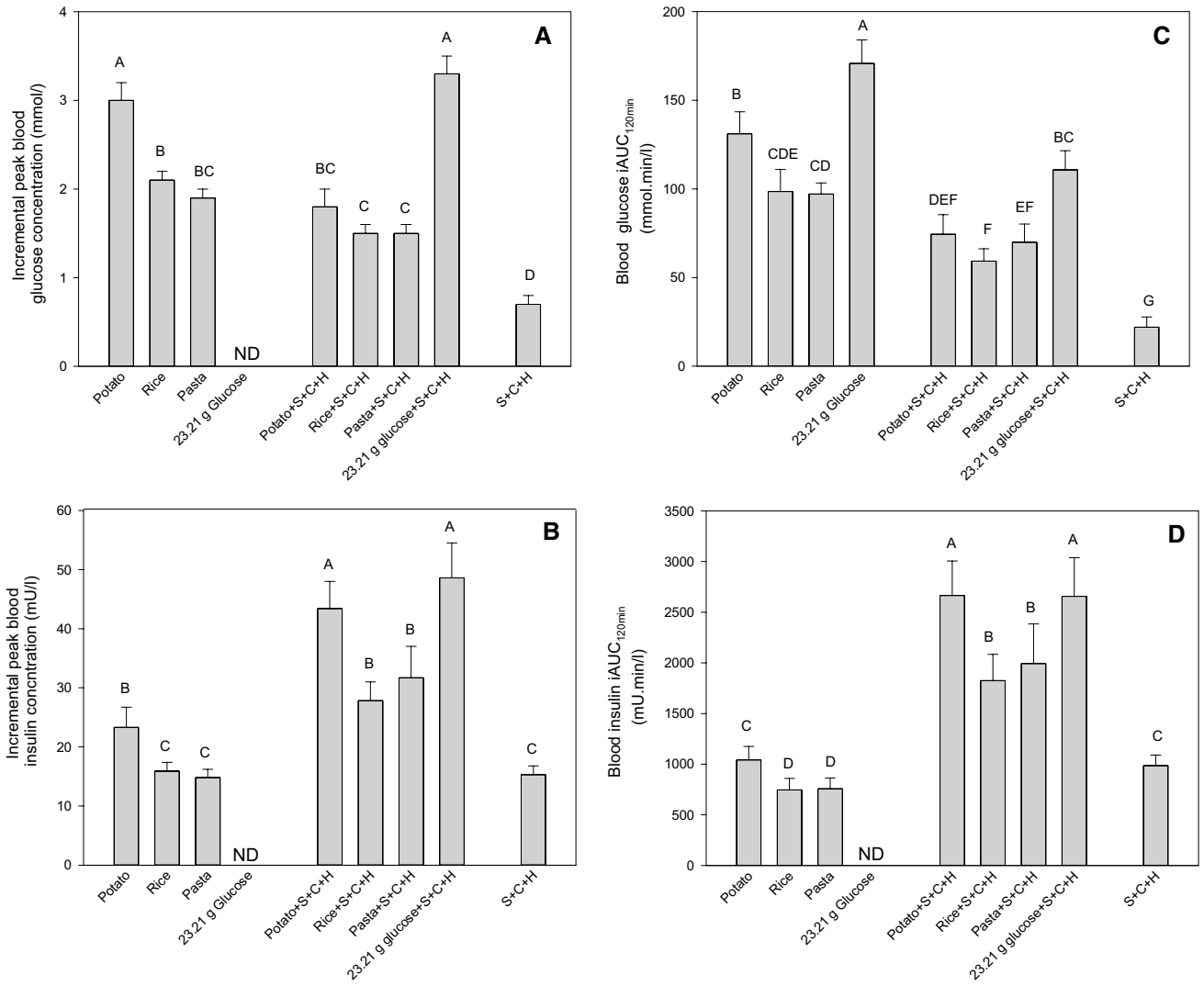

Fig. 2 Incremental peak concentration (**a, b**) and incremental area (**c, d**) under the curves above fasting baseline between 0 and 120 min of capillary blood glucose (top right and top left) and insulin (bottom right and bottom left) in healthy subjects following postprandial consumption of the study foods and mixed meals (mean + SEM). S + C + H is salmon, carrot and herb sauce. $n = 12$ for S + C + H, $n = 14$ for potato and rice alone and as mixed meals, $n = 14$ for glucose drink as part of a mixed meal and $n = 15$ for pasta and glucose alone, and pasta as a mixed meal. Foods and mixed meals that share a letter are not significantly different. *ND* not determined. For the reference food comprising 23.21 g glucose, only the iAUC value (calculated) was displayed in the figure, because the original measurements of iAUC and concentration for glucose and insulin for this sample were based on measurement of the 50 g glucose reference

meals but underwent a similar proportional reduction when consumed with the mixed meal. The mixed meal (salmon, carrot and herb sauce) without further carbohydrate additions not surprisingly had significantly lower RGRs.

Relative insulin responses (RIR) to the carbohydrate-based food alone and with the mixed meal also showed a number of noteworthy significant differences, where again $p < 0.001$ (Figs. 2c, d, 3c, d). For potato, insulin responses were significantly greater than pasta and rice eaten alone. They also underwent large and proportionally similar increases ($iAUC_{120min}$: potato 61%, rice 59%, pasta 62%; incremental insulin peak 46%, rice 43%, pasta 53%) when staples were consumed in mixed meals. The $iAUC_{120mi}$ and peak insulin responses to the mixed meal plus carbohydrate staple were approximately equal to the sum of the separate response to carbohydrate staple and mixed meal.

The CMGIs ranged from 49 for the rice-based mixed meal to 79 for the mixed meal with the glucose drink (Table 2). These values are all markedly greater, by between 21 and 31 GI units, than MMGI. On the other hand, adjusted-CMGI values were a much better predictor of MMGI values (Table 2). The difference between these two parameters were only between 1 and 7 GI units with three of the meals only having a difference of less than 3 GI units. Assessment of

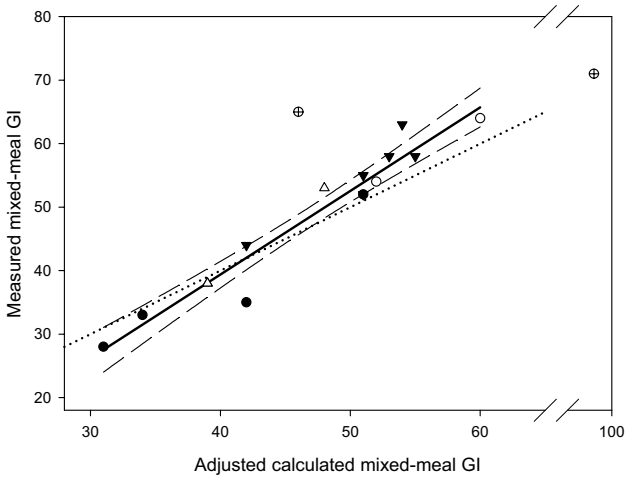

Fig. 3 The performance of adjusted-CMGI in predicting MMGI in healthy subjects. Filled circles are data from this study of carbohydrate staple/glucose with salmon, carrots and herb sauce (Table 3). Open circles are the literature data from four mixed meals with potato and various combinations of oil, chicken, salad and rye bread (open circles) [5, 9]. Filled inverted triangles are calculated from the literature data (Table 3) for combinations of white bread with either light tuna or unsalted butter [14]. Open triangles are also calculated from literature data for rice, spaghetti and potato-based mixed meals [10]. The solid line is the best-fit linear regression line for all data in the plot ($R_2 = 0.94$, standard error of estimate = 2.88) excluding the data represented by open circles with a cross. Large and small dashed lines are the respective 95% confidence and prediction intervals. The dotted line is the line of identity

data from three dinner-type mixed meals evaluated in the study of Dodd et al. [10] (Table 3; Fig. 3) showed a very similar trend.

For white bread with added fat [14] there is almost no difference between calculated GI, adjusted calculated GI and measured GI (Table 3). A maximum difference of only 9 GI units between these different parameters was observed showing that for healthy subject's fat in the form of butter added to bread had a minimal effect on mixed-meal GR. Where protein in the form of tuna was added to white bread there was a reduction of MMGI with an increase in added protein ([14], Table 3) whilst CMGI was constant. At a 50 g added dose of protein the difference between calculated and MMGI was 17 GI units (Table 3). However, when the CMGI was adjusted using specific values for the mean percentage reduction in AUC/g tuna protein [14] to provide an adjusted-CMGI value, this difference was only 2 GI units (Fig. 3; Table 3).

Figure 2 shows the overall performance of adjustment of CMGI as a predictor of MMGI. It also includes additional data taken directly from the literature for a further four dinner-type mixed meals [5, 9]. Two of these mixed meals comprising (1) 362 g mashed potato with 30 g rapeseed oil, 40 g cucumber and 170 ml of water, and (2) 272 g mashed potato with 30 g rapeseed oil, 108 g chicken, 120 g salad,30 g rye bread, 6 g margarine and 90 ml of water were excluded from the regression analysis as outliers. This is due to their apparently large difference (27 and 19 GI units respectively) between measured (see Table 2 in [9]) and adjusted calculated mixed-meal GI (MMGI vs adjusted-CMGI) values (see from Table 1 in [5]). Otherwise, linear regression of the remaining mixed meals ($n = 15$) had an R_2 of 0.94, a slope of 1.316, y-intercept of − 13.27 and a standard error of estimate of 2.88 (Fig. 3). The line of identity was partly inside and outside the 95% confidence interval.

Table 3 Macronutrient content, adjustment factors, CGI, adjusted calculated mixed-meal GI and measured mixed-meal GI from two published clinical studies

	CHO (g)	Fat (g)	Protein (g)	Adj. factor			Overall adj.	CMGI	Adjusted-CMGI	MMGI (mean ± SD)
				Avail. CHO	Fat	Protein				
Meng et al. [14]										
WB + 12.5 g protein	50	0	12.5	1.00	1.00	0.93	0.93	59	55	58 ± 26
WB + 25 g protein	50	0	25.0	1.00	1.00	0.86	0.86	59	51	52 ± 26
WB + 50 g protein	50	0	50.0	1.00	1.00	0.72	0.72	59	42	43 ± 18
WB + 5.6 g fat	50	5.6	0	1.00	0.98	1.00	0.98	55	54	63 ± 18
WB + 11.1 g fat	50	11.1	0	1.00	0.97	1.00	0.97	55	53	58 ± 21
WB + 22.2 g fat	50	22.2	0	1.00	0.94	1.00	0.94	55	51	55 ± 17
Dodd et al. [10][a]										
Potato meal	50	15.9	17.4	1.00	0.97	0.79	0.76	63	48	53
Rice meal	50	12.1	16.5	1.00	0.97	0.79	0.76	51	39	38
Spaghetti meal	50	12.5	19.6	1.00	0.97	0.79	0.76	54	39	38

[a]Protein and fat content of potato, rice and spaghetti mixed meals containing chicken, vegetables and sauce is found in Table 1 of Dodd et al. [10]. In the study of Meng et al., the source of added fat to white bread (WB) was unsalted butter while the source of added protein was canned tuna. For tuna, a value of 0.57 for the mean % reduction in AUC/g protein was used in calculation of the adjustment factor. For all other mean % reductions in AUC a value of 0.29%/g fat and 1.45%/g protein was used

Discussion

Consumption of common carbohydrate staples (rice, pasta or potato) or a glucose drink, as part of a dinner mixed meal with salmon, carrots and herb sauce had a significantly lower postprandial RGR and concurrent higher RIR, than the same amount of staples eaten alone. To different degrees the protein and/or fat component in mixed meals is chiefly responsible for this and has been observed many times before [9, 10].

Adjusted-CMGI appears to predict MMGI in healthy subjects for 15 out of 17 mixed meals studied. Values of about ~ 1.5%/g for mean percent reduction in $iAUC_{120\ min}$ per gram protein added per 50 g carbohydrate [7, 8] for all but two of the chicken-based mixed meals seem appropriate. This value also seems valid in the current study with salmon as the major protein source even when added to mixed meals of lower total available carbohydrate content of 33–34 g as opposed to the usual 50 g.

Yet for other mixed meals, such as those with tuna protein spread on white bread, the effects of protein on $iAUC_{120min}$ reduction appear markedly less [14]. Tuna eaten with potato also had a mild effect on $iAUC_{120min}$ reduction, but a much greater effect when eaten with pasta [12]. Together such observations are in line with the current understanding of a large variability between different types of protein in their capacity to reduce postprandial RGR and stimulate concomitant insulin production [5, 18]. Differences in protein digestibility may explain this, but also other factors may play a role in determining effect size, such as branched-chain amino acid content [4].

Fat appears to have a much smaller effect on RGR reduction than protein in nondiabetic and healthy subjects, when added to a carbohydrate-rich food [7, 8]. Values of ~ 0.3%/g in reduction $iAUC_{120min}$ per gram fat addition to 50 g glucose have been measured for corn oil [7] while for additions of 0–30 g canola oil to 50 g glucose there was no change in $iAUC_{120\ min}$ [8]. Still, there are other studies where fat additions to potato have resulted in much bigger $iAUC_{120min}$ reductions (> 40%) compared to controls without added fat [9, 19, 20]. In a recent study of 22–27 g of different types of fats added to pancake containing 50 g available carbohydrate significant reduction of GR occurred, but it was small ($p = 0.05$) [21]. The majority of studies fail to find a difference in the GR lowering ability of different types of fats [21]. For our current study, and for those assessed from the literature, a value of 0.29%/g reduction iAUC suggested previously [15] was used to calculate an adjusted-CMGI. This seemed to perform fine for bread and potato-based carbohydrate staple mixed meals even in studies where a minor effect of adding fat to carbohydrate was observed [14]. Assuming the effect of fat on $iAUC_{120}$ reduction is negligible, and caution should be exercised, the effect of fat could possibly be ignored altogether in adjusted-CMGI calculations especially where there is a large amount of protein in the meal. Still more work on both different fat and carbohydrate staple combinations is needed to verify this.

The type of available carbohydrate in the mixed meal, whether from starch in semi-solid foods, or glucose in a drink, appears not to have a large impact on the predictive ability of adjusted-CMGI for MMGI. Glucose in a drink consumed with the meal produces a significantly larger peak and incremental peak glucose response than the other meals. This is probably due to the rapid emptying of liquids from the stomach [22] coupled to instantaneous uptake of glucose from the small intestine without the need for enzymatic digestion. These differences in GR are still captured within the 2h window of blood sampling and reinforce $iAUC_{120min}$ as the most appropriate primary physiological response.

Assuming no other confounding dietary factors that may significantly reduce GR in a mixed meal such as a particular type and dose of dietary fibre, phenolic acids, organic acids, then the difference between calculated and measured GI is largely explainable by protein type and its dose. This presumes the value for CMGI is accurate. In turn, this relies on accurate GI values of the foods comprising the mixed meal. GI values from international GI tables may be insufficient because of large differences in published GI values for certain foods with potatoes as a prime example. Further, an accurate measure of macronutrients including available carbohydrate, and correct response/adjustment factors for fat and protein are required. If other confounding factors should be identified that have a significant effect on AUC reduction, and if appropriate 'adjustment factor' for these other factors can be calculated with knowledge of their dose–response effect on GR, it should be possible to extend the adjusted-CMGI model to take other significant factors into account. In reality and at present, meeting all these requirements is no mean feat and this hampers the current practical utilization of the adjusted-CMGI model.

For mixed meals, in particular, we suggest it may not be essential for them to contain an equivalent amount of available carbohydrate to that of the glucose reference, as is current convention for GI determination in foods. If the replicate reference drink contains 50 g glucose, a robust dose–response equation is suggested to calculate the change in $iAUC_{120\ min}$ of any given dose of glucose up to at least 100 g [3]. Such an equation, with near identical rate constant, was found by earlier studies [3] to account for 96–97% of the variability of mean blood glucose responses in heathy and diabetic subjects from four separate postprandial GR studies [23–26]. This was for doses between 0 and 200 g of sugars (glucose, fructose and sucrose) and a range of starchy foods. A corrected iAUC for the reference drink can then be calculated for each subject to match the equivalent and precise available carbohydrate content of the mixed meal. The

fact that adjusted-CMGI closely predicted MMGI for our four mixed-meal dinners where the carbohydrate content was 32–33 g lends support to such a methodological approach. In this way, one can be free from the current restriction in postprandial GR studies that the mixed meal must always contain a fixed 50 g of available carbohydrate. This opens up the possibility to investigate any particular combination and size of mixed meal. Certainly more experiments are required to verify this approach, but at least from a mixed-meal perspective, it seems to make sense.

Although iAUCs for insulin in heathy subjects increases linearly with carbohydrate dose, it has been suggested that because of the non-linear relationship between glucose and insulin responses, a similar model to predict insulin responses from carbohydrate dose and GI is invalid [3]. Still, it could well warrant future investigation especially since hyperinsulinemia is a risk factor for insulin resistance and type 2 diabetes. This is recognized by the European Food Safety Authority (EFSA) who only accept health claims on the reduction of postprandial blood glucose response so long the concomitant insulin response is not disproportionally increased [27].

In conclusion, we show that the adjusted-CMGI model may be a viable approach to predict MMGI in healthy subjects. Our suggestion to use customarily consumed food amounts in study design would increase the relevance and broaden the scope of mixed-meal glycaemic response studies. The adjusted-CMGI model may need further modification or extension to take into account other food factors that may influence GR in healthy subjects. It could be appropriate to have further sub-categories of adjusted-CMGI models that may represent overall meal complexity and differences in size. Division of mixed meals into mealtime categories such as breakfast, lunch, dinner or snack might be necessary. Clearly much more research is still required before the approaches presented here can have practical utility. Ultimately, this could lead to the development of tools that could aid the rational design of healthy mixed meals targeting particular consumer groups and for personalized nutrition. This is important since the majority of carbohydrate foods are eaten as mixed meals and not as individual foods. At the very least, we expect this study should stimulate further discussion on the topic of mixed meals and glycemic health.

Acknowledgements The authors would like to acknowledge the skillful technical assistance of Hanne Zobel, Ingunn Berget and Silje Johansen. We thank Dr. Huicui Meng, Tufts University, Boston, USA, for providing raw data for evaluation from reference [12]. This study is part of Project no. 225148 in The Research Council of Norway with financial support by the Research Funding for Agriculture and the Food Industry in Norway (85%) and Norwegian potato industry (15%). Additional financial support (25% in total) is acknowledged from Project no. 262300 from the Foundation for the Research Levy on Agricultural Products.

Compliance with ethical standards

Ethical standards All human studies have been approved by the appropriate ethics committee and, therefore, have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons participating in the clinical study gave informed consent prior to their inclusion.

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

References

1. Jenkins D, Wolever T, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV (1981) Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 34:362–366
2. Augustin LSA, Kendall CWC, Jenkins DJA, Willett WC, Astrup A, Barclay AW, Björck I, Brand-Miller JC, Brighenti F, Buyken AE, Ceriello A, La Vecchia C, Livesey G, Liu S, Riccardi G, Rizkalla SW, Sievenpiper JL, Trichopoulou A, Wolever TMS, Baer-Sinnott S, Poli A (2015) Glycemic index, glycemic load and glycemic response: an international scientific consensus summit from the International Carbohydrate Quality Consortium (ICQC). Nutr Metab Cardiovasc Dis 25:795–815. https://doi.org/10.1016/j.numecd.2015.05.005
3. Wolever T (2006) The glycaemic index: a physiological classification of dietary carbohydrates. CABI, Wallingford
4. Rietman A, Schwarz J, Tomé D, Kok FJ, Mensink M (2014) High dietary protein intake, reducing or eliciting insulin resistance? Eur J Clin Nutr 68:973
5. Wolever T (2013) Is glycaemic index (GI) a valid measure of carbohydrate quality? Eur J Clin Nutr 67:522
6. Wolever TM, Jenkins DJ (1986) The use of the glycemic index in predicting the blood glucose response to mixed meals. Am J Clin Nutr 43:167–172
7. Moghaddam E, Vogt JA, Wolever TM (2006) The effects of fat and protein on glycemic responses in nondiabetic humans vary with waist circumference, fasting plasma insulin, and dietary fiber intake. J Nutr 136:2506–2511
8. Lan-Pidhainy X, Wolever TM (2009) The hypoglycemic effect of fat and protein is not attenuated by insulin resistance. Am J Clin Nutr 91:98–105
9. Hätönen KA, Virtamo J, Eriksson JG, Sinkko HK, Sundvall JE, Valsta LM (2011) Protein and fat modify the glycaemic and insulinaemic responses to a mashed potato-based meal. Br J Nutr 106:248–253
10. Dodd H, Williams S, Brown R, Venn B (2011) Calculating meal glycemic index by using measured and published food values compared with directly measured meal glycemic index. Am J Clin Nutr 94:992–996
11. Foster-Powell K, Holt SH, Brand-Miller JC (2002) International table of glycemic index and glycemic load values: 2002. Am J Clin Nutr 76:5–56
12. Henry C, Lightowler H, Kendall F, Storey M (2006) The impact of the addition of toppings/fillings on the glycaemic response to commonly consumed carbohydrate foods. Eur J Clin Nutr 60:763–769
13. Monro J, Mishra S (2009) Nutritional value of potatoes: digestibility, glycemic index, and glycemic impact. In: Singh JKL (ed) Advances in potato chemistry and technology. Elsevier, London, pp 395–424

14. Meng H, Matthan NR, Ausman LM, Lichtenstein AH (2017) Effect of macronutrients and fiber on postprandial glycemic responses and meal glycemic index and glycemic load value determinations. Am J Clin Nutr 105:842–853
15. Brouns F, Bjorck I, Frayn K, Gibbs A, Lang V, Slama G, Wolever T (2005) Glycaemic index methodology. Nutr Res Rev 18:145–171
16. Wolever TMS, Brand-Miller JC, Abernethy J, Astrup A, Atkinson F, Axelsen M, Bjorck I, Brighenti F, Brown R, Brynes A, Casiraghi MC, Cazaubiel M, Dahlqvist L, Delport E, Denyer GS, Erba D, Frost G, Granfeldt Y, Hampton S, Hart VA, Hatonen KA, Henry CJ, Hertzler S, Hull S, Jerling J, Johnston KL, Lightowler H, Mann N, Morgan L, Panlasigui LN, Pelkman C, Perry T, Pfeiffer AFH, Pieters M, Ramdath DD, Ramsingh RT, Robert SD, Robinson C, Sarkkinen E, Scazzina F, Sison DCD, Sloth B, Staniforth J, Tapola N, Valsta LM, Verkooijen I, Weickert MO, Weseler AR, Wilkie P, Zhang J (2008) Measuring the glycemic index of foods: interlaboratory study. Am J Clin Nutr 87:247S–257S
17. Wolever TM, Nuttall FQ, Lee R, Wong GS, Josse RG, Csima A, Jenkins DJ (1985) Prediction of the relative blood glucose response of mixed meals using the white bread glycemic index. Diabetes Care 8:418–428
18. Acheson KJ, Blondel-Lubrano A, Oguey-Araymon S, Beaumont M, Emady-Azar S, Ammon-Zufferey C, Monnard I, Pinaud S, Nielsen-Moennoz C, Bovetto L (2011) Protein choices targeting thermogenesis and metabolism. Am J Clin Nutr 93:525–534
19. Collier G, McLean A, O'dea K (1984) Effect of co-ingestion of fat on the metabolic responses to slowly and rapidly absorbed carbohydrates. Diabetologia 26:50–54
20. Welch IM, Bruce C, Hill S, Read N (1987) Duodenal and ileal lipid suppresses postprandial blood glucose and insulin responses in man: possible implications for the dietary management of diabetes mellitus. Clin Sci 72:209–216
21. Clegg ME, Pratt M, Markey O, Shafat A, Henry CJK (2012) Addition of different fats to a carbohydrate food: Impact on gastric emptying, glycaemic and satiety responses and comparison with in vitro digestion. Food Res Int 48:91–97
22. Grimm M, Scholz E, Koziolek M, Kühn J-P, Weitschies W (2017) Gastric water emptying under fed state clinical trial conditions is as fast as under fasted conditions. Mol Pharm 14:4262–4271
23. Christensen NJ, Örskov H, Hansen AP (1972) Significance of glucose load in oral glucose tolerance tests. J Intern Med 192:337–342
24. Wolever TM, Katzman-Relle L, Jenkins AL, Vuksan V, Josse RG, Jenkins DJ (1994) Glycaemic index of 102 complex carbohydrate foods in patients with diabetes. Nutr Res 14:651–669
25. Lee BM, Wolever TMS (1998) Effect of glucose, sucrose and fructose on plasma glucose and insulin responses in normal humans: comparison with white bread. Eur J Clin Nutr 52:924–928
26. Wolever TM, Bolognesi C (1996) Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. J Nutr 126:2798–2806
27. EFSA (2012) Guidance on the scientific requirements for health claims related to appetite ratings, weight management, and blood glucose concentrations. EFSA J 10:2604