Original Research

Cereal Processing Influences Postprandial Glucose Metabolism as Well as the GI Effect

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Key words: ^{13}C-labeled carbohydrates, breakfast cereals, slowly available glucose, healthy subjects, glycemic index, starch, food process

Objective: Technological processes may influence the release of glucose in starch. The aim of this study was to compare the metabolic response and the kinetics of appearance of exogenous glucose from 2 cereal products consumed at breakfast.

Methods: Twenty-five healthy men were submitted to a randomized, open, crossover study that was divided into 2 parts: 12 of the 25 subjects were included in the "isotope part," and the 13 other subjects were included in the "glycemic part." On test days, subjects received biscuits (low glycemic index [GI], high slowly available glucose [SAG]) or extruded cereals (medium GI, low SAG) as part of a breakfast similar in terms of caloric and macronutrient content. The postprandial phase lasted 270 minutes.

Results: The rate of appearance (RaE) of exogenous glucose was significantly lower after consumption of biscuits in the first part of the morning (90–150 minutes) than after consumption of extruded cereals ($p \leq 0.05$). Conversely, at 210 minutes, it was significantly higher with biscuits ($p \leq 0.01$). For the first 2 hours, plasma glucose and insulin were significantly lower after biscuits during the glycemic part. C-peptide plasma concentrations were significantly lower at 90, 120, and 150 minutes after ingestion of the biscuits ($p \leq 0.05$).

Conclusion: The consumption of biscuits with a high content of slowly digestible starch reduces the appearance rate of glucose in the first part of the morning and prolongs this release in the late phase of the morning (210 minutes). Our results also emphasize that modulation of glucose availability at breakfast is an important factor for metabolic control throughout the morning in healthy subjects due to the lowering of blood glucose and insulin excursions.

INTRODUCTION

The quality of carbohydrates in food has been studied for some 30 years, with particular attention being paid to the structure of starch in processed food products [1,2]. The technology and cooking methods applied to cereal products result in various degrees of starch digestion from rapidly (as extruded cereals [3,4]) to slowly digested carbohydrates (e.g., in some types of biscuits or pasta [5]). Numerous studies have compared the physiological effects of starch-based products and showed a correlation between the *in vitro* digestibility of starch and the postprandial plasma glucose and insulin responses [6–12]. Investigation of postprandial metabolism of food starch fractions is generally based upon the monitoring of postprandial changes in circulating plasma glucose and insulin concentrations over a 2-hour period. This approach makes it possible to calculate

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the glycemic [13] and insulinemic indexes of foods [13]. However, these peripheral postprandial markers provide only a partial reflection of the absorption kinetics of starch-derived glucose and give no indications about its absorption kinetics. Although moderate postprandial glucose response may indicate a slow appearance of ingested carbohydrates and slow tissue uptake [14], this response also results from rapid appearance of ingested carbohydrates and rapid uptake by tissue [15]. In the latter case, insulin secretion is enhanced in relation to glycemic response. It is thus necessary to describe metabolic response to carbohydrate ingestion rather than simply the glycemic profile resulting from the difference between incoming and outgoing glucose flow rates whether exogenous from the food or endogenous from the organism. In order to study the kinetics of absorption of carbohydrate rich foods, the double-isotope labeling method is generally used [14,16–19]. This method makes it possible to measure the rates of appearance in plasma of exogenous glucose from the test food only [14]. Most studies are limited to a 120-minute postprandial follow-up and to the ingestion of an isolated tested cereal product. In the present study, the test food was incorporated within a complete breakfast and tested over a longer postprandial duration (270 minutes).

The aim of this study was to compare the kinetics of appearance of exogenous glucose in healthy subjects in response to the ingestion of a breakfast containing different cereal products manufactured from the same ingredients but using 2 distinct technologies (extrusion technology and the rotary molded biscuit process). The 2 cereal products tested had different glycemic index (GI) and contents of slowly available glucose (SAG as determined *in vitro* [20,21]).

SUBJECTS AND METHODS

Subjects

Twenty-five healthy male subjects with no familial history of metabolic disease (non-insulin-dependent diabetes, dyslipidemia, glucose intolerance) or early cardiovascular diseases, no dietary behavior disorders, and no intensive physical activity were selected. The inclusion criteria were age between 18 and 40 years, stable weight over the previous 3 months, a body mass index (BMI) between 20 and 25 kg/m^2, and normal results for biological tests at inclusion. Twelve subjects (aged 25 $\pm$ 1 year) were recruited for the "isotope part" of the study. Subjects presenting natural ^{13}C isotopic enrichment in exhaled $CO_2 \geq -23‰$, determined with a breath test at the preinclusion visit, and subjects consuming high levels of products naturally rich in ^{13}C (e.g., maize, glucose corn syrup) were excluded. Based on unpublished data from CRNH-Rhône-Alpes (Normand, March 2000) comparing the postprandial exogenous appearance rates of 2 cereal products (semolina and pasta), the mean difference between the 2 groups was 1 mg.kg^{-1}.min^{-1} with a variance of 4 mg.kg^{-1}.min^{-1}. The power of the trial was fixed at 80% and alpha risk at 5% in bilateral conditions. Based on these data, the calculated sample size was 12 subjects [22]. This part of the study was approved by the ethics committee of Lyon A, France, and was in accordance with both the French Huriet-Serusclat law and the Second Declaration of Helsinki.

The 13 other subjects were recruited for the "glycemic part." This part of the study was approved by the Human Ethics Review Committee of Sydney University and was performed in accordance with the revised Declaration of Helsinki, Good Clinical Practice (CPMP/ICH/135/95) and the European regulatory requirements (Directive 75/78/CE). Both sites were selected for their expertise in the scientific domain. All volunteers signed an informed consent form before undergoing a medical checkup and starting any experimental test sessions.

Study Design

The study was divided into 2 parts, in which the same breakfasts were consumed.

Part 1: The Isotope Part

This was a randomized, open, crossover study. Subjects consumed the products during 2 periods of 21 days each as habituation periods, each of which was followed by a metabolic study day. During the habituation periods, non-^{13}C-enriched breakfast cereal products were provided to the subjects. One-week semiquantitative food consumption surveys were carried out 3 times during the experimental periods: during the preinclusion period to quantify the food contributions in basal condition and at the end of each habituation period to detect a potential effect of the test breakfasts on the daily dietary intake. Each subject received individual guidance by a trained clinical dietician and was instructed to evaluate and record the amount of food and beverages eaten each day using a 7-day food diary. Ingested food quantities were evaluated using a pictures dietary support elaborated during the SU.VI.MAX (*SUpplémentation en VItamines et Minéraux AntioXydants*) [23]. The macronutrient intakes were calculated using a computerized food database including specific product ingredient lists and recipes for test foods (GENIE by MICRO 6 using CIQUAL table; AFSSA, France). The periods of consumption of each test product were separated by a 14-day washout period.

Part 1 of the study was performed at the Centre de Recherche en Nutrition Humaine Rhône-Alpes (CRNH) at the Hôpital Edouard Herriot (Lyon, France). After a 10-hour overnight fast, on arrival at the metabolic unit (i.e., study days 22 and 57), catheters were inserted into a forearm vein of each subject for blood sampling and isotope infusion. D-[6,6-$^2H_2$] glucose was given in a continuous infusion (0.0475 mg/kg.min^{-1}) over a period of 2 hours prior to the consumption of the test cereal products and over a period of 4.5 hours after the start of ingestion of the test breakfasts. Starch and simple carbohydrates from test cereal products were enriched with ^{13}C. Breakfast was taken over a period of 15 minutes maximum (from T0 to T15). Blood samples were taken at baseline (T-120, T-15, and T0) and every 30 minutes during the 270 minutes of the postprandial period for

measurements of glucose, insulin, C-peptide, nonesterified fatty acids (NEFA) concentrations and deuterium and ^{13}C glucose isotopic enrichments in plasma.

Part 2: The Glycemic Part

Part 2 of the study was led according to a randomized, open, crossover design at the Human Nutrition Unit of the Department of Biochemistry at the University of Sydney. It was done to duplicate the results obtained in part 1 on glycemia and insulinemia, with a more precise evaluation due to the time frame of the blood collection. Subjects consumed each of the 2 breakfast test meals on one occasion. Each subject thus completed 2 separate experimental sessions with at least 3 days between sessions. After a 10-hour overnight fast, subjects consumed the test breakfast in 15 minutes maximum. Arterialized blood was collected via a catheter inserted in the hand after subjects warmed their hands in a warm box. Two fasting blood samples were collected (times −15 and 0 minutes). Blood samples were then withdrawn 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 195, 210, 225, 240, and 270 minutes after participants began eating breakfast for glycemia and insulinemia evaluation.

Test Products

The 2 test cereal products (plain biscuits and extruded cereals) were manufactured on pilot lines at the Danone Research Centre, Palaiseau, France. Their nutritional composition is provided in Table 1. The 2 products differed in terms of carbohydrate digestibility. Their GI, measured according to World Health Organization recommendations [24,25], were 47 for the plain biscuits and 65 for the extruded cereals (unpublished data; Brand-Miller, February 2006). The *in vitro* digestibility characteristics of carbohydrates were determined using the method of Englyst et al. [20]. The SAG (17.2 g and 1.3 g/100 g for plain biscuits and extruded cereals, respectively) and the rapidly available glucose (RAG) contents (43.2 g and 69.7 g/100g for plain biscuits and extruded cereals, respectively) are given in Table 1.

In part 1, during the two 21-day periods preceding the metabolic study, subjects consumed nonlabeled cereal products manufactured with commercially available flour. In part 1 only, they were also instructed to avoid consumption of products naturally rich in ^{13}C. For the 2 metabolic days of part 1, the cereal products consumed were manufactured from wheat flour grown in an atmosphere enriched in $^{13}CO_2$ at the same level of isotopic enrichment as cane sugar (naturally rich in ^{13}C) to obtain the same labeling for both sugars and starch.

Test Breakfasts

For each part of the study, the breakfasts were designed to ensure that the main differences between the 2 meals resulted from the actual cereal products tested, namely, a difference in SAG content and in GI due to the different manufacturing processes used. The 2 breakfasts were designed to be similar in terms of energy content and were composed of one of the cereal products, milk, and a hot beverage (Table 2). The lipid content of the 2 breakfasts was adjusted using half- or full-fat milk in order to ensure an identical content of fatty acids. The energy provided by the 2 breakfasts was between 20% and 25% of the mean daily calorie intake. The carbohydrate content of the breakfast was derived mainly from the cereal product consumed (about 80% of total breakfast carbohydrate content).

Biochemical Analyses

Part 1: The Isotope Part

Plasma glucose and NEFA concentrations were measured with an enzymatic colorimetric method [26,27] on a Cary 50 Bio spectrophotometer (Varian, France) using a BioMérieux Glucose RTU kit (Marcy l'Etoile, France) and a Wako Chemicals NEFA-C kit (Neuss, Germany), respectively. Plasma insulin

Table 1. Nutritional Composition and Characteristics of the Cereal Test Products

	Units	Biscuits (low GI, high SAG content)	Extruded cereals (medium GI, low SAG content)
Energy	kJ/100 g (kcal/100 g)	1917 (458)	1754 (419)
Available carbohydrates	g/100 g (% calories)	72 (63%)	81 (77%)
Starch	g/100 g	50	56
Simple carbohydrates	g/100 g	22	25
Lipids	g/100 g (% calories)	16 (32%)	8 (17%)
Protein	g/100 g (% calories)	6 (5%)	6 (6%)
SAG	g/100 g	17.2	1.3
RAG	g/100 g	43.2	69.7
SAG/total carbohydrates	%	23.2%	1.5%
Resistant starch	g/100 g	3.2	2.3
Fiber	g/100 g	2	2
Glycemic index[1]		47	65
Insulinemic index[1]		47	61

GI = glycemic index, SAG = slowly available glucose, RAG = rapidly available glucose.

[1]Brand-Miller, February 2006 (unpublished data).

Table 2. Nutritional Composition of the Test Breakfasts

		Biscuits breakfast	Extruded cereals breakfast
Foods		70 g of biscuits + 250 ml half-fat milk + hot drink[1] (tea or coffee) with no sugar or sweetener	70 g of extruded cereals + 250 ml full-fat milk + hot drink[1] (tea or coffee) with no sugar or sweetener
Mean nutritional composition			
Energy	kJ (kcal)	1821 (435)	1884 (450)
Carbohydrates	g	62[2]	68.4[3]
Proteins	g	12.0	12.2
Lipids	g	15.4	14.2

[1]Optional, but not more than 300 ml, and identical for both breakfasts for each subject.

[2]That is, 62.0 g carbohydrates, of which 50.5 g was from biscuits and 11.5 g was from milk.

[3]That is, 68.4 g carbohydrates, of which 56.9 g was from extruded cereals and 11.5 g was from milk.

and C-peptide concentrations were determined with a radioimmunoassay kit (respectively, Medgenix Diagnostics, Rungis, France, and Immunotech, Marseille, France).

Part 2: The Glycemic Part

Plasma glucose concentrations were measured in duplicate from 5 μl samples using a Roche/Hitachi 912 automatic centrifugal spectrophotometric analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) using the glucose hexokinase/glucose-6-phosphate dehydrogenase enzymatic assay. Plasma insulin concentrations were measured using a commercial solid-phase antibody-coated tube radioimmunoassay kit with internal standards and controls (Coat-A-Count Insulin kit, Diagnostic Products Corporation, Los Angeles, CA).

Isotope Analysis

D-[6,6-$^2H_2$] glucose (99 mol% excess) was obtained from Eurisotop (Gif-sur-Yvette, France). Chemical and isotopic purity was confirmed by gas chromatography (GC)–selected ion monitoring mass spectrometry (MS) [27]. It was dissolved in sterile isotonic saline solution (9 g NaCl/L) and passed through a 0.22-μm Millipore filter (Millipore Corp., Bedford, MA) before infusion. The preparation was pyrogen free. The concentration of D-[6,6-$^2H_2$]-glucose in the infusate was determined at the end of each test.

Plasma glucose isotopic enrichments were determined on neutralized fractions of deproteinized plasma samples partially purified over sequential anion–cation exchange resins as previously described [28,29]. Plasma D-[6,6-$^2H_2$]-glucose was measured by organic GC-MS (model 5890, Hewlett Packard, Evry, France) on an acetyl-bis-butane-boronyl glucose derivative using the electron-impact mode and selective monitoring of *m/z* 297 and 299 [30].

Plasma ^{13}C glucose enrichment was measured by GC–combustion–isotope ratio MS (SIRA 10, VG Isogas, Middlewich, Cheshire, UK) after derivatization to pentacetyl glucose as described by Dejongh and Hanessian [31].

The ingredients and finished cereal products (biscuits and extruded cereals) were analyzed for highly precise determination of their respective ^{13}C-enrichment levels, determined after enzymatic hydrolysis of biscuits and extruded cereals (with amyloglucosidase and sucrase) using the method of Thivend and Guilbot [32]. The resulting glucose was purified on ion-exchange resins and then derivatized to pentacetyl glucose and analyzed by GC–combustion–isotope ratio MS. The ^{13}C enrichment of the derivatized glucose molecule was -37.03 δ‰ (1.07052 $\pm$ 0.00035 at% ^{13}C) for biscuits and -37.17 δ‰ (1.07037 $\pm$ 0.00064 at% ^{13}C) for extruded cereals (mean $\pm$ SD of 5 measurements).

These levels of enrichments were sufficient for reliable and reproducible measurements of ^{13}C plasma glucose. The precision measurement was in the range of $\pm$ 0.1 δ‰ and in the plasma sample enrichment values as low as 0.001 at% excess were easily measurable.

Determination of Plasma Glucose Kinetics

The rates of appearance and disappearance of total glucose (RaT and RdT) were calculated from plasma D-[6,6-$^2H_2$] glucose enrichment, and the rates of appearance and disappearance of exogenous glucose (RaE and RdE) were determined from plasma [^{13}C] glucose enrichment using Steele's equation for non-steady-state conditions [33,34] as previously described [29]. Endogenous glucose production (EGP) was calculated as RaT $-$ RaE.

Indirect Calorimetry Analysis

An indirect open-circuit calorimeter (Deltatrac Metabolic Monitor, Datex Corp., Helsinki, Finland) was used to measure the respiratory gas exchanges in the basal state and from 15 to 270 minutes after the ingestion of the test meals. Carbohydrate, fat, and protein oxidation rates were calculated from respiratory gas exchanges and urinary nitrogen excretion according to the equation developed by Ferrannini [35].

Statistical Analyses

All statistical analyses were performed with SAS statistical software (ver. 8.2; SAS Institute Incorporated, Cary, NC). Descriptive statistics are presented with group size and means $\pm$

SD or standard error of the mean (SEM). A 2-tailed p value $\leq$ 0.05 was considered statistically significant. Analyses were performed on an intention-to-treat population (all randomized subjects with at least one measurement of the main parameter) with 12 subjects considered to be in part 1 (isotope part) and 13 subjects in part 2 (glycemic part). Raw data or calculated variables were analyzed, including the area under the curve, the incremental area under the curve (iAUC), and the rates of glucose appearance and disappearance.

For each parameter used, the principal adjusted model was the usual Gaussian model for crossover designs with 2 periods. The analysis of variance was performed taking into account subject, test product, study period, and sequence effect (equivalent to the interaction Period $\times$ Test product and the carryover effect). Subject factor was considered as a random effect.

The sequence effect was also calculated and tested using this principal model. No sequence effect was found. The analysis was therefore based on the 2 periods within each part of the study.

RESULTS

Subject Characteristics

The 25 male subjects participating in the study had a mean (SD) age of 24.0 (3.3) years and a mean (SD) body mass index (BMI) value of 22.6 (1.8) kg/m^2. There was no significant difference between mean age and BMI for subjects in the 2 parts of the study (mean [SD]: 25.0 [1.3] vs. 23.5 [1.8] years and 22.0 [0.4] vs. 23.2 [2.0] kg/m^2). No adverse effect attributable to the test products was observed.

Part 1: Isotope Part

Dietary Intake

The type of breakfast did not influence the total daily caloric intake (9398 kJ [SEM = 335], 9381 kJ [SEM = 377] and 9490 kJ [SEM = 398] during preinclusion and after a 20-day ingestion of biscuits and extruded cereal breakfasts, respectively).

The energy distribution between the 3 major meals and collations was not significantly modified by the type of breakfast ingested (percentage of daily energy for biscuits and extruded cereals, respectively: 20% vs. 21% for breakfast, 39% vs. 40% for lunch, 38% vs. 34% for dinner, and 3% vs. 5% for collations), nor was the type of nutrient eaten at lunch (20% vs. 18% for proteins, 42% vs. 40% for lipids and 36% vs. 39% for carbohydrates of lunch energy for biscuits and extruded cereals, respectively).

RaE and RdE of Exogenous Glucose Derived from Cereal Products

After the biscuits breakfast, RaE increased for 60 minutes to reach a plateau, with RaE being significantly lower at 90, 120, and 150 minutes compared to the levels for the extruded cereals breakfast (Fig. 1A). At 210 minutes, the RaE was significantly higher for the biscuits breakfast than for the extruded cereals breakfast ($p \leq 0.01$).

The RdE following consumption of the biscuits breakfast recorded at study times 90, 120, 150, and 180 minutes was significantly lower than for the extruded cereals breakfast (Fig. 1B). Due to these differences, the quantity of exogenous glucose from biscuits appearing during the first half of the morning was lower than for extruded cereals. The cumulated RaE over the entire morning tended to be lower for the biscuits (59.0 g; SEM = 4.1) than for the extruded cereals (70.1 g; SEM = 4.1, $p = 0.05$).

RaT and RdT of Total Glucose

RaT and RdT were significantly lower ($p \leq 0.05$) following ingestion of the biscuits breakfast at 90, 120, and 150 minutes than for the extruded cereals breakfast and at T60 for RaT (Fig. 2A,B).

Endogenous Glucose Production

EGP was partly inhibited over the entire morning following the consumption of both types of breakfasts (Fig. 2C). Slight differences were observed, however. During the first part of the morning, it tended to be less inhibited ($p \leq 0.05$ at 180 minutes) with the biscuits breakfast, whereas at the end of the morning, it tended to be more inhibited after the biscuits breakfast than after the extruded cereals breakfast ($p \leq 0.01$ at 210 minutes).

Plasma Glucose, Insulin, C-Peptide, and NEFA Responses.

Between T0 and T180 minutes, glycemia and insulinemia were not significantly different between breakfasts except at T120 ($p \leq 0.05$) (Fig. 3A,B). At the end of the morning, plasma glucose concentrations were significantly higher 210 and 240 minutes after the biscuits breakfast than after the extruded cereals breakfast ($p \leq 0.05$).

The iAUC for plasma glucose and insulin did not differ significantly between the 2 breakfasts, although they were slightly lower with the biscuits breakfast.

The C-peptide concentration was significantly lower following the consumption of the biscuits breakfast at 90, 120, and 150 minutes ($p \leq 0.05$; Fig. 3C).

NEFA profiles were similar for the 2 test breakfasts with an exception at 150 and 180 minutes, where the concentrations were significantly higher following the biscuits breakfast (Fig. 4).

Total Carbohydrate and Lipid Oxidation

At baseline, there were no significant differences between breakfasts in total carbohydrate oxidation (6.4 g/60 min [SEM = 0.6] for biscuits breakfast and 6.5 g/60 min [SEM = 0.6] for extruded cereals breakfast), or in total lipid oxidation

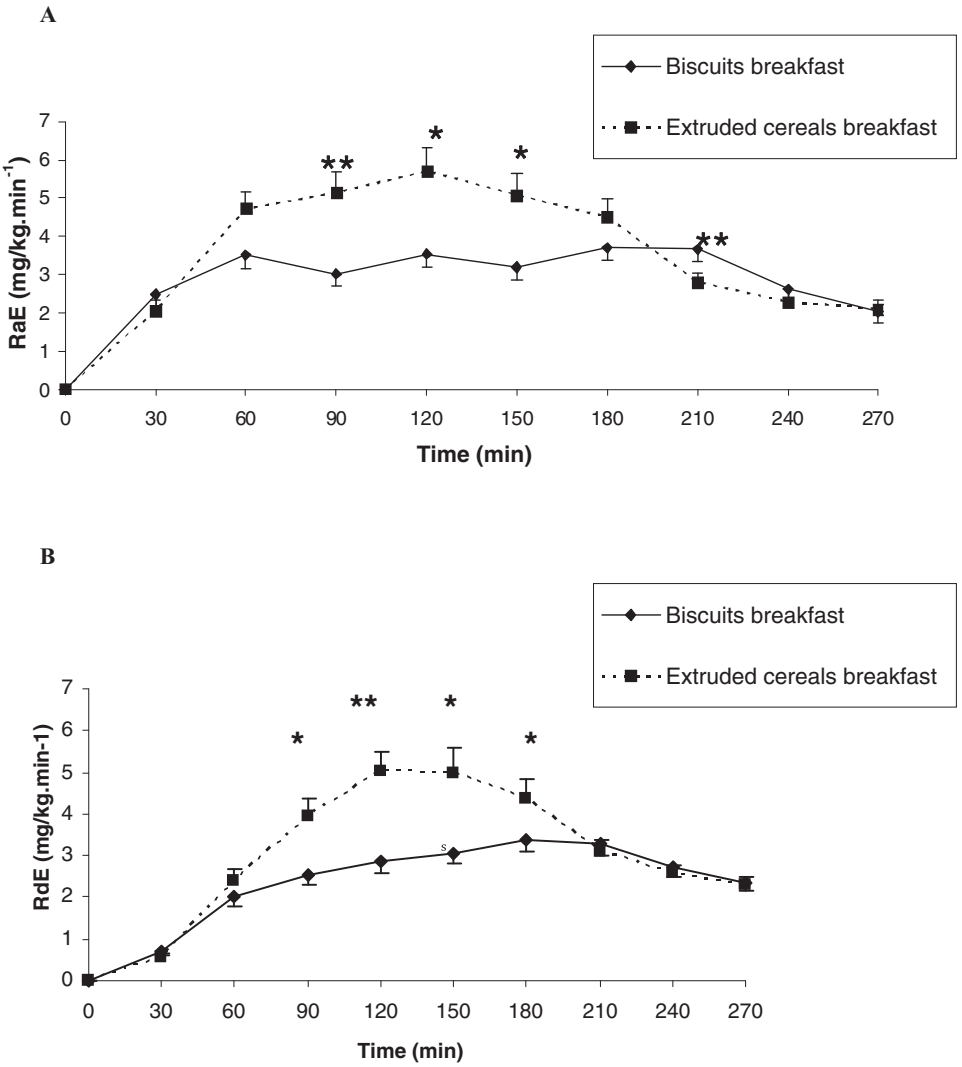

Fig. 1. Part 1: Kinetics for rates of (A) appearance of exogenous glucose (RaE) and (B) disappearance of exogenous glucose (RdE) for biscuits and extruded cereals breakfasts (mean $\pm$ SEM, $n = 12$). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$.

(3.4 g/60 min [SEM = 0.2] for biscuits breakfast and 3.5 g/60 min [SEM = 0.2] for extruded cereals breakfast). Over the entire postprandial period (T0–T270), total carbohydrate oxidation was significantly decreased following consumption of the biscuits breakfast (40.4 g; SEM = 2.1) compared to the extruded cereals breakfast (45.6 g; SEM = 2.1, $p \leq 0.05$). Total lipid oxidation was similar for the 2 breakfasts (12.9 g; SEM = 0.8. and 11.6 g; SEM = 0.8, for biscuit and extruded cereals breakfasts, respectively).

Part 2: Glycemic Part

Following consumption of the biscuits breakfast, glycemia was significantly lower during the first part of the morning (times 30, 40, 60, 75, and 90 minutes) than after the extruded cereals breakfast (Fig. 5A).

The iAUC for plasma glucose during the first 120 minutes after breakfast was significantly lower ($p = 0.01$) following ingestion of the biscuits breakfast than after the extruded cereals breakfast. The iAUC for plasma glucose for the second part of the morning (120–270 minutes) and for the entire morning (0–270 minutes) did not differ significantly. Following ingestion of the biscuits breakfast, plasma insulin was significantly lower during the first part of the morning (times 40, 50, 60, 75, 90, and 105 minutes) than after the extruded cereals breakfast (Fig. 5B). The overall insulinemic response (iAUC 0–270) was significantly reduced by 28% with the biscuits breakfast compared to the extruded cereals breakfast ($p < 0.01$). The reduction in insulinemic response was particularly marked during the first part of the morning (0–120 minutes, $p < 0.01$).

The glycemic and insulin responses did not differ significantly between the isotope and glycemic parts when comparing iAUC during the full postprandial phase (0–270 minutes) or between 0 and 120 minutes, though the time points were not exactly the same between the 2 parts (data not shown).

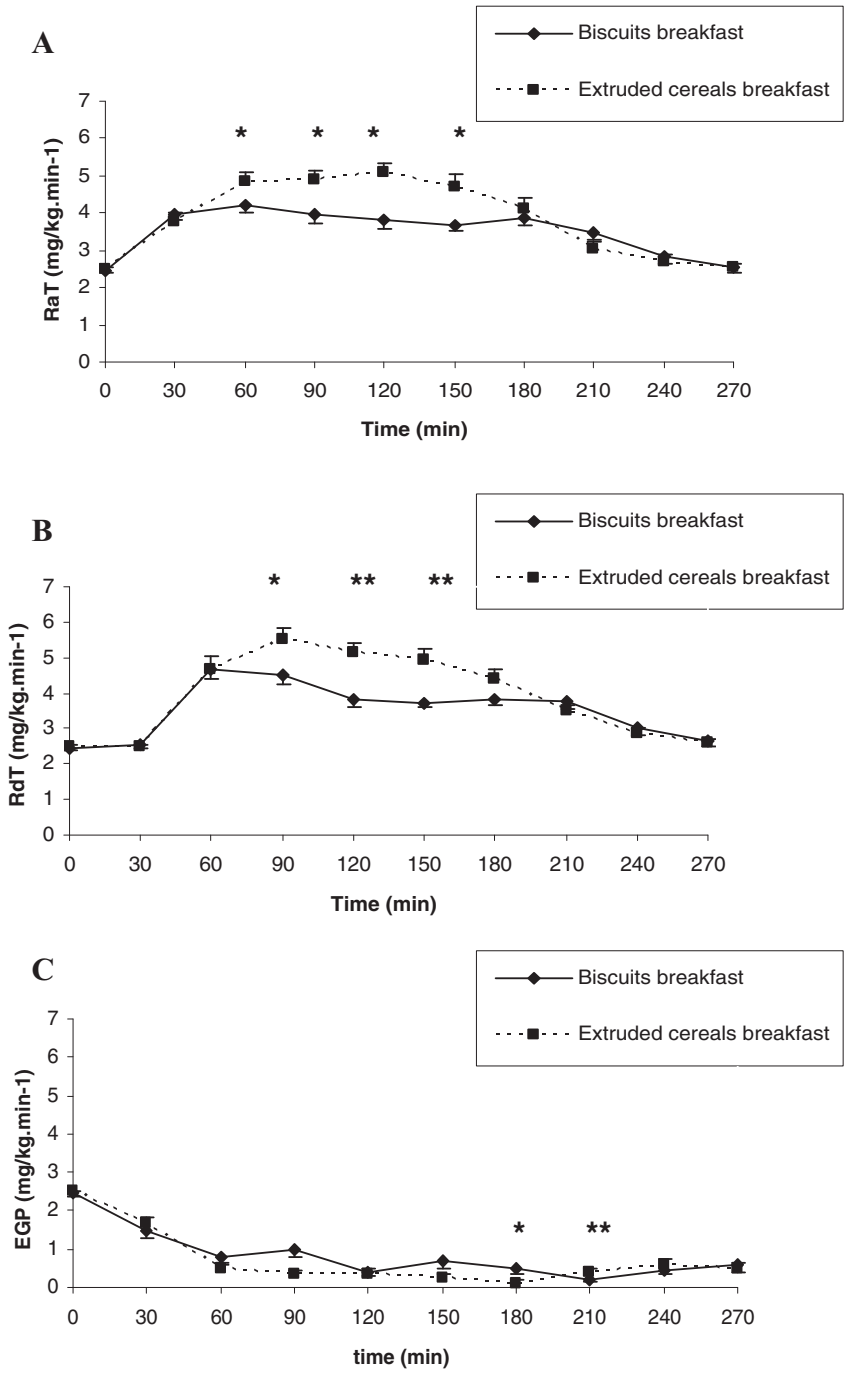

Fig. 2. Part 1: Kinetics for rates of (A) appearance of total glucose (RaT), (B) disappearance of total glucose (RdT), and (C) endogenous glucose production (EGP) for biscuits and extruded cereals breakfasts (mean $\pm$ SEM, $n = 12$). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$.

DISCUSSION

The present study shows that under realistic conditions of breakfast consumption, the kinetic profile for the RaE was slower during the first part of the morning with biscuits (low GI and high SAG content) than with extruded cereals (medium GI and low SAG content; significant differences at 90, 120, 150 minutes). By the end of the morning, RaE from biscuits tended to become greater than RaE from extruded cereals (significant difference at 210 minutes). The disappearance rates of total and exogenous glucose RdT and RdE followed a similar evolution over the postprandial period and were lower after the biscuits breakfast, in association with a lower C-peptide secretion.

Contradictory results were obtained between parts 1 and 2 of the study with regard to glycemic response. In part 1, no significant difference was obtained on the peak of glycemia or

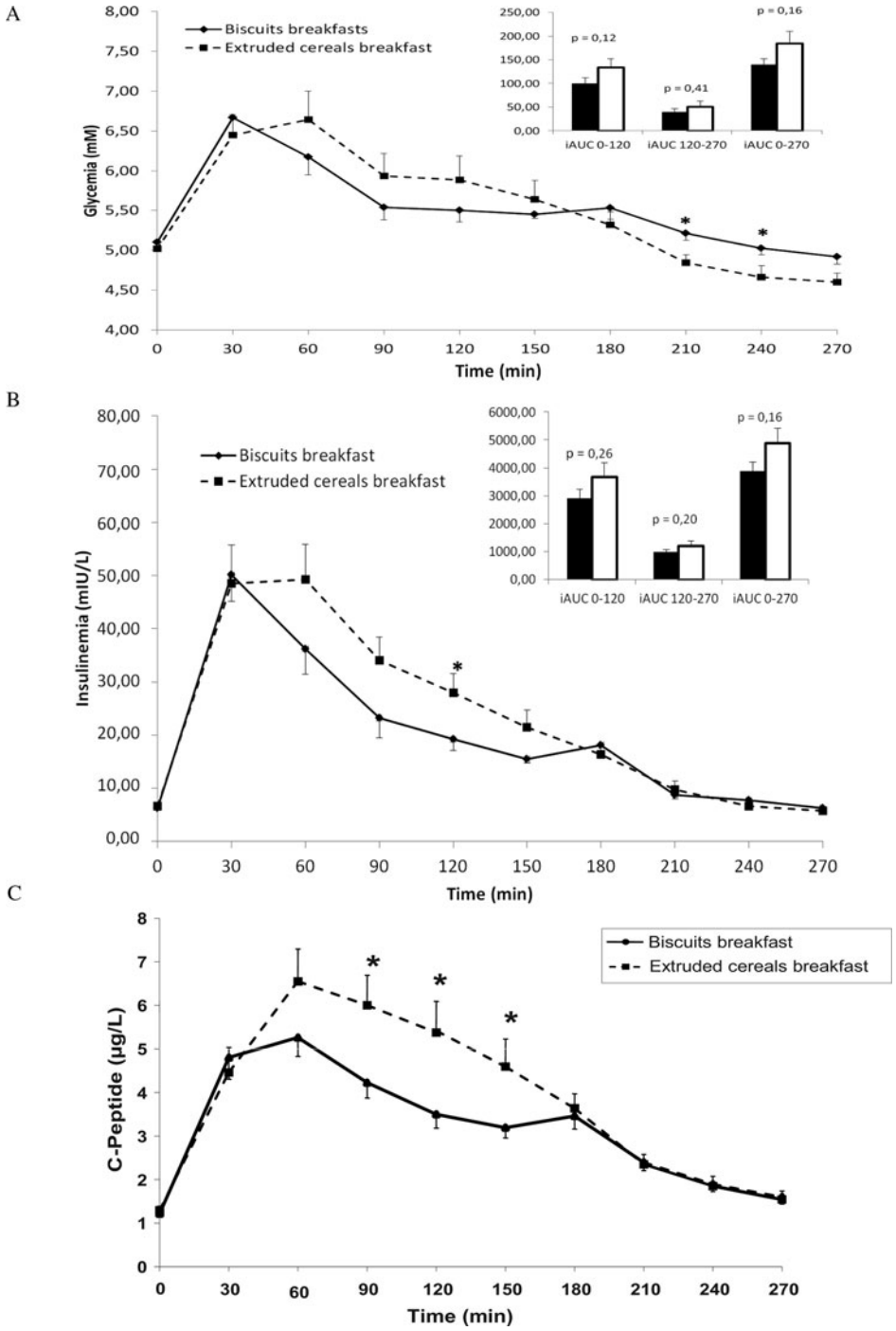

Fig. 3. Part 1: (A) Plasma glucose response and incremental areas under the curve, (B) plasma insulin response and incremental areas under the curve for the first and second parts of the morning and for the entire morning following ingestion of biscuits and extruded cereals breakfasts, and (C) plasma C-peptide response following ingestion of biscuit and extruded cereal breakfasts (mean $\pm$ SEM, $n = 12$). iAUC = incremental area under the curve. $^{*}p \leq 0.05$.

iAUC between the 2 breakfasts, whereas in part 2, significant differences with a reduced peak and lower iAUC of glycemia were obtained after the biscuits breakfast than after the extruded cereals breakfast. The main difference between the 2 parts was the blood sampling interval. In part 1, with a sampling every 30 minutes, we hypothesize that the glycemic peak was missed. In part 2 when the blood sampling was performed every 10 minutes during the first postprandial hour, significant peak and iAUC reduction were obtained after the biscuits breakfast.

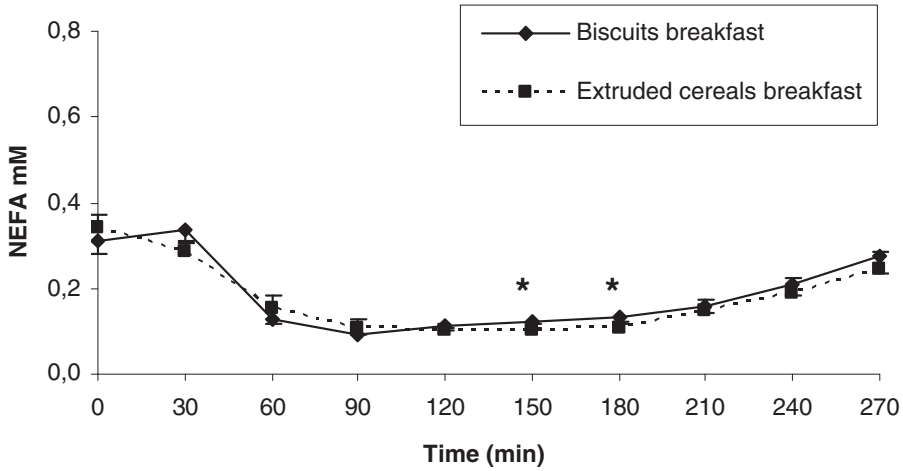

Fig. 4. Part 1: Plasma nonesterified fatty acid (NEFA) response following ingestion of biscuits and extruded cereals breakfasts (mean ± SEM, $n = 12$). $^{*}p \leq 0.05$.

The information on the insulin and C-peptide plasma concentration kinetics reinforced this hypothesis. Though there was no significant difference in insulin response between breakfasts in part 1, C-peptide response, which has a longer half-life than insulin (30 minutes for C peptide [36,37] compared to ∼5 minutes for insulin [38–40]), was reduced after the biscuits breakfast. The difference in the degree of significance of C-peptide and insulin kinetics probably resulted from the higher biological variability induced by the short half-life of insulin compared to that of C-peptide [41]. Moreover, insulin response was significantly reduced (peak and iAUC) after the biscuits breakfast when the interval between blood sampling was 10 minutes (part 2) instead of 30 minutes (part 1).

At the late phase of the postprandial period, glycemia tended to be significantly higher after the biscuits breakfast than after the extruded cereals, especially in part 1 of the study. A work confirmed our results for overweight subjects without metabolic disturbances [39].

Most studies dealing with the quality of carbohydrates are based upon an approach that measures glucose from both exogenous and endogenous origins. However, these parameters are the result of the appearance and disappearance of carbohydrates in the systemic circulation. Previous studies using the stable isotope method have shown that moderate glycemic response may result from an increase in the rate of disappearance of glucose induced by stimulated insulin secretion through higher protein content of cereal products [15]. In addition, Normand et al. demonstrated that the rate of appearance of glucose may be reduced by adding lipids to a meal [14]. In Normand et al. [14] and Schenk et al. [15], the glycemic responses to meals were reduced. Therefore, not all products with a low GI or that induce moderate glycemic response necessarily contain slowly digestible carbohydrates, because low glycemic response may be obtained by other means, such as adding lipids or insulinogenic substrates (i.e., proteins [42–44]). Hence, the concept of low GI may not be routinely associated with the slow appearance of carbohydrates. Therefore, it is not possible to draw a conclusion regarding the metabolic impact and long-term health benefits of a food solely by observing glycemia and insulinemia without giving appropriate attention to glucose kinetics. In the present study, the low GI of biscuits resulted in a slow carbohydrate appearance, as expected from the high SAG content of the product.

The sequence of events following breakfast can be summarized as follows. The RaE was similar for both breakfasts for the first 30 minutes, whereas the RdE was similar only during the first hour. This similarity may be due to the initial release of a part of the RAG from both products. After the first hour, the biscuits breakfast resulted in a slower and more stable RaE compared to the extruded cereals, combined with a slower RdE and a lower insulin demand, corresponding to digestion of the SAG fraction. Finally, at 210 minutes, the RaE from the biscuits breakfast was greater than that from the extruded cereals, whereas the RdE and RdT were similar for the 2 cereal products.

The quantities of exogenous glucose appearing during the total postprandial period were greater than the ingested quantities, irrespective of the breakfast eaten. This result may be ascribed to ^{13}C-glucose recycling, which occurs as a result of labeled glucose synthesized in the liver from ^{13}C-lactate produced from exogenous glucose in the gut or in peripheral tissues [16,45–47]. This phenomenon leads to further appearance of ^{13}C-glucose in the peripheral circulation, which is consequently determined twice. This phenomenon, if it occurred, led to an overestimation of glucose absorption late in the observation period, which should be greater for the extruded cereals than for the biscuits breakfast, because the ^{13}C-glucose rate was higher for extruded cereals during the first part of the postprandial phase. However, RaE from the biscuits breakfast became greater than RaE from extruded cereals at 210 minutes. Hence, the late overestimation of RaE may have a major impact for the extruded cereals

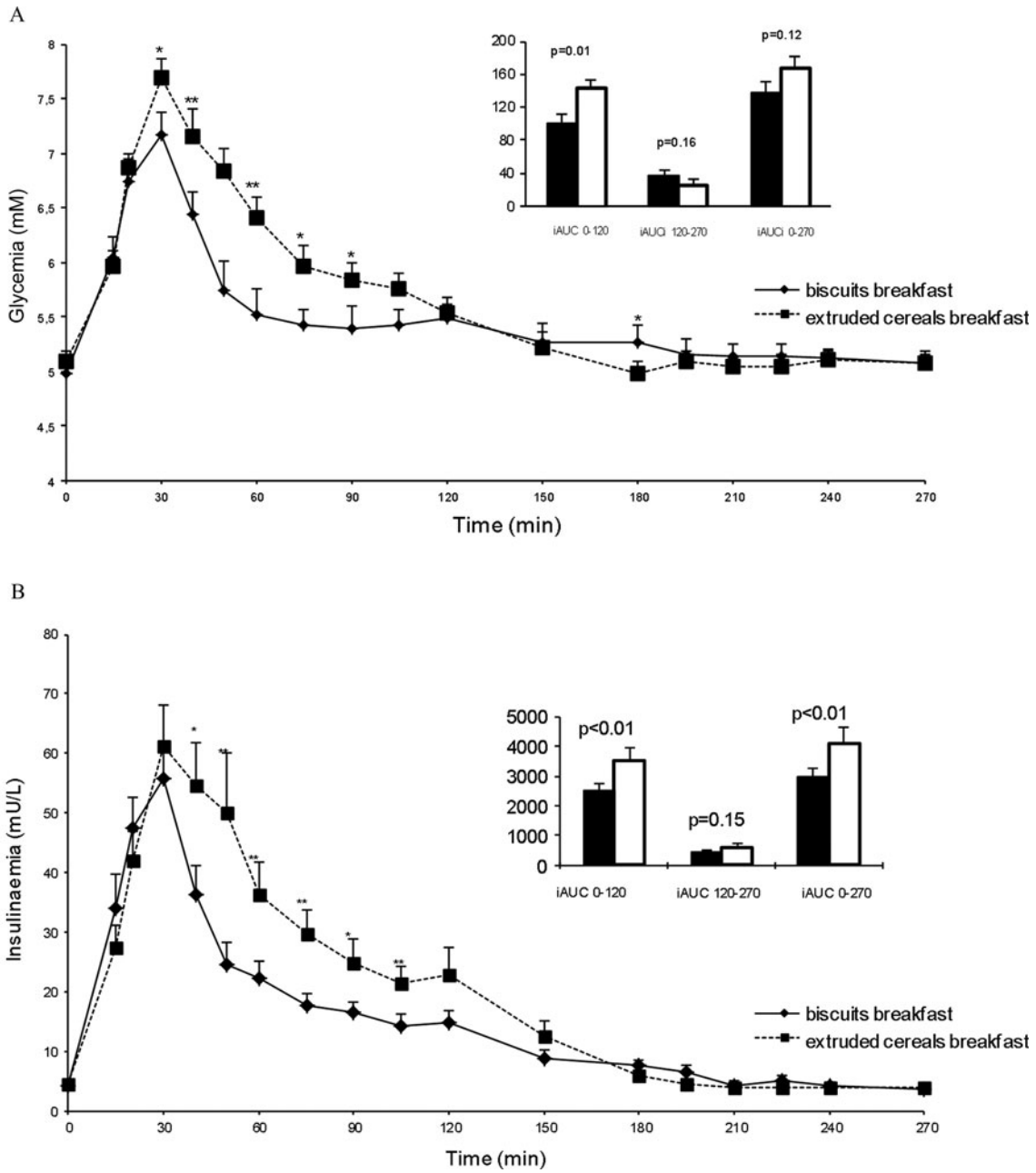

Fig. 5. Part 2: Plasma responses for (A) glucose and incremental areas under the curve and (B) insulin and incremental areas under the curve for the first and second parts of the morning and for the entire morning following ingestion of biscuits and extruded cereals breakfasts (mean $\pm$ SEM, $n = 13$). iAUC = incremental area under the curve. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$.

breakfast and then lead to an underestimation of the differences between the 2 breakfasts during the last hour of the postprandial period.

It also should be noted that the extruded cereals had a higher carbohydrate content (6 g in the 70 g portion); in fact, the caloric content of the breakfasts was adjusted and the addition of full-fat milk to the extruded cereal product allowed adjustment of the lipid content. This could explain the observed difference in RaE and postprandial responses. Based on previous works, a recent paper showed significant differences in RaE and glycemic responses, where the carbohydrate content of the cereal products (and breakfasts) were similar (59 g) but the SAG contents were different (21 g/80 g portion vs. less than 1 g/80 g portion) [48]. In addition, when considering results obtained on GI, where the carbohydrate content was adjusted to 50 g of carbohydrates, it has been shown that SAG is the main parameter explaining the GI of cereal products, before fat and other carbohydrates [6]. The SAG range of the 26 cereal products was between less

than 1 g/portion and 14 g/portion, comparable to the difference observed in the present article. Hence, both carbohydrate parameters (quantity 6 g difference, digestibility 12 g difference) may play a role. However, based on the breakfast composition and previous data, carbohydrate digestibility (evaluated according to SAG content) is an important parameter to be considered in the modulation of RaE and glycemic response.

Recently, a novel method of evaluating glucose fluxes has been developed, the triple-tracer method, which has been compared to the conventional dual-tracer method [49]. The results obtained for 8 subjects confirmed that the conventional method provides relevant results but slightly underestimates RaE compared to the triple-tracer method. Moreover, EGP is more accurate with the triple-tracer method because it is measured directly, in contrast to being deduced from calculations in the dual-tracer method (difference between RaT and RaE).

In our study, EGP tended to be less inhibited during the first part of the morning after the biscuits breakfast than after the extruded cereals breakfast (the opposite trend was observed at the end of the morning). This was coherent with the lower insulin secretion induced by the biscuits breakfast during the first part of the morning. The lower inhibition of EGP after the biscuits breakfast may have hidden the influence of the slower appearance of exogenous glucose on glycemia. However, this result has to be considered cautiously because of the means used for calculation and, thus, its level of accuracy. When comparing the triple-tracer and the double-tracer methods, it has been shown that the main differences for EGP between the 2 methods were observed during the first postprandial stage and that there was a global underestimation of EGP suppression with the dual-tracer method [49]. Hence, further investigations are required to confirm this initial result.

Still, we can assume that the lower total carbohydrate oxidation was related to the lower glucose disappearance rates observed with the biscuits breakfast throughout the morning. These consistent results have not always been observed in previous studies [50].

In conclusion, this study shows that under real conditions of breakfast consumption, in healthy males the RaE from biscuits containing high levels of SAG and a low GI was lower at the beginning and higher at 210 minutes (i.e., more stable over the entire morning period) compared to the extruded cereals containing low levels of SAG and medium GI. Concomitantly, postprandial glucose response and insulin demand were reduced. Furthermore, this study shows that measurement of glucose rates and fate can be precious information to use in evaluating the influence of low-GI foods on metabolic parameters and thus their health interest [51].

In our context, the double labeling of glucose (exogenous and total) allowed the parallel follow-up of endogenous glucose kinetics and indeed highlighted the compensatory lower inhibition of EGP. Consequently, isotope analysis is important in understanding the mechanisms underlying plasma glycemic response. The combined glucose isotope and glycemic analyses showed that if RaE was highly different between groups in response to breakfast, the difference in glycemic response was less pronounced due to modulation of EGP.

Finally, the present findings suggest that the consumption of slowly digestible starch products could contribute to decreasing postprandial glucose excursions and potentially metabolic profiles. This could be of great interest in relation to the genesis of chronic pathologies such as diabetes [52–54].

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AUTHOR CONTRIBUTIONS

S.V., S.N., and M.L. contributed to the conception and the design of the study. S.N., C.L.P., C.M., J.P., and J.B.M. contributed to data collection and analysis. S.V., S.N., J.-A.N., A.M., and M.L. interpreted the data before writing the article. S.V. proposed the first draft of the article. All authors participated in the writing of the final draft of the article. S.V. and A.M. are employees of Kraft Biscuit Europe.

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