Short Communication

Reduced glycaemic and insulinaemic responses following isomaltulose ingestion: implications for postprandial substrate use

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The impact of slow digestible sources of dietary carbohydrate in reducing the risk of developing obesity and related metabolic disorders is unclear. The aim of the present study was to compare the postprandial metabolic response to the ingestion of sucrose v. isomaltulose. We hypothesised that the reduced digestion and absorption rate of isomaltulose would result in lower glycaemic and insulinaemic responses when compared with the ingestion of sucrose, leading to greater postprandial fat oxidation rates. In a randomised, single-blind, cross-over study, ten overweight subjects ingested two different carbohydrate drinks (sucrose and isomaltulose, 75 g carbohydrate equivalents) following an overnight fast (08.40 hours) and with a standardised meal (12.30 hours, 25 % of total energy content was provided as either a sucrose or isomaltulose drink). Blood samples were taken before ingestion and every 30 min thereafter for a period of 3 h, substrate use was assessed by indirect calorimetry and breath samples were collected. Ingestion of carbohydrates with a mixed meal resulted in a lower peak glucose and insulin response and a lower change in area under the curve (ΔAUC) following isomaltulose when compared with sucrose. Together with the lower glucose and insulin responses, postprandial fat oxidation rates were higher (14 %) with isomaltulose when compared with sucrose when ingested with a mixed meal (P=0.02). The attenuated rise in glucose and insulin concentrations following isomaltulose results in reduced inhibition of postprandial fat oxidation. The metabolic response to isomaltulose co-ingestion suggests that this may represent an effective nutritional strategy to counteract overweight-induced metabolic disturbances.

Isomaltulose: Glycaemic response: Substrate use

Over the last two decades the prevalence of obesity and obesity-related disorders has increased rapidly¹. Both genetic and environmental factors play an important role in the aetiology of these chronic metabolic diseases. Obesity develops as a result of an imbalance between energy intake and energy expenditure, resulting in a positive energy balance. Although many factors promote a positive energy balance, there is sound evidence that a high-fat–low-carbohydrate (CHO) diet increases the risk of weight gain due to excess energy intake². On the other hand, high-CHO–low-fat-diets containing a large amount of rapidly available CHO (cooked starches) and added refined sugars (sucrose, high-fructose corn syrup) may be counterproductive to body-weight control because they markedly increase postprandial glycaemia and insulinaemia. The latter may promote fat storage in both adipose and non-adipose tissue, through an inhibitory effect on adipose tissue lipolysis and/or muscle fat oxidation. Greater postprandial fat storage in non-adipose tissue, such as skeletal muscle and liver tissue, has been associated with the development of insulin resistance, whilst postprandial hyperglycaemia per se represents a strong risk factor for the development of type 2 diabetes mellitus and cardiovascular co-morbidities³.⁴. Finally, hyperinsulinaemia may negatively impact on TAG clearance, resulting in higher plasma TAG concentrations. Therefore, the recommendation to ingest a CHO-rich diet, containing a large amount of high-glycaemic CHO, may have a less favourable effect on the blood lipid profile⁵.⁶. Potential negative side effects of high-CHO diets may be counteracted by the use of low-glycaemic index foods. The prolonged use of low-glycaemic index foods has been reported to prevent the risk profile for developing obesity, diabetes and CVD⁷. Brand-Miller et al. hypothesised that the ingestion of

Abbreviations: ΔAUC, change in area under the curve; CHO, carbohydrate.
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slowly digestible CHO attenuates the postprandial rise in glycaemia and insulinemia, and enhances fat oxidation rates. The latter may assist in preventing body-weight gain and insulin resistance(7). As such, slowly digestible CHO may be of relevance in dietary strategies to modulate body weight and improve insulin sensitivity.

The aim of the present study was to compare postprandial hormonal and metabolic responses following the ingestion of sucrose compared with isomaltulose. We hypothesised that the ingestion of isomaltulose would be accompanied by a lower glycaemic and/or insulinemic response, a greater increase in satiety-regulating peptides, less inhibition of postprandial fat oxidation rate and a lower plasma TAG response when compared with sucrose.

Methods

Subjects

Ten healthy, overweight men (n 8) and women (n 2) were recruited to participate in the present study (age 31 (SE 4) years, BMI 27-7 (SE 0-8) kg/m², fasting glucose 5-1 (SE 0-1) mmol/l, fasting insulin 14 (SE 1-9) μU/ml). Subjects with cardiovascular or metabolic disorders, and those using medication, were excluded from the study. The study was reviewed and approved by the Medical Ethics Committee of Maastricht University. All subjects provided written informed consent.

Study design

All subjects were studied following an overnight fast at 08.00 hours on two occasions with an interval of at least 1 week. At the beginning of the experimental day, a Teflon cannula was inserted into an antecubital vein. Two different CHO drinks were ingested (sucrose or isomaltulose), during two different trials, performed using a single-blind, randomised cross-over design. Sucrose and isomaltulose were derived from cane sugar, a natural CHO source with a high natural abundance of 13C. The CHO load consisted of 75 g CHO and was dissolved in 400 ml water, to assess the metabolic response. After baseline measurements all experimental beverages were consumed within 15 min. Blood samples were taken before the consumption of the drinks or meals (t = − 5 min) and at t = 30, 60, 90, 120, 150 and 180 min after ingestion to determine circulating metabolite and hormone concentrations. Energy expenditure and substrate use were measured, immediately before and for 3 h after CHO ingestion (08.40 hours), using a ventilated hood system. Expired breath was collected every 1 h to determine 13CO2 enrichment. These procedures were repeated on the same day before consuming a standardised lunch and for 3 h after lunch (12.30 hours). Lunch had a total energy content equivalent to 50% of calculated 24 h resting energy expenditure. After collection, blood samples were centrifuged immediately and frozen at − 80°C until further analysis. Plasma glucose and NEFA concentration were determined enzymically (ABX Diagnostics, Montpellier, France) as were NEFA concentrations (NEFA-NEFA C kit; Wako, Neuss, Germany) on a semi-automatic analyser (COBAS FARA centrifugal spectrophotometer; Roche Diagnostics, Basel, Switzerland). Insulin was analysed by RIA (Human Insulin RIA Kit; LINCO Research Inc., St Charles, MO, USA), as was total ghrelin (Total Ghrelin RIA kit; LINCO Research Inc., St Charles, MO, Switzerland). Insulin was analysed by RIA (Human Insulin RIA Kit; LINCO Research Inc., St Charles, MO, USA) on a semi-automatic analyser (COBAS FARA centrifugal spectrophotometer; Roche Diagnostics, Basel, Switzerland). Insulin was analysed by RIA (Human Insulin RIA Kit; LINCO Research Inc., St Charles, MO, USA). Subjects were instructed not to consume any food products with a high natural abundance of 13C for at least 1 week before and during the experimental period. In European countries the consumption of native CHO sources with high natural 13C abundance is low(9).

Biochemical analyses

At all time points, 8 ml blood were collected in pre-chilled tubes with 200 μ l 0·2 m-EDTA (Sigma, Poole, Dorset, UK). After collection, blood samples were centrifuged immediately at 4°C for 10 min at 1000 g and frozen at − 80°C until further analysis. Plasma glucose and NEFA concentration were determined enzymically (ABX Diagnostics, Montpellier, France) as were NEFA concentrations (NEFA-NEFA C kit; Wako, Neuss, Germany) on a semi-automatic analyser (COBAS FARA centrifugal spectrophotometer; Roche Diagnostics, Basel, Switzerland). Insulin was analysed by RIA (Human Insulin RIA Kit; LINCO Research Inc., St Charles, MO, USA), as was total ghrelin (Total Ghrelin RIA kit; LINCO Research Inc.). Plasma active glucagon-like peptide-1 concentration was analysed by enzyme-linked immunoradiometric assay (EGLP-35K; LINCO Research Inc.). Breath samples were analysed as 13C:12C ratio by GC isotope ratio MS (Finnigan MAT 252).

Calculations

Metabolic rate was calculated from VO2 (litres/min) and VCO2 (litres/min) according to the equations of Frayn(10). N excretion was calculated based on the assumption that protein oxidation represents 15% of total energy expenditure. Energy expenditure was calculated using the formula of Weir(11):

\[
\text{CHOoxidation} = (4·555 \times \text{VCO}_2) - (3·21 \times \text{VO}_2) - (2·87 \times N).
\]

Fat oxidation = \(1·67 \times \text{VO}_2 - (1·67 \times \text{VCO}_2) - (1·92 \times N)\).

\[N (\text{g}/\text{min}) = ((0·15 \times \text{energy expenditure})/17)/6·25.\]

Energy expenditure (kJ/min) = \(4·187 \times (3·9 \times \text{VO}_2 + 1·1 \times \text{VCO}_2)\).

The isotopic enrichment was expressed as the δ% difference between the 13C:12C ratio of the sample and a known
laboratory reference standard according to the formula of Craig\cite{12}:

\[
\delta^{13}C = \left( \frac{^{13}C/^{12}C\text{ sample}}{^{13}C/^{12}C\text{ standard}} - 1 \right) \times 10^3 \text{ per mil.}
\]

The $\delta^{13}C$ was then related to the international standard Pee Dee belemnite (PDB).

Exogenous CHO oxidation was estimated using the following formula\cite{13}:

Exogenous CHO oxidation = $V_{CO_2} \times \left( \frac{\delta_{Exp} - \delta_{Exp_{bkg}}}{\delta_{Ing} - \delta_{Exp_{bkg}}} \right) \left( \frac{1}{k} \right)$,

in which $V_{CO_2}$ is the volume of expired CO$_2$ per min (litres/min), $\delta_{Exp}$ is the $^{13}C$ enrichment of expired air with CHO ingestion at different time-points, $\delta_{Ing}$ is the enrichment of the CHO in the experimental drinks, $\delta_{Exp_{bkg}}$ is the $^{13}C$ enrichment of

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{Plasma glucose (a), insulin (b) and NEFA (c) concentrations following sucrose (––) or isomaltulose (––) ingestion. Time-course of fat oxidation (d), carbohydrate (CHO) oxidation (e) and ghrelin (f) following sucrose or isomaltulose ingestion. Values are means, with their standard errors represented by vertical bars. * Mean value was significantly different from that following isomaltulose ingestion ($P < 0.05$).}
\end{figure}
expired breath before the intervention (background) and k is the amount of CO₂ (in litres) produced by the oxidation of 1 g glucose (k = 0.7467 litres CO₂ per g glucose). Endogenous CHO oxidation was calculated as the difference between total CHO oxidation and exogenous CHO oxidation. This represents a minimal estimate of exogenous CHO oxidation, as part of the ¹³C will be temporarily fixated in the bicarbonate pool and in the tricarboxylic acid cycle intermediates\(^{(14,15)}\).

Statistics
A computerised statistics program, SPSS 11 for Macintosh (SPSS Inc., Chicago, IL, USA), was used to perform all calculations. All data are expressed as mean values with their standard errors. The total response of parameters after CHO ingestion was expressed as the incremental area under the curve (ΔAUC) and calculated by the trapezoid method. Response is defined in the Results section as ΔAUC, unless mentioned otherwise. Differences between responses to sucrose compared with isomaltulose were analysed by means of the Student’s paired t test. The Student’s paired t test was used to compare differences in peak response between the different CHO.

Results
Plasma glucose, insulin, non-esterified fatty acids and triacylglycerol
Fasting plasma glucose, insulin and NEFA concentrations did not differ between experiments (Fig. 1(a–c)). Peak plasma glucose concentrations were lower after the ingestion of isomaltulose when compared with sucrose both when ingested as a drink (morning) as well as with a meal (afternoon) (Fig. 1(a)). The glycaemic response was lower after the intake of isomaltulose when compared with sucrose during the afternoon (P<0.01) (Table 1).

Peak insulin concentrations were lower after the ingestion of isomaltulose compared with sucrose during the morning (P<0.02) (Fig. 1(b)). The intake of isomaltulose resulted in a lower insulin response as compared with sucrose both during the morning (P=0.03) as well as the afternoon (P<0.01) (Table 1).

The ingestion of isomaltulose resulted in less suppression of NEFA concentrations when compared with sucrose during the afternoon (P=0.01) (Fig. 1(c), Table 1). TAG concentrations were equal between isomaltulose and sucrose (Table 1).

Total fat oxidation
Fat oxidation rates decreased after the CHO drink (and with the drink in combination with a mixed meal) and returned to baseline levels after 3 h during the morning as well as the afternoon. The suppression of the fat oxidation response (ΔAUC) tended to be lower with isomaltulose compared with sucrose during the morning (P=0.10), reaching statistical significance during the afternoon (P=0.018) (Fig. 1(d), Table 1).

Total carbohydrate oxidation
The CHO oxidation rates were different after the ingestion of isomaltulose or sucrose during the morning, but were not significant. The rise in CHO oxidation rate following isomaltulose ingestion was attenuated when compared with sucrose and overall response (ΔAUC) showed less of an increase in combination with a mixed meal (P<0.01) (Fig. 1(e), Table 1).

Thermogenesis and respiratory quotient
The thermogenic response was equal for isomaltulose and sucrose during the morning and afternoon (Table 1).

Exogenous carbohydrate oxidation
No differences were observed in the minimal estimates of exogenous CHO oxidation rates between experiments. The mean percentage of the enriched sugar recovered in breath carbon dioxide excretion was at least 7–10% in all trials during the morning.

Satiety peptides responses
Intake of isomaltulose resulted in lower peak values of ghrelin when compared with sucrose during the morning (P=0.04,

| Table 1. Overview over metabolic responses, expressed as change in area under the curve (ΔAUC), after ingestion of isomaltulose and sucrose |
|---------------------|---------------------|---------------------|---------------------|
|                     | Isomaltulose         | Sucrose             | Isomaltulose         | Sucrose             |
| Glucose (mmol/l)    | 79                  | 59                  | 149                 | 239**               |
| Insulin (µU/ml)     | 3176                | 4726*               | 10 693              | 14 634**            |
| NEFA (mmol/l)       | –54 730             | –59 122             | –22 743             | –43 370*            |
| TAG (mmol/l)        | –7418               | 9740                | 61 386              | 66 804               |
| Fat oxidation (g/min)| –3.59               | –5.58               | –0.56               | –3.99*               |
| Carbohydrate oxidation (g/min) | 12.99            | 18.96               | 8.06                | 18.3*                |
| Respiratory quotient| 8.9                 | 13.0                | 3.2                 | 9.5*                 |
| Energy expenditure (kJ/min) | 72.1              | 89.3                | 124                 | 153                  |
| Ghrelin (pg/ml)     | –16 177             | –14 326             | –21 700             | –34 678              |
| Glucagon-like peptide-1 (mmol/l) | 188              | 43                  | 642                 | 464                  |

Mean value was significantly different from that for isomaltulose: * P<0.05, ** P<0.01.
Fig. 1(f)). Differences were observed between plasma concentrations and ΔAUC of the satiety peptides, ghrelin and glucagon-like peptide-1, during the afternoon which were not significant (Table 1).

Discussion
The present study provides evidence that an attenuated rise in glycaemic and insulinaemic responses following isomaltulose may shift postprandial substrate utilisation towards greater fat use in overweight subjects. The attenuated glycaemic and insulinaemic responses following isomaltulose are attributed to the slower rates at which isomaltulose is digested and absorbed. Several studies have shown that isomaltulose absorption rates are lower when compared with the digestion and/or absorption with sucrose\(^\text{16,17}\). The present study is the first to show that isomaltulose intake attenuates the postprandial rise in plasma glucose and insulin levels in overweight subjects. Although there were no significant differences in the total integrated glycaemic responses following the ingestion of different CHO after an overnight fast (morning), a lower peak plasma glucose concentration was evident after the ingestion of isomaltulose compared with sucrose. The different duration of elevated glycaemia and the absence of a strong rebound effect may explain the lack of difference when considering the ΔAUC. The rebound effect induced hypoglycaemia and low levels of insulin during the morning, which resulted in an increase in NEFA levels after the intake of sucrose as compared with isomaltulose.

Substrate use
Intake of isomaltulose in combination with a mixed meal resulted in an attenuated rise in the plasma glucose and insulin responses when compared with sucrose and subsequently less inhibition of postprandial fat oxidation. The greater postprandial fat use was accompanied by higher circulating plasma NEFA concentrations. The latter is probably attributed to a greater supply of plasma NEFA, resulting from a reduced NEFA concentrations. The latter is probably attributed to a lower peak plasma glucose concentration was evident after the ingestion of isomaltulose compared with sucrose. The different duration of elevated glycaemia and the absence of a strong rebound effect may explain the lack of difference when considering the ΔAUC. The rebound effect induced hypoglycaemia and low levels of insulin during the morning, which resulted in an increase in NEFA levels after the intake of sucrose as compared with isomaltulose.

Satiety regulatory peptides
Contact of nutrients with the small intestine is postulated to be an important mechanism inducing satiety and it has been suggested that a slower CHO digestion rate extends this contact\(^\text{24}\). Circulating ghrelin concentrations rise with fasting and decline following meal ingestion and this primary regulation by food intake is in accordance with a suggested role of ghrelin as a ‘hunger hormone’\(^\text{25}\). In the present study, peak ghrelin levels following isomaltulose were significantly lower when compared with sucrose during the morning. The observation that ghrelin responses following isomaltulose were much less pronounced when ingested as part of a mixed meal indicates that either the total quantity of CHO consumed within the meal, or the interaction with other macronutrients in the meal, may be more important in the overall satiety response.

In conclusion, ingestion of isomaltulose attenuates the postprandial glycaemic and insulinaemic responses when compared with sucrose ingestion. Lower postprandial plasma glucose and insulin concentrations shift postprandial substrate use towards greater fat use, which in the case of isomaltulose was most pronounced when provided in combination with a mixed meal. Additionally, isomaltulose ingestion has an impact on the postprandial ghrelin response. We speculate that exchanging high-glycaemic/insulinaemic CHO in the diet for slowly digestible CHO sources may represent an effective nutritional strategy to counteract overweight-induced metabolic disturbances such as reducing insulin resistance and ectopic fat accumulation.

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