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Short Communication

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

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The proposed impact of slowly digestible sources of dietary carbohydrate in reducing the risk of developing obesity and related metabolic disorders remains unclear. The aim of the present study was to compare the postprandial metabolic response to the ingestion of glucose *v.* trehalose. We hypothesised that the reduced digestion and absorption rate of trehalose is accompanied by an attenuated glycaemic and insulinaemic response, leading to a less inhibited postprandial fat oxidation rate. In a randomised, single-blind, cross-over study, ten overweight subjects ingested two carbohydrate drinks (75 g carbohydrate equivalents of trehalose or glucose) following an overnight fast (08.40 hours) and together with a standardised mixed meal (12.30 hours; 25 % total energy content was provided as either glucose or trehalose). Blood samples were collected before ingestion and every 30 min thereafter for a period of 3 h; substrate use was assessed by indirect calorimetry and expired breath samples were collected. Ingestion of carbohydrates with a mixed meal resulted in a lower peak glucose response and a lower change in area under the curve (ΔAUC) following trehalose when compared with glucose. Differences in peak insulin response and ΔAUC were observed with trehalose when compared with glucose during the morning and afternoon. These differences were accompanied with a reduced carbohydrate oxidation after trehalose when ingested as a drink, whilst no significant differences in fat oxidation between drink were observed.

Trehalose: Glycaemic response: Substrate use

Over the last two decades the prevalence of obesity and obesity-related disorders has increased rapidly[1]. Both genetic and environmental factors (physical activity and diet) 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 may increase the risk for weight gain largely because of excess energy intake[2]. On the other hand, high CHO–low-fat diets contain a large amount of rapidly available CHO (cooked starches) and added refined sugars (sucrose, high-fructose maize syrup) which may be counterproductive to body-weight control and glycaemic control. This is because rapidly available CHO and refined sugars markedly increase postprandial glycaemia and insulinaemia, thereby inhibiting adipose tissue lipolysis and/or muscle fat oxidation and, as such may promote fat storage in both adipose and non-adipose tissue. 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* is a strong risk factor for the development of type 2 diabetes mellitus and cardiovascular co-morbidities[3,4]. Finally, hyperinsulinaemia might affect TAG clearance and liver TAG production, thereby increasing plasma TAG concentrations. Therefore, the recommendation to ingest a CHO-rich diet, containing a large amount of high-glycaemic CHO may actually have a less favourable effect on the blood lipid profile[5,6].

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 CVD[7]. Brand-Miller *et al.* hypothesised that the ingestion of slowly digestible CHO attenuates postprandial glycaemia, reduces insulinaemia and enhances fat oxidation, all of which may assist to prevent body-weight gain and insulin resistance[7]. The latter may be of relevance in dietary strategies to modulate body weight and improve insulin sensitivity.

Abbreviations: ΔAUC, change in area under the curve; CHO, carbohydrate.

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The present study compares the postprandial metabolic response following the ingestion of glucose compared with trehalose. We hypothesised that the ingestion of trehalose would be accompanied by an attenuated glycaemic and/or insulinaemic response, a reduced inhibition of postprandial fat oxidation rate, and a lower plasma TAG concentration when compared with glucose ingestion.

Methods

Subjects

Ten healthy, overweight men (*n* 8) and women (*n* 2) were recruited to participate in the study (age 31 (SE 4) years, BMI 27·7 (SE 0·8) kg/m^2, 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 (glucose or trehalose), during two different trials, performed using a single-blind, randomised cross-over design. Glucose and trehalose were derived from maize, a natural CHO source with a high natural abundance of ^{13}C. The CHO load consisted of 75 g CHO equivalents 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 samples were collected every 1 h to determine $^{13}CO_2$ enrichment. These procedures were repeated on the same day before consuming a standardised lunch together with a beverage containing either glucose or trehalose 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. Lunch macronutrient composition represented 55 % energy as CHO, 30 % energy as fat and 15 % energy as protein; 25 % energy of the total energy content of the meal was provided in the form of a beverage containing either trehalose or glucose. Lunch was consumed within 15 min.

Test products

Trehalose. Trehalose is a disaccharide of glucose with an α-1,1 glycoside linkage. It is a non-reducing sugar that is naturally present in honey, bread, mushrooms and fermented drinks. Trehalose is produced industrially by enzymic conversion using starch as the base material. Its sweetness is 40–45 % compared with that of sucrose. When ingested, trehalose is enzymically hydrolysed in the small intestine by trehalase into two D-glucose molecules, which are subsequently absorbed and metabolised[8,9]. It appears that ingestion, hydrolysis, absorption and metabolism of trehalose is essentially identical to all other digestible disaccharides[8].

Exogenous carbohydrate oxidation. As indicated above, all CHO were derived from naturally ^{13}C-enriched sources: glucose (^{13}C enrichment = −11·17 δ per mil *v.* Pee Dee belemnite (PDB) (δ‰)) and trehalose (^{13}C enrichment = −17·86 δ‰). The ^{13}C enrichment of the experimental beverages was determined by elemental analyser isotope ratio MS (Carlo Erba-Finnigan MAT 252, Bremen, Germany). Subjects were instructed not to consume any food products with a high ^{13}C natural abundance for at least 1 week before and during the experimental period. In European countries the consumption of native CHO sources with a high natural ^{13}C abundance is low[10].

Biochemical analyses

At all time points, 8 ml blood were collected in pre-chilled tubes with 200 μl 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 analysis. Plasma glucose concentrations 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; Roche Diagnostics, Basel, Switzerland). Insulin was analysed by RIA (Human Insulin RIA Kit; LINCO Research Inc., St Charles, MO, USA). Breath samples were analysed for ^{13}C:^{12}C ratio by GC isotope ratio MS (Finnigan MAT 252).

Calculations

Metabolic rate was calculated from VO_2 (litres/min) and VCO_2 (litres/min) according to the equations of Frayn[11]. 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[12]:

$$\text{CHO oxidation} = (4{\cdot}55 \times \text{VCO}_2) - (3{\cdot}21 \times \text{VO}_2) - (2{\cdot}87 \times \text{N}).$$

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

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

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

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

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

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

Exogenous CHO oxidation was estimated using the following formula[14]:

$$\text{Exogenous CHO oxidation} = VCO_2 \times \left(\frac{\delta Exp - \delta Exp_{bkg}}{\delta Ing - \delta Exp_{bkg}}\right)\left(\frac{1}{k}\right),$$

in which VCO_2 is the volume of expired CO_2 per min (litres/min), δExp is the ^{13}C enrichment of expired air with CHO ingestion at different time-points, δIng is the enrichment of the CHO in the experimental beverages, δExp_{bkg} is the ^{13}C enrichment in expired breath before the intervention (background) and k is the amount of CO_2 (in litres) produced by the oxidation of 1 g glucose (k = 0·7467 litres CO_2 per g glucose). This represents a minimal estimate of exogenous CHO oxidation, as part of the ^{13}C will be temporarily fixated in the bicarbonate pool and in the tricarboxylic acid cycle intermediates[15,16].

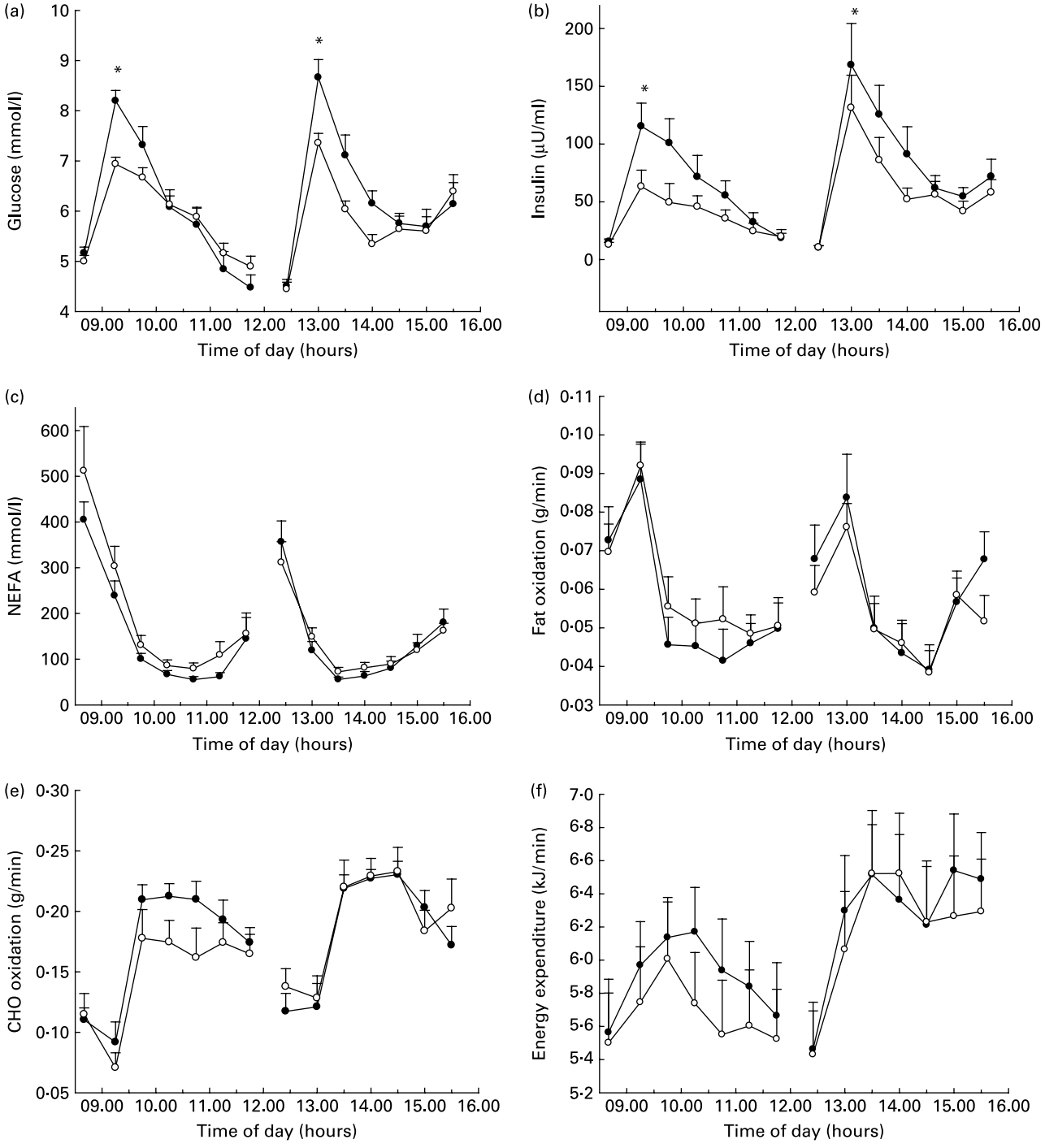

Fig. 1. Plasma glucose (a), insulin (b) and NEFA (c) concentrations following glucose (–●–) or trehalose (–○–) ingestion. Time-course of fat oxidation (d), carbohydrate (CHO) oxidation (e) and energy expenditure (f) following glucose or trehalose ingestion. Values are means, with their standard errors represented by vertical bars. * Mean value was significantly different from that following trehalose ingestion ($P < 0·05$).

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 means with their standard errors. The total response of metabolic parameters after CHO ingestion was expressed as the incremental area under the curve (minus baseline values, change in 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 glucose compared with trehalose 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 trehalose when compared with glucose (Fig. 1(a)). The glycaemic response (ΔAUC) was comparable after the intake of trehalose compared with glucose following an overnight fast ($P=0{\cdot}08$), whilst there was a lower response after trehalose compared with glucose when ingested in combination with a mixed meal ($P<0{\cdot}02$; Table 1).

Peak insulin concentrations and the total response were lower after the ingestion of trehalose compared with glucose during the morning and afternoon (Fig. 1(b) and Table 1). During the morning, circulating NEFA concentrations following the ingestion of trehalose and glucose were equal, whilst there was a trend towards less suppression of NEFA with trehalose during the afternoon ($P=0{\cdot}09$) (Fig. 1(c) and Table 1). The intake of trehalose resulted in a lower increase in TAG concentrations compared with glucose during the morning ($P<0{\cdot}02$) and a higher increase during the afternoon ($P<0{\cdot}05$) (Table 1).

Thermogenesis and respiratory quotient

The thermogenic response (ΔAUC) following trehalose compared with glucose was comparable during the morning and afternoon. There was a tendency towards a lower increase of the respiratory quotient after the intake of trehalose compared with glucose during the morning ($P=0{\cdot}09$), but no significant differences were observed between trehalose and glucose during the afternoon (Table 1).

Total fat oxidation

There were differences in fat oxidation with ingestion of trehalose when compared with glucose during the morning ($P=0{\cdot}1$) and the afternoon, which were not significant (Fig. 1(d)).

Total carbohydrate oxidation

After intake of trehalose the increment in total CHO oxidation was lower as compared with glucose during the morning ($P=0{\cdot}02$). CHO oxidation rates were comparable during the afternoon (Fig. 1(e) and 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 averaged at least 7–10 % in all trials during the morning.

Discussion

The present study provides evidence that attenuated postprandial glycaemic and insulinaemic responses following trehalose ingestion shifts postprandial substrate use towards a lower CHO oxidation in overweight subjects after ingestion of a CHO drink but not when ingested as part of a mixed meal. No significant effects were observed on postprandial fat oxidation. The attenuated glycaemic and insulinaemic responses following trehalose are attributed to the slower rates at which trehalose is digested and/or absorbed. Several studies have shown that the absorption rate of trehalose is slower than glucose[17]. An attenuated rise in blood glucose and insulin levels after the intake of trehalose were observed in trained athletes and healthy subjects[18,19]. The present study is the first to show that trehalose ingestion attenuates the rise in plasma glucose and insulin levels in overweight subjects. Although there were no significant differences in

Table 1. Overview of metabolic responses, expressed as change in area under the curve (ΔAUC), after ingestion of trehalose and glucose

	ΔAUC in the morning over 3 h		ΔAUC in the afternoon over 3 h	
	Trehalose	Glucose	Trehalose	Glucose
Glucose (mmol/l)	178	188	274	360*
Insulin (μU/ml)	4868	9264**	10 693	14 634**
NEFA (mmol/l)	−61 372	−49 332	−32 263	−41 182
TAG (mmol/l)	−6759	16 102*	62 761	40 076*
Fat oxidation (g/min)	−0·99	−3·21	−0·88	−1·95
Carbohydrate oxidation (g/min)	5·18	11·86*	10·1	13·27
Respiratory quotient	7·7	3·8	6·3	4·3
Energy expenditure (kJ/min)	69·5	48·9	156	147·9

Mean value was significantly different from that for trehalose: * $P<0{\cdot}05$, ** $P<0{\cdot}01$.

the total integrated glycaemic responses following ingestion of different CHO after an overnight fast (morning), there was a clearly attenuated rise in peak plasma glucose concentration after the ingestion of trehalose compared with glucose. The differences in the duration of elevated glycaemia and the absence of a strong rebound effect may explain the lack of difference when considering the ΔAUC.

Substrate use

The postprandial hyperglycaemia with ingestion of glucose increases insulin secretion, and higher insulin levels would promote glucose uptake in insulin-sensitive tissues, such as the skeletal muscle, and inhibit adipose tissue lipolysis[20]. Through these mechanisms, interindividual differences in substrate use may play a role in the development of obesity and subsequently type 2 diabetes mellitus. Additionally, a shift towards a greater postprandial fat oxidation rate may attenuate lipid accumulation in non-adipose tissues leading to reduced insulin resistance[21,22]. Lower glucose and insulin levels were observed after the ingestion of trehalose and no significant effects on postprandial fat oxidation were observed, whilst there was a slightly lower increase in CHO oxidation. Thus, the trehalose-induced lower glycaemic and insulinaemic response did not promote fat oxidation in the postprandial period.

Postprandial triacylglycerol concentration

Hyperinsulinaemia may be accompanied by a greater increase in plasma TAG concentrations, which are considered to be risk factors for the development of CVD[5]. Low-glycaemic, low-insulinaemic CHO sources may be used to attenuate the postprandial rise in TAG concentrations, but there is no clear consensus. In the present study, we observed that trehalose resulted in reduced TAG concentrations during the morning, whilst during the afternoon postprandial TAG was slightly increased when compared with glucose.

In conclusion, ingestion of trehalose compared with glucose attenuates the postprandial glycaemic and insulinaemic responses. Attenuated blood glucose and insulin concentrations tended to shift postprandial substrate use towards a reduced CHO oxidation when trehalose was ingested during the morning, but had no significant effects on postprandial fat oxidation or postprandial NEFA concentrations.

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