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Reduced glycaemic and insulinaemic responses following trehalose and isomaltulose ingestion: implications for postprandial substrate use in impaired glucose-tolerant subjects

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Abstract

The impact of slowly digestible sugars in reducing the risk of developing obesity and related metabolic disorders remains unclear. We hypothesised that such carbohydrates (CHO), resulting in a lower glycaemic and insulinaemic response, may lead to greater postprandial fat oxidation rates in subjects with impaired glucose tolerance (IGT). The present study intends to compare the postprandial metabolic responses to the ingestion of glucose (GLUC) *v.* trehalose (TRE) and sucrose (SUC) *v.* isomaltulose (IMU). In a randomised, single-blind, cross-over design, ten overweight IGT subjects were studied four times, following ingestion of different CHO drinks either at breakfast or in combination with a mixed meal at lunch. Before and 3 h after CHO ingestion, energy expenditure, substrate utilisation and circulating metabolite concentrations were determined. Ingestion of CHO drinks with a meal resulted in an attenuated rise in GLUC (−33 %) and insulin (−14 %) concentrations following TRE when compared with GLUC and following IMU, an attenuation of 43 and 34 % when compared with SUC ingestion, respectively. Additionally, there was less inhibition of the rise in NEFA concentrations and less decline in postprandial fat oxidation (22 %) after IMU when compared with SUC, whereas TRE did not differ from GLUC. The attenuated rise in GLUC and insulin concentrations following IMU ingestion attenuated the postprandial inhibition of fat oxidation compared with SUC when co-ingested with a meal. This suggests that exchange of SUC in the diet for IMU may result in a more favourable metabolic response and may help to reduce the risks associated with obesity and type 2 diabetes.

Key words: Trehalose: Isomaltulose: Substrate use

The increasing prevalence of obesity and obesity-related disorders such as type 2 diabetes has become the greatest health problem of the present and coming decades[1]. According to the physiological state where abnormalities in glucose (GLUC) metabolism are present but below the cut-off point for the diagnosis of type 2 diabetes, individuals can be grouped into those who suffer from (1) impaired fasting GLUC or (2) impaired GLUC tolerance (IGT). Individuals with isolated IGT show moderate to severe muscle insulin resistance and suffer from a defect in both the early- and late-phase insulin secretory response to an oral GLUC load. Patients with IGT have a 2- to 5-fold greater risk of developing CVD, compared with age-matched normoglycaemic controls[2]. Each year, about 10 % of the subjects with impaired fasting GLUC and IGT progress to develop type 2 diabetes[3]. Lifestyle intervention, directed towards a healthy diet, i.e. a reduction in saturated fat intake and an increase in low-glycaemic carbohydrate (CHO) intake, and an increase in habitual physical activity level, has proven effective in preventing or delaying the onset of type 2 diabetes in subjects with IGT[4,5]. Interventions to reduce the glycaemic index (GI) and glycaemic load of the daily diet have received much interest in nutritional research[6,7]. So far, numerous studies have reported that diets low in GI or glycaemic load can have beneficial effects on weight loss and/or reduce the risk of developing chronic metabolic disease in human subjects[6,8–10]. Whereas some have suggested that diets high in CHO may have an adverse effect on TAG concentrations

Abbreviations: CHO, carbohydrate; En%, percentage of energy; GI, glycaemic index; GLUC, glucose; iAUC, incremental area under the curve; IGT, impaired glucose tolerance; IMU, isomaltulose; SUC, sucrose; TRE, trehalose.

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and HDL-cholesterol[11], others have failed to confirm those findings. The apparent discrepancy between studies is probably attributed to differences in the duration of the intervention, sex and the use of different types of sugars between studies[12–14].

It has been hypothesised that low-GI foods may affect body-weight control and insulin sensitivity by promoting satiety and stimulating fat oxidation at the expense of CHO oxidation[15]. This increased fat oxidation may reduce fat storage in adipose and non-adipose tissues, thereby promoting insulin sensitivity and an improved metabolic profile. Indeed, animal studies have shown that a reduced GI can shift substrate use in favour of fat oxidation, independent of diet-induced changes in body composition or energy intake[16–18]. We recently showed that a reduced glycaemic response after a mixed meal containing trehalose (TRE) or isomaltulose (IMU) may improve fat oxidation rates at the expense of CHO oxidation in overweight subjects[19,20]. Similar findings[21] have also been observed during exercise conditions.

So far, it is not known whether these beneficial effects on fat oxidation also extend to impaired GLUC-tolerant subjects who show profound disturbances in the capacity to utilise fat as a substrate source during basal fasting conditions as well as in the capacity to switch between CHO and fat oxidation during postprandial conditions[22]. The fact that disturbances in fatty acid uptake and oxidation are already present in the pre-diabetic state suggests a key role in the progression towards type 2 diabetes[23]. Consequently, more work is warranted to assess the impact of low-GI CHO on postprandial substrate use in an obese group with IGT. Therefore, we examined the metabolic response to the ingestion of two slowly digestible CHO sources, TRE and IMU, respectively. TRE is a GLUC disaccharide with an α-1,1 glycoside linkage, whereas IMU is a disaccharide produced by an enzymatic conversion of sucrose (SUC).

We hypothesised that the ingestion of TRE and IMU will be accompanied by a lower glycaemic and/or insulinaemic response, an attenuated inhibition of postprandial lipolysis and fat oxidation rate and a lower plasma TAG response when compared with GLUC and SUC, respectively.

Methods

Subjects

A total of ten overweight men (n 6) and women (n 4), of which two were post-menopausal, with IGT were recruited for the present study. Subjects' characteristics are presented in Table 1. Subjects with type 2 diabetes and/or overt cardiovascular complications, and those using medication for digestive disorders were excluded from the study. All subjects were screened with a standard 75 g oral glucose tolerance test after an overnight fast. IGT was diagnosed based on the WHO criteria. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Medical Ethical Committee of the Maastricht University Medical Centre. All subjects gave written informed consent.

Table 1. Subjects' characteristics
(Mean values and standard deviations)

	Subjects (female n 4, male n 6)	
	Mean	SD
Age (years)	56	8
Weight (kg)	91·3	20·3
BMI (kg/m^2)	30·8	4·9
Fasting glucose (mmol/l)	5·63	0·64
2 h Glucose (mmol/l)	8·78	0·96
Fasting insulin (μU/ml)*	18	8·9
HOMA-IR	4·63	2·53
HbA_{1c} (%)	5·85	0·19
Fasting NEFA (μmol/l)	402	101
Fasting TAG (mmol/l)	1·15	0·47
ALAT (U/l)	30	9·7
Creatinine (μmol/l)	79	15·2

HOMA-IR; homeostasis model assessment-insulin resistance; HbA_{1c}, glycated Hb; ALAT, alanine transferase.
*To convert insulin from μU/ml to pmol/l, multiply by 6.

Study design

Each subject participated in four trials, separated by a 1-week washout period, in which the metabolic response was measured after ingestion of four different CHO drinks. CHO drinks were ingested after an overnight fast (breakfast drink) and in combination with a standardised mixed meal (lunch). The CHO drinks (GLUC, TRE, SUC and IMU) were provided in a single-blind, randomised order.

Protocol

At the beginning of the experimental day, after an overnight fast, a cannula was inserted into an antecubital vein. The CHO load consisted of 75 g CHO equivalents and was dissolved in 400 ml of water. The CHO drink was consumed after an overnight fast at breakfast (08.45 hours) or in combination with a mixed meal at lunch (12.30 hours) within a period of 15 min. Energy expenditure and substrate utilisation were measured, before and for 3 h after ingestion of the meal and/or drink using a ventilated hood system (Omnical)[24]. Gas analyses, recorded every minute, were performed by dual paramagnetic O_2 analysers and dual IR CO_2 analysers (type 1156, 1507, 1520; Servomex), similar to the analysis system described by Schoffelen *et al.*[25]. Blood samples were taken before consumption of the meal/drinks ($t = -5$ min) and then at $t = 30$, 60, 90, 120, 150 and 180 min after CHO ingestion to determine circulating metabolites and hormone concentrations. Expired breath samples were collected each hour to determine $^{13}CO_2$ enrichment. Energy expenditure and substrate use were calculated using the formulas of Weir[26] and Frayn[27].

Lunch had a total energy content equivalent of 50 % of calculated 24 h resting energy expenditure based upon the formula of Harris & Benedict[28]. Lunch macronutrient composition

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represented 55 En% CHO, 30 En% fat and 15 En% protein; 25 En% of the total energy content of the meal was provided in the form of a beverage containing either TRE, IMU, GLUC or SUC.

Test products

Trehalose. TRE is a disaccharide of GLUC with an α-1,1 glycoside linkage. It is a non-reducing sugar that is naturally present in honey, bread, mushrooms and fermented drinks. For our experiment, ^{13}C-enriched TRE was produced by enzymatic conversion using maize starch as the base material. In the human intestine, TRE is exclusively digested by epithelial trehalase into two D-GLUC molecules, which are subsequently absorbed and metabolised[29,30]. Apart from the trehalase action, it appears that ingestion, hydrolysis, absorption and metabolism of TRE are essentially identical to all other digestible disaccharides[29].

Isomaltulose. IMU is a disaccharide produced by an enzymatic conversion of SUC, whereby the 1,2-glycosidic linkage between GLUC and fructose is rearranged to a 1,6-glycosidic linkage. For our experiment, ^{13}C-enriched IMU was produced by enzymatic conversion using cane sugar as the base material. The sucrase–isomaltase complex located on the brush-border membrane of the small-intestinal epithelial cells hydrolyses both IMU and SUC. The resulting monosaccharides, GLUC and fructose, are taken up into the portal blood[31].

Biochemical analyses

At all time points, 8 ml blood were collected in pre-chilled tubes with 200 μl of 0·2 M-EDTA (Sigma). 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 was used for the enzymatic colorimetric quantification of NEFA (NEFA C kit; Wako Chemicals) and TAG (Sigma) on a COBAS FARA centrifugal spectrophotometer (Roche Diagnostica). Plasma GLUC concentration (ABX Diagnostics) was measured enzymatically on a COBAS MIRA automated spectrophotometer (Roche Diagnostica). Plasma insulin was measured with a double antibody RIA (Linco Research). Breath samples were analysed for ^{13}C:^{12}C ratio by GC–isotope ratio MS (Finnigan MAT 252; Finnigan), as described in van Can *et al.*[19,20].

Statistics

A computerised statistics program (SPSS 15 for Windows; SPSS, Inc.) was used to perform all calculations. All data are expressed as means with their standard errors. The total response of parameters after CHO ingestion was expressed as the incremental area under the curve (iAUC) and calculated by the trapezoid method. Response is defined in the Results section as iAUC, unless mentioned otherwise. Differences between responses to GLUC *v.* TRE and SUC *v.* IMU were analysed by means of Student's paired *t* test. Student's paired *t* test was used to compare differences in peak response between the different CHO. The four CHO were not compared with each other due to the fact that they are made out of different CHO sources. Therefore, TRE is compared with GLUC and IMU compared with SUC.

Results

Circulating metabolites

Glucose response. Ingestion of TRE resulted in lower peak GLUC concentrations when compared with GLUC both during breakfast drinks ($P<0·01$) and lunch ($P=0·001$) (Fig. 1(a)). This did, however, not result in a significant difference in glycaemic response, expressed as iAUC (Table 2). GLUC peaks were lower after ingestion of IMU compared with SUC during breakfast ($P=0·01$) and lunch ($P=0·001$) (Fig. 1(b)). There was a reduced incremental glycaemic response after the ingestion of IMU when combined with a mixed meal ($P<0·001$; Table 3).

Insulin response. TRE resulted in lower peak insulin concentrations when compared with GLUC following breakfast ($P=0·003$) and lunch ($P=0·025$; Fig. 1(c)). The iAUC was lower after the ingestion of TRE compared with GLUC during breakfast ($P=0·009$) but not when TRE was ingested with a mixed meal during lunch (Table 2). Insulin responses were reduced after the ingestion of IMU compared with SUC following breakfast (iAUC, $P<0·05$) and lunch (iAUC, $P=0·001$) (Fig. 1(d); Table 3).

NEFA response. As expected, plasma NEFA concentrations decreased after CHO ingestion. Ingestion of either TRE or GLUC resulted in a similar NEFA response pattern, also when ingested in combination with a mixed meal (Fig. 1(e)). There were no significant differences in the integrated decrement between TRE and GLUC (Table 2). Ingestion of IMU in combination with a mixed meal during lunch resulted in a less inhibition of the decline in plasma NEFA concentrations when compared with SUC ($P<0·0001$; Fig. 1(f); Table 3).

TAG response. TAG concentrations increased after the ingestion of the different CHO drinks and when the drinks were ingested in combination with a mixed meal. There were no differences in incremental TAG AUC after the ingestion of TRE compared with GLUC during breakfast and lunch (Fig. 2(a); Table 2). There was a trend towards a lower iAUC when IMU was ingested in combination with a mixed meal ($P=0·06$; Fig. 2(b); Table 3).

Thermogenesis and substrate oxidation

There were no differences in the thermogenic response between the CHO drinks during breakfast or when ingested with a mixed meal (Tables 2 and 3).

There were no differences in the iAUC of the respiratory quotient after TRE ingestion compared with GLUC during breakfast and lunch (Table 2). Intake of IMU did not result in differences in respiratory quotient response compared with SUC during breakfast, whereas IMU ingested in combination with a mixed meal resulted in a reduced respiratory quotient response compared with SUC ($P=0·034$; Table 3).

There were no significant differences in the decrement in fat oxidation rates between TRE and GLUC during breakfast and lunch (Fig. 2(c); Table 2). Fat oxidation did not differ between IMU and SUC during breakfast; interestingly, fat oxidation was significantly less suppressed after IMU when compared with SUC following lunch ($P<0·05$; Fig. 2(d); Table 3).

There were no significant differences in CHO oxidation between TRE and GLUC during breakfast and lunch (Fig. 2(e), Table 2). Intake of IMU did not result in significant differences following breakfast when compared with SUC, whereas the increment in CHO oxidation was lower after the ingestion of IMU when compared with SUC during lunch ($P=0{\cdot}036$; Fig. 2(f); Table 3).

No differences were observed in the minimal estimates of exogenous CHO oxidation rates between the experiments. The mean percentage of the enriched CHO oxidised, as

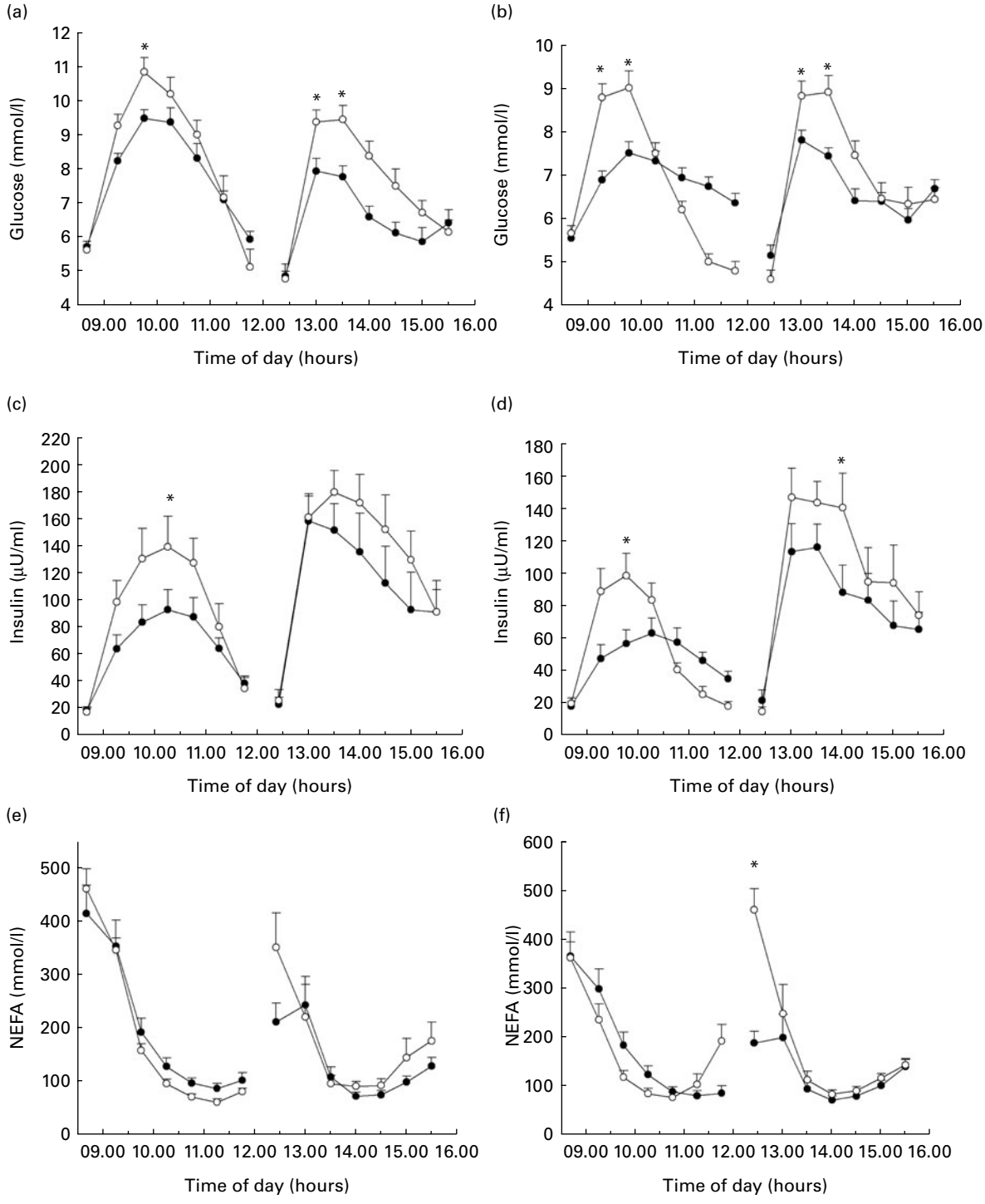

Fig. 1. Time course of the glycaemic response after the intake of (a) trehalose (TRE, –●–) *v.* glucose (GLUC, –○–) and (b) isomaltulose (IMU, –●–) *v.* sucrose (SUC, –○–). Time course of the insulinaemic response after the intake of (c) TRE *v.* GLUC and (d) IMU *v.* SUC. To convert insulin from μU/ml to pmol/l, multiply by 6. Time course of NEFA concentrations after the intake of (e) TRE *v.* GLUC and (f) IMU *v.* SUC. Values are means, with standard errors of the mean represented by vertical bars (n 10). *Mean values were significantly different ($P<0{\cdot}05$).

Table 2. Metabolic responses, expressed as change in area under the curve (iAUC), after ingestion of trehalose and glucose

	iAUC breakfast over 3 h		iAUC lunch over 3 h	
	Trehalose	Glucose	Trehalose	Glucose
Glucose (mmol/l over 3 h)	428	554	374	559
Insulin (μU/ml over 3 h)†	9425**	15 216	17 934	20 875
NEFA (mmol/l over 3 h)	−41 527	−52 838	−15 200	−55 284
TAG (mmol/l over 3 h)	13 182	9158	56 566	6863
Fat oxidation (g over 3 h)	−1·32	−2·66	0·62	0·38
Carbohydrate oxidation (g over 3 h)	6·55	10·11	8·72	9·6
Energy expenditure (kJ over 3 h)	33	53	144	166
Respiratory quotient (over 3 h)	5·47	7·60	1·77	2·68

Mean value was significantly different from that of glucose: ** $P<0·01$.
† To convert insulin from μU/ml to pmol/l, multiply by 6.

calculated by the recovery of $^{13}CO_2$ in the expired breath, was 11 % for TRE, 12 % for GLUC, 15 % for IMU and 19 % for SUC, respectively.

Discussion

Substrate utilisation

The main finding of the present study is that intake of IMU in combination with a mixed meal resulted in an attenuated rise in postprandial plasma GLUC and insulin concentrations and a lesser inhibition of circulating NEFA concentration and fat oxidation compared with SUC ingestion. The reduced inhibition of postprandial fat oxidation could be attributed to a greater supply of NEFA to the fat-oxidising tissue, secondary to a reduced insulin-mediated suppression of lipolysis[32]. The present results seem consistent with other work, highlighting the stimulating effects of IMU ingestion on postprandial fat oxidation and/or lipid deposition when compared with SUC, in rats, healthy and overweight subjects[19,33,34]. The present study shows that IMU ingestion in exchange for SUC has beneficial effects in subjects with IGT and, as such, may help to prevent the progression into type 2 diabetes.

The attenuated postprandial decline in fat oxidation induced by the ingestion of IMU may have implications for body-weight control. Flatt[35] proposed that subjects who continue to oxidise CHO in the post-absorptive state deplete their endogenous glycogen stores, thereby stimulating food intake. Through this mechanism, inter-individual differences in substrate selection may play a key role in the development of obesity. A lower decrement in circulating NEFA and fat oxidation following the ingestion of more slowly digestible CHO may favour fat oxidation above storage, resulting in less fat accumulation in non-adipose tissues with a favourable effect on insulin sensitivity by preventing late hypoglycaemia and the accompanying increase in plasma NEFA concentrations[36]. High NEFA concentrations may be linked with insulin resistance and CVD by increasing muscle ectopic fat promoting lipotoxicity, which may reduce insulin action[37].

Glycaemic and insulinaemic responses

The attenuated glycaemic and insulinaemic responses following TRE and IMU ingestion are attributed to the slower rates at which TRE and IMU are digested and absorbed. Several studies have shown that the absorption rates of TRE and IMU are slower than GLUC and SUC, respectively[38,39]. TRE as well as IMU are absorbed and tolerated well in human subjects[29,31]. Reduced GLUC and insulin concentrations after the intake of TRE or IMU have been observed in trained athletes, healthy subjects, as well as in overweight subjects[19,20,40,41]. The present study is the first to show that intake of TRE and IMU attenuated the postprandial rise in plasma GLUC and insulin concentrations in subjects with IGT. Although there were no significant differences in the integrated glycaemic

Table 3. Metabolic responses, expressed as change in area under the curve (iAUC), after ingestion of isomaltulose and sucrose

	iAUC breakfast over 3 h		iAUC lunch over 3 h	
	Isomaltulose	Sucrose	Isomaltulose	Sucrose
Glucose (mmol/l over 3 h)	248	266	279**	489
Insulin (μU/ml over 3 h)†	5779*	7326	11 726**	17 658
NEFA (mmol/l over 3 h)	−36 137	−38 869	−12 880**	−55 284
TAG (mmol/l over 3 h)	19 802	20 896	40 787	60 680
Fat oxidation (g over 3 h)	−4·22	−5·20	−0·89*	−4·08
Carbohydrate oxidation (g over 3 h)	14·14	16·19	12·27*	23·16
Energy expenditure (kJ over 3 h)	54	55	156	158
Respiratory quotient (over 3 h)	11·14	13·18	4·89*	12·24

Mean values were significantly different from those of sucrose: * $P<0·05$, ** $P<0·01$.
† To convert insulin from μU/ml to pmol/l, multiply by 6.

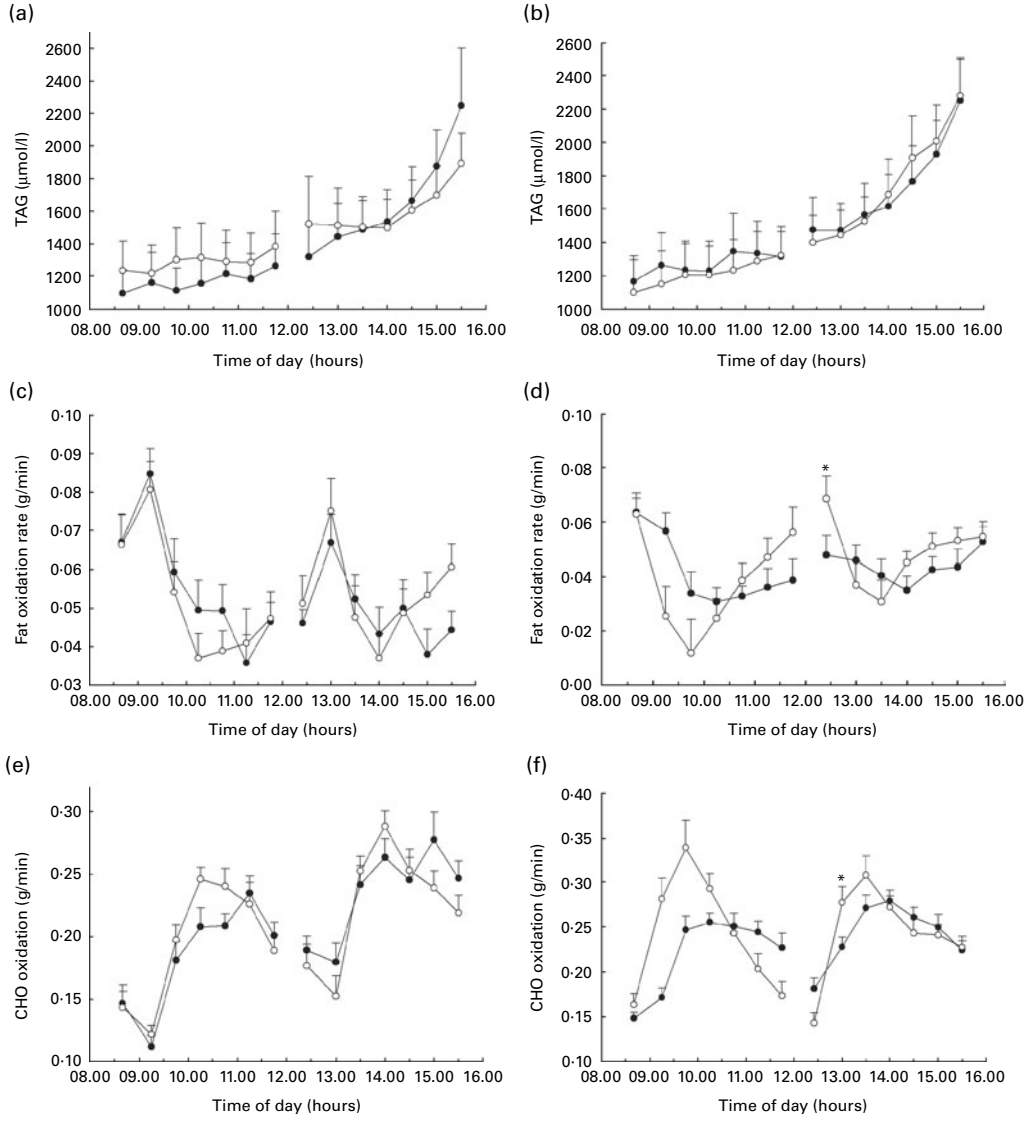

Fig. 2. Time course of TAG concentrations after the intake of (a) trehalose (TRE, –●–) *v.* glucose (GLUC, –○–) and (b) isomaltulose (IMU, –●–) *v.* sucrose (SUC, –○–). Time course of fat oxidation after the intake of (c) TRE *v.* GLUC and (d) IMU *v.* SUC. Time course of carbohydrate (CHO) oxidation after the intake of (e) TRE *v.* GLUC and (f) IMU *v.* SUC. Values are means, with standard errors of the mean represented by vertical bars (*n* 10). *Mean values were significantly different ($P < 0{\cdot}05$).

responses following the ingestion of different CHO after an overnight fast (breakfast), there was a clearly attenuated rise in peak plasma GLUC concentration after the ingestion of IMU compared with SUC and after the ingestion of TRE compared with GLUC (see Fig. 1).

Postprandial TAG concentration

High plasma TAG concentrations are considered to be risk factors for the development of CVD[42]. Low-glycaemic, low-insulinaemic CHO sources may be used to attenuate the postprandial rise in TAG concentrations. However, data show no consensus regarding higher postprandial TAG concentrations following the ingestion of fructose[43,44]. In the present study, we observed a trend towards reduced TAG concentrations with ingestion of IMU in combination with a mixed meal compared with SUC ingestion, whereas no such differences were observed for TRE. In contrast, in healthy, overweight subjects, TRE resulted in reduced TAG concentrations during breakfast[20]. This discrepancy could be explained by the higher age of the subjects in the present study. Animal as well as human studies generally observed more pronounced effects in younger subjects[45,46].

A limitation of the present study is that the number of subjects is rather small. We cannot rule out sex differences, although the cross-over design limits inter-individual variation. The set-up of the study provides a proof of principle on the impact of TRE and IMU in the breakfast setting and under more physiological conditions where the drink is consumed in combination with a mixed meal. Further studies are warranted to investigate the overall response and physiological significance of the observed differences.

In conclusion, ingestion of TRE and IMU results in an attenuated postprandial rise in plasma GLUC and insulin concentrations when compared with GLU and SUC, respectively. Co-ingestion of IMU with a mixed meal resulted in an attenuated decline in plasma NEFA concentrations and postprandial fat oxidation rate when compared with SUC, which may reduce ectopic fat accumulation and improve insulin sensitivity. Thus, exchanging SUC for IMU may be favourable to prevent metabolic disturbances, thereby potentially slowing down the progression to type 2 diabetes. More studies are needed to determine the long-term effects of exchanging rapid for more slowly digestible sugars on body-weight control and the prevention of type 2 diabetes in subjects with IGT.

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