British Journal of Nutrition (2002), **87**, 569–577
DOI: 10.1079/BJN2002586

Quantitative kinetics of glucose appearance and disposal following a ^{13}C-labelled starch-rich meal: comparison of male and female subjects

M. Denise Robertson[1]*, Geoff Livesey[2] and John C. Mathers[1]

[1]*Human Nutrition Research Centre, Dept Biological and Nutritional Sciences, University of Newcastle, Newcastle-upon-Tyne NE1 7RU, UK*

[2]*Independent Nutrition Logic, Pealerswell House, Wymondham, Norfolk NR18 0QX, UK*

(Received 3 May 2001 – Revised 4 October 2001 – Accepted 7 February 2002)

In the UK, starch contributes up to 25 % of energy intake in adults (Gregory *et al.* 1990). The present study investigated the acute response to a starchy meal on whole-body glucose metabolism and assessed insulin sensitivity in men compared with women. Low insulin sensitivity has been postulated to pre-dispose individuals to a cluster of associated abnormalities known to increase the risk of CHD. Metabolic responses to a ^{13}C-labelled meal were determined in conjunction with a primed continuous infusion of D-[6,6-^{2}H]glucose in groups of healthy age- and BMI-matched men and women. Peripheral plasma glucose disposal (Gd) was computed using non-steady state kinetics in a single compartment model, simultaneously with determination of whole-body net glucose oxidation by indirect calorimetry. Insulin sensitivity was derived using cumulative Gd as the dependent variable, and time and the integrated insulin concentration as independent variables. The female group had the higher fractional rate of glucose appearance in plasma from starch ($P=0{\cdot}019$) immediately after ingestion. Females also had a higher rate of plasma Gd and a significantly higher insulin-dependent Gd (6·8 *v.* 5·6 μg glucose/(min.kg) per pmol insulin, $P<0{\cdot}05$) compared with the males. A smaller absolute pool of endogenous glucose in females allowed the rate of exogenous $^{13}CO_2$ production to be significantly higher in the females ($P=0{\cdot}007$) corresponding also to a significantly higher ($P<0{\cdot}05$) carbohydrate oxidation rate obtained by indirect calorimetry. The present study suggests that during the ingestion of a starchy meal, females exhibit higher glucose flux and greater whole-body insulin sensitivity than males.

Starchy foods: Stable isotope: Gender: Insulin sensitivity

Resistance to insulin-mediated glucose (Gd) disposal has been postulated to pre-dispose individuals to a cluster of associated abnormalities known to increase the risk of CHD (Reaven, 1988).

Pre-menopausal women have a 2–3-fold times lower incidence of CHD than men (Kannel & Abbott, 1987) which may be linked to sex-related differences in insulin sensitivity. The overall effects of gender on insulin sensitivity remain controversial. A large epidemiological study by Yip *et al.* (1998) found no effect of gender on fasting plasma glucose concentrations, whereas when insulin sensitivity was assessed by a hyperinsulaemic–euglycemic clamp, women showed a significant 'insulin-advantage' (Nuutila *et al.* 1995; Donahue *et al.* 1996; Nilsson *et al.* 2000). Nothing appears to be known about insulin sensitivity in the postprandial, non-steady state when insulin levels would be much lower than during clamp studies and the glucose concentration would not be constant.

This present study has been designed to investigate the acute effects of consumption of a starchy meal on the kinetics of whole-body glucose metabolism in a group of healthy men and women matched for age and BMI. By utilizing a dual-isotope approach, kinetic modelling and an estimate of insulin sensitivity under non-steady state conditions, we attempted to quantify rates of glucose appearance and disappearance from the plasma in the postprandial period, giving particular attention to possible gender differences.

Abbreviation: Gd, glucose disappearance.

* **Corresponding author:** Dr M. Denise Robertson, fax +44 865 224652, email denise.robertson@oxlip.ox.ac.uk

Materials and methods

Subjects

Twelve subjects (six men and six women, matched for age and BMI, Table 1) participated in this study. All were healthy and none had recently taken medication likely to affect either substrate metabolism or gastrointestinal motility. Female volunteers were studied on day 20 (± 1) of their menstrual cycle. All subjects gave written informed consent to the study which was approved by the Joint Ethical Committee of the University of Newcastle-upon-Tyne and the Newcastle and North Tyneside Health Authorities.

Study protocol

For 7 d before the study, subjects were asked to avoid foods naturally enriched in ^{13}C (maize products, cane sugar and exotic fruits). On the day prior to the study, alcohol and exercise were avoided and subjects were instructed to eat a standardized low-fat evening meal (5·6 g fat, 145 g complex carbohydrate) before fasting overnight (12–14 h). The following morning, subjects were admitted to the Wellcome Research Laboratories, Royal Victoria Infirmary, Newcastle-upon-Tyne. Antecubital and retrograde distal forearm intravenous cannulas were inserted under local anaesthetic (1 % lignocaine), in opposing arms. A primed-constant infusion of D-[6,6$^2H_2$]glucose was started at time zero via the antecubital cannula and arterialized blood samples were taken from the distal forearm cannula at frequent intervals for 11 h. After 120 min of D-[6,6$^2H_2$] glucose infusion, a ^{13}C-enriched test meal of cooked peas was administered. This labelled test meal was followed by unlabelled carbohydrate-free meals at 240 and 480 min. Both carbohydrate-free meals consisted of eggs and cheese; the second meal contained 25 g fat, 15 g protein and provided 1286 kJ and the third meal contained 48·7 g fat, 38·8 g protein and provided 2699 kJ. O_2 consumption and CO_2 production were monitored by indirect calorimetry for a 20 min period at 30 min intervals (Deltatrac Metabolic Monitor Ca, flow rate 40 l/min (Datex Instruments, Helsinki, Finland)). Subjects remained in the supine position for the duration of the study except when required to void urine.

Table 1. Age, BMI and fasting plasma metabolite concentrations of subjects at baseline†

(Mean values with their standard errors for six male and six female subjects)

	Males		Females	
	Mean	SEM	Mean	SEM
Age (years)	35·6	5·4	33·5	3·3
BMI (kg/m^2)	22·2	1·1	21·5	0·6
Body weight (kg)	71·1	2·8	58·6**	2·4
Plasma glucose (mmol/l)	5·1	0·2	4·53	0·2
Plasma triacylglycerol (mmol/l)	1·28	0·15	1·05	0·12
Plasma total cholesterol (mmol/l)	5·06	0·24	4·8	0·35
Plasma insulin (pmol/l)	43·4	5·7	53·3	9·6

† For details of subjects and procedures, see p. 570. Mean value was significantly different from that of male group (Student's *t* test for independent samples): **$P<0·01$.

Materials

Infusate. D-[6,6$^2H_2$]Glucose (99 atom % excess) was obtained from MassTrace, Woburn, MA, USA. The priming dose provided 590 mg D-[6,6$^2H_2$]glucose in an 11 ml bolus and was followed immediately by a continuous infusion of 7 mg D-[6,6$^2H_2$]glucose/min in a volume of 6 ml/h.

^{13}C-Labelled starchy test meal. ^{13}C-Labelled peas (*Pisum sativum*) of a high amylose cultivar designated RRrbrb were prepared by pulse-dosing immature pea plants with $^{13}CO_2$ at 3 d intervals over 30 d (Faulks *et al.* 1994). The labelled peas (50 g dry weight) were presoaked overnight at 4° before being cooked under pressure for 1 h shortly before consumption. This test meal contained 18·6 g starch (700 g amylose/kg) and provided 688 kJ metabolizable energy. The relative ^{13}C enrichment of the test meal was 1·420 atom % excess (30 % above background enrichment) as determined by GC–combustion–isotope ratio MS (PDZ Europa, Sandbach, Cheshire, UK).

Sample collection and analysis

Blood. Arterialized whole blood was collected into sodium fluoroxolate, and glucose concentration measured using the glucose oxidase method (Yellowsprings Instrument Co., Yellow Springs OH, USA). For insulin assays, blood was collected into potassium EDTA, plasma was separated by centrifugation and all samples were analysed together to avoid inter-batch variation. Insulin concentration was determined using unextracted plasma (Hampton, 1984) with a double antibody plus PEG radioimmunoassay. The CV for the glucose and insulin measurements were 2·3 and 9·4 % respectively.

Measurement of the isotopic enrichment of glucose. Plasma glucose samples were prepared for ^2H enrichment analysis by forming butylboronic acid acetate derivatives and rediluting in ethyl acetate (Wieko & Shernam, 1976). Samples were analysed in duplicate by GC–MS using a Hewlett Packard MSD system with connecting 5870 series II GC with autosampler. Ions at m/z (mass divided by charge) 297 and 299 were recorded. For ^{13}C-glucose enrichment measurements, plasma glucose samples were derivatized to sorbitol hexaacetate with excess derivatization products removed by evaporation under N_2 (Pickert *et al.* 1991). Dried-down extracts were redissolved in acetone. Samples were analysed in duplicate by GC–isotope ratio MS using a Hewlett Packard series II GC containing a Restek RTX5 column. Ions at m/z 44, 45 and 46 were recorded.

Analysis of breath samples

Indirect calorimetry. Respiratory exchange measurements were integrated over 20 min periods and the non-protein RER was calculated from V_{O_2}, V_{CO_2} and measured urinary N excretion determined using the Kjeldahl method (Kjeltec analyser, Perstorp Analytic Ltd, Perstorp, Sweden). Total carbohydrate and lipid oxidation were calculated according to the equations developed by Elia & Livesey (1992). Rates of exogenous carbohydrate oxidation were

calculated using the following equation (Normand *et al.* 1992):

$$\text{Rate} = \frac{\delta‰CO_2(t) - \delta‰CO_2(t_0)}{\delta‰\text{Starch} - \delta‰CO_2(t_0)} \times \frac{V_{CO_2}(t) \times 180}{22{\cdot}29 \times 6} \quad (1)$$

where δ‰ starch is the δ value of the starchy food measured by GC–isotope ratioMS, δ‰ CO_2(t) and δ‰ $CO_2(t_0)$ represent the δ value at the sampling time and baseline respectively. $V_{CO_2}(t)$ is expressed in litres/min, 180 is the molar mass of glucose, 22·29 is for the conversion factor for CO_2 (litres) to CO_2 (mol), and 1/6 the conversion factor for C (mol) to glucose (mol).

$^{13}CO_2$ enrichment. Duplicate 10 ml vacutainer samples of expired air were analysed for $^{13}CO_2$ enrichment using the Europa Scientific Continuous Flow ANCA system with a 20–20 MS analyser. $^{13}CO_2$ enrichment represents the excess ^{13}C present compared with background. Differences between measured ^{13}C enrichment in breath CO_2 and the international standard for ^{13}C abundance, Pee Dee Belemnite were calculated and expressed as atoms %.

Calculations

Rate of glucose appearance in plasma. The rates at which glucose appeared in arterialized plasma from the exogenous (oral) and endogenous (hepatic) sources were estimated using the non-steady state equation of Steele (1959) assuming an effective volume of distribution of 230 ml/kg body weight (Livesey *et al.* 1998). Plasma concentrations of D-[6,6^{2}H$_2$]glucose, [^{13}C]glucose and total glucose (labelled and unlabelled) were measured and identical behaviour of labelled and unlabelled glucose molecules was assumed. Using endogenous glucose and [^{13}C] glucose as tracees in the single-compartment model and D-[6,6^{2}H$_2$]glucose as the tracer, the relative fluxes of plasma glucose derived from both the gut and endogenous sources were calculated. The appropriate tracee concentrations were substituted into Steele's (1959) equation:

$$\text{Ra}(t) = \frac{\text{Ra}^*}{\text{a}(t)} - \text{Vs} \times \frac{\text{C}(t)\dot{\text{a}}(t)}{\text{a}(t)}, \quad (2)$$

where Ra(t) was the tracee rate of appearance, Ra* was the tracer ([6,6^{2}H$_2$]glucose) rate of appearance, Vs was the effective volume of the sampled compartment, C(t) was the tracee concentration, a(t) was the ratio of tracer to tracee and å(t) its derivative with respect to time. Validity of the model and pool size for the present circumstance is described in detail elsewhere (Livesey *et al.* 1998).

Rate of glucose disposal from plasma. Plasma Gd was the sum of plasma glucose appearance and the balance of its accumulation in the glucose pool. Gd was calculated using Steele's non-steady state equations (Steele, 1959) for a single-compartment model as modified for volume by Livesey *et al.* (1998). Insulin-dependent and independent disappearance rates were assessed using a formula described in detail elsewhere (Robertson *et al.* 2000):

$$\text{aucG}_d(t) = a + b \times \text{auc(I)}(t) \quad (3)$$

Where, aucGd is the cumulative area-under-the-curve for total plasma Gd reached after time (t), a is a constant representing an individual's insulin-independent Gd rate, b represents the rate of change in the rate of glucose disposal with changing insulin concentration and auc(I)(t) is the cumulative area-under-the-curve for insulin concentration reached after time (t).

Statistics

The time-course of the glucose and hormone concentration responses to the test meal and following the D-[6,6^{2}H$_2$] glucose infusion are displayed in the figures as mean values with their standard errors. Statistical analysis using SPSS (SPSS, Chertsey, Surrey, UK) was performed on time-course and summary data. The statistical significance of differences between the two subject groups was determined using two-factor repeated measures ANOVA with interaction. The normal distribution of the data were assessed using the Shapiro-Wilk test. Summary data was analysed using independent Student's *t* tests.

Results

Plasma glucose

Changes in plasma glucose concentrations throughout the 11 h study are shown in Fig. 1. Fasting glucose was lower in females than males (4·5 *v.* 5·1 mmol/l) although not significantly (P=0·061). However, the postprandial glucose concentrations were significantly lower in the female group (Fig. 1). The concentration of the tracer D-[6,6^{2}H$_2$]glucose increased to approach a steady state of 3·3 (SEM 0·2) and 3·8 (SEM 0·3) atom % for the male and

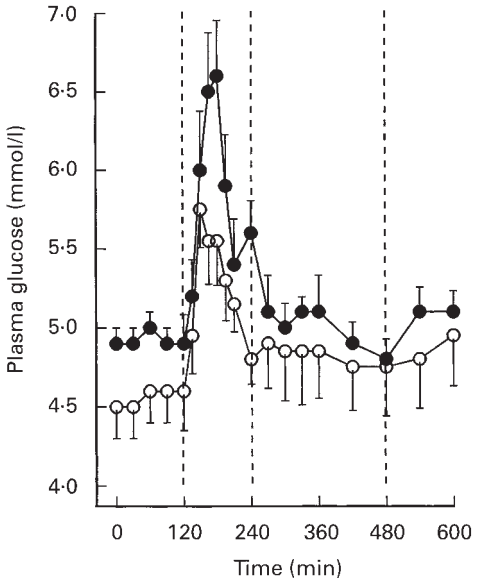

Fig. 1. Plasma glucose response following a starch-rich test meal in groups of male (●) and female (○) subjects. For details of subjects, test meals and procedures, see p. 570. ----, Test meal given. Values are means for six male or six female subjects with standard errors shown by vertical bars. Repeated-measures ANOVA showed a significant time effect (P<0·001) and sex *v.* time interaction (P=0·05).

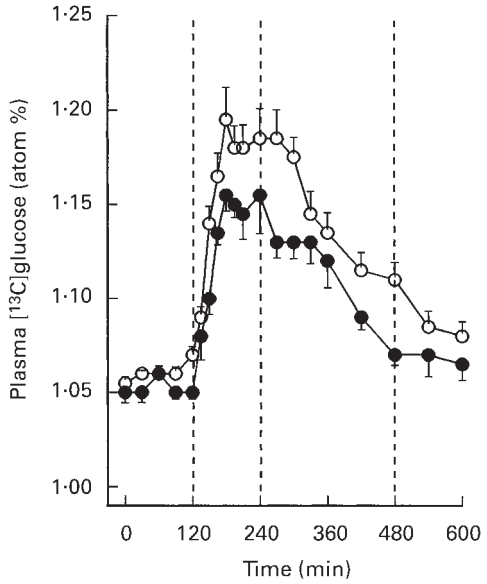

Fig. 2. Plasma [^{13}C]glucose enrichment following a ^{13}C-labelled starch-rich meal ingestion in groups of male (●) and female (○) subjects. For details of subjects, test meals and procedures, see p. 570. ----, Test meal given. Values are means for six male or six female subjects with standard errors shown by vertical bars. Repeated-measures ANOVA showed a significant time effect ($P<0{\cdot}001$), a sex effect ($P=0{\cdot}019$) and a sex *v.* time interaction ($P=0{\cdot}039$).

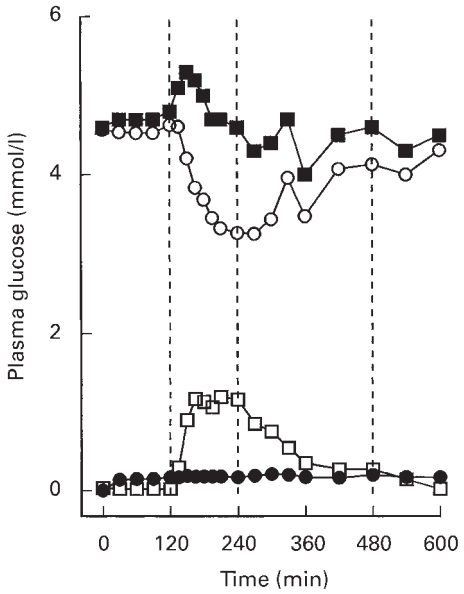

Fig. 3. Glucose provenance graph for a single volunteer showing: (■), the total glucose concentration; (○), the unlabelled-glucose concentration; (□), the [^{13}C]glucose concentration; (●), the D-[6,6^{2}H$_2$]glucose concentration in plasma during the dual-isotope procedure. For details of test meals and procedures, see p. 570. ----, Test meal given.

female groups respectively, during the 2 h immediately after the primed-constant infusion and before the test meal of ^{13}C-labelled peas. Three distinct nadirs in the D-[6,6^{2}H$_2$]glucose concentration following the attainment of the steady state were apparent in both subject groups. However, the time course of these nadirs differed significantly ($P=0{\cdot}036$). The second nadir was earlier (195 *v.* 240 min) and the third nadir was later (420 *v.* 360 min) in the female group compared with the male (results not shown). The tracer concentration remained below the pre-meal level for the duration of the study in all subjects. Fig. 2 shows the ^{13}C enrichment in plasma glucose. This rose rapidly after ingestion of the ^{13}C-labelled starchy meal reaching an initial peak after 60 min. Subsequent peaks in the plasma [^{13}C]glucose occurred in both groups although at different times ($P=0{\cdot}039$). By combining all the glucose data, a glucose provenance graph (Fig. 3) was constructed for each individual subject in which the total glucose concentration in arterialized plasma was, by definition, the sum of the labelled and unlabelled glucose molecules.

Plasma insulin

The pattern of plasma insulin concentration followed closely that of plasma glucose, no significant differences was found between subject groups. There was a biphasic response, with the major sharp peak at approximately 30 min and a second broad peak at approximately 210 min after the cooked-peas test meal (Fig. 4). This second rise in the plasma insulin was less pronounced than the first peak but was observed in all volunteers.

Glucose kinetics

The mean fasting rate of endogenous glucose production was 2·5 (SEM 0·2) and 2·8 (SEM 0·1) mg/min per kg body mass in males and females respectively (Fig. 5). Consumption of the starch-rich meal rapidly suppressed endogenous glucose production by approximately 33 % to 1·6 (SEM 0·2)

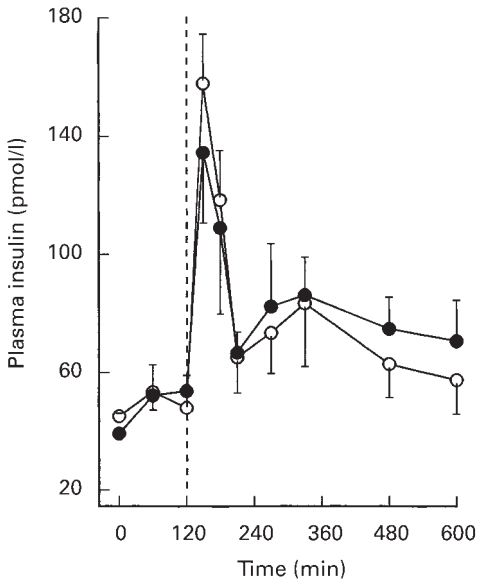

Fig. 4. Plasma insulin response following a starch-rich meal in a group of male (●) and female (○) subjects. For details of subjects, test meals and procedures, see p. 570. ----, Test meal given. Values are means for six male or six female subjects with standard errors shown by vertical bars. Repeated-measures ANOVA showed a significant time effect ($P<0{\cdot}001$) only.

in males and by 29 % to 2·0 (SEM 0·3) mg/(ml.kg) body mass in females by 75 min after the starchy meal. Additional nadirs were observed in all subjects and the timings of these were not significantly different between subject groups. Endogenous glucose production had returned to the baseline fasting level by 6 h after the starchy meal (Fig. 5).

The rate of [^{13}C]glucose appearance in plasma accelerated rapidly to a peak of 1·5 (SEM 0·4) in males and 2·6 (SEM 0·7) mg/min per kg body mass in the female group by 60 min after the starchy meal (Fig. 6). This initial appearance of [^{13}C]glucose was followed by two subsequent peaks of smaller magnitude at approximately 120 and 240 min after the starchy meal in males. A similar pattern was observed in the female group although the final peak occurred later at approximately 300 min post ingestion ($P<0·001$). Labelled glucose uptake remained detectable until the end of the study 8 h after the starchy meal.

The plasma Gd rate rose rapidly after ingestion of the starchy test meal and failed to return to baseline by the end of the study (results not shown). A peak Gd of 4·1 (SEM 0·5) in males and 5·5 (SEM 0·2) mg/min per kg body mass in females group was reached by 75 min post-ingestion, with further peaks at 120 and 240 min. Although there was no sex difference in the total Gd over the whole study period, the insulin-dependent Gd rate was significantly higher in the females. In contrast, estimates of insulin-independent glucose Gd did not differ significantly between groups (Table 2).

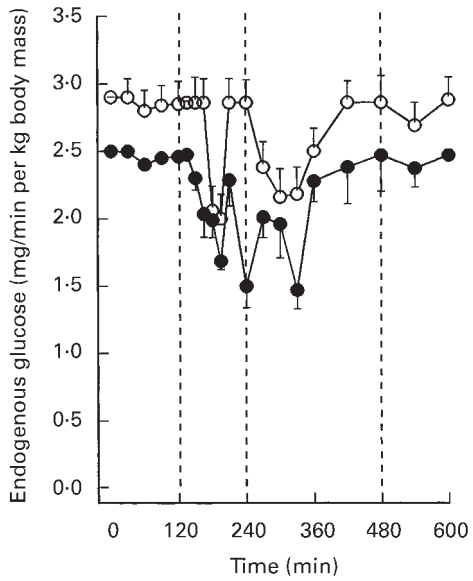

Fig. 5. Endogenous glucose production following a starch-rich meal in a group of male (●) and female (○) subjects calculated using the single-compartment model of Steele (1959) with pool size 230 ml/kg body weight. For details of subjects, test meals and procedures, see p. 570. ----, Test meal given. Values are means for six male or six female subjects with standard errors shown by vertical bars. Repeated-measures ANOVA showed a significant time effect ($P<0·001$) only.

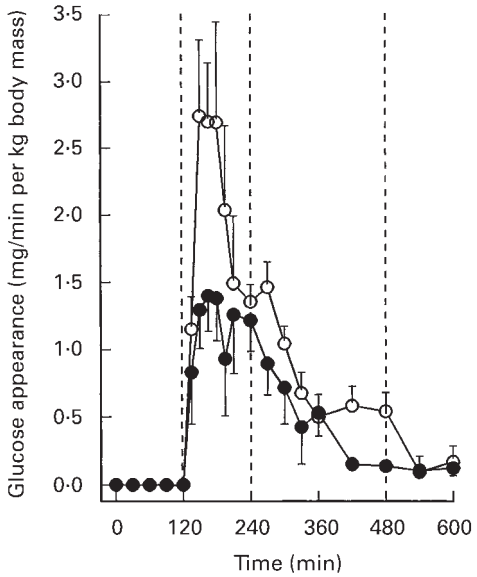

Fig. 6. [^{13}C]Glucose appearance following a ^{13}C-enriched starch-rich meal in a group of male (●) and female (○) subjects calculated using the single-compartment model of Steele (1959) with pool size 230 ml/kg body weight. For details of subjects, test meals and procedures, see p. 570. ----, Test meal given. Values are means for six male or six female subjects with standard errors shown by vertical bars. Repeated-measures ANOVA showed a significant time effect ($P<0·001$) and a sex *v.* time interaction ($P<0·001$).

Carbohydrate oxidation

In the fasting state the rate of total carbohydrate oxidation determined by indirect calorimetry was 1·2 (SEM 0·4) for males and 1·4 (SEM 0·2) mg/min per kg body mass for the female group. The carbohydrate oxidation rate increased after ingestion of the starchy meal in both groups, and by 60 min had reached a maximum rate of 1·8 (SEM 0·4) for males and 2·8 (SEM 0·3) mg/min per kg body mass in the females. The post-ingestive rate of carbohydrate oxidation remained above the fasting value for the whole study. According to indirect calorimetry, the total amount of carbohydrate oxidized during the study was significantly higher in females. There were no gender differences in lipid oxidation (Table 2).

The [^{13}C]glucose oxidation rates were calculated from knowledge of breath $^{13}CO_2$ enrichment and V_{CO_2} assuming no contribution from ^{13}C-labelled protein in the peas. No gender difference was noted in V_{CO_2} between groups when adjusted for body mass (results not shown) but breath $^{13}CO_2$ enrichment differed significantly ($P=0·007$, Fig. 7) being consistently higher in females. The total ^{13}C recovery over 0–8 h was significantly higher in females ($P=0·022$) but cumulative recovery had not yet plateaued, suggesting this total to be an underestimate of eventual ^{13}C starch that would be oxidized.

Discussion

Experimental approach and aims

Studies of whole-body metabolism employing modelling techniques and results obtained using stable isotopes are

Table 2. Estimates of glucose appearance and disappearance and of lipid oxidation following a ^{13}C-labelled carbohydrate test meal†‡

(Mean values with their standard errors for six male and six female subjects)

	Males		Females	
	Mean	SEM	Mean	SEM
Total [^{13}C]glucose appearance in plasma (g)	16·2	2·4	16·6	5·0
Total plasma glucose disposal (g)	115	12	117	16
Total non-oxidative disposal of [^{13}C]glucose load (g)	6·5	1·4	5·1	2·7
Total glucose oxidation by indirect calorimetry (g)	67·8	8·3	80·7*	6·1
[^{13}C]Glucose oxidation (g)				
Total	9·7	0·4	11·5*	0·5
0–300 min	6·2	0·2	7·8**	0·3
300–600 min	3·5	0·2	3·7	0·2
Total lipid oxidation by indirect calorimetry (g)	62·1	8·1	52·6	3·5
Insulin-independent Gd (mg/(min.kg))	2·74	1·2	2·91	0·6
Insulin-dependent Gd (μg/(min.kg) per pmol insulin)	5·6	0·5	6·8*	0·6

Gd, glucose disposal.
Mean values were significantly different from those of the male group (Student's *t* test for independent samples): *$P<0·05$, **$P<0·01$.
† For details of subjects, test meals and procedures, see p. 570.
‡ Estimates were calculated using the single compartment model of Steele (1959).

rapidly becoming established as the methods of choice for investigating the substrate kinetics that underly physiological conditions, and this includes the state of insulin sensitivity. The single-compartment model of glucose kinetics, first used by Steele (1959), has been used successfully following both glucose and starch ingestion from single carbohydrate-rich meals (Tissot *et al.* 1990; Delarue *et al.* 1993; Livesey *et al.* 1998; Robertson *et al.* 2000). In the present study, these methods have been combined to obtain quantitative information on the kinetics of glucose absorption and disposal from a conventional starchy food (cooked peas served as soup). In western societies, the pattern of nutrient intake usually consists of sequential meals taken several hours apart so that much of the day is spent in the postprandial state. We chose to investigate glucose kinetics following ingestion of a starchy-meal at breakfast followed by carbohydrate-free meals at 'lunch' and 'dinner'. We questioned whether glucose kinetics would be different in male and female subjects and whether differences in insulin sensitivity exist that might contribute towards the gender difference in the risk of cardiovascular disease.

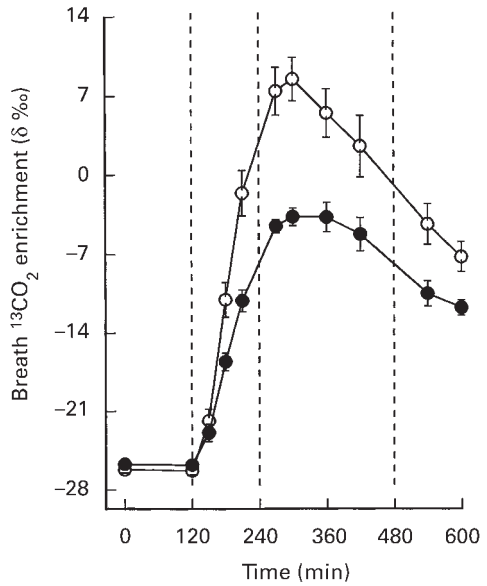

Fig. 7. $^{13}CO_2$ enrichment in (δ‰) of end-expired breath following a ^{13}C-enriched starch-rich meal in groups of male (●) and female (○) subjects. For details of subjects, test meals and procedures, see p. 570. ----, Test meal given. Values are means for six male or six female subjects with standard errors shown by vertical bars. Repeated-measures ANOVA showed a significant time effect ($P<0·001$), a sex effect ($P=0·007$) and a sex *v.* time interaction $P<0·001$).

Kinetics of glucose absorption

Following the starchy pea-soup meal there appeared to be three phases of digestion resulting in three peaks in [^{13}C]glucose appearance in plasma (Fig. 6), each of which corresponded with nadirs in the endogenous glucose production rate (Fig. 5). Neither returned to baseline after 300 min. In this respect both gender groups responded similarly. This pattern contrasts with observations using [^{13}C]glucose as a single meal, which generally yield a single peak and nadir which return to baseline within 300 min (Tissot *et al.* 1990; Livesey *et al.* 1998).

Some studies suggest that only 60–70 % starch-derived glucose appears in plasma (Capaldo *et al.* 1999; Noah *et al.* 2000), lower than that calculated for the present study which was in the range of 85–90 % (Table 2). Recoveries of 90–100 % of an oral glucose load have been demonstrated previously using the present model (Livesey *et al.* 1998). The apparent higher glucose recovery with the present model compared with other studies may be partly explained by our assumption of a larger effective volume of glucose distribution (230 ml/kg), which is appropriate when glucose turnover is slow (for review see Livesey *et al.* 1998). Our recovery rate was lower than expected if all the ingested starch had been digested in the small bowel and absorbed as glucose.

Work with ileostomy subjects using this pea cultivar has shown that 5·9 % or 1·1 g of the starch-load ingested would have escaped digestion in the small intestine (Robertson, 1997) and explains the apparent discrepancy. A further possible source of [^{13}C]glucose would be via gluconeogenesis from ^{13}C-labelled protein within the peas. It is not possible to predict this contribution with certainty. However, it cannot be large, otherwise the estimate of [^{13}C]-glucose absorption from starch would certainly have exceeded the amount expected after correction for losses in digestion (Robertson, 1997). Moreover, such *de novo* [^{13}C]glucose is likely to be stored as liver glycogen after gluconeogenesis rather than be released from the liver in the postprandial period. Finally, we estimate that the maximum amount of [^{13}C]glucose from naturally-enriched protein in the subsequent lunch and dinner would be negligible, and at most 0·2 g even if all the *de novo* glucose was released into the plasma.

The pea cultivar chosen for this study was relatively high in amylose (700 g/kg). The ^{13}C-labelling of plasma glucose up to 8 h postprandially may be due to the recycling of ^{13}C label that had previously been taken up into the peripheral tissues. Studies incorporating [^{13}C]glucose as opposed to starch rarely observe this apparent 'recycling' and during the present study the 'peaks' in [^{13}C]glucose appearance in the plasma coincided with nadirs in the endogenous glucose output (Fig. 5) which is not consistent with the release of significant amounts of [^{13}C]glucose from glycogen stores. An alternative explanation for the persistent labelling of [^{13}C] glucose after the starchy meal may be due to a considerable proportion of the starch in the test meal being only slowly hydrolysed by α-amylase digestion, which would effectively slow the rate appearance of labelled glucose in the circulation (Mathers & Daly, 2001). As such, the kinetics of glucose absorption after our test meal is very different from that observed after a glucose load (Livesey *et al.* 1998). The pattern of sequential peaks in [^{13}C]glucose appearance could potentially be due to the absorption of the different starch fractions as characterized by *in vitro* studies (Englyst *et al.* 1999). Based upon the rates at which glucose becomes available for absorption, starch has been divided into rapidly digestible starch, slowly digestible starch and resistant starch. The magnitude of each of these fractions is derived from glucose release by hydrolysis of food starches in the periods 0–20 min, 20–120 min and post 120 min respectively when digested by a battery of enzymes under specific conditions *in vitro* (Englyst *et al.* 1999). Results shown in Fig. 6 demonstrate that there is significant [^{13}C]glucose appearance post 120 min potentially due to slowly digestible starch which would be 'invisible' to procedures relying on blood concentrations only due to the homeostatic action of insulin and the concomitant drop in the endogenous glucose production.

Glucose disappearance

When expressed per kg body mass the total plasma Gd during the study was similar for the two gender groups, which is similar to findings based on hyperinsulaemic–euglycaemic clamp studies (Kahn *et al.* 1994). Glucose that escapes initial splanchnic extraction is disposed of by both insulin-independent and insulin-dependent mechanisms, and the insulin requirement for this process varies with insulin sensitivity of tissues. Whole-body insulin-mediated Gd in the present non-steady state study was significantly higher in the females than males, again similar to observations in steady-state euglycaemic conditions (Nuutila *et al.* 1995). In the latter study, whole-body insulin sensitivity was 41 % greater in women when measured using positron emission tomography; this corresponded to a 50 % increase in femoral muscle uptake of glucose. There was no effect of gender on the rate in insulin-independent Gd in the present study.

The oxidation of ^{13}C glucose was quantified from $^{13}CO_2$ enrichment in expired air and V_{CO_2}, and was significantly higher in females (Fig. 7). This may be due to the higher insulin sensitivity acting to elevate glucose oxidation (Table 2), but could also be explained by a smaller pool of endogenous glucose in females, due to their lower body mass and higher degree of adiposity. A complicating factor is the method used to produce the original test material. Whilst pulse dosing the pea cultivar with $^{13}CO_2$ it is possible to label the starch with a ^{13}C label, proteins intrinsic to the pea structure will also exhibit a ^{13}C label. Labelled amino acids are then absorbed along with the labelled glucose and have the potential to be oxidized, providing $^{13}CO_2$. This would result in an overestimation in the calculated [^{13}C]glucose oxidation. Boirie *et al.* (1996) have shown that approximately one-third of ingested proteins are oxidized directly and of the remainder, only approximately 70 % can be converted to glucose due to the presence of ketogenic amino acids. From this we can estimate the maximal effect of protein oxidation to be in the region of 2·5 g of the calculated [^{13}C]glucose oxidized. Although ^{13}C-labelled amino acid oxidation is an important source of error in the quantification of [^{13}C]glucose oxidation, the rate of total carbohydrate oxidation (labelled and unlabelled) was still significantly higher in the female group when adjusted for protein oxidation (calculated from the non-protein RER). The colonic fermentation of labelled-starch would have led to be production of ^{13}C capable of bypassing the [^{13}C]glucose pool and appearing 'directly' as $^{13}CO_2$. Colonic fermentation will contribute to the rate of $^{13}CO_2$ production (Robertson *et al.* 1996) especially in the late postprandial period (5–6 h after starch ingestion) and so possible differences in colonic fermentation patterns and the production of $^{13}CO_2$ between the genders must be considered. However, when the time course of $^{13}CO_2$ appearance in breath is examined (Table 2), the significant difference in ^{13}C recovery between the genders occurs in the first 300 min after test meal ingestion, i.e. before there is likely to be significant colonic fermentation of ^{13}C-labelled pea residues.

As expected, there was a time delay between the peak appearance of [^{13}C]glucose in plasma and the peak in [^{13}C]glucose oxidation (Normand *et al.* 1992). Before appearing in breath, $^{13}CO_2$ may enter bicarbonate pools (which turn over relatively slowly) or become fixed into metabolic intermediates (Leijssen & Elia, 1996). Recovery of $^{13}CO_2$ can vary considerably and such variation may

result in errors in the estimated rates of substrate oxidation (Van-Hall, 1999). Whilst there is continued interest in the determination of appropriate correction factors for bicarbonate and acetate (Schrauwen *et al.* 2000) the effects of gender have not been investigated.

Concluding remarks

In summary, glucose kinetics following ingestion of a meal of cooked peas results in the persistent appearance of plasma [^{13}C]glucose up to 8 h after ingestion with three phases of [^{13}C]glucose appearance and suppression of endogenous glucose production. There were significant sex differences, with females exhibiting a higher whole-body insulin sensitivity than males with the same age and BMI. This applies to the physiological relevant post-prandial state.

Acknowledgements

Thanks are due to the BBSRC Institute of Food Research, Norwich for cooperation in the supply of ^{13}C-labelled peas. We thank Drs Mark Walker and Vipin Mishra, Department of Medicine, University of Newcastle for medical cover during clinical procedures. M.D.R. held a BBSRC CASE studentship.

References

Boirie Y, Gachon P, Corny S, Fauquant J, Maubois JL & Beaufrere B (1996) Acute postprandial changes in leucine metabolism as assessed with an intrinsically labeled milk protein. *American Journal of Physiology* **271**, E1083–E1091.

Capaldo B, Gastaldelli A, Antoniello S, Auletta M, Pardo F, Ciociaro D, Guida R, Ferrannini E & Sacca L (1999) Splanchnic and leg substrate exchange after ingestion of a natural mixed meal in humans. *Diabetes* **48**, 958–966.

Delarue J, Normand S, Pachiaudi C, Beylot M, Lamisse F & Riou JP (1993) The contribution of naturally labelled ^{13}C fructose to glucose appearance in humans. *Diabetologia* **36**, 338–345.

Donahue RP, Prineas RJ, DeCarlo Donahue R & Skyler JS (1996) The female 'insulin advantage' in a biracial cohort: Results from the Miami Community Health Study. *International Journal of Obesity* **20**, 76–82.

Elia M & Livesey G (1992) Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. In *Metabolic Control of Eating, Energy Expenditure and the Bioenergetics of Obesity*, pp. 68–131 [AP Simopoulos, editor]. Basel: Karger.

Englyst KN, Englyst HN, Hudson GJ, Cole TJ & Cummings JH (1999) Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. *American Journal of Clinical Nutrition* **69**, 448–454.

Faulks RM, Roe MA & Livesey G (1994) Production of ^{13}C labelled peas for studies of starch metabolism. In *EURESTA: European Commission – Science, Research and Development*, [N-G Asp, JMM Van Amelsvoort and JGAJ Hautvast, editors], EURESTA.

Gregory J, Foster K, Tyler H & Wiseman M (1990) *The Dietary and Nutritional Survey of British Adults*. London: H.M. Stationery Office.

Hampton SM (1984) C-peptide of pro-insulin: its diagnostic use and a possible physiological role. PhD Thesis, University of Survey.

Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP & Porte D Jr (1994) The contribution of insulin-dependent and insulin-independent glucose uptake to intravenous glucose tolerance in healthy human subjects. *Diabetes* **43**, 587–592.

Kannel WB & Abbott RD (1987) Incidence and prognosis of myocardial infarction in women: the Framingham study. In *Coronary Heart Disease in Women*, pp. 208–214 [ED Eaker, B Packard, NK Wenger, TB Clarkson and HA Tyroler, editors]. New York, NY: Haymarket-Doyma.

Leijssen DPC & Elia M (1996) Recovery of $^{13}CO_2$ and $^{14}CO_2$ in human bicarbonate studies: a critical review with original data. *Clinical Science* **91**, 665–677.

Livesey G, Wilson PDG, Dainty JR, Brown JC, Faulks RM, Roe MA, Newman TA, Eagles J, Mellon FA & Greenwood RH (1998) Simultaneous time-varying systematic appearance of oral and hepatic glucose in adults monitored with stable isotopes. *American Journal of Physiology* **275**, E717–E728.

Mathers JC & Daly ME (2001) Food polysaccharides, glucose absorption and insulin sensitivity. In *Advanced Dietary Fibre Technology*, pp. 186–197 [B McCleary and L Prosky, editors]. Oxford: Blackwell Science.

Nilsson PM, Lind L, Pollare T, Berne C & Lithell H (2000) Differences in insulin sensitivity and risk markers due to gender and age in hypertensives. *Journal of Human Hypertension* **14**, 51–56.

Noah L, Krempe M, Lecannu G, Maugere P & Champ M (2000) Bioavailability of starch and postprandial changes in splanchnic glucose metabolism in pigs. *American Journal of Physiology* **278**, E181–E188.

Normand S, Pachiaudi C, Khalfallah Y, Guilluy R, Mornex R & Riou JP (1992) ^{13}C appearance in plasma glucose and breath CO_2 during feeding with naturally enriched ^{13}C-enriched starchy food in normal humans. *American Journal of Clinical Nutrition* **55**, 430–435.

Nuutila P, Knuuti MJ, Maki M, Laine H, Ruotsalainen U, Teras M, Haaparanta M, Solin O & Yki-Jarvinen H (1995) Gender and insulin sensitivity in the heart and in skeletal muscles: studies using positron emission tomography. *Diabetes* **44**, 31–36.

Pickert A, Overkamp D, Renn W, Liebich H & Eggstein M (1991) Selected ion monitoring gas chromatography/mass spectrometry using uniformly labeled ^{13}C glucose for determination of glucose turnover in man. *Biological Mass Spectrometry* **20**, 203–209.

Reaven GM (1988) Role of insulin resistance in human disease. *Diabetes* **31**, 670–673.

Robertson MD (1997) Starch absorption and glucose kinetics in normal and colectomized humans. PhD Thesis, University of Newcastle.

Robertson MD, Livesey G, Hampton SM & Mathers JC (2000) Evidence for altered control of glucose disposal after total colectomy. *British Journal of Nutrition* **84**, 813–819.

Robertson MD, Mathers JC, Mishra V & Livesey G (1996) Is the ileostomist a good model for studies of starch digestion in man? *Proceedings of the Nutrition Society* **55**, 50A.

Schrauwen P, Blaak EE, Van Aggel Leijssen DPC, Borghouts LB & Wagenmakers AJM (2000) Determinants of the acetate recovery factor: Implications for estimation of ^{13}C substrate oxidation. *Clinical Science* **98**, 587–592.

Steele R (1959) Influence of glucose loading and of injected insulin on hepatic glucose output. *Annals of the New York Academy of Sciences* **82**, 420–430.

Tissot S, Normand S, Guilluy R, Pachiaudi C, Beylot M, Laville M, Cohen R, Mornex R & Riou JP (1990) Use of a new gas

chromatograph isotope ratio spectrometer to trace exogenous ^{13}C-labelled glucose at a very low level of enrichment in man. *Diabetologia* **33**, 449–456.

Van-Hall G (1999) Correction factors for ^{13}C labelled substrate oxidation at whole-body and muscle level. *Proceedings of the Nutrition Society* **58**, 979–986.

Wieko J & Shernam WR (1976) Boroacetylation of carbohydrates. Correlations between structure and mass spectral behaviour inmonoacetylhexose cyclic boronic esters. *Journal of the American Chemical Society* **98**, 7631–7637.

Yip J, Facchini FS & Reaven GM (1998) Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. *Journal of Clinical Endocrinology and Metabolism* **83**, 2773–2776.