Influence of dietary fat on postprandial glucose metabolism (exogenous and endogenous) using intrinsically ^{13}C-enriched durum wheat

Sylvie Normand[1,2]*, Yadh Khalfallah[2], Corinne Louche-Pelissier[1], Christiane Pachiaudi[1], Jean-Michel Antoine[3], Stéphane Blanc[1], Michel Desage[1], Jean Paul Riou[2] and Martine Laville[1,2]

[1]*Human Nutrition Research Centre (HRNC), Batiment 1, Edouard Herriot Hospital, Lyon, France*
[2]*INSERM U449, Faculté de Médecine RTH Laënnec, 8 rue G. Paradin, 69372 Lyon Cedex 08, France*
[3]*Danone Vitapole, 15 Avenue Galilée-92350-Le Plessis Robinson, France*

The present study evaluates the influence of different amounts of fat added to starch on postprandial glucose metabolism (exogenous and endogenous). Nine women (24 (SE 2) years old, BMI 20·4 (SE 0·7) kg/m^2) ingested 1 week apart 75 g glucose equivalent of ^{13}C-labelled starch in the form of pasta without (low fat; LF) or with 15 (medium fat; MF) or 40 (high fat; HF) g sunflower oil. During the 7 h following meal consumption, plasma glucose, non-esterified fatty acids, triacylglycerols (TG) and insulin concentrations, and endogenous (using [6,6-^{2}H$_2$]glucose) and exogenous glucose turnover were determined. With MF and HF meals, a lower postprandial glucose peak was observed, but with a secondary recovery. A decrease in exogenous glucose appearance explained lower glycaemia in HF. At 4 h after the HF meal the insulin, insulin:glucose and postprandial blood TG were higher than those measured after the LF and MF meals. Despite higher insulinaemia, total glucose disappearance was similar and endogenous glucose production was suppressed less than after the LF and MF meals, suggesting insulin resistance. Thus, the addition of a large amount of fat appears to be unfavourable to glucose metabolism because it leads to a feature of insulin resistance. On the contrary, the MF meal did not have these adverse effects, but it was able to decrease the initial glycaemic peak.

^{13}C-labelled starch: Polyunsaturated fat: Bioavailability: Postprandial glucose metabolism: Oral load

As obesity, diabetes and cardiovascular risk factors are increasing in industrialised countries, nutritional approaches are being used to limit their development. In addition to energy-restriction programmes, the consumption of low glycaemic index foods has been recommended. As the metabolic syndrome represents a vicious circle whereby insulin resistance leads to hyperinsulinaemia, and thus exacerbates insulin resistance, reducing postprandial glucose and insulin levels could be a goal to reach (Wolever, 2000). One easy way to lower the glycaemic index of carbohydrate nutrients is to add fat; by using scintigraphy it has been shown that this slows gastric emptying (Cecil *et al.* 1999). Many authors have studied the effects of various carbohydrate and fat contents on plasma substrates and postprandial insulin profiles (Collier *et al.* 1984, 1987, 1988; Cunningham & Read, 1989; Van

Amelsvoort *et al.* 1989, 1990; Cohen & Berger, 1990; Gatti *et al.* 1992). Collier *et al.* (1984) have shown that 37·5 g fat added to 75 g carbohydrate as lentils or potatoes were able to flatten the postprandial glucose curves but not the insulin response. Gannon *et al.* (1993), studying the effect of butter added to potatoes, also showed a decreased glucose response but an enhanced insulin postprandial area. Thus, if the decreased postprandial glucose response induced by fat addition is apparently favourable, the effect on insulin secretion appears more controversial, and the hypothesis that it might exert a beneficial effect on insulin sensitivity is not substantiated. Moreover, it has been shown that adding fat increases postprandial triacylglycerolaemia in a dose-dependent manner (Dubois *et al.* 1998), which is also an undesirable side effect. Thus, it appears necessary, as emphasised by Gannon *et al.* (1993), to understand better

---

Abbreviations: AUC, area under the curve; EGP, endogenous glucose production; HF, high-fat meal, 40 g sunflower oil added to pasta; LF, low-fat meal, no fat added to pasta; MF, medium-fat meal, 15 g sunflower oil added to pasta; NEFA, non-esterified fatty acids; RaT, rate of total glucose appearance; TG, triacylglycerols.

* **Corresponding author:** Dr. S. Normand, fax +33 4 78 77 87 62, email snormand@laennec.univ-lyon1.fr

the mechanisms by which fat attenuates the glucose response to carbohydrate. As the influence of fat on glucose metabolism (i.e. exogenous glucose appearance, endogenous glucose production) has never been reported, it is important to investigate the mechanisms by which fat is able to modify carbohydrate metabolism. Previously we have been able (Normand *et al.* 1992) to study glucose metabolism after a carbohydrate load by using naturally ^{13}C-enriched maize. In the present study, we used ^{13}C-enriched durum wheat obtained by culture in a ^{13}CO$_2$-enriched atmosphere, to produce intrinsically labelled pasta. This process allowed us to follow the metabolic fate of the ingested pasta in the presence of different amounts of sunflower oil (0, 15, 40 g). The influence of fat on the appearance of glucose derived from ^{13}C-enriched pasta, on glucose utilisation and on endogenous glucose production was studied in nine normal women. We were able to demonstrate that besides the delay in exogenous glucose appearance, abnormalities of glucose metabolism, such as defects in postprandial endogenous glucose suppression, were induced by high doses of fat.

Methods

Subjects

Nine healthy women of mean age 24 (SE 2, range 20–37) years and of mean weight 57 (SE 2, range 45–67) kg with a mean BMI of 20·4 (SE 0·7, range 16·6–23·3) kg/m^2 volunteered for the study. None had a family history of diabetes mellitus, gastrointestinal disease or were taking any medication. All subjects gave their written consent after being informed of the nature, purpose and possible risks of the study. The ethical committee of 'Hospices Civils de Lyon' approved the experimental protocol according to the Huriet Law (20th January 1988, modified 25th July 1994, no. 94-630).

Materials

The macronutrient composition of the test meals is given in Table 1. These meals consisted of starchy food and the amount ingested was calculated as 75 g glucose equivalent (94·7 g crude product containing 720 g starch/kg). The

Table 1. Macronutrient composition and energy content of the test meals*

Test meal	Energy		Carbohydrate (%)	Lipid (%)	Protein (%)
	KJ	kcal			
LF	1384	331	80·0	4·6	15·4
MF	1997	473	56·0	33·2	10·8
HF	2964	709	37·4	55·4	7·2

LF, low fat; MF, medium fat; HF, high fat.
* The amount of starchy food ingested was 94·7 g crude pasta, which correspond to 260 g cooked pasta (63·4 g carbohydrates, 12·8 g protein and 1·6 g lipid). For the three meals the amount of carbohydrate and protein ingested was fixed, and the fat content was increased (LF 1·6 g, MF 16·6 g, HF 41·6 g). Fat:carbohydrate was 2·5:100, 26:100 and 66:100 for LF, MF and HF respectively. The composition of sunflower oil was (g/100 g) 65 linoleic acid (18:2, polyunsaturated), 23·8 oleic acid (18:1, monounsaturated), 10·1 saturated acids (palmitic (16:0) and stearic (18:0)), 1·1 other fatty acids, and was glycerol free.

starchy food used was pasta (noodles) which was manufactured by Commissariat à l'Energie Atomique (CEA Cadarache, France) and Crealis (Groupe Danone, Brive, France). No eggs were added and the product was free of soluble sugar.

Pastas were manufactured with starch derived from a preparation of 75 g milled durum wheat cultivated in a ^{13}CO$_2$-enriched atmosphere (^{13}C isotope abundance 11 %) mixed with 11 kg milled natural durum wheat (^{13}C isotopic abundance 1·08 %). Noodles were cooked in 950 ml boiling mineral water (Evian; Evian, France) salted with 7 g NaCl/l for 7 min. The sunflower oil was added to the cooked product.

D-[6,6-^{2}H$_2$] glucose (99 mol % excess) was obtained from Commissariat à l'Energie Atomique (Gif-sur-Yvette, France); chemical and isotopic purity was confirmed by GC–selected-ion-monitoring MS; it was dissolved in sterile isotonic saline (9 g NaCl/l) and passed through a 0·22 μm Millipore filter (Millipore Corp., Bedford, MA, USA) before infusion. The preparation was pyrogen free. The actual concentration of [^{2}H]glucose in the infusate was determined at the end of each test.

Experimental protocol

At 1 week before the tests, nutrients known to be enriched in ^{13}C (maize starch and oil, cane sugar, exotic fruits and tinned foods) were excluded from the diet, and 24 h before the study the subjects were asked to limit extreme physical activity, to drink no alcohol and to eat a usual evening meal.

Each subject was submitted to three different test meals in which ^{13}C-enriched pasta was ingested without (low fat; LF), or with 15 (medium fat; MF) or 40 (high fat; HF) g sunflower oil. The order of the test was randomised with a Latin square design, with an interval of at least 1 week between each test.

All tests were performed between 07.00 and 08.00 hours in the post-absorptive state, 12 h after the last evening meal. Intravenous catheters were inserted into forearm veins in both arms for tracer infusion on one side, and blood sampling on the other side. A primed continuous infusion of D-[6,6-^{2}H$_2$]glucose (0·04 mg/kg per min) was started 150 min before the meal ingestion, and was maintained during the next 7 h to determine the total rate of glucose appearance (RaT). The priming dose was eighty times the infusion rate over 1 min. At time 0, subjects ate the meal in 15 min, and drank 250 ml mineral water. Blood samples were taken sequentially each 15 or 30 min over the 7 h following ingestion and were used for analysis of glucose, triacylglycerols (TG), non-esterified fatty acids (NEFA) and insulin concentrations, and isotopic enrichments [^{2}H]- and [^{13}C]glucose). This sequential sampling procedure allowed detection of very small and transient changes in plasma concentrations. Blood was immediately centrifuged (10 min at 3500 rpm at 4°C) and plasma was stored at −20°C until analysed.

Analytical procedures

Metabolites and hormones. Plasma glucose was determined in HClO$_4$-neutralised plasma extracts by enzymic

methods (Bergmeyer, 1974a,b) and plasma insulin levels by radioimmunoassay (Medgenix Diagnostics, Rungis, France). Enzymic methods were used to measure plasma NEFA (Wako, Freiburg, Germany; Laville et al. 1983) and TG (Sigma Diagnostics, St Quentin Fallavier, France; Bergmeyer, 1974b) levels.

Isotope analysis. Plasma glucose isotopic enrichments were determined on neutral fractions of deproteinised plasma samples partially purified over sequential anion–cation exchange resins as described previously (Tissot et al. 1990; Normand et al. 1992). Plasma [6,6-^{2}H$_2$]glucose was measured by organic GC–MS (model 5890; Hewlett Packard, Evry, France) on acetyl-bis-butane-boronyl glucose derivative, using an electron-impact mode and a selective monitoring of *m:z* 297 and 299 (Bier et al. 1977). Plasma ^{13}C glucose enrichment was measured by GC–combustion–isotope-ratio MS (SIRA 10; VG Isogas, Middlewich, Cheshire, UK) after derivatisation to penta-cetyl glucose as described by Dejongh (1965).

The ^{13}C enrichment of ingested starch was determined after enzymic hydrolysis (with amyloglucosidase) using the Thivend method (Thivend et al. 1972) and the glucose
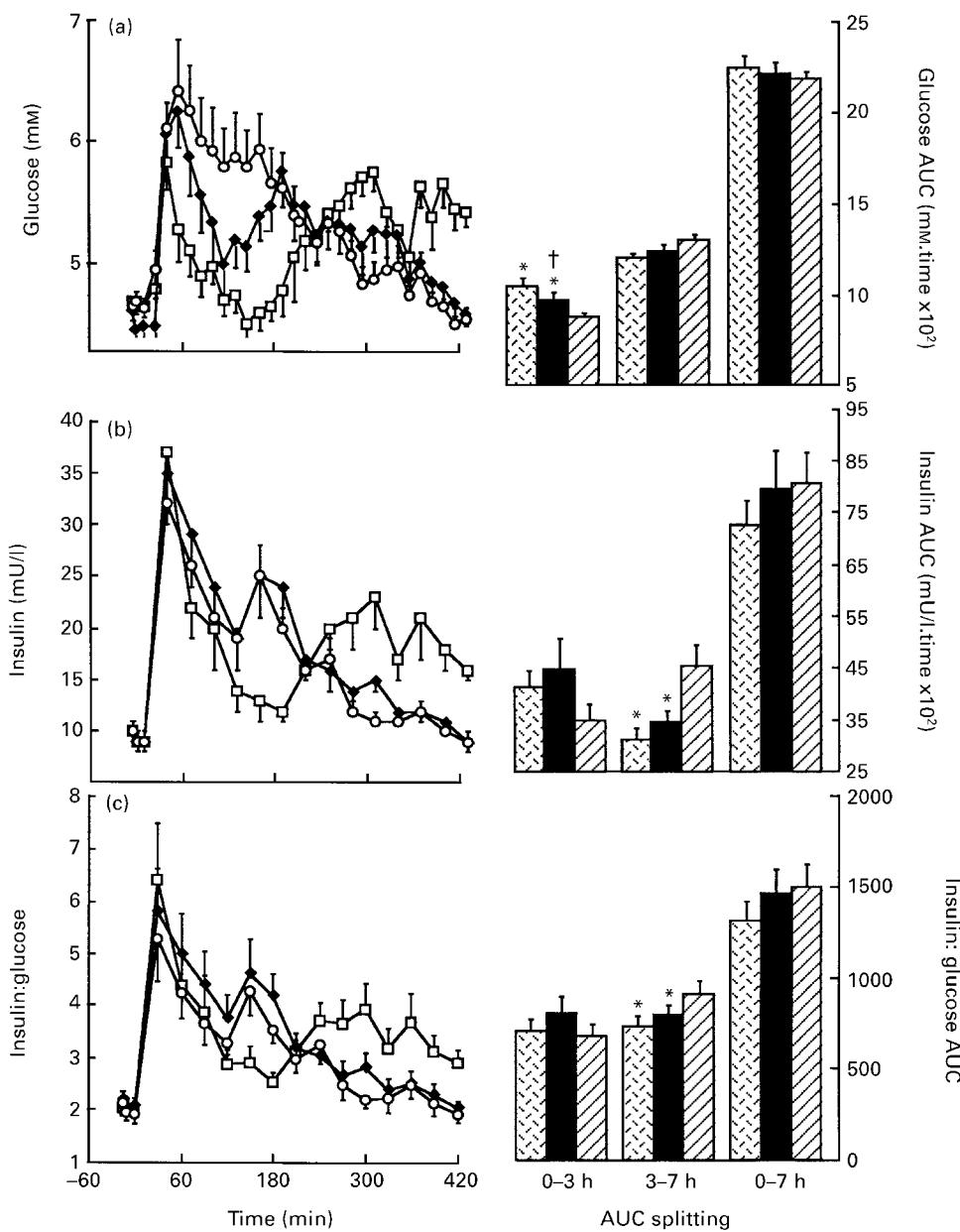
Fig. 1. Plasma glucose (a), insulin (b) and insulin:glucose (c) response (kinetics and area under the curve (AUC) to 75 g glucose equivalent given to healthy women in the form of pasta together with 15 (medium fat (MF); ◆, ■) 40 (high fat (HF); □, ▨) or ○ (low fat (LF); ○, ▥) g fat. For details of test meals and procedures, see Table 1 and p. 4. Values are means with their standard errors represented by vertical bars for nine subjects. Mean values were significantly different from those for HF meal; *$P < 0.05$. Mean value was significantly different from that for the LF meal: †$P < 0.05$.

obtained was purified by sequential anion–cation exchange chromatography before derivatisation as glucose penta-acetate and analysed as described previously (Normand *et al.* 1992). The ^{13}C enrichment of the derivatised glucose molecule was −15·54 (SD 0·31) δ^{13}C‰ (1·09416 (SD 0·00034) atom % ^{13}C; mean of four measurements).

Calculations

RaT and the rate of total glucose disappearance were calculated from plasma [6,6-^{2}H$_2$]glucose enrichment and from plasma [^{13}C] glucose enrichment the rates of exogenous glucose appearance and disappearance, using Steele's equation for non-steady-state conditions (De Bodo *et al.* 1963; Proietto *et al.* 1987) as described previously (Tissot *et al.* 1990). Endogenous glucose production (EGP) was calculated as RaT − RaE (exogenous glucose appearance).

Statistical analysis

The results are expressed as mean with their standard errors. Pasta data were analysed using Latin square ANOVA followed when significant (all ANOVA $P < 0.05$) by the Fisher's least significant difference pair wise for multiple comparison test. Fisher *post hoc* tests were used to identify significant differences $P < 0.05$ when ANOVA yielded a significant F ratio. Postprandial data were also assessed as area under the curve (AUC) calculated by using the trapezoidal method and integrated throughout the experimental period (0–180 min (first 3 h), 180–420 min (last 4 h) or 0–420 min (7 h postprandially)). Such an AUC splitting was selected to allow (1) a comparison with the classical 3 h oral glucose tolerance tests performed in the clinic and (2) an accurate estimation of the kinetic variables.

Results

Metabolites and insulin responses

The responses in kinetics and AUC of glucose, insulin and insulin:glucose to the three test meals are shown in Fig. 1. The HF meal resulted in an earlier (30 min) but significantly lower ($P = 0.037$) increase in blood glucose level than did the MF and LF meals. After a decrease in glycaemia, a second peak was observed 180 min after the MF meal, and 300 min after the HF meal. When compared
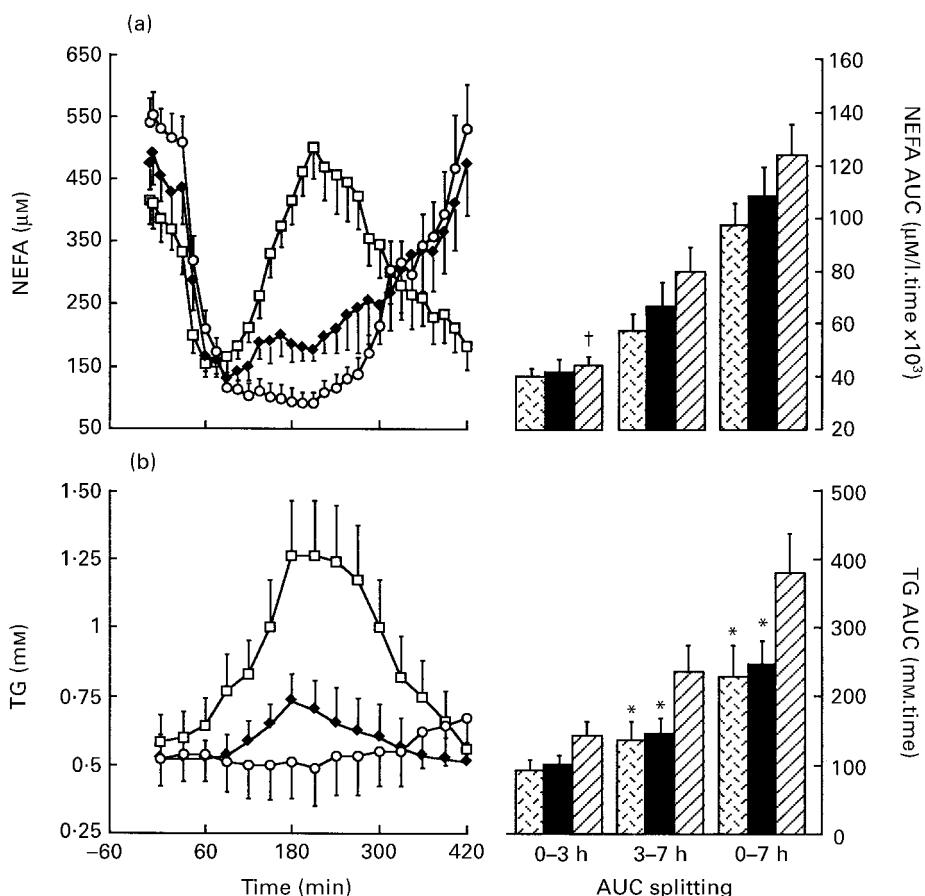
Fig. 2. Plasma non-esterified fatty acids (NEFA) and triacylglycerol (TG) response (kinetics and area under the curve (AUC) to 75 g glucose equivalent given to healthy women in the form of pasta together with 15 (medium fat; ◆, ■), 40 (high fat (HF); □, ▨) or ○ (low fat; ○, ▨) g fat. For details of test meals and procedures, see Table 1 and p. 4. Values are means with their standard errors represented by vertical bars for nine subjects. Mean values were significantly different from those for the HF meal: *$P < 0.05$. Mean value was significantly different from that for the LF meal: † $P < 0.05$.

with the LF and MF meals, glycaemia did not return to the basal level 7 h after the HF meal, and was significantly higher at 420 min ($P = 0.0001$). The first 3 h AUC were significantly different between the three meals ($P = 0.0003$), with the HF meal being lower than MF and LF meals ($P < 0.01$) and MF meal lower than LF meal ($P = 0.027$). The last HF meal 4 h AUC was significantly higher than that of the LF meal ($P = 0.007$). The total 7 h AUC was not significantly different between the three meals.

Insulin secretion was essentially unaffected by the addition of fat for the first early sharp peak 30 min after ingestion of the three test meals. A biphasic lipid dose-dependent response was then observed. Compared with the LF and MF meals, insulinaemia 7 h after ingestion of the HF meal did not return to basal values ($P = 0.0001$ at 420 min). The last HF meal 4 h AUC was significantly higher than that for the LF ($P = 0.0015$) and MF meals ($P = 0.0095$) and so was the last 4 h AUC of insulin:glucose when compared with LF meal $P = 0.005$ and when compared with MF meal $P = 0.023$.

NEFA and TG responses to the three test meals are shown in Fig. 2. Plasma NEFA concentration decreased during the first 90 min after consumption of all three meals. After ingestion of the LF meal, a plateau was observed from 120 to 270 min and then the concentration increased steadily until the end of the test. For the MF meal at 150 min after ingestion a weak peak value was noted before a return to the fasting level. However 1 h after ingestion of the HF meal NEFA concentration rose rapidly until 210 min and then declined until the end of the test. The first NEFA 3 h AUC was significantly higher after the HF meal ($P = 0.007$) compared with the LF meal.

A large increase in plasma TG concentration occurred after HF meal ingestion only, with a plateau from 180 to 240 min. The last 4 h AUC was significantly higher after the HF meal ($P = 0.002$), as was the 7 h AUC ($P = 0.0001$); there was no difference between the LF and MF meals.

Thus, the HF meal induces a lower glucose peak in the first period of the test but alters the second part profoundly by increasing glycaemia, insulinaemia and TG concentration.

Glucose turnover

Fig. 3 shows the changes in RaT, exogenous glucose appearance and EGP during pasta meals. The kinetics of RaT were not affected significantly by lipid addition. Overall glucose appearance increased to a peak value 30 min after ingestion of the three pasta meals. After a subsequent decrease, a biphasic response was observed, with a second peak 150 min after ingestion of the LF meal and 180 min after ingestion of the MF meal, whereas values almost stabilised at lower values between 60 and 180 min after ingestion of the HF meal. Between 210 and 270 min RaT was similar (3.79 (SE 0.07) mg/kg per min) after the three meals, but during the last hour RaT approached basal levels after the LF and MF meals while being maintained at a higher level after the HF meal.

The overall rate of disappearance of glucose (data not
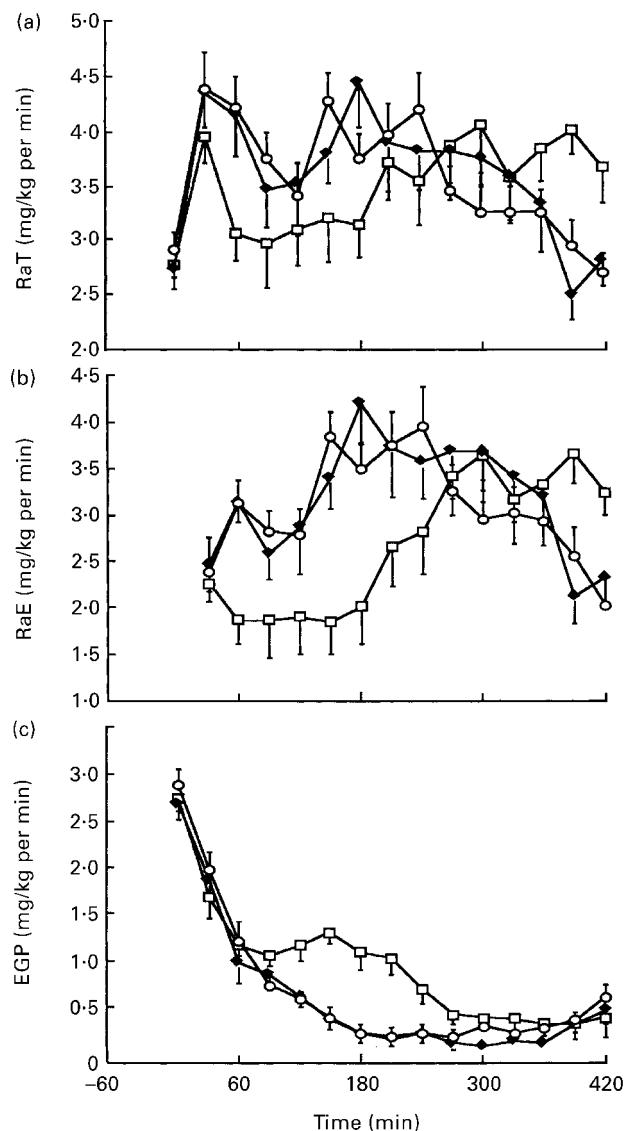
Fig. 3. Changes in total glucose appearance (RaT; a), exogenous glucose appearance (RaE; b) and endogenous glucose production (EGP; c) in healthy women after ingestion of pasta together with 15 (medium fat; ◆, ■), (40 high fat; □, ▨) or (0 low fat; ○, ▨) g fat. For details of test meals and procedures, see Table 1 and p. 4. Values are means with their standard errors represented by vertical bars for nine subjects.

shown) peaked 60 min after the three meals, then, as for RaT, values were lower between 90 and 270 min and increased to values higher than the fasting level after the HF meal.

As shown in Table 2, the RaT was unchanged throughout the experiment: 86 (SE 3)g (1.51 (SE 0.05) g/kg), 83 (SE 3) g (1.46 (SE 0.05) g/kg), and 84 (SE 4) g (1.47 (SE 0.07) g/kg) for LF, MF and HF meals respectively; the overall rate of glucose disappearance was also unchanged. By taking into account the [²H] enrichment values measured by organic MS and the [¹³C] enrichment values of plasma glucose measured by isotope-ratio MS, the kinetics of exogenous glucose metabolism were calculated (Fig. 3). The change was similar after the LF and MF meals. The rate of

Table 2. Cumulated 7 h glucose fluxes (g) in healthy women during the ingestion of the three test meals comprising 75 g glucose equivalent together with 15 (medium fat; MF), 40 (high fat; HF); 0 (low fat; LF) g fat†

(Mean values with their standard error)

Test meal	LF		MF		HF	
	Mean	SE	Mean	SE	Mean	SE
Total glucose appearance	86	3	83	3	84	4
Exogenous glucose appearance	70*	3	70*	3	60	5
Endogenous glucose production	15*	2	12*	1	22	2
Total glucose disappearance	85	3	82	3	82	4
Exogenous glucose disappearance	64*	3	63*	4	52	5

Mean values were significantly different from those for the HF meal:
  * $P < 0.05$.
† For details of test meals and procedures, see Table 1 and p. 4.

exogenous glucose appearance reached a peak at 60 min of 3·11 (SE 0·27) and 3·13 (SE 0·22) mg/kg per min after the LF and the MF meal respectively, then the maximum was reached between 150 and 240 min for the LF and MF meals, before decreasing until the end of the test. However, after the HF pasta meal, no first peak was observed; instead there was a lower plateau phase lasting for about 3 h, that was followed by a further increase that was maintained until the end of the test.

The rate of exogenous glucose disappearance (data not shown) was similar after the LF and MF meals, with a progressive increase until 180–240 min and then a diminution until 420 min. After the HF meal, the rate of exogenous glucose disappearance increased steadily for 7 h. In all cases, no return to baseline values was observed 7 h postprandially.

The overall rate of exogenous glucose appearance integrated over 420 min was 70 (SE 3) g (1·23 (SE 0·05) g/kg) for the LF and MF meals and 60 (SE 5) g (1·05 (SE 0·09) g/kg) for the HF meal ($P = 0.040$) and it accounted for 93 % (LF and MF) and 80 % (HF) of ingested equivalent glucose (75 g or approximately 1·31 g/kg).

After the three meals EGP declined rapidly during the first 60 min. After the LF and MF meals, there was a similar decrease that almost plateaued until the end of the test, whereas after the HF meal a recovery of EGP was observed between 90 and 330 min. For the three meals, EGP remained suppressed 7 h postprandially.

Overall EGP accounted for 17 % (LF 15 (SE 2) g) and 14 % (MF 12 (SE 1) g) of RaT. It accounted for 26 % (22 (SE 2) g) of RaT after the HF meal ($P = 0.005$) suggesting lower inhibition of EGP by the HF meal compared with the LF and MF meals.

Discussion

Addition of fat to carbohydrate nutrients could be used to decrease postprandial glycaemia; however, the mechanism of interaction between fat and postprandial glucose metabolism (exogenous and endogenous) has not previously been studied in detail. We have studied the metabolic fate of a starchy food by using stable-isotope tracers (D-[6,6-^{2}H$_2$]glucose and starch naturally labelled with ^{13}C). The analysis of [^{13}C] enrichment in glucose

allows the appearance of exogenous glucose to be determined. We were able to demonstrate that the addition of 40 g sunflower oil delayed the appearance of exogenous glucose (Fig. 3). Moreover, the quantity of exogenous glucose appearing during the 7 h of the study was decreased (60 (SE 5) g for the HF meal compared with 70 (SE 3) g for the LF and MF meals; $P = 0.04$). There are considerable data indicating that adding a large amount of fat to a standard carbohydrate load reduces the glycaemic curve (Estrich *et al.* 1967; Collier *et al.* 1984; Gulliford *et al.* 1989). The most likely hypothesis concerning this reduced postprandial blood glucose response is slower gastric emptying (Cunningham & Read, 1989; Houghton *et al.* 1990; Latgé *et al.* 1994) resulting in a delay in carbohydrate absorption. Thus, our data are in accordance with those studies that show a fast-induced delay in gastric emptying; however there could be other mechanisms that influence starch absorption.

The use of D-[6,6-^{2}H$_2$]glucose allowed determination of total glucose turnover. RaT was not affected significantly by the addition of fat; RaT (g) during the 7 h experimental period was 86 (SE 3), 83 (SE 3) and 84 (SE 4) for LF, MF and HF meals respectively. During this period, the overall rate of exogenous glucose appearance accounted for 81, 84 and 71 % of the RaT after LF, MF and HF meals respectively. There are no previous reports on glucose turnover after starchy food ingestion with fat, but our result of 81 % overall systemic appearance of exogenous glucose after the LF meal is consistent with the range of values obtained by other researchers (Jackson *et al.* 1986; Kelley *et al.* 1988; Tissot *et al.* 1990) for a pure glucose load (70–90 %).

After meal ingestion, EGP was decreased after all meals but lower inhibition was observed after the HF meal. This finding suggests that a hepatic insulin resistance state was induced by the HF meal. A biphasic effect was observed in the glycaemic response: a decrease in glucose response during the 0–3 h period was induced in a dose-dependent manner by fat addition, highlighted by the significant decrease in 0–3 h glucose AUC after MF and HF meals ($P < 0.05$); a recovery effect was observed during the second part of the test, with an increase in the 3–7 h glucose AUC after the HF meal. Nevertheless, after the HF meal, a lower inhibition of EGP occurred that minimised the influence on glycaemia of the delayed appearance of exogenous glucose. Following the MF meal a small decrease in the first phase of the glucose response was observed, whereas no significant effect on exogenous glucose appearance was noted and EGP was suppressed normally. The small decrease in glycaemia could be explained only by the occurrence of a small delay in exogenous glucose appearance but which did not reach significance in our small sample. When comparing our data with that of previous studies with an oral glucose load (Ferrannini *et al.* 1980, 1983, 1985; Acheson *et al.* 1985; Jackson *et al.* 1986; Kelley *et al.* 1988), we may conclude that the still substantial appearance of [^{13}C]glucose in blood 7 h after the three meals could be related to the existence of late glucose absorption by the intestine, slower gastric emptying or significant recycling, with the release of label from glycogen stores built up early after meal

absorption. It has been shown that the rate of recycling during an oral glucose load represents 10 % of the metabolism of this load (Ferrannini *et al.* 1985; Jackson *et al.* 1986; Kelley *et al.* 1988).

Surprisingly, the second glycaemic peak is not present in other studies using other types of lipid. These studies include results obtained with 50 g butter (Himaya *et al.* 1997), 50 g margarine (Flatt *et al.* 1985) or 80 g saturated lipids (Griffiths *et al.* 1994). Rasmussen (1993) showed that butter increases the insulin response more than olive oil (monounsaturated fat) in subjects with non-insulin-dependent diabetes mellitus fed a starchy meal of 300 g mashed potato. The reason for the different response with sunflower oil (polyunsaturated fat) compared with saturated or monounsaturated fat is not clear, but it could reflect some specificity of the action of lipases. Previous studies have shown that the fatty acid chain length and the degree of saturation may influence fatty acid oxidation (Jones *et al.* 1985; Rasmussen *et al.* 1996). However, a multitude of enzymes, cofactors and gastrointestinal hormones play a role in fat absorption, and the data observed in the present study may have resulted from other changes in the digestive environment and potential changes in gastrointestinal motility.

A massive increase in plasma TG and NEFA concentrations was also observed after the HF meal. The enhanced postprandial TG concentrations observed after consuming HF pasta corroborates the findings of Gannon *et al.* (1993) and Rasmussen *et al.* (1996), who reported a dose-dependent increase in TG after adding increasing amounts of butter to potatoes. As observed previously by Cohen *et al.* (1988) and Dubois *et al.* (1994), in an upper range of dietary TG (40–120 g) blood TG response is proportional to the amount of fat ingested. The increased level of NEFA after the HF meal in the second part of the study could be due to the hydrolysis of ingested dietary TG, as shown by Griffiths *et al.* (1994) and Binnert *et al.* (1996); it could also be related to a defect in lipolysis suppression.

The insulin response was not modified by fat during the first 0–3 h of the study. During the last part of the protocol (180–420 min), we found that insulin and insulin:glucose AUC were significantly higher after HF meal ingestion, indicating higher insulin secretion. All these results are consistent with the well-documented insulin resistance associated with high dietary fat intakes (Himsworth, 1935; Beck-Nielson *et al.* 1978) and the data of Boden *et al.* (1994), who suggested that an increased plasma NEFA concentration may increase blood glucose by inducing insulin resistance at the peripheral and hepatic levels. In our study a defect of EGP suppression was observed after the HF meal that could be related to this mechanism. Previous studies have shown that in normal subjects there is an inverse relationship between TG levels and insulin sensitivity (Olefsky *et al.* 1974; Tobey *et al.* 1981), and some investigators have found that insulin resistance and hypertriacylglycerolaemia were strictly interrelated (Reaven, 1988). Ferrannini *et al.* (1983) clearly showed an inhibition of insulin-stimulated glucose utilisation in the presence of physiological elevations of plasma NEFA concentrations. Thus raised plasma NEFA concentrations, at least in some circumstances, may contribute to insulin resistance (Ferrannini *et al.* 1983; Fraze *et al.* 1985), reduced glucose oxidation (Bevilacqua *et al.* 1990), and increased hepatic glucose (Ferrannini *et al.* 1983; Fraze *et al.* 1985) and TG production (Havel *et al.* 1970; Barter *et al.* 1972).

Conclusion

These data provide for the first time a comprehensive picture of glucose metabolism after ingestion of starchy food. Taken together, these results show that the addition of a large amount of lipid (40 g) to a starchy meal initially delays exogenous glucose absorption but leads to a secondary recovery of glycaemia. Moreover, it is associated with high levels of TG and NEFA, higher insulin: glucose level and lower inhibition of EGP, suggesting insulin resistance. Such adverse effects were not observed with the MF meal, whereas the beneficial glycaemic lowering effect did occur. Thus, the addition of large amounts of fat to starch should not be advised, but a moderate amount (e.g. 15 g oil), as in our study, could be encouraged.

References

Acheson KJ, Thelin A, Ravussin E, Arnaud M & Jequier MD (1985) Contribution of 500 g naturally labelled ^{13}C dextrin maltose to total carbohydrate utilization and the effect of the antecedent diet in man. *American Journal of Clinical Nutrition* **41**, 881–890.

Barter PJ, Nestel PJ & Carroll KF (1972) Precursors of plasma triglyceride fatty acid in humans: effects of glucose consumption, clofibrate administration and alcoholic fatty liver. *Metabolism* **21**, 117–124.

Beck-Nielsen H, Pedersen O & Sorensen NS (1978) Effects of diet on the cellular insulin binding and the insulin sensitivity in young healthy subjects. *Diabetologia* **15**, 289–296.

Bergmeyer HU (editor) (1974a) In *Methods of Enzymatic Analysis*, 3rd ed., vol. 3, p. 1228: D-glucose. New York: Academic Press.

Bergmeyer HU (editor) (1974b) In *Methods of Enzymatic Analysis*, 3rd ed., vol. 4, p. 1831: Triglycerides. New York: Academic Press.

Bevilacqua S, Buzzigoli G, Bonadonna R, Brandi LS, Oleggini M, Boni C, Geloni M & Ferrannini E (1990) Operation of Randle's cycle in patients with NIDDM. *Diabetes* **39**, 383–389.

Bier DM, Leake RD, Haymond MW, Arnold KJ, Gruenke LD, Sperling MA & Kipnis DA (1977) Measurement of 'true' glucose production rates in infancy and childhood with 6-6 dideuteroglucose. *Diabetes* **26**, 1016–1023.

Binnert C, Pachiaudi C, Beylot M, Croset M, Cohen R, Riou JP & Laville M (1996) Metabolic fate of an oral long-chain triglyceride load in humans. *American Journal of Physiology* **270**, E445–E450.

Boden G, Chen X, Ruiz J, White JV & Rossetti L (1994) Mechanisms of fatty acid-induced inhibition of glucose uptake. *Journal of Clinical Investigation* **93**, 2438–2446.

Cecil JE, Francis J & Read NW (1999) Comparison of the effects of a high-fat and high-carbohydrate soup delivered orally and intragastrically on gastric emptying. *Physiology and Behavior* **67**, 299–306.

Cohen JC & Berger GM (1990) Effects of glucose ingestion on post-prandial lipemia and triglyceride clearance in humans. *Journal of Lipid Research* **31**, 597–602.

Cohen JC, Noakes TD & Benade AJ (1988) Serum triglycerides responses to fatty meals: effect of meal fat content. *American Journal of Clinical Nutrition* **47**, 825–827.

Collier GR, Greenberg GR, Wolever TMS & Jenkins DJA (1988) The acute effect of fat on insulin secretion. *Journal of Clinical Endocrinology and Metabolism* **66**, 323–326.

Collier G, McLean A & O'Dea K (1984) Effect of co-ingestion of fat on the metabolic response to slowly and rapidly absorbed carbohydrates. *Diabetologia* **26**, 50–54.

Collier GR, Wolever TH & Jenkins DJA (1987) Concurrent ingestion of fat and reduction in starch content impairs carbohydrate tolerance to subsequent meals. *American Journal of Clinical Nutrition* **45**, 963–969.

Cunningham KM & Read NW (1989) The effect of incorporating fat into different components of a meal on gastric emptying and postprandial blood glucose and insulin responses. *British Journal of Nutrition* **61**, 285–290.

De Bodo RC, Steele R, Altszuler N, Dunn A & Bishop JS (1963) On the hormonal regulation of carbohydrate metabolism: studies with 14C glucose. *Recent Progress in Hormone Research* **19**, 445–448.

Dejongh DC (1965) Mass spectrometry in carbohydrate chemistry. A cyclic peracetates of pentoses and hexoses. *Journal of American Chemical Society* **30**, 453–458.

Dubois C, Armand M, Azais-Braesco V, Portugal H, Pauli AM, Bernard PM, Latge C, Lafont H, Borel P & Lairon D (1994) Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipidic adults. *American Journal of Clinical Nutrition* **60**, 374–382.

Dubois C, Beaumier G, Juhel C, Armand M, Portugal H, Pauli AM, Borel P, Latge C & Lairon D (1998) Effects of graded amounts (0–50g) of dietary fat on postprandial lipemia and lipoproteins in normolipidic adults. *American Journal of Clinical Nutrition* **67**, 31–38.

Estrich D, Ravnik A, Schlierf G, Fukayama G & Kinsell L (1967) Effects of coingestion of fat and protein upon carbohydrate induced hyperglycemia. *Diabetes* **16**, 232–237.

Ferrannini E, Barrett EJ, Bevilacqua S & DeFronzo RA (1983) Effect of free fatty acids on glucose production and utilization in man. *Journal of Clinical Investigation* **72**, 1737–1747.

Ferrannini E, Bjorkman O, Reichard GA, Alessandro JR, Olsson M, Wahren J & DeFronzo RA (1985) The disposal of an oral glucose load in healthy subjects. *Diabetes* **34**, 580–588.

Ferrannini E, Wahren J, Felig P & DeFronzo RA (1980) The role of fractional glucose extraction in the regulation of splanchnic glucose metabolism in normal and diabetic man. *Metabolism* **29**, 28–35.

Flatt JP, Ravussin E, Achreson KJ & Jequier E (1985) Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *Journal of Clinical Investigation* **76**, 1019–1024.

Fraze E, Donner CC, Swislocki ALM, Chiou YAM, Chen YDI & Reaven GM (1985) Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance. *Journal of Clinical Endocrinology and Metabolism* **61**, 807–811.

Gannon MC, Nuttall FQ, Westphal SA & Seaquist ER (1993) The effect of fat and carbohydrate on plasma glucose, insulin, C-peptide, and triglycerides in normal male subjects. *Journal of the American College of Nutrition* **12**, 36–41.

Gatti E, Noe D, Pazzucconi F, Gianfranceschi G, Porrini M, Testolin G & Sirtori CR (1992) Differential effect of unsaturated oils and butter on blood glucose and insulin response to carbohydrate in normal volunteers. *European Journal of Clinical Nutrition* **46**, 161–166.

Griffiths AJ, Humphreys SM, Clark ML, Fielding BA & Frayn KN (1994) Immediate metabolic availability of dietary fat in combination with carbohydrate. *American Journal of Clinical Nutrition* **59**, 53–59.

Gulliford MC, Bicknell EJ & Scarpello JH (1989) Differential effect of protein and fat ingestion on blood glucose responses to high-and low-glycemic-index carbohydrates in non-insulin-dependant diabetic subjects. *American Journal of Clinical Nutrition* **50**, 773–777.

Havel RJ, Kane JP, Balasse O, Segel N & Basso LV (1970) Splanchnic metabolism of free fatty acids and the production of triglycerides of VLDL in normotriglyceridemic and hypertriglyceridemic humans. *Journal of Clinical Investigation* **49**, 2017–2035.

Himaya A, Fantino M, Antoine JM, Brondel L & Louis-Sylvestre J (1997) The satiety power of dietary fat: a new appraisal. *American Journal of Clinical Nutrition* **65**, 1410–1418.

Himsworth HP (1935) The dietetic factor determining the glucose tolerance and sensitivity to insulin in healthy men. *Clinical Science* **2**, 67–94.

Houghton LA, Mangnall YF & Read NW (1990) Effect of incorporating fat into a liquid test meal on the relation between intragastric distribution and gastric emptying in human volunteers. *Gut* **31**, 1226–1229.

Jackson RA, Roshania RD & Hava MI (1986) Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: an analysis based on simultaneous use of the forearm and double isotope techniques. *Journal of Clinical Endocrinology and Metabolism* **63**, 541–549.

Jones P, Pencharz P & Clandinin M (1985) Absorption of ^{13}C-labeled stearic, oleic and linoleic acids in humans: application to breath tests. *Journal of Laboratory and Clinical Medicine* **105**, 647–652.

Kelley D, Mitrakou A, Marsh H, Schwenk F, Benn J, Sonnenberg G, Arcangeli M, Aoki T, Sorensen J, Berger M, Sonksen P & Gerich J (1988) Skeletal muscle glycolysis, oxidation and storage of oral glucose load. *Journal of Clinical Investigation* **81**, 1563–1571.

Latgé C, Thouvenot P & Kedzierewicz F (1994) The influence of a lipid loading on gastric emptying and glycemia. *American Journal of Clinical Nutrition* **59**, Suppl., S782.

Laville M, Riou JP, Beylot M, Perrot L, Odeon M, Berthezene F & Mornex R (1983) Mesure des acides gras libres par méthode enzymatique (Measurement of free fatty acids by enzymic method). *Lyon Medical* **249**, 67–70.

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 ^{13}C-enriched starchy food in normal humans. *American Journal of Clinical Nutrition* **55**, 430–435.

Olefsky JM, Farquar JW & Reaven GM (1974) Reappraisal of the role of insulin in hypertriglyceridemia. *American Journal of Medicine* **57**, 551–560.

Proietto J, Rohner-Jeannenaud F, Ionescu E & Terretaz J (1987) Non steady state measurement of glucose turnover in rats by a one-compartment model. *American Journal of Physiology* **252**, E77–E84.

Rasmussen O (1993) Dose-dependency of the glycemic response to starch-rich meals in insulin-dependant diabetic subjects: studies at constant insulinemia. *Metabolism* **42**, 710–713.

Rasmussen O, Lauszus FF, Christiansen C, Thomsen C & Hermansen K (1996) Differential effects of saturated and monounsaturated fat on blood glucose and insulin responses in subjects with NIDDM. *American Journal of Clinical Nutrition* **63**, 249–253.

Reaven GM (1988) Role of insulin resistance in human disease. *Diabetes* **37**, 1595–1607.

Thivend P, Mercier C & Guilbot A (1972) Determination of starch with glucoamylase. In *Methods in Carbohydrate Chemistry*, vol. 6, pp. 100–105 [RL Whisler, editor]. New York and London: Academic Press.

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 mass spectrometer to trace the exogenous 13C labelled glucose at a very low level of enrichment in man. *Diabetologia* **33**, 449–456.

Tobey TA, Greenfield M, Kramer F & Reaven GM (1981) Relationship between insulin resistance, insulin secretion, VLDL kinetics, and plasma TG levels in normotriglyceridemic man. *Metabolism* **30**, 165–171.

Van Amelsvoort JMM, Van Stratum P, Kraal JH, Lussenburg RN & Dubbelman GP (1990) Minor difference in postprandial responses of men between starch and sugar when replacing fat in a normal meal. *British Journal of Nutrition* **63**, 37–51.

Van Amelsvoort JMM, Van Stratum P, Kraal JH, Lussenburg RN & Houtsmuller UMT (1989) Effect of varying the carbohydrate:fat ratio in hot lunch on postprandial variables in male volunteers. *British Journal of Nutrition* **61**, 267–283.

Wolever TM (2000) Dietary carbohydrates and insulin action in humans. *British Journal of Nutrition* **83**, Suppl. 1, S97–S102.