Comparison of carbohydrate utilization in man using indirect calorimetry and mass spectrometry after an oral load of 100 g naturally-labelled [^{13}C]glucose

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1. Carbohydrate (CHO) oxidation was measured simultaneously in a group of five normal subjects after an oral load of 100 g naturally-labelled [^{13}C]glucose, using indirect calorimetry and mass spectrometry.

2. CHO utilization, calculated from the results of indirect calorimetry, increased 30 min after the glucose load to reach a peak at 90 min. It then decreased to reach basal values at 380 min. Cumulative total CHO oxidation at 480 min was 83 ± 8 g, and CHO oxidized above basal levels, 37 ± 3 g.

3. Enrichment of expired carbon dioxide with ^{13}C began at 60 min and maximum values were observed at 270 min. At 480 min, cumulative CHO oxidation measured by use of [^{13}C]glucose was 29 g. The difference from calorimetric values can be attributed in part to the slow isotopic dilution in the glucose and bicarbonate pools.

4. Thus, approximately 30% of the glucose load was oxidized during the 8 h after its ingestion and this accounts for a significant part of the increased CHO oxidation (37 g), as measured by indirect calorimetry.

There are two major sources of metabolic fuel, glucose and free fatty acids (FFA) whose energy is used for vital activities. Measurement of over-all utilization of carbohydrate yields useful information on the fate of this fuel in normal and pathological cases. Two different approaches are usually used to estimate carbohydrate utilization, i.e. indirect calorimetry and isotopic methods. Continuous indirect calorimetry, based on the measurement of oxygen and carbon dioxide in the expired air, yields information on over-all carbohydrate (CHO) and lipid utilization, particularly under dynamic conditions such as during oral glucose tolerance tests (OGTT). However, unlike isotopic methods, indirect calorimetry cannot be used to differentiate between the endogenous or exogenous origin of the substrate (Gomez *et al.* 1972; Müller-Hess *et al.* 1975; Felber *et al.* 1977). Radioisotopes have been used for in vitro and in vivo animal studies (Searle *et al.* 1956; Eaton & Steinberg, 1961; Steele, Winkler *et al.* 1968), but since their use is limited in humans, interest has been focused on the use of non-radioactive isotopes. The non-radioactive isotope of carbon, ^{13}C, can be used for human investigation. There are some natural sugars, e.g. maize and cane sugar, in which the ratio $^{13}C:^{12}C$ is higher than that of other sugars (Smith & Epstein, 1971). If such a sugar is used for OGTT, it is possible to collect the expired CO_2 and measure the variations in the ratio $^{13}C:^{12}C$ during the experiment. These variations reflect the fate of the exogenous glucose (Lefebvre *et al.* 1975; Mosora *et al.* 1976).

The purpose of this study was to try to quantify endogenous and exogenous CHO oxidation in control subjects by using the two methods simultaneously.

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MATERIALS AND METHODS

Five healthy subjects, one man and four women between 18 and 24 years of age, volunteered for this study (Table 1). All were within 15% of their ideal body-weight according to normal values from Tables 2 and 3 of the Metropolitan Life Insurance Company (1959) and none had any family history of diabetes.

OGTT with naturally-labelled [^{13}C]glucose (100 g)

The normal diet of the subject did not include maize or cane sugar during the month preceding the test.

The subjects, who were asked to collect their overnight urine, arrived at the hospital at 07.00 hours after a 12 h fast and were allowed to rest for approximately 1 h before the test began. After baseline measurements lasting 30 min, they drank a glucose solution of 100 g naturally-labelled [^{13}C]glucose (Fluka, Buchs, Switzerland) in 400 ml water. Continuous indirect calorimetric measurements were made for another 4 h and then at regular intervals until the eighth hour.

A second urine collection was made at the end of the 4 h continuous measurements.

Indirect calorimetry was performed as described by Gomez *et al.* (1972) and Felber *et al.* (1977) of which some details are given later. A transparent-plastic, ventilated hood was lowered over the subject's head and secured around his neck with a piece of air-tight cloth. A slight negative pressure was maintained in the hood to prevent expired air leaking out. The air-flow through the hood was measured using a pneumotachograph and samples of the outflowing air were continuously taken to monitor O_2 and CO_2 concentrations, using a paramagnetic and infrared analyser respectively. Using the calorimetric results and the urinary nitrogen, it was possible to calculate the non-protein respiratory quotient (NP-RQ) and then the oxidation rates of CHO and lipid, using the tables of Lusk (Lusk, 1924).

Integration of the continuous measurements was recorded every 5 min for the whole duration of the test. The CHO oxidation rate was determined every 30 min, from −30 to 240 min, each 30 min period being the mean of six integrated periods of 5 min.

Blood measurements

Blood samples were taken at −30, 0, 30, 60, 90, 120, 150, 180, 210, 240, 360 and 480 min for glucose, insulin and FFA determinations. Blood glucose was measured using the hexokinase method (Slein, 1965) and plasma insulin by radioimmunoassay according to Hales & Randle (1963). Plasma FFA were extracted using the method of Dole & Meinertz (1960) and determined according to the method of Heindel *et al.* (1974).

Isotopic method

To determine the ratio $^{13}C:^{12}C$ in the expired CO_2, a known fraction of the expired air was bubbled through 60 ml 1 M-sodium hydroxide (Titrisol®; Merck, Darmstadt, Germany) to absorb the CO_2. The samples were then kept at −20° until analysed.

The 1 M-NaOH sample was thawed and 2–5 ml were taken and put into an evacuating flask. The flask was connected to a vacuum line to de-gas the sample and then put in liquid N_2. The vacuum was broken so that 3 ml 3 M-sulphuric acid could be added. This froze in a layer immediately above the NaOH. The flask was re-evacuated on the vacuum line, after which it was warmed to allow the frozen reagents to thaw and react together to produce CO_2. Finally, the evacuated flask was put into an ethanol and dry-ice bath at

Table 1. *Details of subjects*

Subjects	Sex	Age (years)	Height (m)	Wt (kg)	Body-wt (% ideal body-wt)*
B.F.	♀	20	1·795	70·5	106
J.E.	♀	24	1·690	55·0	93
L.J.	♂	22	1·770	67·7	98
M.C.	♀	20	1·680	50·0	86
R.C.	♀	18	1·650	59·2	106

* Metropolitan Life Insurance Company (1959).

−80° to freeze the water which was formed and prevent its introduction into the mass spectrometer (MS 20 AEI).

Analysis of mass spectrometry

Using the information obtained for each sample it was possible to calculate the following:

$$\text{atom \% sample} = \frac{^{13}\text{C sample}}{^{12}\text{C sample} + ^{13}\text{C sample}} \times 100, \quad (1)$$

$$\text{atom \% standard} = \frac{^{13}\text{C standard}}{^{12}\text{C standard} + ^{13}\text{C standard}} \times 100, \quad (2)$$

$$\text{atom \% excess} = \text{atom \% sample} - \text{atom \% standard}. \quad (3)$$

The standard was a potassium carbonate liquid reference (Fluka). For each sample taken, at time t, the atom % excess was referred to that taken at zero time (the time when no exogenous glucose was present in the organism) to obtain the change in ($\triangle$) atom % excess:

$$\triangle \text{ atom \% excess} = \text{atom \% excess}_t - \text{atom \% excess}_0. \quad (4)$$

There is no major difference between these calculations and those used by Lefebvre *et al.* (1975), where enrichment is expressed as $^{13}C‰$ according to Craig's (1957) formula. This formula is mathematically related to the atom % excess value used in the present work.

Quantification of the mass spectrometry results

In order to compare the calorimetric results with those from the mass spectrometer, it was necessary to convert atom % excess (%) into glucose oxidation mg/min.

It can be established that:

$$\frac{\dot{Q} \text{ exogenous glucose oxidized}}{Q \text{ glucose load}} = \frac{\dot{Q}^{13}C_{(CO_2)}}{Q^{13}C_{(load)}},$$

where $\dot{Q}^{13}C_{(CO_2)}$ is the amount of ^{13}C g/min in the expired CO_2 resulting from the oxidation of exogenous glucose.

$$\dot{Q}^{13}C_{(CO_2)} = \frac{\dot{V}_{CO_2} \times 12}{22{\cdot}26} \times \triangle \text{ atom \% excess} \times \frac{1}{100},$$

where $\dot{V}_{CO_2}$ is the volume of CO_2 produced/min, 12 is the atomic weight of C, 22·26 is the volume of 1 mol CO_2 (l; standard temperature and pressure). The $Q^{13}C$ of the load is the amount of ^{13}C (g) in 100 g of the glucose load.

Thus:

$$\dot{Q}^{13}C_{(load)} = 100 \times 0{\cdot}4 \times \text{atom \% excess glucose} \times \frac{1}{100},$$

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Table 2. *Volume of carbon dioxide produced* ($\dot{V}_{CO_2}$; *l/min*) *and atom % excess* (*at % excess*) *values for each subject* during oral glucose tolerance test with a glucose load of* 100 *g naturally-labelled* [^{13}C]*glucose*

Subject ...	B.F.		J.E.		L.J.		M.C.		R.C.	
Period after dose (min)	$\dot{V}_{CO_2}$	at % excess	$\dot{V}_{CO_2}$	at % excess	$\dot{V}_{CO_2}$	at % excess	$\dot{V}_{CO_2}$	at % excess	$\dot{V}_{CO_2}$	at % excess
0	0·208	−0·00087	0·207	−0·00364	0·238	−0·00175	0·217	−0·00222	0·179	0·00008
30	0·229	−0·00118	0·219	−0·00406	0·249	−0·00237	0·232	−0·00209	0·173	−0·00057
60	0·281	−0·00178	0·247	−0·00331	0·269	−0·00192	0·248	−0·00122	0·224	0·00000
90	0·276	−0·00033	0·240	−0·00233	0·302	−0·00187	0·282	−0·00046	0·232	0·00127
120	0·269	0·00070	0·239	−0·00084	0·303	0·00057	0·241	0·00092	0·208	0·00273
150	0·255	0·00196	0·235	0·00000	0·315	0·00134	0·239	0·00180	0·207	0·00353
180	0·252	0·00310	0·228	0·00087	0·280	0·00287	0·250	0·00325	0·193	0·00488
210	0·251	0·00305	0·230	0·00145	0·259	0·00321	0·250	0·00367	0·224	0·00429
240	0·264	0·00302	0·236	0·00166	0·285	0·00277	0·263	0·00359	0·190	0·00518
270	—	—	0·256	0·00341	0·235	0·00427	0·192	0·00497	0·223	0·00726
300	0·233	0·00589	0·256	0·00403	0·249	0·00264	0·185	0·00496	0·197	0·00551
330	—	—	0·203	0·00284	0·242	0·00337	0·191	0·00479	0·198	0·00488
360	0·249	0·00385	0·198	0·00238	0·281	0·00121	0·178	0·00402	0·187	0·00361
420	0·245	0·00233	0·190	0·00000	0·241	0·00193	0·164	0·00210	0·163	0·00376
480	0·207	0·00149	0·210	−0·00130	0·240	0·00226	0·198	0·00062	0·204	0·00262

* For details, see Table 1.

where 0·4 represents the fractional weight of C in glucose. From the above equations, it follows that:

$$\dot{Q} \text{ exogenous glucose oxidized} = \frac{\frac{\dot{V}_{co_2} \times 12}{22{\cdot}26} \times \triangle \text{ atom \% excess}}{0{\cdot}4 \times \text{atom \% excess glucose}}.$$

The mean $\dot{V}_{co_2}$ (l/min) and the atom % excess per period, for each subject is presented in Table 2.

RESULTS

OGTT with [^{13}C]glucose in five normal subjects

Fig. 1 illustrates CHO and lipid oxidation rates as well as plasma glucose, insulin and FFA levels during the 8 h test.

The mean plasma glucose value at zero time was 830 ± 30 mg/l. This increased after the load and reached a peak (1190 ± 50 mg/l) at 30 min. It then decreased and reached basal levels (810 ± 70 mg/l) at 180 min. Insulin levels increased from 12 ± 2 μU/ml at zero time to a peak value (87 ± 13 μU/ml) 30 min later. Its return to basal values was more delayed than that for blood glucose. At 240 min after the glucose load, plasma immunoreactive insulin was 29 ± 14 μU/ml and at 360 min, 15 ± 3 μU/ml. Basal FFA levels ranged from 190 to 680 μmol/l with a mean (± SEM) of 416 ± 80 μmol/l. After the glucose load, FFA levels decreased and reached minimum values (134 ± 17 and 117 ± 17 μmol/l) between 90 and 120 min. They then increased progressively until the end of the test (774 ± 121 μmol/l). In each instance, the increase in FFA occurred when insulin had returned to its basal level. Table 2 shows the CO_2 production rate and the △ atom % excess for each subject.

The CHO oxidation rate at the beginning of the test, calculated from the indirect calorimetry results, was 97 ± 14 mg/min. After the load, CHO utilization increased significantly after 60 min (214 ± 22 mg/min) and reached maximum values between 90 and 180 min (250 and 228 mg/min respectively). It then decreased gradually to basal levels by the end of the test.

In contrast, a significant increase in the utilization (18·4 ± 6·2 mg/min) of [^{13}C]glucose was only observed at 90 min. Peak utilization (115·3 ± 7·3 mg/min) was reached at 270 min and at the end of the test it was 45 ± 6·7 mg/min.

Lipid oxidation was 56 ± 5 mg/min at zero time. This rapidly decreased to 30 ± 13 mg/min at 60 min. Minimum values oscillated between 20 ± 6 and 16 ± 7 mg/min from 90 to 180 min and then increased progressively until the end of the test (59 ± 2 mg/min).

Cumulative CHO utilization

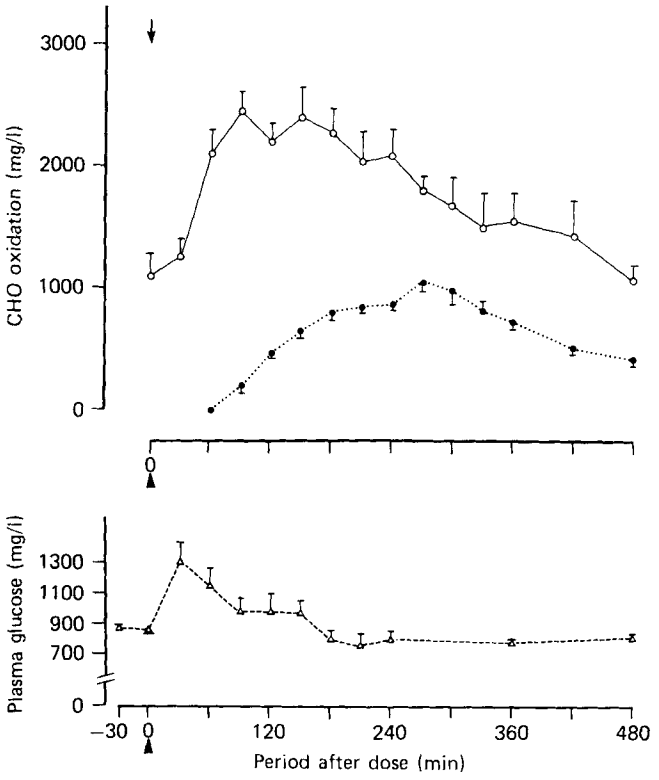
Cumulative values for CHO oxidation during the 480 min OGTT are shown in Table 3 and Fig. 2. Total utilization was 38 ± 3 g after 180 min and 83 ± 8 g after 480 min. If the basal value is subtracted, 21 ± 1 g and 37·2 ± 2 g CHO were oxidized at 180 and 480 min respectively. Mass spectrometry gave values of 6·4 ± 0·6 g at 180 min and 29·2 ± 1·5 g after 480 min.

Energy expenditure

The energy produced by the oxidation of the three substrates was calculated for periods of 30 and 60 min (Fig. 3).

The mean (± SEM) basal energy expenditure was 4·86 ± 0·21 kJ/min and the substrate contribution was 34 ± 4, 45 ± 3 and 21 ± 5% for CHO, lipid and protein respectively.

Maximal expenditure occurred from 60 to 90 min (5·77 ± 0·29 kJ/min), at which time



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74 ± 3% of the energy came from CHO oxidation, 13 ± 3% from lipid and 13 ± 3% from protein.

The CHO contribution increased to 77% and that of lipid decreased to 9% between 120 and 150 min, after which there was a progressive return to normal values at 480 min (4·69 ± 0·25 kJ/min, with a relative contribution of 35 ± 3% CHO, 50 ± 1·5% lipid and 15 ± 3% proteins).

DISCUSSION

A delay in the rate of CHO utilization with respect to the peak in blood glucose has been previously reported using different methods, i.e. [^{14}C]glucose (Baker *et al.* 1954; Searle *et al.* 1956), [^{13}C]glucose (Lefebvre *et al.* 1975) and indirect calorimetry (Felber *et al.* 1977). This delay is a result of several factors such as: (1) the slow switch over, in muscle tissue, from FFA oxidation in the fasting state to glucose oxidation in the fed state; (2) the delay due to glucose polymerization to glycogen which would precede oxidative degradation, as suggested by Beloff-Chain *et al.* (1955) in their study on rat diaphragm muscle.

The cumulative values of the CHO oxidized (Table 3), measured by indirect calorimetry, are of the same order of magnitude as the total splanchnic glucose output reported by Felig *et al.* (1975). Values in the present study which show that 38 ± 3 g CHO, expressed as glucose, were used during the 3 h after the 100 g glucose load, agrees with the 40 ± 3 g reported by Felig *et al.* (1975). The concordance between these results suggests, on the

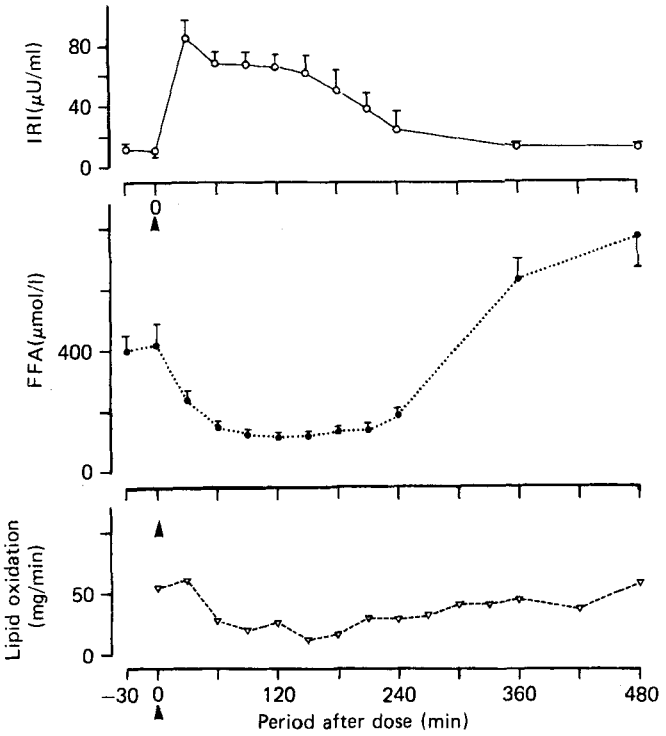

Fig. 1. Measurement, in five healthy adult subjects, of carbohydrates (CHO) and lipid utilization rates (mg/min) by indirect calorimetry (○—○), and glucose oxidation by mass spectrometry (●---●) after 100 g naturally-labelled [^{13}C]glucose load, corresponding plasma glucose (mg/l), immunoresponsive insulin (IRI) and free fatty acid (FFA; μmol/l) levels. ↓, glucose load. Points represent mean values, with their standard errors represented by vertical bars.

one hand, that the major part of the hepatic glucose output is oxidized, and on the other, that little glucose is oxidized in the liver.

A slight increase in the total energy expenditure was observed with a maximum between 60 and 90 min (5·77 ± 0·29 kJ/min *v.* 4·86 ± 0·21 kJ/min in the basal state).

From the results, it can be seen that there is a delay in CHO utilization measured by mass spectrometry when compared to that measured by indirect calorimetry. This cannot be interpreted as a preferential oxidation of endogenous glucose over that of exogenous origin, since Steele, Bjerknes *et al.* (1968), de Bodo *et al.* (1963), Searle & Chaikoff (1952) and Madison *et al.* (1963) have demonstrated in the dog that there is a reduction in hepatic glucose output after a glucose load, as a result of increased insulin secretion, and that when glucose is taken orally hepatic glucose output is reduced over the period of active glucose absorption, i.e. approximately 3 h (Steele, Bjerknes *et al.* 1968). Therefore, there must be an early oxidation of exogenous glucose which bypasses the liver as well as oxidation of endogenous glucose in the peripheral tissues.

This delay can be accounted for by dilution of the administered [^{13}C]glucose in the endogenous glucose pool (Steele *et al.* 1956) and further dilution of $^{13}CO_2$ in the bicarbonate pool after glucose oxidation (Steele *et al.* 1959).

In the resting state, it took 4·5 h to reach peak $^{13}CO_2$ enrichment. Whilst correction factors have been determined for this non-steady state period in dogs (Steele, Winkler *et*

Table 3. *Cumulative amount (g) of oxidized carbohydrate (CHO) in healthy adult subjects* given an oral load of 100 g naturally-labelled [^{13}C]glucose measured by indirect calorimetry (total values and values above basal utilization level) and by mass spectrometry*

Procedure ...	Indirect calorimetry													
	CHO oxidized							CHO oxidized above basal values						
Subject ... Period after dose (min)	B.F.	J.E.	L.J.	M.C.	R.C.	Mean	SE	B.F.	J.E.	L.J.	M.C.	R.C.	Mean	SE
30	4·6	3·6	3·9	3·6	1·9	3·5	0·4	1·6	0·4	0·5	0	0·5	0·6	0·3
60	13·1	10·3	8·6	10·6	7·2	9·9	1·0	7·1	3·9	1·9	3·2	4·5	4·1	0·9
90	21·3	17·3	16·3	19·3	13·0	17·4	1·4	12·4	7·8	6·2	8·1	9·0	8·7	1·0
120	28·8	24·2	23·0	26·3	18·0	24·1	1·8	16·9	11·5	9·6	11·3	12·7	12·4	1·2
150	35·9	30·9	32·6	34·0	22·9	31·2	2·2	21·0	15·0	15·8	15·1	16·3	16·6	1·1
180	42·7	38·1	40·1	41·9	27·6	38·1	2·7	24·9	19·0	20·0	19·3	19·6	20·6	1·1
210	49·5	44·0	44·5	49·9	32·8	44·2	3·1	28·7	—	21·0	23·5	23·6	24·2	1·6
240	56·9	49·9	51·4	58·0	36·5	50·5	3·8	33·1	24·5	24·5	27·7	25·9	27·2	1·6
300	66·2	63·0	62·3	68·3	41·5	60·3	4·8	36·5	31·2	28·7	30·4	28·3	31·0	1·5
360	75·2	71·2	76·2	74·7	46·6	68·8	5·6	39·5	33·0	35·8	30·4	30·7	33·9	1·7
420	81·5	79·5	89·1	79·5	51·3	76·2	6·5	—	35·0	42·0	—	—	—	—
480	87·9	86·3	102·0	84·5	55·9	83·3	7·5	40·4	37·4	42·0	30·4	34·8	37·0	2·1

Procedure ...	Mass spectrometry [^{13}C]glucose oxidized						
Subject ... Period after dose (min)	B.F.	J.E.	L.J.	M.C.	R.C.	Mean	SE
30	0	0	0	0·1	0	0·01	0·01
60	0	0·2	0	0·6	0	0·2	0·1
90	0·02	0·9	0	1·7	0·6	0·6	0·3
120	1·0	2·4	1·6	3·4	1·8	2·0	0·4
150	2·6	4·3	3·7	5·6	3·4	3·9	0·5
180	4·8	6·6	6·6	8·6	5·5	6·4	0·6
210	7·0	9·2	9·5	11·9	7·6	9·0	0·8
240	9·3	12·0	12·4	15·3	9·8	11·7	1·1
300	16·3	20·4	18·0	21·4	15·7	18·4	1·1
360	21·6	26·0	22·6	26·8	19·3	23·3	1·4
420	25·1	29·1	26·6	30·0	22·0	26·5	1·4
480	27·3	31·3	30·9	32·5	24·3	29·2	1·5

* For details, see Table 1.

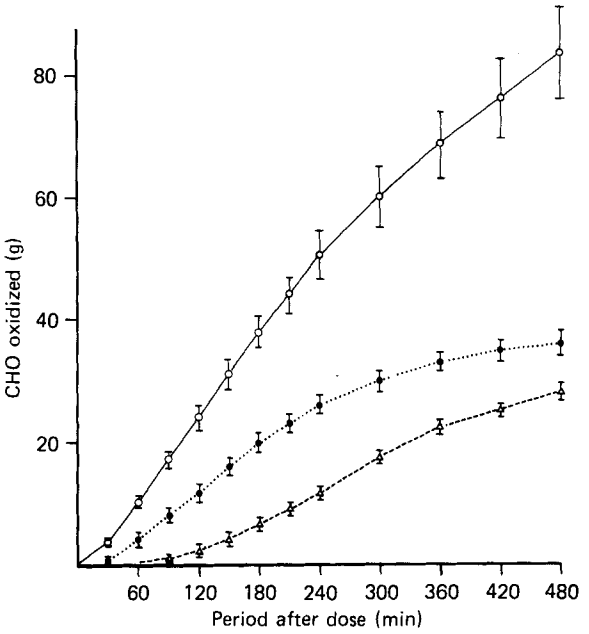

Fig. 2. Cumulative values for carbohydrate (CHO) oxidation (g) after 100 g oral [^{13}C]glucose load in five healthy adult subjects. (○—○), cumulated CHO oxidation values measured by indirect calorimetry; (●····●), cumulated CHO oxidation values above basal oxidation levels, measured by indirect calorimetry; (△ – – △), cumulated [^{13}C]glucose oxidation measured by mass spectrometry. Points represent mean values, with their standard errors represented by vertical bars.

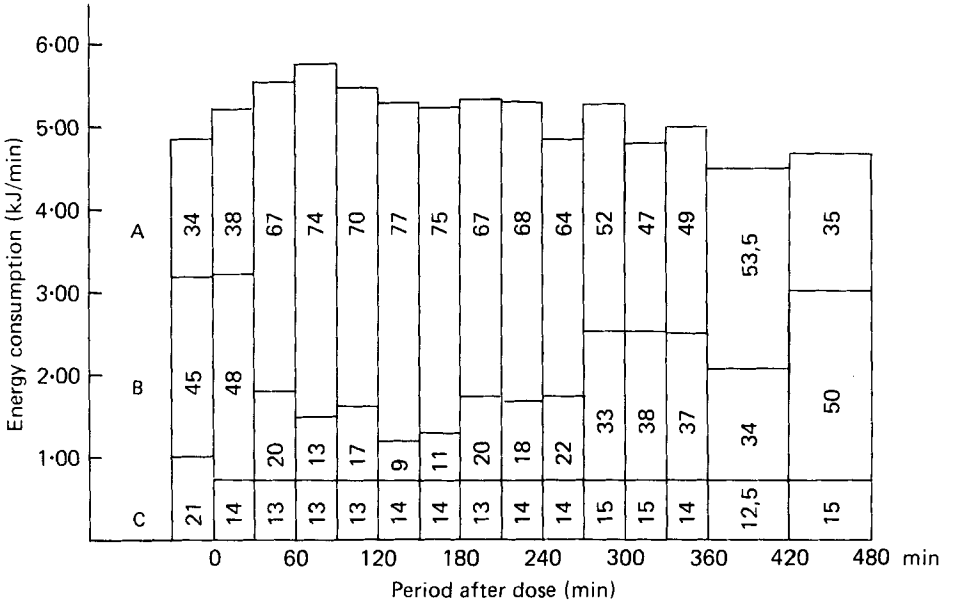

Fig. 3. Relative contribution (%) of proteins (A), lipids (B), and carbohydrates (CHO) (C), to energy consumption (kJ/min) for periods of 30–60 min before and after a 100 g oral glucose load. Values were calculated from the urinary nitrogen and non-protein respiratory quotient.

al. 1968), their application to human studies is questionable. It is possible to accelerate the turnover and mixing of these pools by exercise (Vranic & Wrenshall, 1969).

After its peak at 270 min, [^{13}C]glucose oxidation runs parallel to the total CHO oxidation. Cumulative values show that exogenous glucose provides approximately one-third of the total CHO oxidized and three quarters of the CHO oxidized above basal values.

Indirect calorimetry showed a return of total CHO oxidation to its baseline value at 480 min, at which time the total amount of glucose oxidized above basal values was 37 g. At 480 min, [^{13}C]glucose oxidation was 28 g, but baseline values had not been reached. If the experiments had been extended so that the $^{13}CO_2$ concentration had returned to its baseline value, it would have been possible to calculate the number of [^{13}C]glucose-C atoms which had become $^{13}CO_2$-C atoms. At such a time the error due to $^{13}CO_2$ mixing in the bicarbonate pool has been corrected, since the pool has regained its original composition.

A criticism of the method of indirect calorimetry in evaluating substrate utilization, is the influence of lipogenesis on the NP-RQ.

Whilst it is unlikely that lipogenesis occurred under the conditions of the test, in which the glucose load was preceded by a 10–12 h fast, it is of interest to attempt to clarify this point.

It has been shown since the early 1900s that NP-RQ values exceeding unity are possible (Lusk, 1924) and that they represent net conversion of CHO to lipid. The NP-RQ is only greater than 1·0 when lipogenesis exceeds lipolysis, i.e. when there is a net transfer of C atoms from CHO to lipids. When the NP-RQ is high but less than unity, it can be shown that CHO and lipid oxidation may take place at the same time as lipid synthesis. Lusk (1924) ignored the possibility that lipogenesis may occur at an NP-RQ of less than unity when he derived his equations for calculating CHO and lipid oxidation. Recent stoichiometric calculations for the energy release within the range of respiratory quotient values from 0·7 to 1·0 yield identical values whether CHO and lipid occur in the absence or in the presence of lipogenesis (J. P. Flatt, personal communication). The equations of Lusk (1924) are thus valid in the presence or in the absence of lipogenesis up to NP-RQ values of 1·0.

Thus it is possible to conclude that of a 100 g (naturally-labelled) [^{13}C]glucose load, approximately 30% are oxidized and 70% stored during the 8 h after ingestion. The stimulation of CHO oxidation by the load is largely due to that part of the load which is oxidized, i.e. 29 g [^{13}C]glucose of 37 g CHO oxidation above basal values.

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