Carbohydrate utilization in obese subjects after an oral load of 100 g naturally-labelled [^{13}C] glucose

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1. Total carbohydrate (CHO) and ingested glucose oxidation was measured in five obese subjects with normal glucose tolerance after an oral load of 100 g naturally-labelled [^{13}C]glucose using indirect calorimetry and mass spectrometry respectively.

2. CHO utilization rate (107 ± 14 mg/min in the post-absorptive state) increased 30 min after the glucose load to reach a plateau (245 ± 25 mg/min) between 90 and 120 min. It then decreased to basal values at 330 min. Cumulative CHO oxidation over 480 min was 66 ± 7 g and the CHO oxidized above basal levels was 26 ± 7 g.

3. Enrichment of expired carbon dioxide with ^{13}C began at 45 min and maximum values were observed between 210 and 300 min. At 480 min, cumulative oxidation of the ingested glucose was 24 ± 2 g.

4. Compared with controls, the obese subjects exhibit an impairment of CHO utilization which precedes glucose intolerance. This impairment can be explained by an increased availability of free fatty acids which favours lipid oxidation at the expense of ingested [^{13}C]glucose oxidation.

Although alterations of carbohydrate (CHO) metabolism in obesity have been frequently reported (Ogilvie, 1935; Morse *et al.* 1960; Shreeve, 1965; Pittet *et al.* 1976), the disposal of ingested glucose has been poorly studied (Lefèbvre *et al.* 1975). The use of naturally enriched [^{13}C]glucose allowed us to measure the oxidation rate of ingested glucose in a group of five obese subjects. Measurements were performed as previously described on control subjects by Ebiner *et al.* (1979) by continuous indirect calorimetry and mass spectrometry.

Continuous measurements of respiratory exchange yield results on over-all CHO and lipid oxidation (Pittet *et al.* 1976) but cannot be used to differentiate between the endogenous or exogenous origin of the substrate oxidized. The fate of the ingested glucose can only be studied by using ^{14}C- or ^{13}C-labelled glucose. For ethical reasons, compounds naturally enriched in ^{13}C, the stable non-radioactive isotope of C, are of great interest. It has been shown that the ^{13}C content of maize glucose is greater than that of other sugars (Smith & Epstein, 1971) and that it is present in the expired carbon dioxide of man or animals eating their usual diet. Thus the oxidation rate of ingested maize glucose can be calculated from measurements of the ^{13}C enrichment of expired CO_2.

By comparison with results on control subjects (Ebiner *et al.* 1979) the aim of the study was to investigate during an 8 h oral glucose tolerance test whether alterations in the utilization rate of ingested glucose could be demonstrated in obese subjects with normal glucose tolerance. In these subjects, the question arises whether a defect in CHO utilization might precede the development of glucose intolerance.

MATERIALS AND METHODS

Five obese males (Table 1) with a mean excess weight of 49 % (Metropolitan Life Insurance Company, 1959), aged between 23 and 38 years, volunteered for this study; the protocol had been previously accepted by the hospital ethical committee. All obese subjects

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Table 1. *Characteristics of the subjects*

Subjects	Age (years)	Height (m)	Wt (kg)	Percentage ideal body-wt*
E.S.	29	1·76	95	146
A.M.	28	1·66	93	160
C.W.	32	1·85	105	147
A.T.	38	1·81	100	146
C.T.	23	1·74	93	146
Mean	30	176·4	97·2	149
SE	2·5	3·2	2·3	

* Metropolitan Life Insurance Company (1959) values.

presented a normal glucose tolerance according to Fajans & Conn (1965) criteria but they exhibited a tendency to insulin resistance, as illustrated by a greater insulin response to the glucose load.

After an overnight fast, baseline measurements lasting 45 min were performed on the supine subject. He then ingested 100 g naturally-enriched [^{13}C]glucose dissolved in 400 ml water. Continuous respiratory exchange and isotopic measurements were made for a further 8 h. At 4 h and 6 h the subject was allowed out of the ventilated hood for 20 min. Urine was collected at 4 h and 8 h. Blood was taken at regular intervals from -30 to 480 min for glucose (Slein, 1965), insulin (Hales & Randle, 1963) and free fatty acids (FFA) (Heindel *et al.* 1974) determinations.

The respiratory exchange was continuously measured as previously described (Felber *et al.* 1977; Ravussin *et al.* 1979) using a ventilated-hood open-circuit indirect calorimeter. From the O_2 consumption and CO_2 production, the non-protein respiratory quotient was calculated after taking into account the amount of nitrogen excreted in the urine (Kjeldahl method). The tables of Lusk (1924) were then used to determine CHO and lipid oxidation rates.

A constant volume of the outflowing air was bubbled for 15 or 30 min through 60 ml sodium hydroxide to trap CO_2. After the required collection time, the solution was frozen to facilitate handling and storage. For determination of the ^{13}C-enrichment, samples were defrosted and degassed on a vacuum line. CO_2 was collected in a N_2 trap by acidification of the sample and was then introduced into the double-collector inlet of a mass spectrometer (MS 20; AEI).

^{13}C-enrichment of the sample, mean $\dot{V}_{CO_2}$ during the collection period and the excess ^{13}C content of the glucose load were then used to calculate the ingested glucose oxidation rate ($\dot{G}_{in_{ox}}$) as previously reported (Ravussin *et al.* 1979).

$$\dot{G}_{in_{ox}} = \frac{[(\dot{V}_{CO_2} \times 12)/22{\cdot}26] \times \Delta \text{ atom } \% \text{ excess}}{0{\cdot}4 \times \Delta \text{ atom } \% \text{ excess}_{glucose}}$$

RESULTS

Blood measurements

The mean blood glucose value at 0 time was $4{\cdot}6 \pm 0{\cdot}2$ mmol/l (Fig. 1). After the glucose load this value increased and reached a peak of $6{\cdot}8 \pm 0{\cdot}4$ mmol/l after 60 min. It then slowly decreased and reached basal levels ($4{\cdot}4 \pm 0{\cdot}6$ mmol/l) at 210 min. The mean plasma insulin level was 17 ± 3 μU/ml at 0 time, after which it rose to a plateau of 110 ± 15 μU/ml from 30 to 90 min. Its return to basal values was more delayed than for blood glucose;

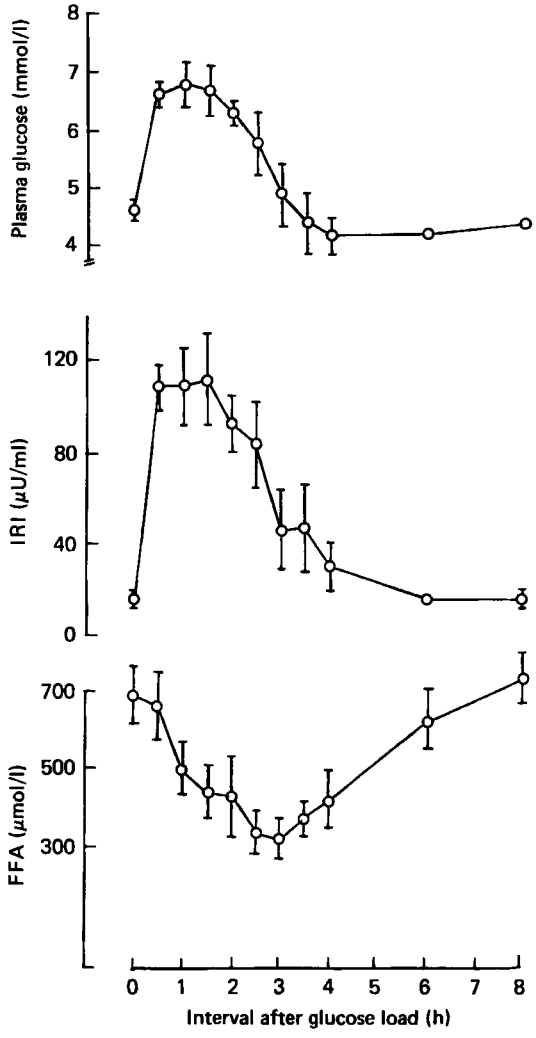

Fig. 1. Plasma glucose (mmol/l), immunoreactive insulin (IRI; μU/l) and free fatty acid (FFA; μmol/l) levels after a glucose load (zero time) in five obese subjects. Points represent mean values with their standard errors represented by vertical bars.

240 min after the glucose load, plasma insulin was 31 ± 11 μU/ml and 17 ± 2 μU/ml at 360 min. The mean basal FFA was 685 ± 72 μmol/l; it fell slowly after the glucose load and reached a minimum value (331 ± 51 μmol/l) at 180 min. It then increased progressively until the end of the test (734 ± 62 μmol/l). For each subject, the rise in FFA occurred after his insulin level had returned to the basal value. Compared with control subjects (Ebiner *et al.* 1979), immunoreactive insulin ($P < 0.05$) and FFA ($P < 0.01$) levels were significantly increased in the obese subjects during the 4 h after the glucose load, whilst changes in blood glucose were similar.

Substrate oxidation rates

Before glucose ingestion, CHO oxidation was 107 ± 14 mg/min (Fig. 2). After glucose ingestion, CHO utilization increased to a plateau (245 ± 25 mg/min) between 90 and 120 min. It then fell gradually below basal levels by the end of the test.

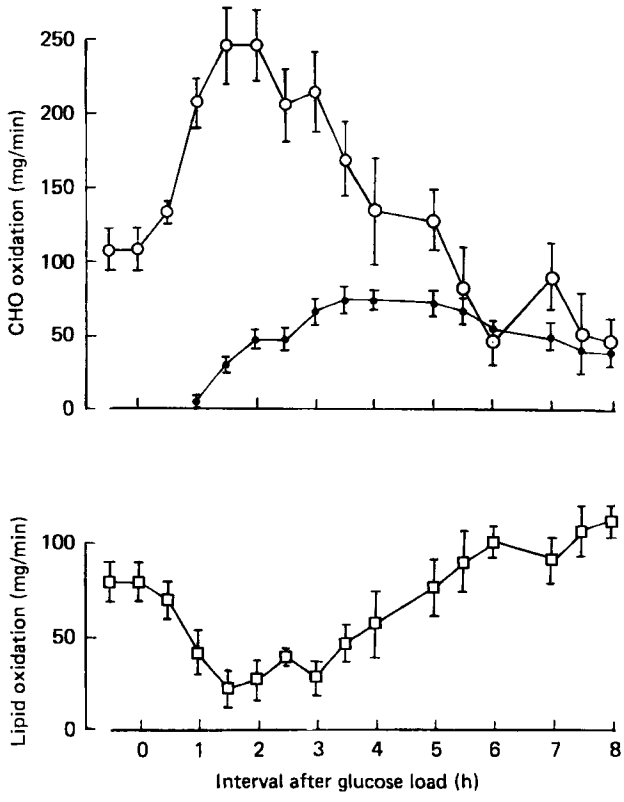

Fig. 2. Measurement of carbohydrate (CHO) (○–○) and lipid (□–□) utilization rates (mg/min) by indirect calorimetry and ingested glucose oxidation (●–●) by mass spectrometry after 100 g naturally-labelled [^{13}C]glucose load in five obese subjects. Points represent mean values with their standard errors represented by vertical bars.

Lipid oxidation was 79 ± 10 mg/min at 0 time. This decreased to 41 ± 12 mg/min from 60 min and then oscillated between 22 ± 10 and 46 ± 10 mg/min from 90 to 210 min. It then progressively rose reaching 112 ± 8 mg/min by the end of the test.

[^{13}C]glucose oxidation rate was first observed at 45 min and reached a peak (74 ± 8 mg/min) at 210 min. It then decreased progressively during the remainder of the test to 39 ± 8 mg/min.

Protein oxidation rate was 68 ± 6 mg/min during the first 4 h and 49 ± 18 mg/min for the remainder of the test.

Cumulative CHO utilization

Cumulative values for CHO oxidation during the 480 min oral glucose tolerance test are presented in Fig. 3. Total utilization was $65{\cdot}7 \pm 6{\cdot}7$ g after 480 min. When the basal value was subtracted, $26{\cdot}0 \pm 6{\cdot}8$ g in excess of the basal value were oxidized. Mass spectrometry results gave a value of $23{\cdot}7 \pm 1{\cdot}8$ g ingested glucose oxidized during 8 h. Compared with results on control subjects (Table 2) obtained in this laboratory with the same methods, one can conclude that total CHO oxidation ($83{\cdot}3 \pm 7{\cdot}5$ g for controls and $65{\cdot}7 \pm 6{\cdot}7$ g for obese subjects) and oxidation in excess of the basal value ($37{\cdot}0 \pm 2{\cdot}0$ g and $26{\cdot}0 \pm 6{\cdot}8$ g respectively) presented a tendency to diminish in the obese subjects but this was not statistically significant. Ingested [^{13}C]glucose oxidation, however, was significantly reduced in the

Table 2. *Cumulative carbohydrate (CHO) oxidation values (g) during 8 h for obese* and control† subjects*

(Mean values with their standard errors)

	Controls		Obese	
	Mean	SE	Mean	SE
Total CHO oxidation (g)	83·3	7·5	65·7	6·7
Amount in excess of basal CHO oxidation (g)	37·0	2·0	26·0	6·8
[13C]glucose oxidation (g)	29·2	1·5	23·7	1·8

* For details, see Table 1.
† Values taken from Ebiner *et al.* (1979).

obese ($29{\cdot}2 \pm 1{\cdot}5$ g in controls and $23{\cdot}7 \pm 1{\cdot}8$ g in obese subjects; $P < 0{\cdot}05$). If these three values (total, in excess of the basal value and ingested CHO oxidation) are expressed as a function of body surface area, they are all significantly lower ($P < 0{\cdot}01$) in the obese.

CHO balance

Figure 4 illustrates the carbohydrate balance in the obese and control subjects in the post-absorptive state (0 hour) and 8 hours after the glucose load. At this time, obese subjects presented a net positive carbohydrate balance ($34{\cdot}3 \pm 6{\cdot}8$ g) which was higher than that of the controls ($16{\cdot}6 \pm 7{\cdot}5$ g).

DISCUSSION

When glucose is offered as a substrate to the organism, lipid utilization is reduced and CHO oxidation increases (Randle *et al.* 1963). As in the controls (Ebiner *et al.* 1979) these changes are associated with an increase in glycaemia and a fall in plasma FFA. Therefore, in spite of their higher weight and higher metabolic rate, the obese subjects demonstrated a lower rate of oxidation of total CHO and ingested glucose than the controls. Whilst these results are in agreement with those of other studies (Shreeve, 1965; Shreeve *et al.* 1968; Pittet *et al.* 1976) where the impairment of total CHO utilization has been observed, they are not supported by those of Lefèbvre *et al.* (1975) who found no difference in ^{13}C-enrichment in the expired CO_2 between control and obese subjects after ingestion of [13C]glucose. However, the latter authors made no quantitative calculations of ingested glucose oxidation since the CO_2 production was not measured.

In the present study, the lower CHO oxidation rate of ingested glucose can be related to the glucose fatty acid cycle (Randle *et al.* 1963). According to Randle *et al.* (1963), an increased availability of FFA in heart muscle leads to an inhibition of glucose oxidation and a stimulation of lipid utilization. Whereas this concept has been challenged in skeletal muscle (Jefferson *et al.* 1972; Goodman *et al.* 1974), recently Rennie & Holloszy (1977) have shown that the availability of fatty acids exerts an inhibitory effect on glucose uptake and glycogen utilization in well-oxygenated red skeletal muscle. Furthermore, Gomez *et al.* (1972) observed a decrease in CHO utilization when they performed an oral glucose tolerance test in the presence of artificially-elevated FFA levels. In our obese subjects where FFA levels were approximately twice as high as in the controls ($P < 0{\cdot}01$), an increased release of FFA from the enlarged adipose tissue fat stores could impair glucose metabolism in muscle; FFA are offered to muscle as an alternative fuel to glucose and their increased oxidation inhibits glucose utilization.

Comparison of cumulative CHO oxidation above basal values with [13C]glucose oxidation after 8 h showed that the stimulation of CHO oxidation after a glucose load was entirely

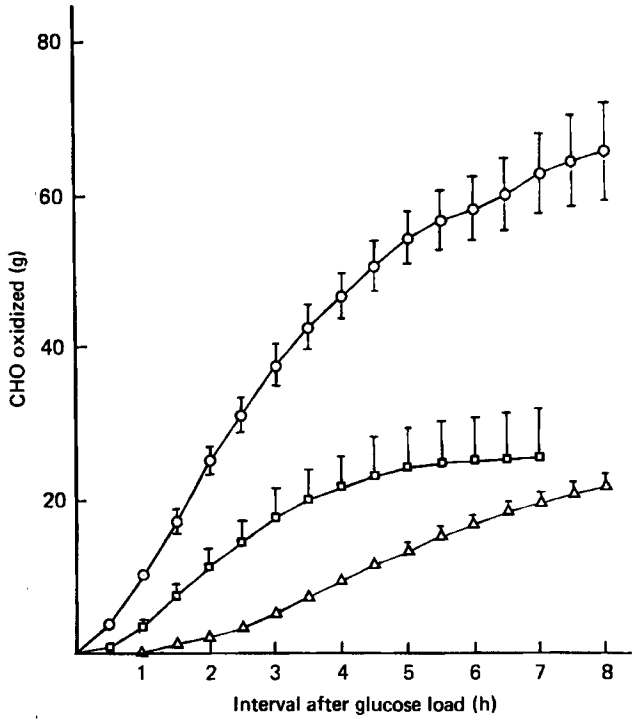

Fig. 3. Cumulative values for carbohydrate (CHO) oxidation (g) after 100 g [^{13}C]glucose load in five obese subjects. (○–○), cumulated CHO oxidation values measured by indirect calorimetry; □–□, cumulated CHO oxidation in excess of basal levels, measured by indirect calorimetry; △–△, cumulated [^{13}C]glucose oxidation measured by mass spectrometry. Points represent mean values with their standard errors represented by vertical bars.

due to utilization of ingested glucose. The delay observed between these two curves (Fig. 3) can be explained by a dilution of $^{13}CO_2$ in the bicarbonate pool. Thus, in calculating the exogenous glucose oxidation, this dilution phenomenon probably induces a slight underestimate of the net glucose oxidation rate before the ^{13}C enrichment peak (4·5 h); later on, this underestimate is partially compensated for by an inverse phenomenon, i.e. a slight over-estimate of the ingested glucose oxidation rate.

A criticism of the method for evaluating the oxidation of naturally-labelled [^{13}C]-glucose is the possibility of the recycling of ^{13}C in the form of lipid (via lipogenesis) or glucose (via the Cori cycle). As demonstrated by Krebs *et al.* (1966) and discussed by others (Vranic, 1973; Streja *et al.* 1977), many metabolic pathways share common metabolic pools and crossing-over of label can occur. However, even after a large intake of CHO (Passmore & Swindells, 1963; Björntorp & Sjöström, 1978; Acheson *et al.* 1979) the conversion of CHO into fat seems to be of minor importance. Recycling of ^{13}C in the form of glucose can also be excluded since Felig *et al.* (1975) demonstrated that glucose ingestion resulted in complete inhibition of splanchnic lactate uptake, thus inhibiting the Cori cycle. It can therefore be concluded that the calculated [^{13}C]glucose oxidation represents the net conversion of the ingested glucose into CO_2 and H_2O, irrespective of the metabolic pathways involved.

Considering the total CHO balance after 8 h (Fig. 4), it can be seen that the obese subjects have a greater positive CHO balance than the controls (34·4 ± 6·8 g and 16·7 ± 7·5 g respectively) due to a decreased CHO utilization. The question now arises as to where and

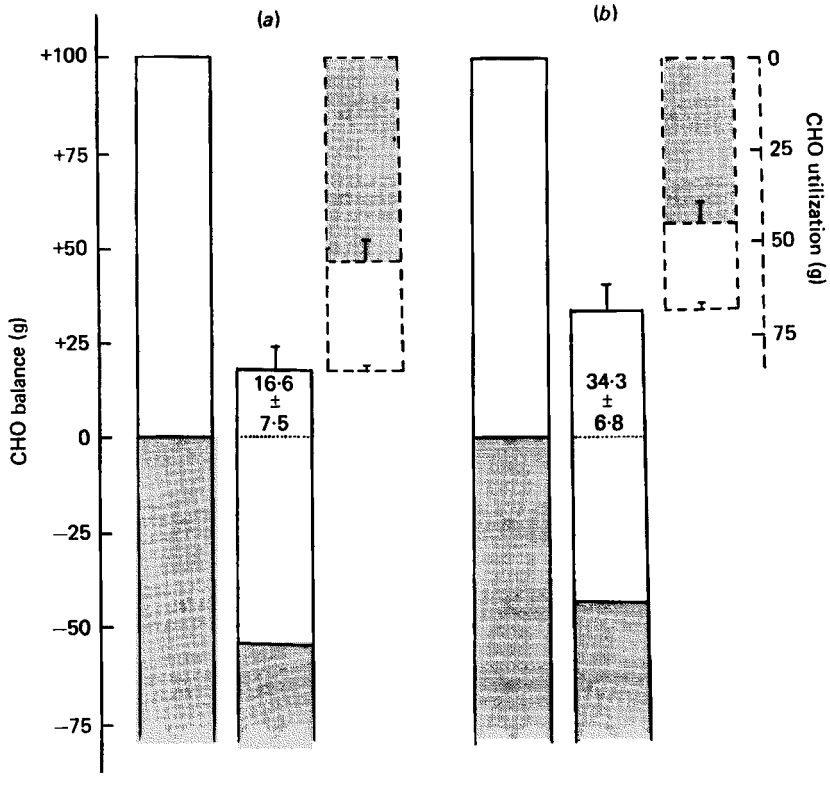

Fig. 4. Representation of carbohydrate (CHO) balance in (*a*) five normal-weight (Ebiner *et al.* 1979) and (*b*) five obese subjects at 0 time and 8 h after the 100 g [13C]glucose load. □, glucose load; ▨, endogenous CHO stores. ⬚, mean oxidation of [13C]glucose; ▩, endogenous CHO oxidation during the 8 h test. The net gain in the CHO content is 16·6 ± 7·5 g and 34·3 ± 6·8 g for control and obese subjects respectively. Values are means, with their standard errors represented by vertical bars.

in what form this excess carbohydrate is stored? Several possibilities exist: (1) due to the elevated insulin levels, the glucose could be stored as glycogen in the liver and muscles; (2) glucose could also be transformed into lipid, but net lipogenesis during the 8 h test can be excluded since at no time did the non-protein RQ exceed unity; (3) as suggested by Passmore & Swindells (1963) there could be a delay before its conversion into fat, the immediate disposal of excess CHO being stored as glycogen, mostly in muscle; (4) assuming that the glycogen stores in the obese subjects and controls are similar, the utilization of CHO is delayed.

Whilst our results do not enable us to answer the previously-mentioned question, they do show that the obese subjects in the resting state have a decreased rate of CHO utilization.

In conclusion, this study supports the concept that the development of glucose intolerance in the obese subject is preceded by an impairment of CHO utilization. This impairment is probably the consequence of an increased availability of FFA. The decreased rate of CHO oxidation will later favour hyperglycaemia and glucose intolerance.

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