Carbohydrate and Lipid Oxidation in Normal and Diabetic Subjects

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SUMMARY

Carbohydrate and lipid oxidation was measured in normal and diabetic human beings by means of continuous indirect calorimetry in the course of a 100-gm. oral glucose tolerance test.

After the glucose load, the carbohydrate (CHO) oxidation rate of 10 control subjects gradually rose, from 30 to 180 minutes, during the decline of plasma glucose and immunoreactive insulin (IRI). The lipid oxidation rate decreased during the same period.

Diabetics were divided into two groups. In a group of six nonobese maturity-onset diabetics with a slight IRI response to glucose load and a fall in plasma free fatty acid (FFA) levels, the CHO oxidation rate was found to be of the same order of magnitude as in normal subjects, but this occurred at a high plasma concentration of glucose. The lipid oxidation rate decreased as in normal subjects.

Conversely, in a group of four juvenile-type diabetics with no IRI response to glucose load and no fall in FFA levels, the CHO oxidation rate was markedly diminished and the lipid oxidation rate presented only a slight fall after glucose load.

In the group of maturity-onset diabetics, a slight insulin secretion seemed to be sufficient to prevent lipolysis and to allow normal rates of lipid and CHO oxidation in response to glucose load. On the other hand, in the group of juvenile-type diabetics, the lack of endogenous insulin secretion seemed to be responsible for increased lipolysis, leading to decreased responses of lipid and CHO oxidation to glucose load.

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The complexity of glucose disposal after oral intake is well recognized. While a large part of the ingested glucose enters the liver,[1] another part goes to the peripheral tissues, particularly muscle, where it is oxidized as a source of energy. For its oxidative processes, the muscle utilizes substrates that essentially derive from glucose and free fatty acids (FFA). The purpose of the present work was to investigate carbohydrate (CHO) and lipid oxidation in normal and diabetic subjects. Several studies have been published on the measurement of glucose oxidation in diabetes by means of ^{14}C- or ^{13}C-labeled glucose.[2-7] The utilization of ^{14}C-glucose presents the ethical problem of giving a radioisotope to human beings. The possibility of employing a nonradioactive isotope, ^{13}C-labeled glucose, has aroused great hopes, but all the methods that use isotopes, ^{14}C- as well as ^{13}C-labeled glucose, are confronted with problems of compartmental kinetics, especially the dilution of labeled glucose in the glucose pool and the mixing of CO_2 produced in the CO_2 pool. Moreover, the use of ^{13}C-labeled glucose is limited by the fact that, up to the present time, it has not been possible to detect it in sufficient amounts in the blood. Studies on glucose oxidation[8,9] in the forearm have yielded useful information, but they are, by definition, limited to one segment of the body.

Indirect calorimetry measured in a continuous system offers the great advantage of being innocuous and of simultaneously measuring both carbohydrate and lipid oxidation. In the present study, these were measured together with plasma glucose, insulin, and FFA in the course of a 100-gm. oral glucose tolerance test.

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MATERIALS AND METHODS

The study was undertaken with 10 control subjects and 10 patients with clinical diabetes. The group of control subjects (nos. 1 to 10) consisted of volunteers of both sexes aged 20 to 36 years, nonobese, and with no family or personal history of diabetes.

The diabetic patients were all nonobese (table 1), all being less than 14 per cent above their desirable weight range for their height according to the Metropolitan Life Insurance Tables. They all presented a marked glucose intolerance, since their level of plasma glucose exceeded at least 300 mg./100 ml. during the two hours after the oral glucose load (100 gm.). None of them had received insulin before the test. They were divided into two groups according to the severity of the disease. In the first group, six maturity-onset diabetics, the rise in blood glucose was accompanied by a slight rise in plasma immunoreactive insulin (IRI) and a fall in FFA. In the second group, of four juvenile-type diabetics, the rise in blood glucose was accompanied by no rise in plasma IRI and no fall in FFA.

The subjects received a 100-gm. glucose load. Venous blood was withdrawn every 30 minutes for measurement of plasma glucose, IRI, and FFA during the first two hours of the test and after three hours. Carbohydrate (CHO) and lipid oxidation rates were determined by indirect calorimetry. Urine was collected during the three hours of the test for measurement of urinary nitrogen. The subjects emptied their bladders completely before and at the end of the collection.

Indirect calorimetry was conducted as previously described[10] and performed by an open-circuit system. Ventilation was measured with a pneumotachograph. A constant fraction of the flow of expired air was automatically sampled for analysis. The oxygen content was determined in a paramagnetic oxygen analyzer and carbon dioxide in an infrared analyzer. Respiratory quotient (RQ) and metabolic rate were calculated by means of these data and simultaneously corrected for gas pressure, temperature, and moisture. The nonprotein RQ was determined by subtracting the amount of protein consumed as calculated from urinary nitrogen. The tables of Lusk[11] were used to obtain the amount of carbohydrate and lipid oxidized.

Indirect calorimetry has limitations that must be taken into account in order to interpret data. Sources of error include unsteady respiratory conditions and utilization of metabolic pathways other than carbohydrate, lipid, and protein oxidation. If these limitations are known and taken into consideration, indirect calorimetry makes it possible to measure carbohydrate and lipid oxidation in most subjects with diabetes.

Although the RQ can be modified by changes in ventilation over short periods (i.e. hypo- or hyperventilation), its integration over 10-minute periods corrects these changes and results in a mean RQ that is obviously an index of the food consumed. In addition, it is well known that RQ increases during the respiratory compensation of metabolic acidosis; the diabetic patients in this study were not ketotic except for two patients mentioned later.

The second cause of error in indirect calorimetry is due to lipogenesis from glucose, which may result in an RQ higher than unity. This is due to decarboxyla-

TABLE 1

Classification and characteristics of the diabetics

Subjects no.	Age	Sex	Height (cm.)	Weight (kg.)	Desirable weight* (kg.)	% above desirable weight
Maturity-onset diabetics						
11	38	F	148	56.2	52.4	7
12	58	M	172	77.8	74.8	4
13	33	F	158	62.4	57.4	9
14	62	F	149	52.8	58.2	−9
15	79	F	155	60.5	60.8	−1
16	56	F	158	70.0	62.4	12
Juvenile-type diabetics						
17	58	M	176	70.2	77.8	−10
18	38	M	179	60.4	75.5	−20
19	28	M	187	93.0	81.7	14
20	24	M	177	70.5	70.6	0

*According to the Metropolitan Life Insurance Company Tables (1959).

tion via the pentose-phosphate shunt, which is activated during lipogenesis. Pyruvate decarboxylation to acetyl-CoA followed by fatty acid synthesis also results in a higher RQ. The latter two causes of error might lead to misinterpretation of high glucose oxidation rates if a significant amount of lipogenesis occurred after the glucose uptake. Recent studies[12-14] have shown that fatty acid synthesis de novo is of little importance after glucose uptake. It seems, therefore, justified to use indirect calorimetry in these studies, provided the data obtained on respiratory gas exchanges are continuously measured and the mean values of oxygen consumption and carbon dioxide production are averaged over a period of 10 minutes.

The contribution of protein oxidation during acute experiments cannot be accurately estimated. Mean urinary nitrogen excretion measured during the test period provides an index of the mean amino acid oxidation rate. Although the latter is not likely to remain constant after an acute load, the assumption of a constant amino acid oxidation rate does not significantly affect the calculation of carbohydrate and lipid oxidation.

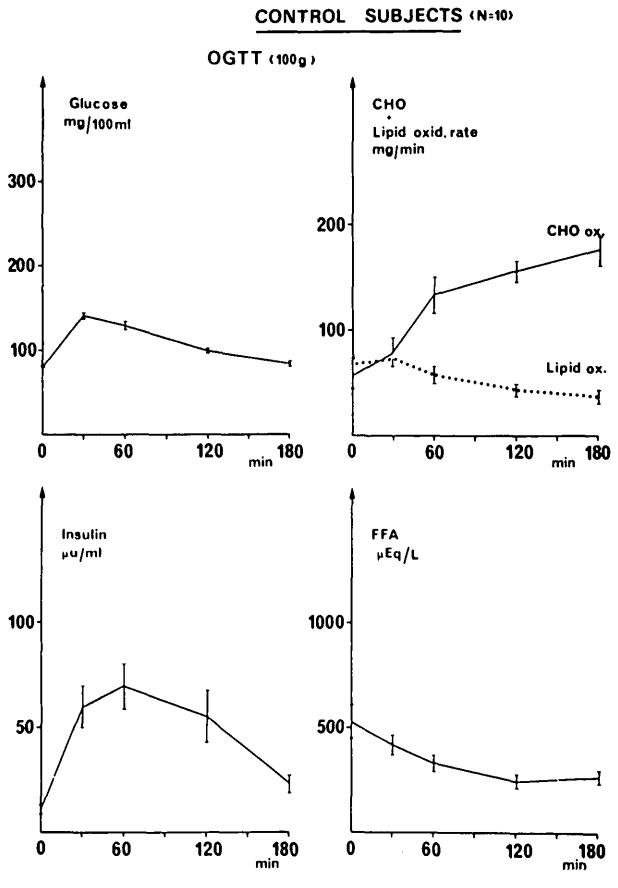

FIG. 1. Plasma glucose, insulin, and FFA and CHO + lipid oxidation rates in a group of 10 normal subjects following a 100-gm. oral glucose load.

The total amount of CHO and lipid oxidized during the three hours of the test is calculated by addition of the oxidation rate values measured for each 10-minute period. Similarly, the total amount of CHO oxidized above basal value is calculated after subtraction of the basal value from the amount of CHO oxidized during each period.

Plasma glucose was measured by the O-toluidine method[15] as modified by Michod and Frei,[16] plasma immunoreactive insulin (IRI) by the radioimmunoassay method described by Hales and Randle[17] using human insulin as a standard, plasma FFA by the method of Dole and Meinertz,[18] and ketone bodies in urine by means of test tapes (Acetest, Ames).

RESULTS

In the group of 10 normal subjects (figure 1, table 2), the CHO oxidation rate remained almost unaltered for the first 30 minutes after the 100-gm. glucose load, in contrast to the rise in plasma glucose and IRI levels. Then the CHO oxidation rate increased, reaching its highest value at the end of the test, when plasma glucose and IRI had returned nearly to fasting levels. Although FFA levels had started to fall immediately after the glucose load, the lipid oxidation rate did not begin to decline until 30 minutes after the glucose load. Cumulative measurement of oxidized CHO during the three hours of the test showed oxidation of the equivalent of 14.29 ± 1.31 S.E.M. gm. glucose over the basal value (table 3).

In the group of six maturity-onset diabetics (figure 2, table 2), fasting blood glucose was elevated as well as the blood glucose curve. In response to the glucose load, plasma IRI levels were markedly diminished. FFA levels fell during the test, as they did in the group of normal subjects. CHO and lipid oxidation rates were almost identical with those observed in the normal subjects. During the 180 minutes of the test, these subjects oxidized a quantity of glucose (20.6 ± 2 S.E.M. gm.) almost equivalent to the amount oxidized by the normal subjects (24.5 ± 2 S.E.M. gm.). The total CHO oxidized over basal value during the same period was equivalent to 13.51 ± 1.72 S.E.M. gm. of glucose (table 3).

In the group of four juvenile-type diabetics (figure 3, table 2), plasma glucose levels were higher than in the previous group. No IRI response and no fall in FFA were observed after the glucose load. In the fast-

TABLE 2

Subjects no.	CHO ox. rate (mg./min.)					Lipid ox. rate (mg./min.)					Total amount oxidized during 180 min.	
	0	30	60	120	180	0	30	60	120	180	CHO (gm.)	Lipids (gm.)
Control subjects												
1	71	102	160	163	148	73	74	58	48	55	25.93	10.33
2	9	67	76	110	121	88	71	79	64	66	16.32	12.37
3	40	27	137	194	240	92	129	94	57	32	26.20	13.56
4	24	70	139	147	149	84	71	54	53	57	21.17	10.56
5	81	18	44	176	215	41	84	77	36	23	21.49	9.36
6	40	50	83	115	127	89	92	78	66	52	16.98	12.92
7	65	85	113	124	215	76	82	68	54	54	23.23	11.39
8	132	170	228	217	238	36	59	38	33	13	37.97	6.51
9	62	86	176	141	147	47	52	31	24	27	26.28	5.06
10	58	115	182	177	180	58	28	6	6	2	29.41	2.24
$\bar{x}$	58	79	134	156	178	68	74	58	44	38	24.49	9.43
S.E.M.	11	14	18	11	14	7	8	8	6	7	1.99	1.17
Nonobese, maturity-onset diabetics												
11	32	14	86	155	95	78	93	62	39	57	15.71	11.37
12	50	41	86	100	188	46	67	52	54	19	18.16	8.70
13	27	34	90	102	115	81	58	46	43	40	15.49	8.57
14	52	64	147	180	160	77	106	58	43	34	24.04	10.32
15	28	63	96	163	180	97	26	28	17	0	22.50	4.06
16	48	87	160	203	119	65	55	32	6	23	27.85	4.19
$\bar{x}$	39	50	111	150	142	74	65	46	34	29	20.62	7.89
S.E.M.	4	10	14	17	16	7	12	6	8	8	2.03	1.26
Juvenile-type diabetics												
17	28	35	10	67	77	124	125	151	103	98	8.52	21.92
18	28	0	79	92	68	63	118	70	60	72	10.42	12.72
19	36	66	67	35	36	113	118	119	138	141	8.81	23.25
20	10	18	49	89	80	108	124	91	67	79	11.20	15.85
$\bar{x}$	25	29	51	71	65	102	121	108	92	97	9.73	18.43
S.E.M.	5	14	15	13	10	13	2	17	18	15	0.64	2.49

ing state, lipid oxidation was much higher than CHO oxidation and did not fall much after the glucose load. The CHO oxidation rate was lower than in the other group and in the normal subjects. The total CHO oxidized above the basal value was 5.14 ± 1.55 S.E.M. gm. of glucose during the three hours of the test (table 3). Moderate ketosis was found in two of these patients.

DISCUSSION

In the group of 10 normal subjects, cumulative measurement showed an amount of 14.3 gm. glucose oxidized after a 100-gm. glucose load. This was measured in the resting state within the three hours of the test; this value reflects glucose oxidation exceeding the basal value. It is close to the value of 15 per

TABLE 3

Total amount of CHO oxidized above basal value during 180 minutes (gm.)

Control subjects		Maturity-onset diabetics		Juvenile-type diabetics	
Subjects no.	CHO oxidized	Subjects no.	CHO oxidized	Subjects no.	CHO oxidized
1	13.15	11	9.95	17	3.48
2	14.70	12	9.16	18	5.38
3	19.00	13	10.63	19	2.33
4	19.55	14	14.66	20	9.40
5	6.91	15	17.46		
6	9.78	16	19.21		
7	11.53				
8	14.21				
9	15.12				
10	18.97				
$\bar{x}$	14.29		13.51		5.14
S.E.M.	1.31		1.72		1.55

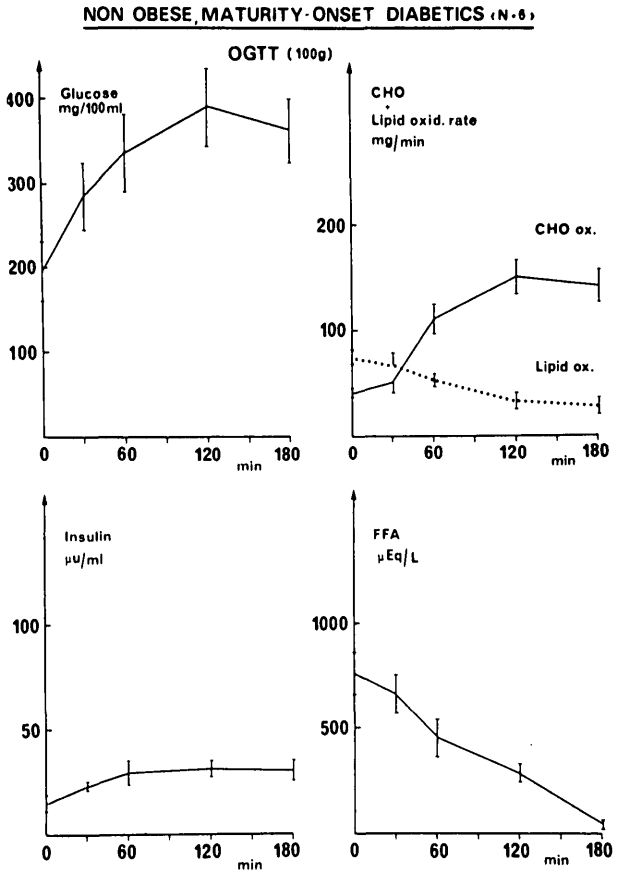

FIG. 2. Plasma glucose, insulin, and FFA and CHO + lipid oxidation rates in a group of six maturity-onset diabetics with a slight rise in plasma IRI and a fall in FFA after a 100-gm. oral glucose load.

cent reported for the ingested glucose available for disposal by the peripheral tissues by Felig et al.[1] in their study of splanchnic exchange of glucose. However, it corresponds solely to oxidized glucose and does not include any other metabolic fate of glucose. These values are lower than those in the total peripheral glucose utilization estimated by other authors in forearm glucose uptake[8,19] or from comparison between glucose infusion and the 100-gm. oral glucose tolerance curve.[20]

In the normal group (figure 1), the slow rise in CHO oxidation rate for the first 30 minutes, when glycemia and insulinemia reached their peaks, and the subsequent increasing CHO oxidation rate, with its highest value after 180 minutes, when glycemia and insulinemia had almost returned to fasting levels, confirm the delayed peak of CO_2 production reported in studies using ^{13}C-glucose in normal human subjects.[5] These findings are compatible with an initial storage of glucose under the influence of insulin and with oxidized CHO originating mainly from glucose stored as glycogen. This transient storage agrees well with the study of Beloff-Chain et al.[21] in isolated rat diaphragm muscle, giving evidence that polymerization of glucose is one of the first reactions that glucose undergoes on entering the muscle cell, which normally precedes oxidative degradation. Another explanation would be that delayed CHO oxidation might result from glucose being initially stored in the liver and released thereafter.

Even though the patients in the first group of diabetics presented glucose intolerance and a markedly increased IRI response to the glucose load (figure 2), the observation that glucose oxidation was maintained at normal rates with the same amount of glucose equivalent oxidized over the three hours of the test first appears to be paradoxic. However, it confirms the previous results of Shreeve et al.,[2] which were obtained by means of ^{14}C-glucose. These investigators mentioned that "the normal rate of glucose

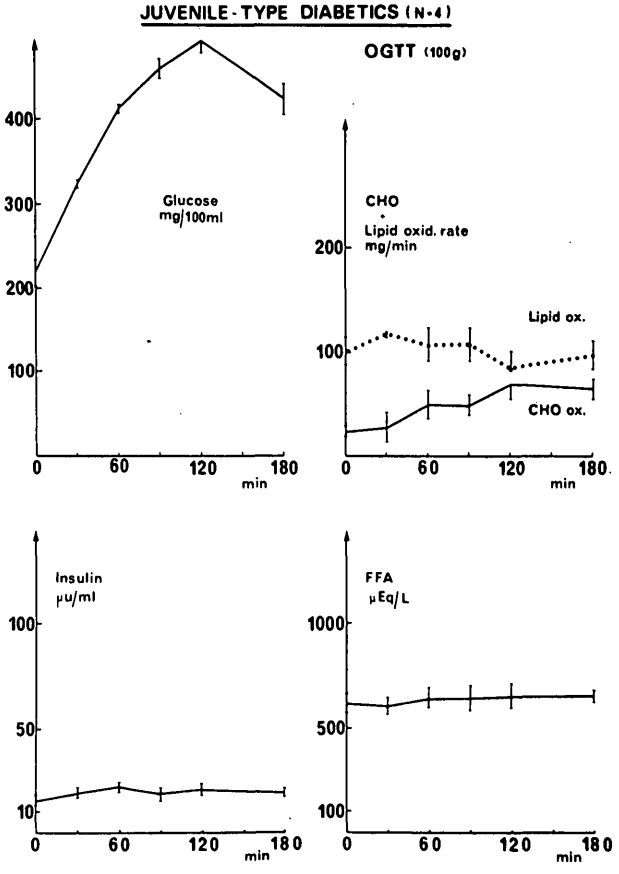

FIG. 3. Plasma glucose, insulin, and FFA and CHO + lipid oxidation rates in a group of four juvenile-type diabetics with no rise in plasma IRI and no fall in FFA after a 100-gm. oral glucose load.

oxidation in the stable diabetics suggests that if any potential impairment of oxidation exists, it might be offset by the presence of hyperglycemia." Differing from the normal group (figure 1), where CHO oxidation took place at a low plasma glucose concentration, it occurred here at a high plasma glucose concentration, perhaps suggesting glucose penetration along a concentration gradient.

This is supported by the results of Chevaux et al.,[22] who observed in normal subjects, an increased CHO oxidation rate caused by hyperglycemia itself during pharmacologic inhibition of endogenous insulin secretion.

The slight concentration of insulin still secreted in response to the glucose load is likely to be sufficient in these cases to prevent an increase in lipolysis, which might inhibit CHO oxidation.

The situation appears to be completely different in the four other cases presenting no IRI response and no fall in FFA levels after the glucose load (figure 3). In these cases, CHO oxidation was decreased, and cumulative measurement showed that only 5.1 gm. of glucose equivalent was oxidized during the three hours of the test, as against the 14.3 gm. in the normal group and 13.5 gm. in the group of maturity-onset diabetics. CHO oxidation appeared to be inhibited in spite of the very high plasma glucose levels that followed the glucose load. It is likely that CHO oxidation was partially prevented by the high lipid oxidation deriving from increased plasma FFA. The presence of a moderate degree of ketosis in two patients does not invalidate the results, since ketosis would increase rather than decrease the RQ, therefore artifically increasing CHO and decreasing lipid oxidation rates.

On the basis of observations made by Williamson and Krebs[23] and Shipp et al.,[24] Randle et al.[25] have described the inhibition of glucose oxidation by increased fatty acid oxidation. The decreased glucose tolerance resulting from experimentally elevated FFA levels in man has been reported by several groups.[26-28] More recently, Gomez et al.[10] have observed by means of indirect calorimetry decreased CHO oxidation together with a decreased glucose tolerance in spite of increased plasma insulin levels during the experimental rise in FFA leading to increased lipid oxidation. The study was performed in a group of eight normal subjects in the course of an oral glucose tolerance test, and FFA levels were enhanced through neutral fat infusion.

Fatty acid utilization seems to be related to plasma FFA levels. Armstrong et al.[29] have indeed demonstrated in dogs that changes in FFA uptake are simple mass-action effects of changes in FFA concentration. Using rat skeletal muscle in vitro, Eaton and Steinberg[30] have shown that the rate of oxidation of palmitate-1-^{14}C to $^{14}CO_2$ was increasing as a function of the concentration of FFA in the medium. However, it is important to remember that plasma FFA levels are not the only factors governing fatty acid utilization. Schonfeld and Kipnis[31] have in fact shown, in their experimentation in rat diaphragm in vivo, that the tissue fatty acid moiety rather than the plasma FFA levels is the major determinant in glucose—fatty acid interactions in striated muscle.

Comparison of CHO oxidation rates in both groups of diabetics shows an inverse relationship between CHO oxidation on one hand and lipid oxidation and plasma FFA levels on the other. It confirms a similar inverse relationship observed by Paul and Bortz[32] between plasma FFA and one hand and glucose turnover and oxidation on the other.

Conversely, plasma insulin levels do not seem to have any major direct effect on CHO oxidation, as shown by the normal rate of CHO oxidation in the group of maturity-onset diabetics, in spite of the very low insulin response to glucose. An indirect action of insulin, through its antilipolytic effect, is, however, evident. In the group of juvenile-type diabetics the decreased CHO oxidation rate seems to be related to the high lipid oxidation rate resulting from the increased lipolysis subsequent to the lack of endogenous insulin.

This situation is analogous to that observed in pancreatectomized animals. Searle et al.[3] have noted a marked decrease in ^{14}C-glucose oxidation in totally pancreatectomized dogs, as Shipley et al.[33] did in alloxan-diabetic rats. The main effect of insulin in CHO oxidation seems therefore to be indirect, through its antilipolytic action.

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