

Effect of glucose ingestion on the metabolism of free fatty acids in human subjects

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ABSTRACT The rate of appearance of $^{14}\text{CO}_2$ in expired air after the injection of a single dose of $\text{NaH}^{14}\text{CO}_3$ has been determined in normal individuals both in the fasted and fed states. These data were combined with previously obtained results on the rate of disappearance of injected palmitate- ^{14}C from the bloodstream, to give a multicompartamental analysis of free fatty acid oxidation and esterification.

The results confirm that glucose feeding promptly inhibits the rate of free fatty acid oxidation to CO_2 . The "irreversible disposal rate," or irreversible flux of free fatty acids from the plasma, was also consistently reduced by glucose feeding. The diminution in irreversible disposal, not accounted for entirely by reduction of direct oxidation, must indicate suppression of other disposal mechanisms, including net esterification of free fatty acids. An average drop of 49% in "net esterification" when glucose was given may be compared with the 65% inhibition of rapid free fatty acid oxidation.

SUPPLEMENTARY KEY WORDS compartmental analysis · bicarbonate injection · CO_2 excretion · free fatty acid oxidation · esterification · fasting · fed · human subjects

THE EFFECT of glucose ingestion on FFA oxidation to CO_2 in normal subjects has been studied by Waterhouse and Kemperman (1). Values for the rates of oxidation given by these authors were based on calculations in which the body bicarbonate pool was treated as a single, slowly-turning-over compartment, as in the model

proposed earlier by Baker et al. (2). We have now reevaluated and extended the earlier analysis by using a model in which a more complex treatment of the body bicarbonate pools (3-8) was included. In this model the assumption is made (6) that metabolically produced CO_2 has the same disposal pattern as CO_2 derived from a single intravenous NaHCO_3 injection, and takes into account the rapid component of CO_2 expiration that is present prior to complete mixing of the newly formed CO_2 with the total bicarbonate pool. Thus, we first obtained complete curves for $^{14}\text{CO}_2$ expiration by three normal subjects after $\text{NaH}^{14}\text{CO}_3$ administration, both in the fed and the fasted states. These curves, combined with our original data, were used to carry out multicompartamental analysis for each subject in each dietary state. Glucose feeding has been found to inhibit both the rates of FFA oxidation and of esterification. Quantitative aspects of these data are compared with earlier studies on FFA metabolism in fed and fasted human subjects (9).

METHODS

The subjects used in this study were metabolically normal. The tracer studies were done under two conditions, namely after an overnight 16 hr fast and during sustained oral ingestion of glucose. We attempted to attain a "steady state," as manifested by constant circulating glucose levels, in the second type of experiment by starting the ingestion of glucose at a rate of 5 g every 15 min 1.5 hr before the injection of tracer, and continuing at the same rate throughout the experimental period. Palmitate- $1\text{-}^{14}\text{C}$ complexed to albumin was

Abbreviations: FFA, free fatty acid(s).

injected intravenously and serial samples of blood and breath were collected over a 2.5 hr experimental period, at the time intervals indicated in Table 1. Concentration as well as radioactivity of the plasma FFA were determined. From blood samples obtained after the first 10 min, plasma FFA were isolated by silicic acid column chromatography (1). The rate of CO_2 expiration was determined by collection of timed expired air samples in Douglas bags, extraction of the contained CO_2 into 1 N NaOH, and precipitation as barium carbonate. Radioactivity was determined on a weighed aliquot of the barium carbonate (1).

The new experimental data presented here, which determine the early $^{14}\text{CO}_2$ excretion after the injection of $\text{NaH}^{14}\text{CO}_3$, were obtained by collection of CO_2 in Douglas bags at very frequent time intervals. The patients breathed into the collection apparatus continuously for the first 15 min, samples being collected at 0–2, 3–5, 6–8, 9–11, and 13–15 min; later samples were collected for 3-min intervals at 30, 60, 90, and 120 min after the injections.

Multicompartmental analysis was carried out by means of a digital computer and the SAAM program of Berman, Weiss, and Shahn (10). The first step was to fit the plasma FFA disappearance data for each subject to a two-compartment model with a single exit. Then the $^{14}\text{CO}_2$ data for each subject after injection of tracer bicarbonate were fitted to a three-compartment model. We then combined the two models, allowing for an indeterminate pool between them. Part of the outflow from plasma FFA was directed to the intermediate pool while the rest was allowed to leave the system. The computer then searched for the unknown rates that would reproduce the $^{14}\text{CO}_2$ data determined after injection of labeled palmitic acid. The accuracy of these values for pool sizes and rates is no greater than $\pm 10\%$ in any case. In the following analysis of the results the symbol λ is always used for rate constants or fractional turnover rates, while k designates the flux or the rate of flow in terms of millimoles per unit time.

RESULTS

Rates of Disappearance of FFA- ^{14}C from Plasma and Appearance of $^{14}\text{CO}_2$ (Original Data)

In Table 1 we present the data originally obtained (1) on four of the five subjects; the original fifth subject, I.W., is not included since his $^{14}\text{CO}_2$ excretion after injection of $\text{NaH}^{14}\text{CO}_3$ (lowest third of table) was not measured.

$^{14}\text{CO}_2$ Excretion After Injection of $\text{NaH}^{14}\text{CO}_3$ (New Data)

Table 2 contains data from which we can deduce the early rapid component of $^{14}\text{CO}_2$ excretion after $\text{NaH}^{14}\text{CO}_3$.

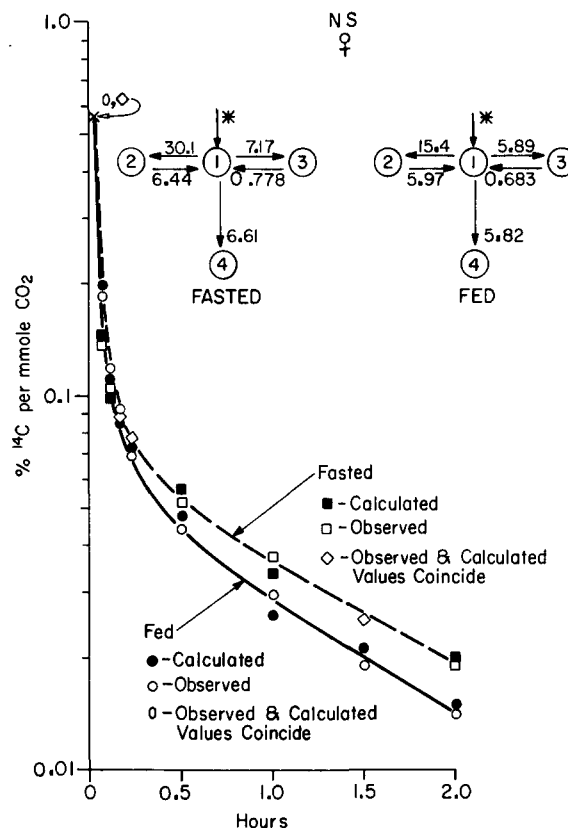


FIG. 1. $^{14}\text{CO}_2$ excretion in subject N. S. after $\text{NaH}^{14}\text{CO}_3$ injection.

Compartment 1 = HCO_3^- in the injected compartment, which is blood and perhaps some extravascular bicarbonate that equilibrates rapidly with blood bicarbonate.

Compartments 2 and 3 = HCO_3^- pools in tissues other than blood.

Compartment 4 = cumulative expired CO_2 .

Numbers on the arrows are rate constants, λ_{ij} , for each process. λ_{ij} = fraction per hr of substance in compartment j going to compartment i .

CO_3 in normal individuals, in both the fasting and the glucose-fed states. These experimental data were used to obtain a set of parameters for a three-pool bicarbonate model as shown in Figs. 1, 2, and 3, for each subject under each condition. The only dependence relationship imposed on this three-compartment analysis was that the rate constant from the injected compartment to expired air times the calculated number of millimoles in the injected compartment was equal to the measured rate of CO_2 production. In addition, both two- and four-compartment analyses were tried, but the former did not allow good fit and the four-compartment fit was not sufficiently better than that of the three-compartment analysis to warrant the addition of its complexity. The values for this multicompartmental analysis are shown in Table 3 along with the size of the injected compartment, Q_1 , and the cumulative excretion of label at 2.5 hr. The value of Q_1 was calculated by dividing the injected

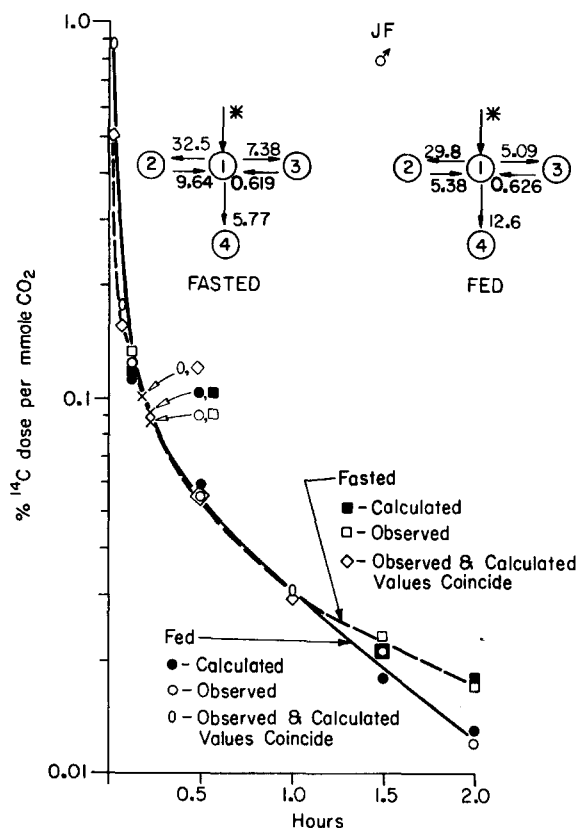


FIG. 2. $^{14}\text{CO}_2$ excretion in J. F. after $\text{NaH}^{14}\text{CO}_3$ injection. See legend for Fig. 1.

dose of radioactivity by the specific activity of CO_2 at $t = 0$, the latter being determined by the curve-fitting.

We wished to use complete bicarbonate curves with our original data in order to determine rate constants and Q_1 values, but we had obtained values in these subjects only at 15 min and later (Table 1). We therefore determined the mean value (in dpm/mmmole of CO_2) for the first four points on the new curves ($n = 3$ for each condition) and divided these by the specific activity at the 15 min point. The 15 min value for each original subject was then multiplied by the appropriate factor to obtain the normalized value at 1, 4, and 10 min. Multicompartmental analysis of these data, carried out in a similar fashion to that done on data from the three newly studied individuals, gave the rate constants and Q_1 values shown in Table 4.

Several features of these data are of intrinsic interest. It will be seen that the size of the bicarbonate pool in the injected compartment is quite variable (Tables 3 and 4) but roughly equivalent to the amount of bicarbonate normally found in blood. This is in contrast to data obtained in animal experimental work (7) and in that obtained in human beings performing heavy exercise (11), where rates of turnover are high and the injected bicarbonate pool size is greater. The injected pool size

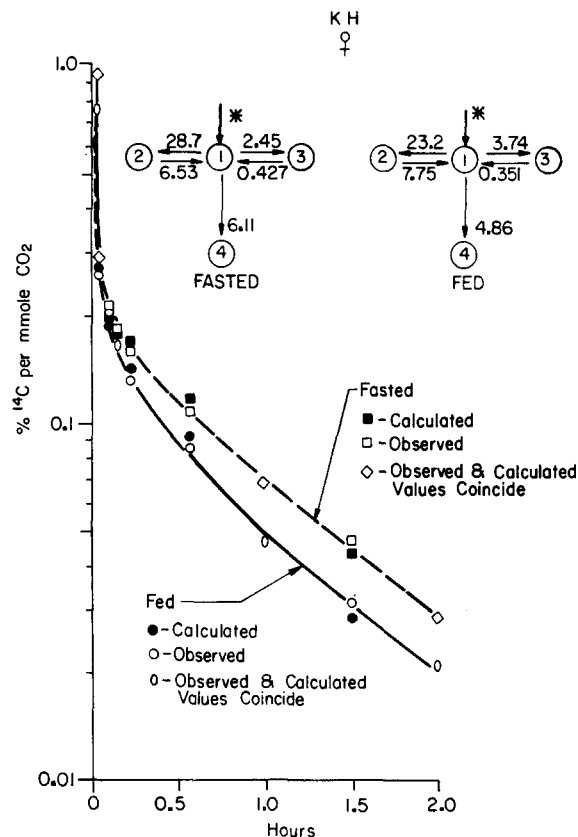


FIG. 3. $^{14}\text{CO}_2$ excretion in K. H. after $\text{NaH}^{14}\text{CO}_3$ injection. See legend for Fig. 1.

is not affected in any consistent way by the process of glucose feeding, even though an increased CO_2 production is usually seen with glucose loading. Although an increased transition rate between blood and expired CO_2 was not conclusively demonstrated with glucose feeding by the present analysis (see λ_{41} , Tables 3 and 4), it would be of considerable interest to measure pulmonary A-V pCO_2 difference under conditions of carbohydrate feeding in order to determine whether the extraction of CO_2 per single passage was increased under this condition. The cumulative excretion of $^{14}\text{CO}_2$ averaged about 70% in 2.5 hr and showed no real difference in the fasted or fed states.

Compartmental Analysis of the Original Data Including Total $^{14}\text{CO}_2$ Curves

Using the values determined above for the excretion of metabolically produced CO_2 , we reexamined the original data by the SAAM program as a seven-compartment model as shown in Fig. 4. Two constraints were placed on these data. The sum of λ_{71} and λ_{01} (the latter represented by the arrow arising from compartment 1 but going to no other compartment) was made equal to the irreversible disposal rate constant, as determined from the plasma FFA disappearance curve [$\text{IDR constant} =$

TABLE 1 RATE OF DISAPPEARANCE OF FFA-¹⁴C AFTER A SINGLE INJECTION OF PALMITATE-1-¹⁴C

Time	E. S.		H. D.		P. R.		R. D.	
	Fasting	Glucose Loaded	Fasting	Glucose Loaded	Fasting	Glucose Loaded	Fasting	Glucose Loaded
<i>mi</i>	<i>fraction of injected dose in plasma</i>							
0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
3	0.437	0.268	0.43	0.205	0.475	0.435	0.310	0.197
5	—	—	0.238	0.075	0.284	0.244	0.152	0.072
6	0.220	0.090	—	—	—	—	—	—
7	—	—	0.154	0.041	0.205	0.154	0.096	0.032
10	0.085	0.032	—	—	—	—	—	—
15	0.040	0.016	—	—	—	—	0.024	0.013
30	0.012	0.009	0.020	0.008	0.025	0.013	0.004	0.003
60	0.007	0.007	0.011	0.008	0.018	0.011	0.004	0.003
90	0.007	0.007	0.010	0.007	0.014	0.011	0.003	0.003
150	0.005	0.006	0.008	0.006	0.013	0.012	0.003	0.002
210	0.004	0.006	0.007	0.006	0.011	0.010	0.002	0.002
	<i>Instantaneous Rate of ¹⁴CO₂ Excretion After Palmitate-1-¹⁴C Injection</i>							
	<i>% of injected dose/hr</i>							
15	0.055	0.056	0.060	0.045	0.049	0.036	0.042	0.024
30	0.063	0.054	0.067	0.051	0.054	0.038	0.058	0.026
60	0.060	0.046	0.060	0.049	0.052	0.033	0.051	0.022
90	0.055	0.039	0.055	0.039	0.044	0.028	0.045	0.018
120	0.050	0.033	—	—	—	—	—	—
150	0.047	0.028	0.044	0.029	0.036	0.021	0.032	0.013
	<i>Specific Activity of Respiratory CO₂*</i>							
	<i>dpm/mole</i>							
15	6120	8910	9770	7250	9200	10200	10700	8540
30	5710	1610	6210	5950	7870	7410	8900	7570
45	—	—	—	—	7110	5525	—	—
60	4440	5420	3980	3780	5990	5020	5840	4660
90	3080	3700	2960	2370	4020	3690	4340	3100
120	—	—	—	—	—	—	3280	2030
150	1820	1720	1570	1018	2500	1820	2380	1410

* Injected dose, 13.4×10^6 dpm of $\text{NaH}^{14}\text{CO}_3$.

TABLE 2 ¹⁴CO₂ EXCRETION AFTER $\text{NaH}^{14}\text{CO}_3$ INJECTION IN THREE NEW SUBJECTS

Time of Sample	N. S. 117.60 kg		J. F. 67.76 kg		K. H. 45.10 kg	
	Fasting	Glucose Loaded	Fasting	Glucose Loaded	Fasting	Glucose Loaded
<i>min</i>	<i>dpm/mole</i>					
0-2	37,300	37,600	33,800	59,100	62,900	50,800
3-5	9,270	12,350	10,200	11,750	18,600	17,000
6-8	7,160	7,940	8,930	8,340	14,100	13,530
9-11	5,810	6,180	6,840	7,010	12,200	10,700
13-15	5,040	4,600	5,700	5,770	10,400	8,460
29-32	3,470	2,930	3,700	3,720	7,130	5,710
59-62	2,480	1,945	2,020	2,070	4,610	3,240
89-92	1,700	1,393	1,510	1,380	3,070	2,080
119-122	1,240	968	1,150	829	1,790	1,340
	<i>mmoles/hr</i>					
Total CO ₂ production	591	677	562	666	362	393

* Injected dose = 6.71×10^6 dpm of $\text{NaH}^{14}\text{CO}_3$.

$1/\int_0^\infty q(t)dt$, where $q(t)$ equals the percentage of the injected radioactivity in the sampled pool at time t]. Parameters λ_{12} and λ_{21} were fixed, as they were also determined from the FFA disappearance curve. With

these constraints the rate constants shown in Table 5 were obtained. The computer-derived curves with these rate constants gave an excellent fit with the determined ¹⁴CO₂ curve, the average deviation being 3-6%. Table 6 shows the fluxes. k_{21} and k_{12} must signify the rates of

TABLE 3 RATE CONSTANTS OF BICARBONATE METABOLISM IN THREE NEW SUBJECTS

	λ_{12}^*	λ_{13}	λ_{21}	λ_{31}	λ_{41}	Q_1	Cumulative- $^{14}\text{CO}_2$ Excretion at 2.5 hr †
	<i>mmoles</i>						
N. S.							
Fasting	6.4	0.78	30.1	7.2	6.6	90	69
Glucose-fed	6.0	0.68	15.4	5.8	5.8	116	71
J. F.							
Fasting	9.6	0.62	32.6	7.4	5.8	97	64
Glucose-fed	5.4	0.63	29.6	5.5	12.7	53	85
K. H.							
Fasting	6.5	0.42	28.7	2.4	6.1	59	78
Glucose-fed	7.8	0.35	23.2	3.7	4.9	81	67
Mean							
Fasting	7.5	0.60	30.5	5.7	6.2	82	70
Glucose-fed	6.4	0.55	22.8	5.0	7.8	83	74
Combined ‡							
Fasting	7.6	0.60	30.7	7.0	5.9	86	71
Glucose-fed	5.8	0.43	21.8	5.0	6.6	87	70

* λ_{ij} = Fraction per hr of substance in compartment j going to compartment i . Such fractional turnover rates are in units of hr^{-1} ; Q_1 = mmoles of bicarbonate in compartment 1, into which tracer was injected.

† Percentage of injected ^{14}C ($\text{NaH}^{14}\text{CO}_3$).

‡ All "fasting data" combined to obtain a single curve which represented the "least squares fit."

TABLE 4 RATE CONSTANTS OF BICARBONATE METABOLISM IN FOUR PREVIOUSLY STUDIED SUBJECTS

	λ_{12}	λ_{13}	λ_{21}	λ_{31}	λ_{41}	Q_1	Cumulative- $^{14}\text{CO}_2$ Excretion at 2.5 hr*
	<i>mmoles</i>						
E. S.							
Fasting	5.2	0.07	31.6	2.7	2.5	191	40
Glucose-fed	4.2	0.07	25.8	1.8	5.2	110	67
H. D.							
Fasting	6.8	0.36	28.6	5.5	5.0	115	58
Glucose-fed	4.6	0.07	25.9	3.2	5.2	129	59
P. R.							
Fasting	5.5	0.24	31.0	3.0	3.4	125	52
Glucose-fed	5.0	0.47	23.2	4.5	6.0	94	69
R. D.							
Fasting	5.9	0.46	30.4	3.0	4.8	107	67
Glucose-fed	4.6	0.11	26.1	2.3	5.6	112	68

* Percentage of injected ^{14}C ($\text{NaH}^{14}\text{CO}_3$).

exchange of some FA pool, perhaps in adipose tissue, with plasma FFA, whereas k_{01} , the rate of an irreversible process other than oxidation, might represent storage or long-term esterification. k_{71} would be the amount of free fatty acid oxidized per hr. The sum of k_{01} and k_{71} would equal the irreversible disposal rate. The data show that the fractional rate of oxidation, λ_{71} , is usually reduced with glucose feeding and that the total flux to the oxidative pathway (k_{71}) is always reduced by at least 50%. The irreversible disposal rate is also consis-

tently reduced by glucose feeding, as a result of the decrease both of the rate of oxidation (k_{71}) and of "irreversible esterification" (k_{01}).

DISCUSSION

Both the present multicompartmental analysis and the previous analysis of the oxidative pathway (1) rely on curve-fitting techniques in which estimates of transition rates are based upon the disappearance of tracer FFA-

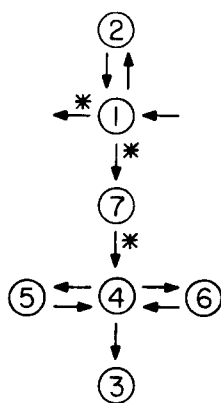


FIG. 4. Model for compartmental analysis.

* = unfixed parameters; $\lambda_{01} + \lambda_{71}$ = fractional rate of irreversible exit from compartments 1 and 2 as determined from FFA

disappearance curve $1/\int_0^{\infty} q(t)dt$.

Compartment 1 = plasma FFA.

Compartment 2 = extraplasma fatty acids which may exchange with plasma FFA.

Compartment 3 = cumulative expired CO_2 .

Compartment 4 = HCO_3^- in the injected compartment, which is blood and perhaps some extravascular bicarbonate that equilibrates rapidly with blood bicarbonate. This is the analogue of compartment 1 in Figs. 1-3.

Compartments 5 and 6 = HCO_3^- pools in tissues other than blood (compartments 2 and 3 in Figs. 1-3).

Compartment 7 = intermediate fatty acid pool of undetermined site, presumably intracellular (compartment 4 in Figs. 1-3).

Compartment 0 = compartment not labeled since it indicates any site of irreversible loss from the system except via CO_2 .

While the model indicates three unfixed parameters, the knowledge of the sum of two of the parameters (λ_{01} and λ_{71}) allows computer search for only two unknowns, λ_{71} and λ_{47} .

^{14}C from the circulation and the appearance of $^{14}\text{CO}_2$ in expired air. Two fundamental differences exist in the handling of the data. First, the current analysis fixes rates of reversible and irreversible exit from the plasma FFA pool on the basis of the FFA- ^{14}C disappearance

curves, whereas the previous analysis allowed any type of reversible and irreversible exit from the plasma FFA without fixing specific rates. Second, the current analysis assumes that metabolically produced CO_2 immediately enters plasma as bicarbonate and shows subsequent kinetic behavior identical with that of the latter. The earlier analysis viewed metabolically CO_2 as already distributed and thus as a single, slowly-turning-over pool, probably an unrealistic simplification.

The fractional rate of oxidation (λ_{71}) as determined by the multicompartmental analysis was consistently higher than that originally determined, but only by a factor of 1.4. It is not surprising that these rates are in fairly good agreement since, over a long period, λ_{71} determines the percentage of tracer that is oxidized. Since the fit of the $^{14}\text{CO}_2$ data is the end point of both analyses and the experimental data cover an extended period of time, this value should be relatively insensitive to the model used (12, 13). The difference in treatment of the bicarbonate pools in the two analyses does result in a significant difference in the intermediate compartment (Fig. 4, compartment 7), which had to be introduced between plasma FFA and total body or plasma bicarbonate to allow good fit of the data. This compartment tends to act as a "buffer," and as long as no restraints are placed on its size or fractional turnover rate, it may be assigned values in a computer analysis which compensate for any differences introduced in the bicarbonate compartments in the model. Thus, it is possible to obtain excellent fits for all of the data at all times using either the previously published or the present model. While both models should give approximately the same value for λ_{71} , one or both of the models will yield erroneous values for Q_7 and for λ_{47} (Fig. 4). Furthermore, if either Q_7 or λ_{47} were known, it would not be possible to fit the present data with both the previously published and the present models.

TABLE 5 RATE CONSTANTS OF FFA METABOLISM

	λ_{01}	λ_{12}	λ_{21}	λ_{34}	λ_{45}	λ_{46}	λ_{47}	λ_{54}	λ_{64}	λ_{71}	Cumulative $^{14}\text{CO}_2$ Excretion at 2.5 hr*
E. S.											
Fasting	4.9	0.46	5.1	2.5	0.07	5.2	2.1	2.7	31.6	4.8	13.6
Glucose-fed	7.6	0.41	12.9	5.2	0.07	4.2	2.8	1.8	25.8	3.0	10.4
H. D.											
Fasting	5.4	0.57	8.1	5.0	0.36	6.8	1.7	5.5	28.6	3.4	13.6
Glucose-fed	3.8	0.34	22.8	5.2	0.07	4.6	2.0	3.2	25.9	4.2	10.0
P. R.											
Fasting	3.6	0.55	8.5	3.4	0.24	5.5	3.1	3.0	31.0	2.4	11.1
Glucose-fed	2.2	0.27	13.0	6.0	0.47	5.0	2.1	4.5	23.2	1.5	7.2
R. D.											
Fasting	11.5	0.37	5.3	4.8	0.47	5.9	1.6	3.0	30.4	3.5	10.9
Glucose-fed	16.2	0.27	12.6	5.6	0.11	4.6	1.7	2.3	26.1	2.2	4.9

* Percentage of injected palmitate-1- ^{14}C .

TABLE 6 FLUX OF FFA AS DETERMINED BY COMPARTMENTAL ANALYSIS

	Q_1^*	$k_{01} \dagger = \lambda_{01} Q_1$	$k_{21} = \lambda_{21} Q_1$	$k_{71} \ddagger = \lambda_{71} Q_1$	$IDR \S = k_{01} + k_{71}$
	<i>mmoles</i>	<i>mmoles/hr</i>		<i>mmoles/hr</i>	
E. S.					
Fasting	2.32	11.3	11.8	11.9	23.1
Glucose-fed	1.0	7.58 (33%)	13.0	3.01 (75%)	10.6
H. D.					
Fasting	3.16	17.0	25.6	10.8	27.8
Glucose-fed	1.30	4.98 (71%)	29.6	5.51 (49%)	10.5
P. R.					
Fasting	2.01	9.2	21.9	6.29	15.6
Glucose-fed	1.05	2.32 (75%)	13.7	1.56 (75%)	3.88
R. D.					
Fasting	2.01	23.1	10.6	6.99	30.1
Glucose-fed	1.19	19.3 (17%)	15.0	2.61 (62%)	22.0

* Plasma FFA pool size = plasma FFA concentration times the initial volume of distribution of the tracer.

† Net outflow (mmoles/hr) of plasma FFA other than by direct oxidative pathways—assumed to represent irreversible rate of esterification (7).

‡ Amount of FFA oxidized per hr.

§ Irreversible disposal rate—net esterification plus oxidation.

|| Percentage of inhibition in parentheses.

As reported previously (1), glucose feeding inhibited the rate of FFA oxidation to CO₂. To some degree this was evident from the diminished cumulative output of ¹⁴CO₂ after injection of palmitate-¹⁴C in these glucose-fed subjects (Table 5). However, the fractional rate of oxidation (λ_{71}) (Table 5) was inhibited to a smaller degree than the net rate of oxidation expressed as amount of FFA oxidized to CO₂ per hr ($k_{71} = Q_1 \lambda_{71}$) (Table 6). The latter flux was inhibited an average of 65% in the four subjects studied. Although the inhibition of FFA oxidation by dietary glucose is well-known (9), the dramatic and rapid effect of glucose feeding would have been underestimated if a mathematical analysis had not been carried out, for only when changes in plasma FFA pool sizes were taken into account were the effects of glucose on FFA oxidation seen to be pronounced and consistent.

There are other pieces of information not included in the original paper which become apparent from this analysis of the data. The irreversible disposal rate, which includes losses other than that from oxidation, is consistently reduced by glucose feeding (Table 6). This signifies that net plasma FFA production is promptly decreased by the ingestion of glucose, a fact well established by the work of others (14). Our analysis indicates that a direct proportionality may exist between pool size and irreversible disposal rate, such as has been noted by Baker and Rostami in rats (15) and by Armstrong et al. in dogs (16). Earlier studies of fed and fasted humans under different conditions failed to indicate such a relationship (9).

There is some evidence that the irreversible loss that is not accounted for by oxidation represents irreversible esterification or net esterification (7). One might entertain the possibility of a steady state such that newly esterified fatty acids replace other esterified fatty acids that are being oxidized, and that this value (k_{01}) is an indirect, crude indicator of the oxidation rate of the large pools of esterified fatty acids (7). One cannot measure the rate of oxidation of these esterified fatty acids directly since they give rise to CO₂ with very low specific activity under the present experimental conditions. However, if one accepts the above speculation the data indicate that glucose feeding inhibits the oxidation of esterified fatty acids (see k_{01} , Table 6, with average inhibition of 49%) as well as that of FFA. It is of interest that calculation of the oxidation of unlabeled fat stores by an entirely different means (1) gave nearly identical values, with a mean for the fasting state of 13.5 meq/hr and for the glucose-loaded state of 8.54 meq/hr. The calculated decrease in the rate of plasma FFA esterification shown here appears paradoxical to the known increase of FFA esterification with glucose administration to adipose tissue (17). It seems most likely that the plasma FFA in intact subjects are not subject to the same control mechanism as those which regulate esterification of intracellular fatty acids *in vitro*.

Finally, a question of some theoretical interest is posed, and that involves the size and turnover rate of the intermediate pool between extracellular FFA and plasma bicarbonate. By inference, this pool should include the portion of intracellular FFA that is available for oxida-

tion. According to our calculations, the size of this pool in the fasting state is 2–6.4 meq and in the fed state 0.74–2.7 meq [in the original calculations (1), the size of this pool was about 1 meq in the fasting state and 0.2–0.6 meq in the fed state]. Glucose feeding seemed to induce a marked reduction in the size of the intermediate pool in each individual subject. There is no way in which our currently available data from human beings can be used to determine directly the nature and actual size of the intermediate pool.

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