Oxidation of Plasma FFA in Lean and Obese Humans

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The rate of oxidation of plasma FFA (palmitate, oleate and linoleate) was measured in 9 lean and 14 obese humans by infusing palmitate-1-^{14}C at a constant rate for 3–7½ hours. The ratio $^{14}CO_2$ specific activity divided by the specific activity of FFA rose in the form of an exponential curve which could be described by an equation consisting of three terms. This equation was established with the help of $NaH^{14}CO_3$ administered to lean and obese subjects in the form of a single dose or in constant rate infusions for 8 hours. Using the asymptotic values, it was shown that in lean persons the per cent contribution of FFA to the exhaled CO_2 rose with the plasma level of FFA in a straight line fashion. In 7 of the obese individuals, the values lay above the one standard deviation of the regression line. There was no evidence in support of an impaired oxidation of plasma FFA in obesity. The $^{14}CO_2$ output divided by the infusion rate of the label (the per cent recovery of radioactivity represents the fraction of turnover rate which is immediately oxidized. This is rather constant, independent of the plasma FFA level, and it cannot be used as a measure of the rate of oxidation of FFA. (Metabolism 17: No. 1, January, 62–73, 1968)

^{14}C-LABELLED SUBSTRATES (glucose or free fatty acids) have often been used to estimate the rate of their oxidation in men and animals by measuring the rate of appearance of $^{14}CO_2$ in the exhaled air. However, the evaluation of the $^{14}CO_2$ output and the computation of the rate of oxidation present certain difficulties. Both the specific activity of CO_2 ($^{14}CO_2$ SA) and, in steady-state, $^{14}CO_2$ output rise in the form of an exponential curve approaching an asymptotic value. This rise is due to a slow exchange between the labelled CO_2 and the unlabelled bicarbonate pool. The smaller the animal, the higher the rate of exchange or the fractional turnover rate of the pool. The most unfavorable situation arises in humans in whom the CO_2 production (mmole/min.) represents a smaller fraction of the body's bicarbonate pool than in the dog or the cat. This problem was sometimes completely overlooked, and the

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exhaled radioactivity was simply expressed in per cent of the administered dose. Baker et al.[1] studied the rate of oxidation of glucose in humans after a single injection of glucose-^{14}C. The calculations were based on the assumption that the arising $^{14}CO_2$ entered a single bicarbonate pool, the fractional turnover rate of which was either measured with the help of Na $H^{14}CO_3$ or computed from the fractional turnover rate of plasma glucose. Havel et al.[2] infused albumin-bound palmitate-1-^{14}C at a constant rate for 4 hours and estimated the oxidation of plasma free fatty acids (FFA) from the $^{14}CO_2$ output measured at the end of the experimental period. More recently, Shreeve[3] attempted to circumvent the problem by measuring not only the exhaled $^{14}CO_2$ derived from the oxidation of glucose-1-^{14}C but also by estimating the total body water after an injection of tritiated water and assuming a bicarbonate pool of 20 mmole per liter of body water. The sum of the amount of $^{14}CO_2$ expired in the breath and of that present in the HCO_3^- pool constituted a percentage of the dose of glucose-1-^{14}C. It was concluded that the "production of $^{14}CO_2$ among obese patients is only 50 per cent that of non-obese subjects."

The most detailed studies of the problem of interpretation of $^{14}CO_2$ output comes from experiments on cats[4] and dogs.[5] Steele[4] and Steele et al.[5] found in both species that the retention of an intravenous dose of $NaH^{14}CO_3$ can be expressed as a sum of 3 exponential terms. "This means that there are three compartments in the HCO_3^- pool whose separate influences on $^{14}CO_2$ excretion can be distinguished."[5] The equation provided for the dog was applied successfully in studies using glucose-^{14}C[5,6,7] or palmitate-1-^{14}C[6,7] in the form of constant rate infusions to calculate the asymptotic value of the specific activity of $^{14}CO_2$. This asymptote ($^{14}CO_2SA\,t = \infty$) represents the ideal situation when the SA of HCO_3 pool is equal to the SA of CO_2 arising from tissue oxidation. No such equation is available for studies on humans. The aims of the present investigations were: using $NaH^{14}CO_3$ in the form of a single dose and also in continuous infusion to establish the equation which can describe the curve of recovery of $^{14}CO_2$, using radiopalmitate in form of constant rate infusion of 5–7½ hours' duration to find the equation which can be applied when the $^{14}CO_2$ arises from tissue oxidations, and then to compare the rate of oxidation of FFA found in lean persons with that in obese individuals.

Methods

Subjects. Fourteen lean male subjects (body weight 65–88 Kg.) and 20 obese subjects (4 males and 16 females) participated in this study. The body weight of the obese subjects ranged from 93 to 183 Kg. This represented 60–130 per cent overweights (according to data of the Metropolitan Life Insurance Company). The weight/height ratio (W/H) of the lean individuals was 0.38–0.48 and that of the obese persons ranged from 0.58–0.95. Eleven of the lean subjects were living on a standard diet of 3200 Cal./day with 72 Gm. protein. Three persons who participated only in experiments with $NaH^{14}CO_3$ were on an uncontrolled diet, and they reported at our Metabolic Ward the day before the experiment. The obese subjects were either on a standard diet or on various hypocaloric diets with 800 Cal./day and 40 Gm./day protein as previously described.[8] All subjects except those lean persons who received palmitate-1-^{14}C infusion entered the experiment after 18 hours' fasting. In the lean group, fasting of various duration (12, 18, 36, 48, and 72 hours) was used to obtain a wide range of plasma FFA suitable to study the correlation between plasma concentration and oxidation of FFA.

Experimental procedure. In a highly ventilated, constant temperature room (20° C) the subject lay on a bed, and a polyethylene catheter was placed in the antecubital vein; when radiopalmitate was used as a tracer, a catheter was also placed in the femoral artery. A plastic hood with a polythene curtain was placed over the head of the subject and an air flow (50–60 l./min.) accurately measured by a flow meter (precision rotameter) was maintained by a pump. At time intervals, duplicate samples of the diluted expired air were collected in Douglas bags and analyzed for O_2 and CO_2 content by means of a calibrated Noyons diaferometer.[9]

Two types of experiments were conducted with $NaH^{14}CO_3$. At zero time ($t = 0$) about 100 μc. (specific activity 4.7–4.8 μc./mmole) were infused for two minutes with the help of a calibrated Harvard infusion pump. Following this, the catheter was removed and air samples were collected at $t = 10, 20, 30, 45, 60$ minutes as shown in Fig. 1. The $NaH^{14}CO_3$ was infused at a constant rate of about 0.2 μc./min. for eight hours, and air samples were collected every 30 minutes for the first three hours, then every hour until the end of the infusion. In experiments with radiopalmitate, the procedure was the same as described previously.[8] At $t = 0$, a priming dose of albumin-bound palmitate-1-^{14}C (specific activity about 10 μc./μEq.) was given intravenously followed by the constant rate infusion for 3–7½ hours. The ratio between the priming dose (mμc). and infusion rate (mμc./min.) was always 10:1, but the total amount administered was adjusted to the duration of the experiment so that it should not exceed 80 μc.

When the experiment lasted for only 3–4 hours, air and arterial blood samples were collected at 30-minute intervals. In long experiments, samples were collected every 60 minutes during the first four hours and every 30 minutes for the duration of the experiment.

Measurements. The specific activity of CO_2 in the expired air was measured according to the method of Fredrickson and Ono[10] adapted to our conditions as previously described.[11] After the readings on the Noyons diaferometer, the diluted air samples were bubbled through hydroxide of hyamine; 1 ml. of this was then titrated with 0.1 N HCl and 2 ml. dissolved in toluene containing PPO and dimethyl POPOP as scintillators. Three or four times during the experiments, room air samples were collected to serve as blanks for both titration and counting. When $NaH^{14}CO_3$ was the tracer, the exact amount of radioactivity given was measured by adding 50 μl. infusate to the hyamine-toluene mixture with the help of an ultra micro-syringe.

In experiments with radiopalmitate, the plasma FFA concentration[12] and the radioactivity in the FFA fraction were measured as described in our previous paper.[8] All radioactivities were counted in a Tri-Carb liquid scintillation counter and, using toluene ^{14}C as internal standard, they were corrected for detection (quenching plus counting, efficiency. In all but three experiments, the plasma was extracted with Folch reagent (2 parts chloroform:1 part methanol v/v) and the fatty acids were separated on silicic acid column according to McCarthy and Duthie.[13] After methylation, the FFA composition of each sample was estimated by means of a Barber-Coleman gas liquid chromatographic unit.[14]

It was assumed that the palmitate-1-^{14}C was a representative tracer for the three major free fatty acids, palmitate, oleate, and linoleate, the fractional turnover rate of which was found to be very similar by Fredrickson and Gordon.[15] Therefore, the radioactivity in the FFA fraction of the plasma was related to the sum of these three fatty acids and the SA was expressed as mμc./μEq.

In an average of 21 experiments, these three FFA represented 81.5 per cent (SD = ±6) of the total titratable FFA; this figure was used in those three experiments where no gas-chromatographic analysis was carried out.

Calculations. In steady state, the infusion rate of the radiopalmitate (mμc./min.) divided by the FFA SA gives the turnover rate (mEq./min.) of the three above-mentioned fatty acids. The estimation of the rate of oxidation must be based on the asymptotic value of CO_2SA ($t = \infty$). Once this is known, $CO_2SA_t = \infty$ multiplied by 1.7 (assuming C_{17} as an average of C_{16} and C_{18} fatty acids) and divided by the FFA SA, we obtain the per cent CO_2 derived from the immediate oxidation (via the most direct pathways) of the three fatty acids. This value multiplied by the CO_2 output and divided by 1700 gives the rate of oxidation of FFA (mmole/min.), which can then also be expressed as per cent of the turn-

over rate. This latter value can also be obtained from the ratio 100 × $^{14}CO_2$ output divided by the infusion rate of the label; it represents the per cent recovery of radioactivity in the exhaled air.

Results

Experiment with $NaH^{14}CO_3$

Two lean subjects received $NaH^{14}CO_3$, 98 or 102 μc., respectively, in the form of a rapid, 2-minute infusion, and the $^{14}CO_2$ output was measured for 480 minutes. When the fraction of the dose (I) recovered (R) was plotted against the time (t), an exponential curve was obtained (Fig. 1A) which, for any practical purpose, could be described by the general formula:

$$\frac{R}{I} = 1 - K_1e^{-\lambda^1 t} - K_2e^{-\lambda^2 t} - K_3e^{-\lambda^3 t}.$$

By plotting the natural logarithm of the amount of radioactivity present in the body $\left(1 - \frac{R}{I}\right)$ at any given time against t, it became obvious that the part of the curve obtained in the last 3 hours (between 300 and 480 minutes) was essentially defined only by the third term of the equation, λ_3 being the slope constant and K_3 the anti-ln of the Y intercept (Fig. 1B). Then the values of the third term can be calculated for the entire length of the experiment and subtracted from $1 - \frac{R}{I}$. The procedure was then repeated to obtain K_2 and λ_2. Theoretically, if K_2 and K_3 are known, K_1 can be calculated from $K_3 = 1 - K_2 - K_3$ (at $t = 0$, $\frac{R}{I} = 0$) and the slope constant (λ_1) of the first term can be determined, or one may follow the method of subtraction to obtain K_1 and λ_1. It should be noted that this latter procedure leads to a slightly lower value of K_1 because the general formula represents only a close approximation of the exponential curve which could be defined more accurately by more than three terms. The continuous line shown in Fig. 1A was based on the average values obtained on the two lean persons (x), and the equation which defines the curve has been given in the legend of Fig. 1. For the sake of comparison, Fig. 1A also shows the recovery curve obtained in the dog after a single injection of $NaH^{14}CO_3$ (dotted line) as constructed from the equation of Steele et al.[5] The difference is quite striking. Due to the higher fractional turnover rate of than in the human. Two experiments of this type were carried out on obese the HCO_3^- pool, the asymptote is approached in the dog much more rapidly subjects (o). In one obese subject, the measured values closely followed the line obtained on lean persons; in the other, the recovery of radioactivity proceeded more slowly than required by the equation. In the second half of the experiments, one obese subject had values slightly above, the other slightly below, the asymptotic curve.

When the $NaH^{14}CO_3$ was infused throughout the 480 minutes at a constant rate, the course of the average recovery curve (Fig. 2, curve 1) was very similar to that obtained with the single-dose technique. In this case, R represented the rate of $^{14}CO_2$ output and I was the infusion rate, both in mμc./min. Although in this experiment the ratio $\frac{R}{I}$ rose slightly faster and at the eighth hour

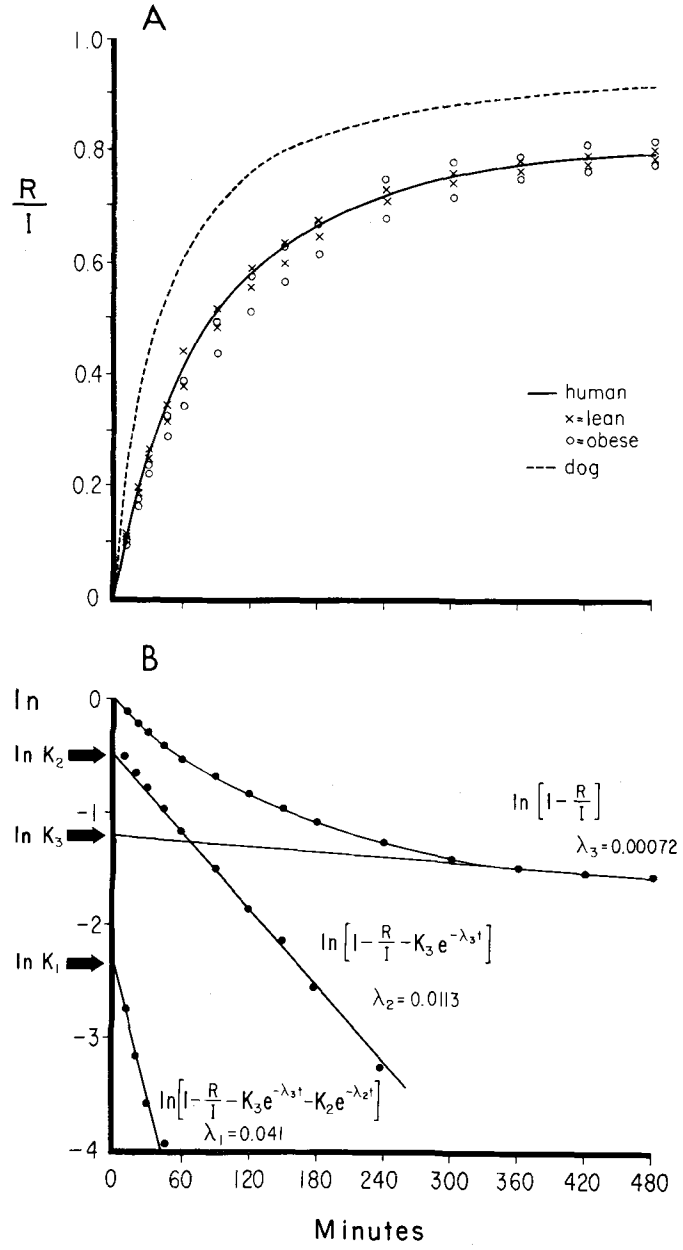

Fig. 1.—Recovery of $^{14}CO_2$ in the exhaled air following the intravenous injection of 100 μc. $NaH^{14}CO_3$. (A) Ordinate $\frac{R}{I}$ is the fraction of the dose recovered. Continuous line: average of two experiments on lean subjects (x), B.W.: 60 and 64.3 Kg., respectively. Values obtained on two obese subjects (B.W.: 96 and 102 Kg.) shown by circles (o). Broken line: recovery curve in the dog based on the equation of Steele et al.[5]: $\frac{R}{I} = 1 - 0.096e^{-0.26t} - 0.684e^{-0.019t} - 0.2204e^{-0.0019t}$. (B) Curve analysis of the continuous line shown in (A). Ordinate: natural logarithm. For details, see text. The

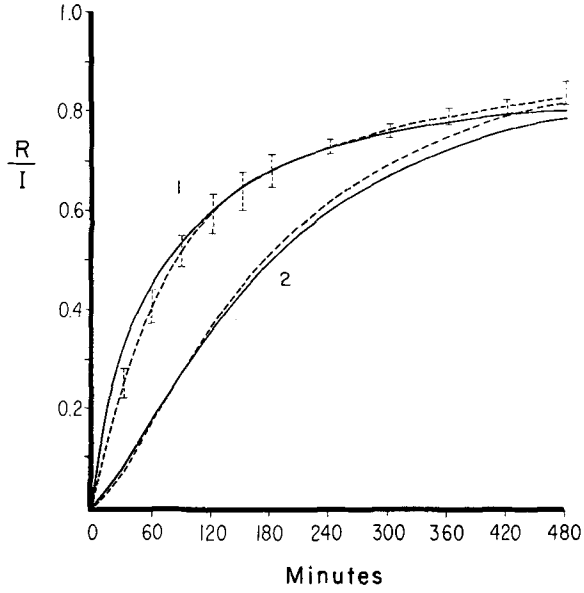

Fig. 2.—Curve 1: recovery of $^{14}CO_2$ in the exhaled air during the infusion of $NaH^{14}CO_3$ at a constant rate of 0.2 mμc./min. for 8 hours. $\frac{R}{I}$ is the fraction of infusion rate exhaled. Continuous line represents the average of experiments on three lean subjects. Broken line represents the means and the standard deviations $\left(\begin{matrix}\text{T}\\ \text{I}\\ \text{I}\\ \text{I}\\ \text{I}\end{matrix}\right)$ obtained on four obese persons. For the lean subjects (from $t = 30$ to $t = 480$): $\frac{R}{I} = 1 - 0.512e^{-0.0144t} - 0.360e^{-0.0013t}$. For the obese subjects: $\frac{R}{I} = 1 - 0.590e^{-0.015t} - 0.390e^{-0.0018t}$. Curve 2 shows the course of $^{14}CO_2$ SA in the exhaled air during the constant rate infusion of palmitate-1-^{14}C. At $t = 0$, 10 times the infusion rate was given as priming dose. $\frac{R}{I} = {}^{14}CO_2\ SA_t/{}^{14}CO_2\ SA\ t=\infty$. Continuous line: mean of five lean subjects $\frac{R}{I} = 1 + 0.343e^{-0.0185t} - 0.983e^{-0.0081t} - 0.360e^{-0.0013t}$. Broken line: mean of four obese subjects $\frac{R}{I} = 1 + 0.311e^{-0.0215t} - 0.921e^{-0.0082t} - 0.390e^{-0.0018t}$. For details and curve fitting, see text and Table 1.

reached a value (0.81) slightly higher than that obtained with a single dose (0.79), the difference was not significant. There was no marked difference between the lean group (3 subjects) and the obese group (4 subjects). Apart from the first 3 hours, during the rest of the experiments the average recovery curve of the lean group was within the standard deviations (dotted vertical

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approximate equation of the curve in (A): $\frac{R}{I} = 1 - 0.097e^{-0.041t} - 0.597e^{-0.0113t} - 0.299e^{-0.00072t}$

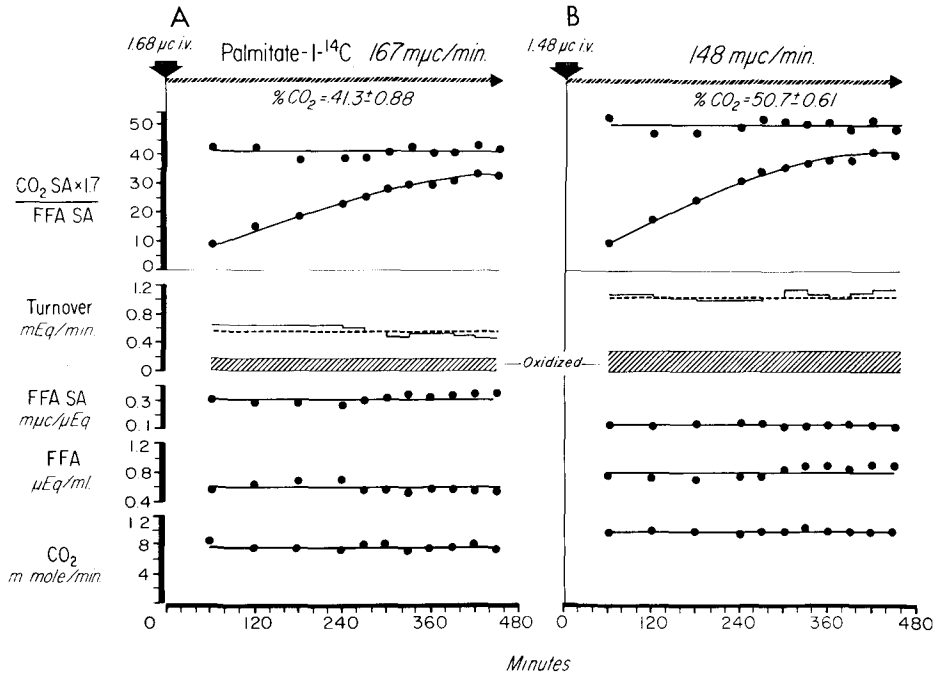

Fig. 3.—Estimation of FFA turnover, the per cent contribution of FFA to the exhaled CO_2, and the rate of oxidation of FFA. (A) lean subject (B.W. 74 Kg.); (B) obese subject (B.W. 119 Kg.).

lines in Fig. 2) of those obtained in obese persons. Nevertheless, the exponential equations which defined the recovery curves between the 30th and the 480th minutes were calculated separately for the two groups of subjects (see the legend of Fig. 2). Since no measurements were made between $t = 0$ and $t = 30$, these equations have only the second and third terms of the general formula as they were calculated by the method of subtraction described above.

Experiments with Radiopalmitate Infusions

Unlike the experiments with $NaH^{14}CO_3$, here the influx of $^{14}CO_2$ into the bicarbonate pool is not known, and this depends not only on the rate of oxidation of FFA but also on their specific activity in the plasma. If the latter is reasonably constant (± 10 per cent), the ratio CO_2 SA/FFA $\overline{SA}$ can be plotted against time, and the resulting curve (Fig. 3) rises in the first 3 hours almost in a straight line; then it begins to approach an asymptotic value. In order to estimate the asymptote, it was necessary to assume that after $t = 450$, the third term of the equation defines the shape of the exponential curve and that this curve is the same as found with the constant rate infusion of $NaH^{14}CO_3$. At this advanced phase of equilibrium (when the hourly increment of CO_2SA is already close to the limit of error), the fraction of $^{14}CO_2$ influx which is recovered in the exhaled air can approach but it cannot exceed the value of $\frac{R}{I}$ obtained when the $^{14}CO_2$ influx was provided by $NaH^{14}CO_3$ infusion. It should be mentioned that this phase was reached in the dog at about $t = 200$ and that

the third term of the equation was found to be the same whether the $^{14}CO_2$ arose in the blood compartment or in the "soft tissue compartment of the body bicarbonate pool" from the oxidation of labelled glucose.[5] This assumption then set $\frac{R}{I}$ at $t = 450$ at a maximum of 0.80 in the lean and at 0.83 in the obese subjects. Therefore, $I = \frac{CO_2SA_{t=450}}{0.8 \times FFA\,\overline{SA}}$ and $\frac{R}{I}$ can be calculated for each measurement at any given time. Then by plotting the natural logarithm of the expression $\left(1 - \frac{R}{I} - K_3 e^{-\lambda_3 t}\right)$ against t, one obtains a straight line for the intervals from $t=180$ to $t=420$. When λ_2 and K_2 are calculated, it becomes evident that K_1 must be positive ($K_2 + K_3 > 1$). This may indicate that in certain compartments with high fractional turnover rate of the HCO_3^- pool (tissues with high metabolic rate), the SA of CO_2 rises more rapidly than in others, and the $^{14}CO_2$ enters not only the exhaled air but also compartments with a relatively slow turnover rate. The result is a straight-line increase of CO_2SA in the first 3 hours (Fig. 2, curve 2).

Once the equation is known (see legend of Fig. 2), the $CO_2SA_{t=\infty}$ can be calculated from each measurement of CO_2SA made at any t:

$$CO_2SA_{t=\infty} = \frac{CO_2SA_t}{\left(\frac{R}{I}\right)_t}$$

and the mean can be used to calculate the per cent CO_2 derived from the direct oxidation of FFA (see above).

Figure 3 shows two long experiments, one on a lean individual and one on an obese subject. Four samples were collected in the first 4 hours and 7 between hours 4–7½. The ratio $1.7 \times CO_2SA_{t=\infty} / FFA\,\overline{SA}$ (= per cent CO_2) was calculated for each sample, and the overall average was used to estimate the rate of oxidation of plasma FFA. In the lean person, at an average FFA level of 0.6 μEq./ml. 41.3 ± 0.9 per cent CO_2 derived from the immediate oxidation of about 0.19 mEq./min. FFA which represented 34.5 per cent of the turnover rate. The corresponding values for the obese individual were plasma FFA:0.81 μEq./ml., per cent CO_2 from FFA: 50.7 ± 0.6, the rate of oxidation of FFA: 0.29 mEq./min. or 27.4 per cent of the turnover.

Table 1 summarizes the results obtained in nine experiments of this type. As a measure of curve fitting (validity of the two equations in the legend of Fig. 2), the table compares the per cent CO_2 calculated from measurements made in the first 4 hours with the measurement obtained after the fourth hour. The mean difference proved to be 1 per cent CO_2 with a standard deviation of ±2.7.

On the basis of these findings, four additional experiments of 3–4 hours' duration were conducted on lean subjects and ten more on obese individuals. These experiments are more comfortable for the subjects and have the additional advantage that the radiopalmitate can be infused at a higher rate and that the chances of achieving a constant level and SA of FFA in the plasma are better than in long experiments. The disadvantage of having to make a rather large correction to obtain the asymptotic value could be reduced by cal-

Table 1

		FFA*		$1.7 \times CO_2SA_{t=\infty}$/FFA SA					CO_2 Output	FFA Oxidized	
Weight Kg.	W/H Kg./cm.	Level μEq./ml.	Turnover mEq./min.	1-4 hr. mean	(a) ±SE	4-7½ hr. mean	(b) ±SE	a−b	mmole/min.	mEq./min.	Per cent of Turnover
Lean Subjects											
73.9	0.41	0.60	0.55	41.4	2.37	41.2	0.63	−0.2	7.64	0.19	34.5
86.7	0.46	0.61	0.59	36.7	0.39	38.1	0.21	−1.4	11.00	0.24	41.4
71.3	0.41	0.92	0.90	56.3	1.90	52.6	0.43	+4.3	8.93	0.29	31.7
65.5	0.38	0.92	0.80	61.1	0.56	63.9	0.62	−2.8	6.63	0.24	30.0
88.1	0.46	1.21	1.21	65.7	0.51	66.7	0.84	−1.0	10.19	0.40	33.2
Obese Subjects											
125.7	0.78	0.60	0.85	60.4	1.57	58.6	1.30	+1.8	10.57	0.37	43.0
96.6	0.59	0.80	0.66	50.0	0.77	54.3	0.94	−4.3	6.31	0.20	29.6
118.6	0.76	0.81	1.07	49.8	1.39	51.2	0.55	−1.4	9.82	0.29	27.4
96.0	0.60	1.01	0.82	51.9	2.17	55.9	0.51	−4.0	7.71	0.24	29.8
								Mean	−1.00		
								SE	±0.91		

*Palmitate, oleate and linoleate.

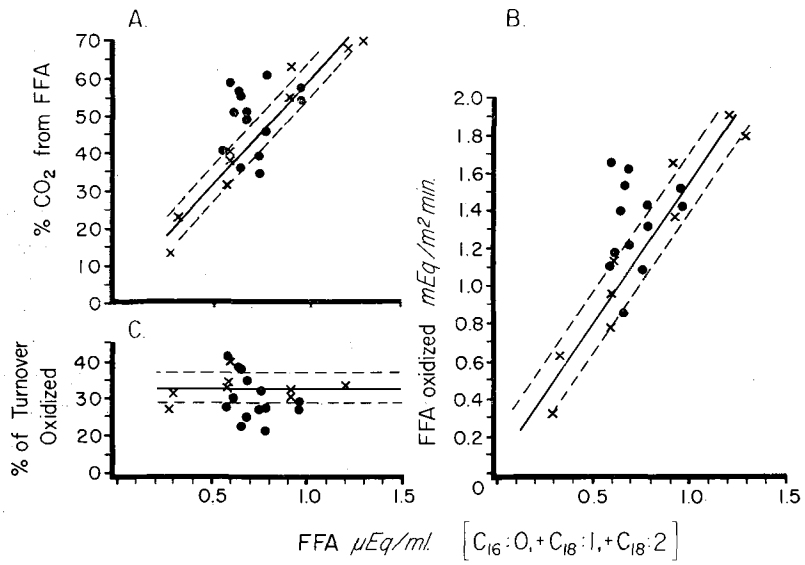

Fig. 4.—(A) Correlation between plasma FFA level (palmitate, oleate, and linoleate) and per cent CO_2 derived from FFA. Regression line obtained in lean subjects (x) $Y = 3.03 + 55.4x$. Standard error (SE) of the slope constant: 55.4 ± 5.04, $p < .001$. Broken line: one standard deviation (SD) of the estimation (± 4.8%). Circles (•) represent the values of obese subjects. (B) Correlation between plasma FFA level and the rate of oxidation of FFA. Regression line refers to the lean subjects (x); $Y = 0.005 + 0.148x$, SE of the slope constant: ± 0.017; SD of the estimation (broken line): ± 0.015 mEq./m^2 min. (•): obese subjects. (C) Per cent of turnover oxidized plotted against plasma level of FFA. The lines represent the mean ± SD obtained in lean subjects (x); values of obese persons are shown by circles (•).

culating the per cent CO_2 from at least six measurements. The mean was then accepted when its standard error did not exceed ±2 per cent CO_2 from FFA.

In humans as in dogs,[9] the contribution of FFA to the exhaled CO_2 greatly depended on the plasma FFA level (Fig. 4A). Applying the method of least squares to the data obtained on lean subjects (x), the regression coefficient (slope constant) of the straight line correlation between the per cent CO_2 and the plasma FFA proved to be 55.4 ± 5.05 (SE). When the rate of oxidation expressed as mEq./m^2 surface area/min. was plotted against the FFA concentration (Fig. 4B), the resulting line had a regression coefficient of 0.148 ± 0.017. In both cases, the value of $p < .001$ for the null hypothesis that there was no correlation between the two variables.

The fraction of FFA turnover which is immediately oxidized seems to be rather constant and independent of the plasma FFA level (at least within the range studied). For the lean group, the mean ±SE proved to be 32.6 ± 1.03 per cent, and for the obese subjects, 30.5 ± 1.4; the difference is not significant (Fig. 4C).

Since in obese subjects the turnover rate is significantly higher than in the lean persons at the same plasma level of FFA,[8] it is not surprising that in 7 out

of the 14 obese subjects the values of per cent CO_2 from FFA have been greater and outside the standard deviation of the regression line obtained on the lean group.

Discussion

The slow rise of CO_2SA during the infusion of radiopalmitate clearly demonstrates that the calculation of the rate of oxidation must be based on the asymptote; otherwise it would lead to erroneously low values. The interaction of more than a single bicarbonate pool is also evidenced by the course of the CO_2SA, and the graphic analysis showed that the introduction of three terms provided an acceptably accurate description of the exponential curve. This finding is consistent with the three compartmental kinetic model suggested by Steele[4] and Steele et al.[5] for the cat and the dog. Because of the great variations in the body composition of our subjects, the equations were established by using $NaH^{14}CO_3$ in the form of a constant rate infusion and by applying the method of curve fitting rather than by giving mathematical considerations to the kinetics of $H^{14}CO_3^-$ in the various pools.

In order to estimate the rate of oxidation, it is also necessary to know and to keep reasonably constant the SA of the substrate in the plasma. Therefore, the single injection technique is not suitable to measure oxidation. After the injection of the label, the SA of the substrate rapidly decreases and the resulting curve of $^{14}CO_2$ SA reflects not only the mixing procedures but also the changes in the substrate SA. The constant rate infusion technique clearly showed that the amount of radioactivity in the exhaled air divided by the infusion rate of the label, i. e., the per cent recovery of radioactivity, is not a measure of the rate of oxidation as often believed. It represents the per cent of turnover oxidized, and this can be the same whether 40 μEq./m^2 min. or 190 μEq./m^2 min. is the rate of oxidation (Fig. 4B).

In contrast to the single injection method,[16] the constant rate infusion technique did not provide any evidence for a diminished oxidation of FFA in obese subjects. In the man as in the dog,[7,9] the rate of oxidation of FFA seems to be the function of the plasma concentration, which in turn depends on the rate of release of FFA from the adipose tissue.[8,17] If obese subjects deviate from these correlations, it seems to be in the upward direction, probably due to their large mass of adipose tissue.

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