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Metabolism of Free Fatty Acids in Obese Humans.* (30002)

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Obesity in humans is of great clinical importance because of its frequency and association with diseases such as diabetes mellitus and arteriosclerotic heart disease. The frequency of familial association and the difficulty in achieving lasting weight reduction have led to suggestions that there is a metabolic defect in human obesity(1). No evidence is presently available to indicate that a metabolic defect is etiologic for obesity in humans but there is evidence that metabolic deviations are present in obese humans. Obese humans, compared to normals, tolerate fasting or a high fat diet with less ketonemia, less nitrogen loss and a slower fall in blood glucose(2). The insulin concentration in the blood of fasting obese subjects is higher than normal and the blood insulin response to a glucose load is exaggerated(3). Glucose uptake by the forearm tissue is elevated but is less responsive to insulin infusion than in normals(4). From studies of recovery of $C^{14}O_2$ in expired air, it has been concluded that oxidation of glucose to CO_2 may be depressed(5). The following studies were undertaken to provide further information on some aspects of FFA metabolism in obese humans. They indicate that in most obese subjects, there is a difference from normal in the disposal of a tracer dose of FFA following glucose administration.

Materials and methods. Studies were conducted on 8 normal volunteers and 5 obese but otherwise normal women (Table I). The obese subjects were studied while in the hospital on a metabolic ward whereas only one of the normal subjects was studied while under hospital observation. No difference was observed between the studies on this subject and the other normal subjects.

Palmitic acid-1-C^{14}† was bound to human serum albumin‡ in a molar ratio of 2.5:1 by addition of KOH to the isotope, heating to 40°C and addition of albumin which resulted in a clear solution. The final concentration of palmitate was approximately 10 μc per ml. Sterility was accomplished by filtration and established by culture. Analysis of the isotope by gas-liquid chromatography revealed only palmitic acid and caprylate; all the radioactivity was present in the palmitate.§

The palmitate oxidation study was performed by continuous measurement of all expired $C^{14}O_2$ for one hour after intravenous injection of 10 μc of 1-C^{14} palmitate(6). A helmet was placed over the head of the subject; a stream of air carried expired air through an ionization chamber and infrared CO_2 analyzer. The specific activity of expired $C^{14}O_2$ calculated for each 10-minute period was expressed as radioactivity (millivolt-minutes) per millimole of CO_2 and plotted against time after injection of the isotope.

Blood samples were collected at intervals of one hour from an antecubital vein before and after the oxidation study. FFA were ti-

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‡ Cutler human serum albumin (salt poor) contains 0.02 M sodium caprylate.

§ Analysis kindly performed by Dr. James Mead.

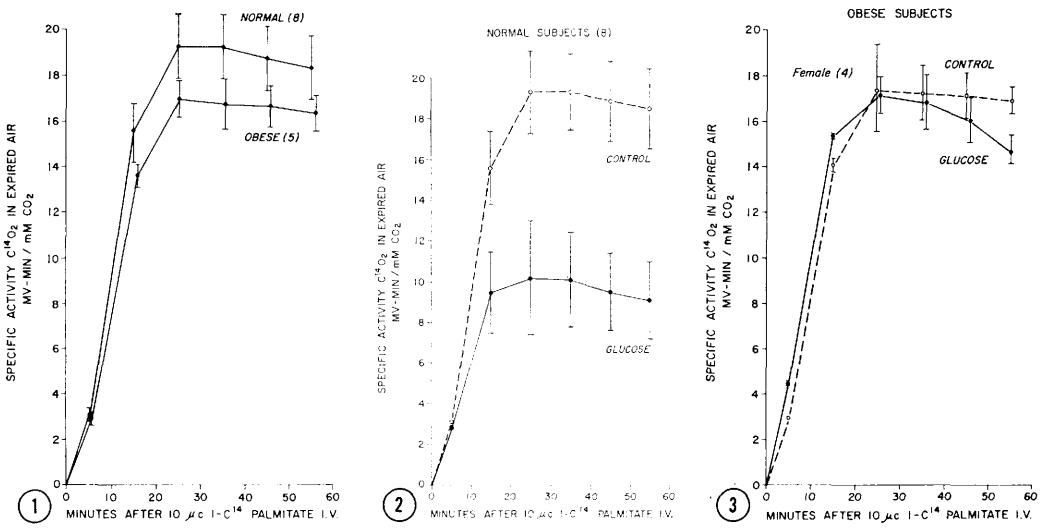

FIG. 1. Specific activity of $C^{14}O_2$ in expired air in normal and obese subjects during the hour following intravenous injection of 1-C^{14} palmitate injected in the post-absorptive state. Each point is mean and standard error of mean for the group of subjects calculated in 10-min periods.

FIG. 2. Specific activity of $C^{14}O_2$ in expired air in normal subjects during the hour following intravenous injection of 1-C^{14} palmitate. Upper curve (control) was obtained in the morning, lower curve 5-6 hr later during 3rd hour of glucose administration. Each point is mean and standard error of mean for the group of subjects calculated in 10-min periods.

FIG. 3. Specific activity of $C^{14}O_2$ in expired air in 4 obese patients during the hour following intravenous injection of 1-C^{14} palmitate. Curves labeled control were obtained in the morning in the post-absorptive state and those labeled glucose, 5-6 hr later during 3rd hour of glucose administration. Each point is mean and standard error of mean for the group of subjects calculated in 10-min periods.

trated by the method of Dole and Meinertz (7) and glucose was measured using glucose oxidase.

The usual experimental design was to perform an oxidation study early in the morning after a standard overnight fast. This will be designated as the control study. A period of 2-3 hours was then permitted for expired $C^{14}O_2$ to fall to a low level. Glucose was then given over a period of 3 hours, 100 g orally during the first hour and 100 g intravenously during the final 2 hours. The second oxidation study was performed during the final hour of glucose administration.

Results. The $C^{14}O_2$ specific activity curves obtained in the morning after a standard overnight fast in normal subjects and obese patients are shown in Fig. 1. Each point is the mean specific activity of the group during each 10-minute period following intravenous injection of 10 μc of albumin bound 1-C^{14} palmitate. There is no significant difference between the curve representing the mean of values for 8 normal subjects compared to that of 5 obese women who averaged 204 lb in weight (Table I).

Fig. 2 compares the $C^{14}O_2$ specific activity curves before and during glucose administration in the normal subjects. The shape of the curve is unchanged but the specific activity of expired CO_2 is greatly lowered by glucose. In Fig. 3 are the specific activity curves before and during glucose for the obese subjects. The curves give mean values for 4 obese women and show that glucose did not significantly lower the peak specific activity. The mean CO_2 specific activity in the interval from 50-60 minutes after palmitate administration appears to be lower after glucose administration but the difference is not significant. In the fifth obese woman, aged 22, who weighed 200 lb, there was a normal response to glucose.

In Table II the range and mean values for total C^{14} recovered in expired air in the hour following injection of C^{14} palmitate are given. The control values for the obese subjects are not different from the normals.

TABLE I. Mean Age and Weight of Obese Patients and Normal Controls. Serum FFA and glucose measured during first 1 hr study in the morning (control) and 5-6 hr later during 3rd hr of glucose.

	Age (yr)	Wt lb	Serum FFA, μEq/l Control	Serum FFA, μEq/l Glucose	Serum glucose, mg/100 ml Control	Serum glucose, mg/100 ml Glucose
Normal (8) Male 5—female 3	27	148	810 ± 92*	330 ± 31	80 ± 21	245 ± 19
Obese women (5)	35	204	1092 ± 76	471 ± 150	82 ± 5	231 ± 48

* Mean ± S.E.M.

After glucose, total recovery of C^{14} did not change in the obese group compared to a decrease of 34% in the normal subjects. Thus in 4 of the 5 obese subjects, glucose administration did not significantly alter the $C^{14}O_2$ specific activity in expired air nor the per cent of injected C^{14} recovered, in contrast to the results in all normal subjects.

Blood glucose levels resulting from glucose administration during the second palmitate oxidation study (Table I) were not different in the 5 obese women compared to the normals nor was mean serum FFA concentration significantly different during glucose administration.

Discussion. The phenomenon of inhibition of fatty acid oxidation following administration of large amounts of glucose was first demonstrated after injection of an emulsion of palmitic acid-1-C^{14} into rats(8) and later was confirmed with albumin-bound palmitate (9). The effect is not restricted to the liver since it can be demonstrated in eviscerated rats(10). Unoxidized fatty acids are held in ester form, mainly as triglyceride, in the liver with a variable amount in muscle and a minor amount in adipose tissue(10,11,12). In fasted animals, very little of injected FFA remains in muscle.

Direct *in vitro* confirmation of the phenomenon of glucose inhibition of fatty acid oxidation has been difficult to obtain(13).

TABLE II. Total Recovery of C^{14} in Expired Air from Injected 1-C^{14} Palmitate.

Subjects	% C^{14} in expired air in 1 hr Control	Glucose	% change
Obese (4)	6.5-10.3 (8.3)*	6.5-11.0 (8.8)*	+ 6
Obese (1)	6.8	4.5	-44
Normal (7)	5.7-10.7 (8.0)*	1.5- 6.8 (5.3)*	-34

* Range and mean of the group.

Some evidence has been obtained with rat diaphragms incubated with palmitate-1-C^{14}. Several days of starvation result in greatly increased oxidation of palmitate-1-C^{14} to $C^{14}O_2$ and addition of glucose to the medium returns this toward normal(14). In diaphragms from younger rats fasted for only 24 hours, glucose concentrations above the physiological range also decreased oxidation of C^{14} labeled albumin-bound palmitate to $C^{14}O_2$(15). The ease of demonstration of inhibition of fatty acid oxidation by glucose *in vivo* is in great contrast to the difficulty of such demonstrations *in vitro*. Prefeeding glucose to rats, however, was followed by inhibition of fatty acid oxidation by liver slices (10). Thus, there seems little question that administration of large amounts of glucose *in vivo* diverts fatty acids to storage sites in liver and muscle and to a minor degree in adipose rather than into an oxidative pathway. The results of glucose administration obtained in normal subjects given here are consistent with findings in experimental animals and with results previously reported in human subjects(16).

The reason for the absence in these obese women of the usual inhibition of fatty acid oxidation by glucose is not apparent. Glucose administration is followed by a fall in circulating FFA indicating normal inhibition of FFA release from adipose tissue. The tracer of palmitate is thus mixed in a smaller pool of circulating FFA in both groups. Disposal of the tracer of palmitate after entry into the muscle cells and liver will determine the amount of the tracer appearing in expired air. In the obese subjects, glucose administration did not reduce recovery of the tracer suggesting that there is a defect in the mechanism by which glucose diverts FFA from oxidation to storage in muscle and liver. A

similar lack of response has been noted in normal subjects following growth hormone administration(6). Clinical observations that obese humans have lower respiratory quotients and tolerate fasting and high fat diets better than normals would be consistent with adaptation to more efficient fat utilization. It is suggested that the phenomenon observed in this study is related to adaptation to fat utilization and might explain decreased glucose oxidation observed in some obese subjects designated "metabolic obesity"(5). Accelerated turnover of fatty acids in starvation, diabetes and following growth hormone administration has been associated with inhibition of glycolysis at the phosphofructokinase step in perfused rat heart and incubated diaphragm(17-20).

An explanation for a normal response to glucose in one obese subject is obscure. The only apparent difference from the remainder of the group is age. She is 19 years younger than the remainder of the women. A study of glucose tolerance in obese women revealed that tolerance is usually normal if obesity has been present for less than 11 years but decreased glucose tolerance becomes more frequent up to 18 years and is universally abnormal thereafter(21).

Whether the abnormal response to glucose is truly a characteristic of obesity or an effect produced by negative caloric balance is not certain. The obese subjects were brought into the hospital and studied immediately without prior observation of steady state caloric balance. It is possible that decreased food intake prior to the study not apparent to the investigator could produce the effects seen. Further study will be necessary to clarify this question.

Summary. To study the metabolism of FFA in obese patients compared to normal subjects, measurements were made of recovery of expired $C^{14}O_2$ from oxidation of 10 μc of albumin-bound 1-C^{14} palmitate. Specific activity of $C^{14}O_2$ was normal in 5 moderately obese women. The response to glucose administration was abnormal in 4 of 5 obese patients who failed to show the usual marked decrease in $C^{14}O_2$ specific activity. In contrast, glucose elicited the usual fall in serum FFA. These findings suggest that the adipose tissue response to glucose is normal but there is a defect in the mechanism by which glucose diverts FFA from oxidation to storage in muscle and liver. It is suggested that adaptation to fat utilization might explain the defect.

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