Factors affecting fatty acid oxidation in fat cells isolated from rat white adipose tissue

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Abstract Fat cells isolated from rat epididymal adipose tissue were incubated with albumin-bound [^{14}C]palmitate. Incorporation of ^{14}C into $^{14}CO_2$ and glycerides was measured. Some evidence is presented to suggest that the exogenous palmitate pool is in isotopic equilibrium with intracellular precursors for these metabolic processes. Precautions were taken to minimize dilution of the exogenous palmitate pool by fatty acids released from the cells. $^{14}CO_2$ production from [1-^{14}C]palmitate was 3 times that from [16-^{14}C]palmitate. Octanoate increased this differential oxidation of palmitate carbons and also inhibited palmitate oxidation without similarly affecting esterification. Glucose increased palmitate esterification in cells from fed or starved rats. Insulin potentiated this effect of glucose. Glucose influenced palmitate oxidation in a more complex manner, dependent upon the glucose concentration. Both the observation that esterification constitutes 99% of the metabolic flux of fatty acid and the manner in which glucose, insulin, or starvation influence palmitate esterification and oxidation suggested that factors controlling esterification may alter oxidation as a secondary effect, but not vice versa. It is suggested that oxidation and esterification compete for a single intracellular precursor, possibly extramitochondrial long chain fatty acyl CoA.

Supplementary key words esterification · insulin · octanoate · palmitate · starvation

The importance of white adipose tissue in the overall fatty acid metabolism of the body is well established. Most investigations have concentrated upon the main role of the tissue, the esterification and mobilization of fatty acids. Less attention has been paid to the oxidation of fatty acids, presumably because observed rates of fatty acid oxidation are low compared to esterification. This disparity in the two processes, however, in part reflects the extraordinarily high capacity of adipose tissue to synthesize glycerides and does not necessarily suggest that fatty acid oxidation is a process of no consequence.

There is evidence that fatty acid oxidation in adipose tissue responds to changes in physiological state. The Respiratory Quotient (RQ) of adipose tissue in vitro is significantly decreased by prior starvation (1) and the proportion of CO_2 that arises through oxidation of endogenous substrates (presumed to be fatty acids) decreases with administration of insulin (2) and increases with prior starvation (3). It has been suggested that in starvation adipose tissue has a decreased ability to restrain the oxidation of endogenous fatty acids and that these changes in fatty acid oxidation may have profound effects on lipogenesis from carbohydrate precursors, thus forming one of the bases for the large reduction in fatty acid synthesis observed after starvation (3).

Alterations in two main processes may be considered as being capable of influencing fatty acid oxidation in adipose tissue after starvation or some other physiological change. First, we considered possible adaptations in the activity of a possible rate-controlling step in fatty acid oxidation, carnitine acyltransferase in particular (4). The liver mitochondrial specific activity of carnitine acyltransferase has been found by several workers to be increased in conditions associated with increased fatty acid oxidation (5–8), although some reports are at variance with this (9, 10). In addition, the ratio of long chain acylcarnitine to free carnitine concentrations has been found to be increased 10-fold in adipose tissue after 48 hr of starvation (11). We recently found however that fat cell mitochondria from starved rats do not show a comparable adaptivity in carnitine acyltransferase specific activity and that mitochondria from starved rats have unchanged activity in carnitine-dependent oxidation of palmitoyl CoA and also have decreased activity in palmitoyl carnitine oxidation (8). Second, we have considered the possibility that adaptation of fatty acid esterification processes, which presumably compete with oxidation for intracellular fatty acyl CoA, may

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influence adipose tissue fatty acid oxidation in starvation. Fatty acid oxidation and esterification appear to be inversely regulated in rat liver (12, 13). Also esterification of fatty acids in whole adipose tissue (14), in adipose tissue cell-free extracts (15), and glyceride synthesis in fat cells (16) are observed to be decreased in starvation.

It was the purpose of the present study to attempt to test the effects of various physiological agents simultaneously upon fatty acid oxidation and esterification in fat cells, and in particular to investigate the adaptations undergone by the fat cell in starvation.

In previous studies assessments have been made of the oxidation of endogenous fatty acids in incubated fat pieces (2, 3). Using the techniques employed in these studies, it is extremely difficult to obtain accurate measurements of this process. We have therefore attempted to quantitate fatty acid oxidation in fat cells by measuring $^{14}CO_2$ production when the cells were presented with ^{14}C-labeled fatty acid in the surrounding medium. This approach leads to certain problems in interpretation of the results. In some cases, these problems appear to be partially resolved. Resolution of other problems awaits further investigation.

MATERIALS AND METHODS

Chemicals

Collagenase (*Clostridium histolyticum*) was from Boehringer Corp. (London) Ltd (London, UK). Triton X-100 and calf thymus DNA (type V, sodium salt, highly polymerized) were obtained from Sigma Chemical Co., Ltd (London, UK). Sodium octanoate and sodium palmitate were from Nu Chek Prep (Elysian, Minn.). Bovine insulin (6 × recrystallized) was obtained from Boots Pure Drug Co Ltd (Nottingham, UK) and anti-insulin serum from Wellcome Research Laboratories (Beckenham, UK). Radiochemicals were from The Radiochemical Centre (Amersham, UK) with the exception of [16-^{14}C]palmitic acid which was from N.E.N. Chemicals GmbH (Frankfurt/Main, Germany). Hyamine hydroxide was from Nuclear Enterprises Ltd (Edinburgh, UK) and 2,5-bis-(5-*t*-butylbenzoxazol-2-yl)-thiophen from CIBA (A.R.L.) Ltd (Duxford, UK). Sodium D-3-hydroxybutyrate was a gift from Professor A. L. Greenbaum of University College London.

Animals

These were either Wistar or Sprague Dawley male rats. Fed animals were maintained throughout on a standard cube diet and weighed 140–200 g at death. Starved animals were within this weight range at the time of withdrawal of food. Animals were supplied with water at all times.

Chemical methods

Bovine serum albumin (fraction V) from Armour Pharmaceutical Co. Ltd. (Eastbourne, UK) was treated with charcoal at pH 3 to remove fatty acids (17), neutralized, dialyzed several times against 10 volumes of distilled water, and lyophilized. Albumin used in all experiments was subjected to this initial treatment.

[^{14}C]Palmitic acid, which was supplied by the manufacturer dissolved in either hexane or benzene, was subjected to a treatment that resulted in appreciable reduction in counting backgrounds. The labeled palmitate was extracted into 50 mM sodium bicarbonate in 1:1 (v/v) ethanol–water. The pH was then adjusted to 3.0 by addition of 2.5 M HCl and the palmitic acid was extracted into hexane, which was then removed by evaporation under N_2 at 50–60°C. The palmitic acid was finally converted to the sodium salt and associated with albumin.

Sodium palmitate (labeled and unlabeled) was associated with defatted bovine serum albumin in 0.15 M NaCl by the method of Evans and Mueller (18). Palmitate used in all experiments was subjected to this initial treatment.

Preparation and incubation of fat cells

Fat cells were prepared by the technique of Rodbell (19). All incubations were carried out at 37°C with shaking in 4-ml volumes of Krebs-Ringer bicarbonate, previously gassed with O_2–CO_2 (95:5), containing albumin and other additions as indicated in individual tables and figures. Where appropriate, [^{14}C]palmitate, [^{14}C]octanoate, or [^{14}C]glucose was present at 0.25 μCi/ml of incubation medium. In all cases incubation flasks were gassed with O_2–CO_2 (95:5) for the first 2–3 min of incubation.

When $^{14}CO_2$ was collected incubations were carried out in sealed 50 ml siliconized Erlenmeyer flasks fitted with glass center wells. All other incubations were in sealed 25-ml siliconized flasks.

Analytical procedures

Collection and estimation of $^{14}CO_2$ was as described by Saggerson and Greenbaum (20). Appropriate blanks were performed in parallel with each experiment to correct for the trapping in hyamine of ^{14}C-labeled substrates made volatile by acidification (21).

When incorporation of ^{14}C into fat cell glycerides

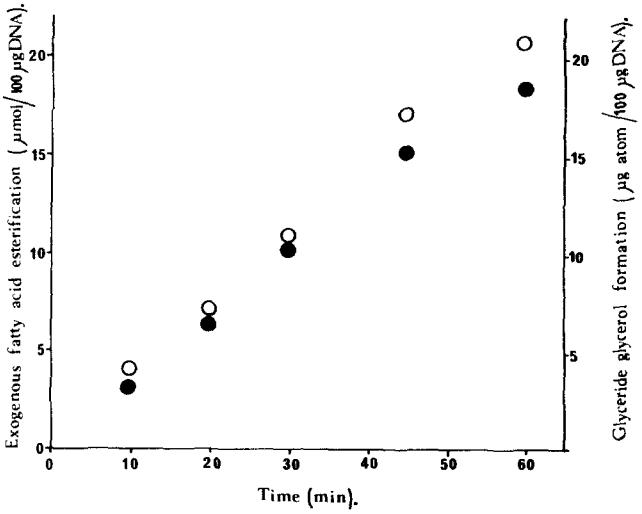

Fig. 1. Time course of glyceride synthesis from exogenous fatty acids. Fat cells from fed rats were incubated with 34 mg/ml albumin, insulin (20 munits/ml) and either 5 mM [U-^{14}C]glucose with 0.5 mM sodium palmitate or 5 mM glucose with 0.5 mM sodium [U-^{14}C]palmitate. The results are the means of three determinations. The incorporation of fatty acid was corrected for changes in specific activity as described in the text. The mean fat cell DNA/ml of incubation flask contents was 3.1 μg. ○, Exogenous fatty acid esterification; ●, glucose incorporation into glyceride-glycerol.

was estimated, lipids from aliquots of fat cells and incubation medium were extracted into hexane (22). The hexane extracts were washed twice with 2 volumes of freshly prepared 50 mM sodium bicarbonate in 1:1 (v/v) ethanol–water to remove labeled free fatty acid (23). Measurement of ^{14}C in glyceride fatty acids or glyceride glycerol was then performed (20, 22). Appropriate blanks were run in parallel with each experiment.

The specific activity of free fatty acids in incubation media was determined after fat cells had been removed by brief centrifugation. Fatty acids were extracted into hexane (22) and aliquots of these extracts were either taken for scintillation counting or were evaporated at 50–60°C, the residues dissolved in chloroform, and estimated for free fatty acid (24) using palmitic acid as a standard.

For determination of fat cell DNA, aliquots of nonincubated cells (equivalent to approximately 1 g dry wt) were extracted with 5 ml cold 5% (v/v) $HClO_4$ and 5 ml diethyl ether. After brief centrifugation the resulting pellet was washed once with 10 ml of acetone and once with 10 ml of diethyl ether (25). The pellet was then dispersed in 2.5 or 3.0 ml of 5% (v/v) $HClO_4$, heated at 70°C for 20 min, cooled, briefly centrifuged and 1 ml of the resulting supernatant was assayed by the method of Burton (26) using hydrolyzed calf thymus DNA as a standard.

Expression of results

In every case where several determinations of a particular parameter are reported, each determination was made in a separate cell preparation. Where presented, statistical significance of results was determined by Student's *t* test.

RESULTS AND DISCUSSION

General considerations

It is a long-established observation that, in vitro, white adipose tissue can both oxidize (14, 27–29) and esterify (14, 30, 31) exogenous ^{14}C-labeled fatty acids. These can therefore potentially be used to label the intracellular precursor pool(s) permitting measurement of rates of esterification and oxidation. There are technical advantages in such use of an external pool, since its size can be determined and monitored to suit experimental requirements. However, this approach requires that the relationship between the specific activities of the extracellular and the intracellular precursor pools can be established. It is known that the intracellular content of fatty acids in adipose tissue is heterogeneous and that only a small fraction of these fatty acids rapidly exchange with extracellular fatty acids (30, 32, 33). This causes difficulty in assessing the specific activity of the intracellular precursor pool used by oxidation and esterification processes.

There is some evidence from studies with incubated fat pieces that the precursor pool for esterification is in isotopic equilibrium with extracellular fatty acids (30, 34, 35). However, with fat pieces, the specific activity of the incubation medium fatty acids does not necessarily reflect that of the immediate extracellular pool unless isotopic equilibration is achieved between the medium and the interstitial spaces of the tissue mass. Use of isolated cells greatly simplifies this problem.

The experiment summarized in **Fig. 1** was performed to establish whether, under our chosen experimental conditions, it was indeed acceptable to assume that the specific activity of medium fatty acids approximated that of an intracellular precursor pool. It may be seen that the incorporation of glucose carbon atoms into glyceride–glycerol closely correlated with the number of moles of exogenous fatty acid incorporated into neutral lipids. Over the 60-min incubation period the specific activity of the incubation medium fatty acid decreased by 31%. The time course of fatty acid incorporation was therefore corrected as follows.

Between each time interval (0–10, 10–20, 20–30, 30–45, and 45–60 min) the increment in [^{14}C]palmitate esterification was determined and divided by the mean specific activity of the medium fatty acid in that time interval to give a corrected increment of fatty acid esterification. Since, under conditions similar to those employed here, approximately 85% of exogenous palmitate label incorporated into fat cell lipids is in triglyceride and most of the remainder is in diglycerides (36), the results shown in Fig. 1 indicate that dilution of the esterification precursor pool by intracellular unlabeled fatty acids must have been negligible and that the specific activity of the extracellular pool can satisfactorily be used to indicate that of the intracellular precursor pool.

Although desirable, it is not possible to apply the same treatment to establish directly that the extracellular fatty acid pool is in isotopic equilibrium with the intracellular precursor pool for fatty acid oxidation. This requires both measurement of the specific activity of CO_2 produced by the cells and an estimation of the CO_2 production that can be attributed to fatty acid oxidation. Calculation of the total amount of CO_2 produced from substrates other than glucose is possible in a bicarbonate-buffered medium, but is complicated, and likely to be inexact (3). We have therefore, of necessity, assumed that the fatty acid oxidation and esterification processes use intracellular precursor pools that are near to isotopic equilibrium or are in fact the same pool. This assumption may be acceptable since palmitoyl group oxidation by fat cell mitochondria is carnitine-dependent (8), implying that fatty acids metabolized in both the esterification and oxidation processes are activated in the extramitochondrial spaces of the cell.

Since, even in the absence of lipolytic agents, incubated fat cells liberate unlabeled fatty acids that can progressively dilute the label of the extracellular pool, the following conditions were generally followed. (*a*) A relatively short incubation time was employed; and (*b*) a high total concentration of palmitate (albumin-bound + free) was used. It was established that, in the presence of albumin, the total extracellular concentration of palmitate could be taken to high levels, apparently without impairment of the cells' ability to esterify or oxidize this substrate (see Fig. 4 and Table 2). (*c*) The fat cell concentration was kept as low as possible to minimize factors that tend to reduce the utilization of exogenous ^{14}C-labeled fatty acids (37). The effect of cell concentration on the oxidation of an initial low concentration of [^{14}C]palmitate is seen in **Fig. 2.** It is uncertain whether this effect is due solely to the release of unlabeled fatty acids that lower the specific activity of the ^{14}C-labeled extracellular precursor as discussed previously (37). The profiles are hyperbolic and permit extrapolation to infinite dilution if so required (37).

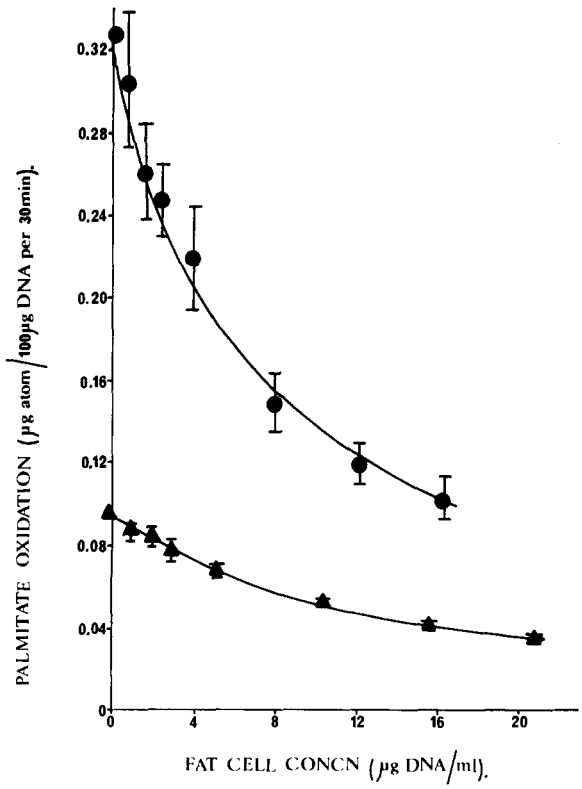

Fig. 2. Effect of cell concentration on the oxidation of palmitate. Fat cells from fed and 48-hr starved rats were incubated at the indicated concentrations for 30 min with 16 mg/ml albumin and 70 μM sodium [U-^{14}C]palmitate. No other substrate was added. The results are the means ± SEM of four and five determinations, respectively, for cells from fed and starved animals. No correction was applied for change in specific activity of the exogenous fatty acid pool. The points on the ordinate (infinite cell dilution) are graphically extrapolated values (37). ●, Fed; ▲, starved.

Palmitate esterification is affected in the same manner, but the ratio of esterification to oxidation is unaffected by cell concentration (37). Fat cells and adipose tissues from starved rats (incubated with glucose and insulin) have higher rates of basal lipolysis (glycerol release) than those from fed animals (16, 38). The results shown in Fig. 2 were therefore surprising since the degree of inhibition of palmitate oxidation due to increasing cell concentration was greater with fed than with starved cells. It may however be possible to reconcile this observation with the presumption that the profiles shown in Fig. 2 are largely due to dilution of extracellular [^{14}C]palmitate, since the starved cells contained less unesterified fatty acid (653 ± 40 nmole/

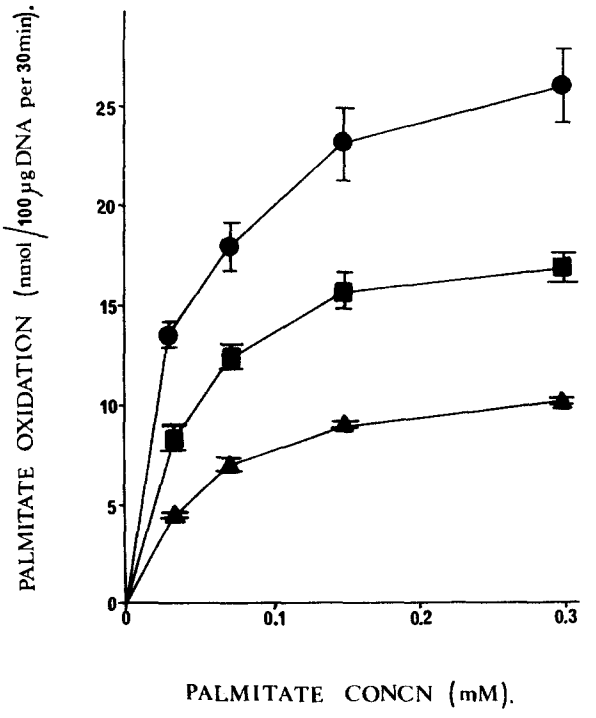

Fig. 3. Effect of octanoate on palmitate oxidation. Fat cells from fed rats were incubated for 30 min with 16 mg/ml albumin and the indicated concentration of sodium [1-^{14}C]palmitate and sodium octanoate, where appropriate. The results are the means ± SEM of four determinations. The mean fat cell DNA/ml of incubation flask contents was 2.4 µg. ●, Palmitate alone; ■, palmitate + 50 µM octanoate; ▲, palmitate + 100 µM octanoate.

100 µg of DNA) than the fed cells (2018 ± 16 nmole/100 µg of DNA) immediately prior to incubation. Dilution of the extracellular label is presumably dependent both upon the rate of basal lipolysis and upon the initial quantity of unesterified fatty acids in the cells that may be released.

There is no possibility that the profiles shown in Fig. 2 result from depletion of the available palmitate during incubation at higher cell concentrations. Even with the highest concentration of fed cells less than 30% of the available [^{14}C]palmitate was utilized. In addition, measurement of the total unesterified fatty acids in incubation media at the end of these experiments showed that there was a small net eflux of fatty acids from the cells under these conditions. The utilization of ^{14}C-labeled-fatty acid may therefore decrease through dilution, but not through depletion of substrate concentration.

For operational reasons the experiments shown in Fig. 2 were performed in the absence of glucose and insulin. Essentially similar effects are seen when glucose is present[3], but the removal and esterification of extracellular fatty acid is far more rapid, and it becomes very difficult, even at moderate cell concentrations, to perform precise experiments with low concentrations of fatty acids. When insulin is present in addition to glucose it is virtually impossible to perform experiments of this kind. Fig. 2 must therefore serve to exemplify the necessity of controlling cell concentration.

In summary, ideal incubation conditions involve low cell concentrations (only 2–3 µg of fat cell DNA/ml), short incubation times (30 min), and high extracellular total fatty acid concentrations (usually 0.5 mM or greater). Under these conditions it was unnecessary to correct data for changes in the extracellular fatty acid specific activity since these were negligible.

The effects of octanoate, caproate, acetate and D-3-hydroxybutyrate on palmitate oxidation

Octanoate, used as a representative medium chain length fatty acid, significantly inhibited palmitate oxidation by fat cells (**Fig. 3**). Double reciprocal plots of these data indicated that the inhibitory effect of octanoate on oxidation of carbon-1 in palmitate was essentially noncompetitive with respect to palmitate. A K_i value of 77 ± 14 µM for the effect of octanoate on palmitate oxidation was determined (this refers to *total* octanoate concentration in the presence of 16 mg/ml albumin). In further experiments (results not shown) it was found that caproate also inhibited the oxidation of carbon-1 in palmitate whereas acetate and D-3-hydroxybutyrate (both tested from 100–600 µM) had no effect upon the formation of $^{14}CO_2$ from [1-^{14}C]palmitate.

In other experiments in which fat cells were similarly incubated with labeled palmitate in the absence of a carbohydrate substrate, it was observed that octanoate (50–200 µM) stimulated palmitate esterification by approximately 10%. Caproate (50–200 µM), acetate (100–400 µM), and D-3-hydroxybutyrate (100–600 µM) had no effect on palmitate esterification. Medium chain length fatty acids therefore appear to selectively inhibit palmitate oxidation without appreciably influencing esterification. It is unlikely that this effect results from interference with palmitate entry into the cells since the rate of fatty acid esterification greatly exceeds the rate of oxidation. It is therefore concluded that this is a mitochondrial phenomenon. As a speculation it may be suggested that octanoylcarnitine and palmitoylcarnitine generated by medium chain and long chain carnitine acyltransferases respectively (39, 40) may compete for reaction with CoASH within the space(s) bounded by the mitochondrial inner membrane. If

[3] E. D. Saggerson, unpublished results.

TABLE 1. Comparison of the oxidation of palmitate carbon-1 and carbon-16 by fat cells from normal and starved rats[a]

Dietary Status	Fat Cell Conc. μg DNA/ml	Further Additions	[1-^{14}C]Palmitate	[U-^{14}C]Palmitate × 1/16[c]	[16-^{14}C]Palmitate	Ratio 1-carbon/ 16-carbon in $^{14}CO_2$
Fed	1.4	None	6.53 ± 0.88[b]	3.57 ± 0.53	1.92 ± 0.39*[d]	3.54 ± 0.29
		Octanoate (0.25 mM)	2.27 ± 0.64	1.14 ± 0.39	0.46 ± 0.23	6.19 ± 1.25
Starved (48 hr)	1.2	None	2.36 ± 0.26	1.39 ± 0.11	0.73 ± 0.07†	3.22 ± 0.25
		Octonoate (0.25 mM)	0.93 ± 0.02	0.43 ± 0.02	0.18 ± 0.03‡	5.43 ± 1.05

[a] Fat cells from fed or 48-hr starved rats were incubated for 30 min in Krebs-Ringer bicarbonate containing 16 mg/ml albumin together with 0.5 mM sodium [1-^{14}C]-, [16-^{14}C]-, or [U-^{14}C]palmitate. Sodium octanoate was added where indicated.

[b] The results are means ± SEM of three determinations and are expressed as μg atom carbon converted to CO_2/10 mg DNA per 30 min.

[c] The results obtained using [U-^{14}C]palmitate were multiplied by 1/16 to make them comparable with those using [1-^{14}C]- or [16-^{14}C]palmitate.

[d] *, †, ‡ indicate $P < 0.02$, < 0.01, and < 0.001, respectively, for comparisons of the oxidation of palmitate C-16 with C-1.

the transfer of palmitoyl groups from cytosol to mitochondria is the rate-limiting step in palmitoyl group oxidation, apparent noncompetitive kinetics may be expected. These inhibitory effects of octanoate must be quite complex since octanoate also accentuates the tendency for palmitate carbon-1 to be preferentially oxidized over carbon-16 (**Table 1**). This effect is observed in cells from fed and starved animals. Differential oxidation of palmitate carbons in fat pads has been reported previously (41).

Oxidation of octanoate by incubated rat fat pieces has been demonstrated previously (27) and we have observed [1-^{14}C]octanoate oxidation by fat cells (results not shown). In the presence of 16 mg/ml albumin, a half-maximum rate of octanoate oxidation was observed at a total octanoate concentration of approximately 80 μM. High concentrations of octanoate (0.4–0.6 mM) appeared to inhibit oxidation and it is presumed that this is due to cell damage.

Since in the rat, as in many other species, the major proportion of shorter chain fatty acids is absorbed from the diet via the portal circulation (42) and is therefore likely to undergo modification in the liver, it is uncertain whether the oxidation of octanoate and its effects on long chain fatty acid oxidation observed in isolated fat cells is of physiological importance.

The effect of starvation on palmitate oxidation

Prior starvation for 48 hr considerably reduced both the oxidation and the esterification of palmitate by fat cells incubated in the absence of any other substrate (**Fig. 4**). Milstein and Driscoll (28) reported increased oxidation of exogenous [1-^{14}C]palmitate by adipose tissue from starved rats (no other substrate present), although this apparent effect of fasting may have arisen through expression of the results per unit wet weight of tissue. Fasting drastically reduces the fat content and hence the wet weight of the tissue. In accord with Shapiro, Chowers, and Rose (14), starvation decreased esterification more than oxidation (Fig. 4). The effects of starvation shown in Fig. 4 did not appear to be due to greater dilution of the extracellular [^{14}C]palmitate by fatty acids of endogenous origin since the effects of starvation are the same at both 0.05 and 0.5 mM palmitate and the low cell concentrations should preclude dilution of the extracellular pool.

It is of course possible that there is more exten-

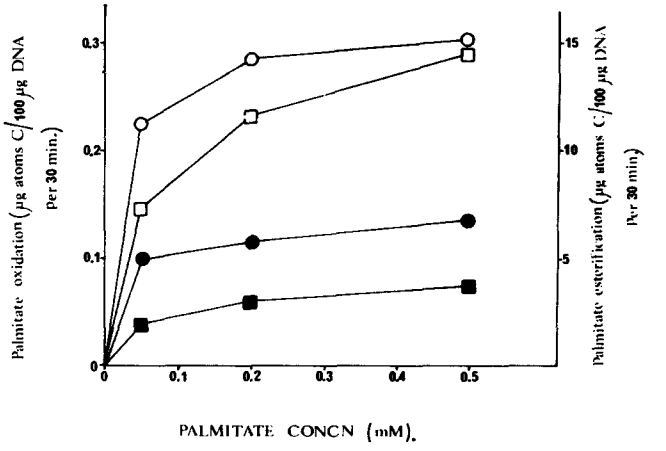

Fig. 4. Effect of starvation on the oxidation and esterification of palmitate. Fat cells from fed or 48-hr starved rats were incubated for 30 min with 16 mg/ml albumin and the indicated concentrations of sodium [U-^{14}C]palmitate. The results are means of three determinations and are expressed as μg atoms of palmitate carbon/100 μg DNA per 30 min. The mean fat cell DNA/ml of incubation flask contents was 1.2 μg for fed cells and 1.0 μg for starved cells. ○, Fed, oxidation; □, fed, esterification; ●, starved, oxidation; ■, starved, esterification.

TABLE 2. The effects of glucose concentration and insulin on [1-^{14}C]palmitate oxidation and esterification by fat cells from normal and 48-hr starved rats

Dietary Status	Conc. Glucose (mM)	Fed		Starved (48 hr.)	
		[1-^{14}C]Palmitate Oxidation	[1-^{14}C]Palmitate Esterification	[1-^{14}C]Palmitate Oxidation	[1-^{14}C]Palmitate Esterification
With anti-insulin serum	0	27.1 ± 1.9	853 ± 268	15.9 ± 1.0	314 ± 27
	0.05	32.7 ± 1.2	1237 ± 307	20.1 ± 1.74	488 ± 117
	0.5	26.0 ± 2.4	3718 ± 825	26.3 ± 1.3	1516 ± 54
	1.0	21.2 ± 1.3	6013 ± 972	25.8 ± 0.3	1964 ± 212
	2.0	18.3 ± 1.8	6541 ± 1127	19.9 ± 0.5	2553 ± 100
	5.0	13.2 ± 0.7	7645 ± 1277	15.5 ± 1.0	3112 ± 74
	10.0	10.1 ± 1.1	8199 ± 1210	16.1 ± 1.1	3411 ± 92
With insulin	0	30.0 ± 3.2	836 ± 271	19.5 ± 3.1	454 ± 88
	0.05	34.3 ± 2.2	1616 ± 380	24.3 ± 2.4	769 ± 88
	0.5	20.7 ± 2.1	6709 ± 423	24.5 ± 1.4	2135 ± 148
	1.0	14.8 ± 0.8	9121 ± 1024	19.5 ± 2.0	2902 ± 218
	2.0	11.4 ± 2.4	9630 ± 843	15.4 ± 1.3	3423 ± 187
	5.0	7.6 ± 1.6	10590 ± 1176	13.3 ± 0.4	3770 ± 160
	10.0	8.2 ± 1.2	10484 ± 493	11.2 ± 1.6	3597 ± 26

Fat cells from fed or 48-hr starved rats were incubated for 30 min in Krebs-Ringer bicarbonate containing 21 mg/ml albumin together with 0.75 mM sodium [1-^{14}C]palmitate, the indicated concentrations of glucose, and either anti-insulin serum (10 mU/ml) or insulin (20 mU/ml). The results are means ± SEM of three determinations and are expressed as nmole/100 μg DNA per 30 min. The mean fat cell DNA/ml of incubation flask contents was 2.3 μg for fed cells and 1.8 μg for starved cells.

sive intracellular dilution of palmitate label in the starved cells. It is not possible to investigate this directly at present. However, as discussed above, starved cells contained a lower total concentration of unesterified fatty acids and appeared to dilute the extracellular pool less than fed cells (Fig. 2). There seems to be no reason to infer that the intracellular precursor pool(s) should be more prone to dilution in starved than in fed cells. The effects of starvation are discussed more fully below.

The effects of glucose and insulin on palmitate oxidation and esterification

Table 2 shows results obtained in experiments in which cells from fed or starved rats were incubated in the presence of a high total concentration of palmitate (0.75 mM, which gives a palmitate: albumin molar ratio of 2.4). Glucose concentration was varied over the range 0–10 mM, with or without insulin. Increasing the glucose concentration resulted in enhancement of palmitate esterification in accord with the observations of Baily et al. (29). This presumably is brought about through increases in the level of glycerol phosphate. Insulin had no appreciable effect on palmitate esterification in the absence of glucose but increased esterification at any given concentration of glucose. The effect of insulin reflects the ability of this hormone to increase glycerol phosphate concentrations in adipose tissue. (20, 25).

The effect of glucose on palmitate oxidation was more complex. In cells from fed animals addition of a very low concentration of glucose slightly increased palmitate oxidation. Above 0.05 mM glucose, palmitate oxidation progressively decreased with increasing glucose concentration, while esterification progressively increased. Fatty acid oxidation and esterification are therefore essentially inversely related over a wide range of glucose concentrations in cells from fed animals. In cells from starved animals, however, enhancement of palmitate oxidation by addition of glucose was observed over a wider range of glucose concentrations. Above 0.5 mM glucose, fatty acid oxidation and esterification were again essentially inversely related in starved cells as in fed cells. These results clearly demonstrate that measurement of palmitate oxidation in the absence of glucose does not give a true indication of the relative ability of fed and starved cells to oxidize fatty acids. Under these conditions fatty acid oxidation appears to be restrained and this restraint is greater in starved cells.

It is unclear whether the 'peak' rates of palmitate oxidation observed (at 0.05 mM glucose + insulin in fed cells, and at 0.5 mM glucose in starved cells) relate to the maximum capacities of the cells to oxidize fatty acids. If they do, the fatty acid oxidation capacity is reduced by 23% after 48 hr of starvation. In this respect it is noteworthy that carnitine palmitoyl transferase 1 and 2 activities are decreased by 22% and 37% respectively in mitochondria from starved fat cells (8). These observations must be reconciled with those of Flatt

(3) who showed that oxidation of endogenous fatty acids is increased in starved adipose tissue when glucose is present. It can be seen that these results are not at variance with those of Flatt (3) when it is considered that glucose exhibits opposing stimulatory and inhibitory effects on fatty acid oxidation and that the glucose concentration dependence of these effects varies with dietary state.

The glucose-dependent inhibition of palmitate oxidation is possibly attributable to at least two effects: (*a*) glucose oxidation substituting for palmitate oxidation to supply the energy requirements of the cell, and (*b*) glucose enhancement of esterification leading to a decreased availability of extramitochondrial fatty acyl CoA. These compounds are common substrates for the esterification process and for carnitine palmitoyltransferase 1, the presumed first committed step in the oxidation process. It would seem unlikely that mechanism (*a*) could account for the difference in response to glucose found in starved and fed cells or for the observation that insulin diminishes fatty acid oxidation in the presence of glucose. Citric acid cycle oxidation of glucose-derived carbon in incubated adipose tissues is unaffected by starvation (38) and is only slightly increased by insulin (20). Other adipose tissue processes that oxidize glucose-derived carbon (hexose monophosphate pathway, triose phosphate oxidation and pyruvate decarboxylation) and that are greatly influenced by starvation and insulin appear to be closely related to reductive biosynthetic activities and not to the energy requirements of the tissue (20).

The second proposal (*b*), namely that regulation of fatty acid oxidation may be secondary to controls influencing the rate of esterification, is attractive since the rate of esterification greatly exceeds that of oxidation under all conditions (Table 2). Regulation of the total flux through the extramitochondrial acyl CoA pool will almost totally reside in the esterification process. The converse, ie., that alterations in the rate of oxidation should be capable of causing changes in esterification rate, is not expected.

The glucose-dependent stimulation of palmitate oxidation at low glucose concentrations is also possibly explained by several effects. (*a*) Glucose may act as a precursor for oxalacetate and thus permit greater oxidation of palmitate to CO_2. Palmitoyl group oxidation in fat cell mitochondria is dependent on oxalacetate provision (8). (*b*) The energy demand by the cell may be greater when palmitate esterification is increased by glucose. (*c*) It is conceivable that in the absence of glucose some of the restraint on palmitate oxidation may be imposed by fatty acyl CoA inhibition of carnitine palmitoyl transferase 1 (43). Promotion of esterification by glucose could reduce the concentration of extramitochondrial acyl CoA, which is both substrate and inhibitor for this enzyme that may control palmitoyl group entry into β-oxidation. It is unknown whether appropriate metabolite levels pertain in the cells for this mechanism to operate.

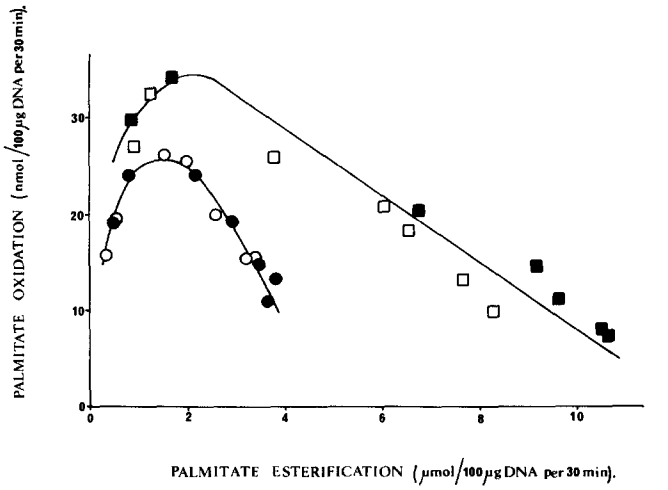

Fig. 5. Relationship between palmitate oxidation and esterification. The data are taken from Table 2. □, Fed + anti-insulin serum; ■, fed + insulin; ○, starved + anti-insulin serum; ●, starved + insulin.

Fig. 5 is an alternative presentation of the data of Table 2 and shows that palmitate oxidation is related to esterification by a biphasic relationship. This is seen particularly well with starved cells. Also, in starved cells at least, insulin clearly does not perturb the relationship between oxidation and esterification. In fed cells it is not possible with the existing data to assess whether or not insulin alters this oxidation versus esterification profile.

In **Table 3** the two extreme conditions of zero and 10 mM glucose are considered in more detail. Additional data were obtained and added to some of those from Table 2 to permit statistical evaluation of effects. In the absence of glucose, palmitate oxidation was severely decreased in starved cells in accord with the observations shown in Figs. 2 and 4. The experimental condition of zero glucose is of course unphysiological and, as discussed above, this apparent decrease in palmitate oxidation does not necessarily reflect a real decrease in the capacity of the cells to oxidize fatty acid. In the presence of 10 mM glucose (Table 3), the phenomenon noted by Flatt (3) is seen, i.e., starvation has led to a change that apparently lessens the ability of the cells to suppress fatty acid oxidation. It is suggested that this change arises from the negative adaptation in esterification found in starvation. Although,

TABLE 3. The effects of starvation, glucose and insulin on [1-^{14}C]palmitate oxidation and esterification by fat cells[a]

Dietary Status	Without Glucose				With Glucose (10 mM)			
	[1-^{14}C]Palmitate Oxidation		[1-^{14}C]Palmitate Esterification		[1-^{14}C]Palmitate Oxidation		[1-^{14}C]Palmitate Esterification	
	With Anti-insulin Serum	With Insulin	With Anti-insulin Serum	With Insulin	With Anti-insulin Serum	With Insulin	With Anti-insulin Serum	With Insulin
Fed	26.82 ± 0.91 (9)	31.95‡ ± 1.34 (9)	817 ± 146 (6)	846 ± 134 (6)	11.94*[b] ± 1.12 (6)	7.23*‡ ± 0.91 (6)	6145* ± 1067 (6)	9603*†[c] ± 545 (6)
Starved (48 hr)	16.76§§§ ± 0.90 (6)	18.19§§§ ± 1.65 (6)	354§[d] ± 28 (6)	397§ ± 49 (6)	19.89§§ ± 1.82 (6)	15.46§§ ± 2.07 (6)	3340*§ ± 171 (6)	3897*§§§ ± 191 (6)

[a] Fat cells from fed or 48-hr starved rats were incubated for 30 min in Krebs-Ringer bicarbonate containing 21 mg/ml albumin together with 0.75 mM sodium [1-^{14}C]palmitate and where appropriate glucose (10 mM), anti-insulin serum (10 mU/ml) or insulin (20 mU/ml). The results are means ± SEM and are expressed as nmole/100 μg DNA per 30 min. The mean fat cell DNA/ml of incubation flask contents was 2.6 μg (9 detns.) or 2.8 μg (6 detns.) for fed cells and 1.9 μg for starved cells. The numbers in parentheses indicate the number of determinations made.

[b] * Indicates $P < 0.001$ for comparison of cells incubated with glucose against the appropriate glucose-free controls.

[c] †, ‡ Indicate $P < 0.02$, < 0.01 respectively for comparison of cells incubated with insulin against the appropriate insulin-free controls.

[d] §, §§, §§§ Indicate $P < 0.05$, < 0.01, < 0.001 respectively for comparison of starved cells against the appropriate fed controls.

at 10 mM glucose, an esterification rate is achieved that is on the 'descending limb' of the oxidation–esterification profile, the starved cells cannot sustain a rate of esterification sufficient to restrain oxidation to the level found in fed cells.

It may also be seen in Table 3 that, if only the two extremes of 0 and 10 mM are considered, glucose apparently causes a large decrease in palmitate oxidation in fed cells but has no apparent effect on this process in starved cells. Reference to Fig. 5, however, shows that this experimental conclusion is an oversimplification. Changes in glucose concentration may influence fatty acid oxidation in starved cells as they do in fed cells, but the effect is dependent on the chosen 'starting point'. The more compressed shape of the curve seen in starvation (Fig. 5) may be explained both by the lowered esterification capacity of the cells and also, perhaps, by limitation of the permissible size of the total fatty acyl CoA pool available to be shared as substrate between the two processes. The total CoASH content of adipose tissue decreases considerably in starvation (20).

At present the contention that oxidation and esterification compete for a common extramitochondrial substrate, thus permitting esterification to indirectly regulate oxidation in some circumstances, must remain speculatory since direct measurement of the entry of palmitoyl groups into β-oxidation is required to establish its correctness. Ideally $^{14}CO_2$ formation from [1-^{14}C]palmitate should measure this. Unfortunately there is no satisfactory way of assessing the extent to which acetyl CoA derived from palmitate oxidation is utilized in processes other than tricarboxylic acid cycle oxidation. The present data support the above explanation only if these alternative fates of palmitoyl-derived acetyl CoA are negligible or represent an approximately constant proportion of acetyl CoA metabolism under all the tested conditions. The present results may however be satisfactorily related to earlier observations of endogenous fatty acid oxidation (2, 3) since these were concerned with measurement of CO_2 production and not with the actual rate of β-oxidation.

The effect of glucose (+ insulin) on the concentration-dependence of palmitate utilization

In the absence of glucose, palmitate oxidation and esterification showed little sensitivity to the concentration of palmitate. At 0.75 mM palmitate (palmitate: albumin molar ratio of 2.4), as was used in the experiment of Table 2, the cells are essentially metabolizing palmitate under V_{max} conditions. This is evident both from Fig. 4 and from the top section of **Table 4.** Table 4 summarizes an experiment in which glucose + insulin, at fixed concentrations, were either absent or present and the concentration of palmitate was varied. Albumin was used at 1 mM and therefore the palmitate:albumin ratio varied from 0.5–4. When glucose + insulin were added the measured rates of palmitate esterification and oxidation became strongly dependent on the fatty acid concentration in a hyperbolic

manner. Double reciprocal plots indicated composite K_m values for palmitate esterification and oxidation of 0.71 mM and 1.89 mM, respectively. These values are obviously far higher than those expected in the absence of glucose (37). Under V_{max} conditions in the presence of glucose + insulin the esterification: oxidation ratio would be approximately 1100. Three conclusions were made from these observations. *1*. Rates of palmitate oxidation and esterification in the experiment of Table 2 will deviate gradually from V_{max} conditions as glucose concentration is raised. This leads to a small overestimation of the esterification:oxidation ratio at high glucose concentrations. This should not severely affect the validity of the findings. *2*. The change in the concentration dependence of palmitate oxidation and esterification presumably resulted from the increased metabolic flux of palmitate and implied that the relationship between the external fatty acid concentration and the concentration of an intracellular precursor of these processes had changed. Increasing disequilibration with increasing flux may possibly occur either at sites of fatty acid traverse of the plasma membrane or at the acyl CoA synthetase reaction. *3*. The degree to which addition of glucose (+ insulin) both increases esterification and decreases oxidation is linearly related to the logarithm of the palmitate concentration (**Fig. 6**). This inverse relationship between the effects of glucose on oxidation and esterification is again suggestive of a situation in which the two processes compete for the same precursor, although it is possible that glucose may have mutually independent effects on oxidation and esterification.

TABLE 4. The effect of glucose + insulin on the oxidation and esterification of palmitate by fat cells from fed rats

Palmitate Conc. (mM)	Additions to Incubation Medium	Incorporation of [U-^{14}C]palmitate into:	
		$^{14}CO_2$	^{14}C-labeled Glycerides
0.5	None	2.14	144
1.0		2.32	171
2.0		2.58	187
4.0		2.63	194
0.5	Glucose (5 mM)	0.43	974 (8.5)
1.0	+ insulin	0.74	1361 (6.0)
2.0	(20 mu/ml)	1.09	1746 (3.8)
4.0		1.40	1977 (2.2)

Fat cells from fed rats were incubated for 30 min in Krebs-Ringer bicarbonate containing 67 mg/ml albumin together with the indicated concentrations of sodium [1-^{14}C]palmitate and glucose (5 mM) with insulin (20 mU/ml) where appropriate. The results are the means of three determinations and are expressed as μg atoms/mg DNA per 30 min. The mean fat cell DNA/ml of the incubation flask contents was 0.7 μg. The figures in parentheses represent the mean percentages of the initial palmitate concentration utilized in the course of each experiment.

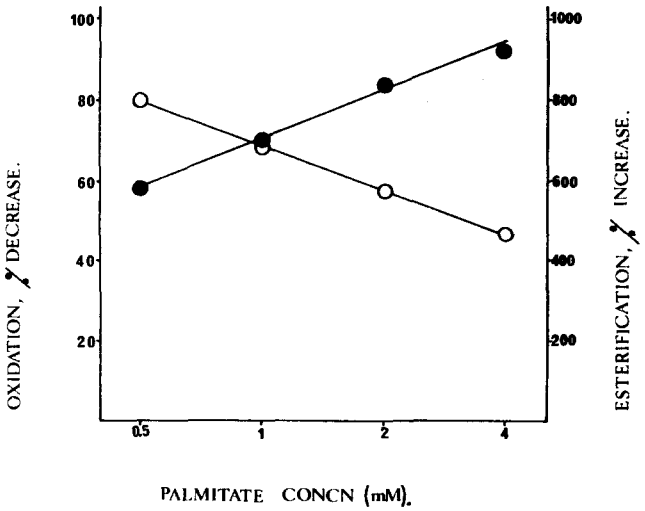

Fig. 6. Dependence of effects of glucose (+ insulin) on palmitate concentration. The data are derived from Table 4 and represent the percentage differences in palmitate metabolism observed in cells incubated with palmitate and glucose (+ insulin) compared with cells incubated with palmitate alone. ○, Oxidation; ●, esterification.

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