Calculation of lipolysis and esterification from glycerol metabolism in rat adipose tissue

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Abstract Pieces of epididymal fat pad from fed and 48-hr-fasted rats were incubated for various periods of time in Krebs-Ringer bicarbonate containing [1-^{14}C]glycerol. The radioactive substrate taken up by the tissue increased linearly with time in both groups and was mainly converted to glyceride-glycerol and CO_2. The slopes of the regressions of ^{14}C-labeled glyceride-glycerol with time were not different between the groups, while those of $^{14}CO_2$ were smaller in the fasted than in the fed animals. Because the radioactive glycerol in the medium is being continuously diluted with the glycerol coming out of the tissue, it is necessary to take account of this factor in calculating the actual amount of glycerol utilized by the tissue. The glycerol produced by the tissues is higher in the fasted than in the fed animals, and in both groups it increases hyperbolically with time. As negligible amounts of the ^{14}C-labeled glycerol taken up by the tissue recirculates to the medium, the rates of glycerol release (lipolysis), esterification, and oxidation to CO_2 were calculated.

Supplementary key words glycerol utilization

We have previously shown that the utilization of glycerol by rat adipose tissue incubated in vitro is considerably higher than previously thought (1). Unless this utilization is taken into account, it is likely that calculations of lipolysis and esterification may be in error. In the present work we have tried to quantify the rate of the removal of ^{14}C-labeled glycerol from the incubation media of rat epididymal fat pads incubated in vitro, correcting for the continuous dilution of the radioactivity by the glycerol coming out of the tissue. Mathematical analysis of the data has allowed us to determine what we think are the true rates of lipolysis and esterification. These rates are considerably different from those calculated on the basis of the net production of glycerol and free fatty acids.

MATERIALS AND METHODS

Rats

Male rats of the Wistar strain, weighing 164–228 g, were used. They were fed a Purina chow diet ad lib. When animals were fasted, food was withheld 48 hr before they were killed but they had free access to water. The rats were killed by cervical fracture without anesthesia.

Reagents

[1-^{14}C]Glycerol (15.4 mCi/mmole) was purchased from the Radiochemical Centre, Amersham, England, and its purity was determined in two ways. (*a*) Portions of the radioactive preparation were passed through microcolumns containing Dowex 1 (X2-400)–Duolite A-4 (OH^- form)–Dowex 1 (X2-400), prepared and processed as previously described (2). It was found that 99.8–100.9% of the [1-^{14}C]glycerol added was recovered in the eluates. On the other hand, when the portions were incubated with sufficient glycerokinase and cofactors to transform all the glycerol into glycerol-3-phosphate before the passage of the mixture through the columns, less than 0.3% of the radioactivity present was recovered in the eluates. (*b*) When the radioactive preparation was chromatographed on paper in the upper phase of butan-1-ol–water–methanol–90.7% (w/v) formic acid 320:320:80:1 (by volume), more than 99.7% of the radioactivity recovered on the strip was found in the glycerol spot.

Enzyme preparations were obtained from Boehringer-Mannheim, Mannheim, West Germany. Adenine and pyridine nucleotides were purchased from Sigma, St. Louis, Mo. Organic solvents were "spectroanalyzed" grade.

In vitro incubation of adipose tissue

Immediately after the rats were killed, epididymal fat pads were removed and placed in Krebs-Ringer bicarbonate buffer, pH 7.4 (3), at room temperature. The pads were cut into small pieces of approximately 10 mg each, rinsed twice with the same buffer, blotted lightly on Whatman no. 1 paper, and weighed on a torsion balance. 5–6 pieces of tissue (56–70 mg) were placed in rubber-sealed 20-ml vials each containing 1 ml of the buffer. At zero time, 1 ml of the buffer containing 0.5 μCi of [1-^{14}C]glycerol was injected into each of the vials, and these were gassed for 5 min with O_2–CO_2 (95:5) and incubated with agitation (100 cycles/min in a Dubnoff metabolic shaker) at 37°C. The incubation was stopped by injecting 250 μl of Hyamine 10X hydroxide into small polyethylene cups suspended from the cover of the vial and 1 ml of 10% (w/v) $HClO_4$ into the medium. The $^{14}CO_2$ evolved was trapped in the Hyamine by gentle shaking at room temperature for 60 min. In three experiments, after the incubation in Krebs-Ringer bicarbonate buffer containing ^{14}C-labeled glycerol, free radioactive glycerol was removed from the tissues by washing the tissue at 37°C in 2 ml of buffer containing carrier glycerol (6.25×10^{-5} M). This operation was repeated twice, and the washed tissues were finally placed into the media of vials incubated for the same time with tissue from the same rat but without the addition of labeled glycerol. After gassing for 5 min with O_2–CO_2 (95:5), the vials were incubated again as described above.

Extraction and purification of lipids

Lipids were extracted from the incubated tissue in chloroform–methanol by the method of Folch, Lees, and Sloane Stanley (4). The chloroform–methanol lipid extract was washed once with saline and three times with 1 M glycerol to remove the radioactive free glycerol bound to the tissue. Portions of the total lipid extract were saponified in 5 M KOH in 95% (v/v) ethanol for 2 hr at 100°C. Fatty acids and nonsaponifiable lipids were extracted with heptane after acidification, and radioactivity in the glyceride-glycerol was calculated from the difference between radioactivities in the total lipid and in the heptane layer.

Determinations in the incubation media

The medium was neutralized with $KHCO_3$ and, after sitting in ice for 15 min, it was centrifuged at 4°C to remove $KClO_4$. Portions of the supernatant solution were taken for measurement of radioactivity, for determination of glycerol by the enzymatic method described by Garland and Randle (5), and for the isolation of ^{14}C-labeled glycerol by ascending paper chromatography in the upper phase of butan-1-ol–water–methanol–90.7% (w/v) formic acid 320:320:80:1 (by volume). The formation of glycerol was calculated from the difference between the amount of glycerol in the medium of the experimental vial and that in the standard vial without tissue.

Radioactive assay

The Hyamine solutions ($^{14}CO_2$), appropriate spots of chromatograms, and aliquots of the medium were counted in a liquid scintillation mixture containing 15 g of 2,5-diphenyloxazole, 150 mg of *p*-bis[2-(5-phenyloxazolyl)]-benzene, and 240 g of naphthalene in 3000 ml of xylene–dioxane–95% ethanol 5:5:3 (by volume). Aliquots of the lipid extracts were counted in a liquid scintillation mixture containing 12% methanol, 0.4% 2,5-diphenyloxazole, and 0.005% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene. Standards were counted in aliquots of media that had been incubated without tissue (i.e., "blank media").

Radioactivity measurements were expressed as percentages of the total [1-^{14}C]glycerol added to each vessel, related to the initial wet weight of the tissue per 100 mg of tissue, and calculated as micromoles as a function of the specific activity of the appropriate counting standards.

Mathematical analysis of the data

Linear and nonlinear regressions were determined using standard methods (6). Statistical comparisons between two groups of data were performed by Student's *t* test.

Calculations were carried out in an Olivetti electronic calculator (Programma 102), with the help of a Friden 130. Comprobation of all the regressions was performed in an IBM 7090 computer.

RESULTS AND DISCUSSION

Glycerol formation by adipose tissue

Pieces of epididymal fat pads from fed and 48-hr-fasted rats were incubated with [1-^{14}C]glycerol. The incubations were carried out in the absence of albumin in the medium to minimize experimental variables and because it has recently been shown (7) that lipolysis is not abolished in these conditions. At different times the incubation was stopped and the amount of glycerol (*g*) in the medium was determined. The formation of glycerol was calculated from the difference between the amount of glycerol in the medium of the experimental vial and that in the standard vial incubated under the same conditions but without tissue. The results obtained are summarized in Fig. 1. At all times studied, with the exception of 120 and 150 min of incubation, the amount

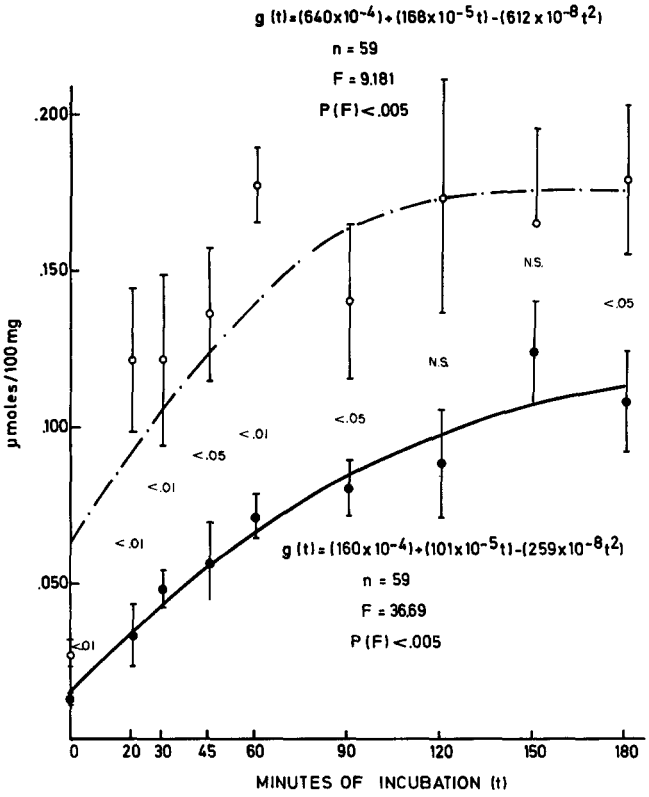

FIG. 1. In vitro glycerol (g) formation by epididymal fat pads from fed (●) and 48-hr fasted (○) rats. Each value is the mean ± SEM of six to eight incubations. The numbers between the points are the P values that correspond to the differences between fed and 48-hr fasted groups at a given time (N.S., not significant, i.e., $P > 0.05$). The regressions obtained from all the data of each group are shown near each curve. Other details are given in the text.

of glycerol formed was significantly higher in the fasted than in the fed animals. From the experimental data it is possible to obtain a nonlinear regression as a function of time, $g(t)$, obtaining a second-degree equation of the type:

$$g(t) = c + b \cdot t + a \cdot t^2 \qquad \text{Eq. 1}$$

The regression obtained from the data from experiments with fed rats is:

$$g(t) = (160 \times 10^{-4}) + (101 \times 10^{-5}\, t) - (259 \times 10^{-8}\, t^2)$$

and that from the 48-hr-fasted rats is:

$$g(t) = (640 \times 10^{-4}) + (168 \times 10^{-5}\, t) - (612 \times 10^{-8}\, t^2)$$

As may be seen in Fig. 1, both regressions fit the experimental data well ($P[F] < 0.05$ in both groups). In both experimental groups, after an initial rise in the formation of glycerol, a plateau was reached after prolonged incubation. This phenomenon can be explained in one of two ways: (a) lipolysis decreased with the time of incubation, or (b) the tissue continued to hydrolyze glycerides, but part of the glycerol that was coming from the tissue into the medium was being reutilized. To decide which of the explanations was more likely, we studied the utilization of [1-^{14}C]glycerol by the tissue during the period of incubation.

Uptake and utilization of [1-^{14}C]glycerol

At all times of incubation studied, the amount of radioactivity transferred to lipids and CO_2 was practically the same as the amount of ^{14}C-labeled glycerol that disappeared from the medium (98.6 ± 0.6% for all the times and both experimental conditions), but the data of the first expression (radioactivity transferred to lipids and CO_2) were better grouped and more reproducible; consequently, this was assumed to be the amount of ^{14}C-labeled glycerol taken up by the tissue. This uptake was not statistically different in fed and 48-hr-fasted rats except after 20 and 150 min of incubation ($P < 0.02$ and < 0.05, respectively); in both groups, uptake increased with the time of incubation (Fig. 2). From the data of the uptake of ^{14}C-labeled glycerol (G) we can obtain the linear regressions as a function of time ($G[t]$):

$$G(t) = A + Bt \qquad \text{Eq. 2}$$

The regression obtained from the data for fed rats is:

$$G(t) = (-871 \times 10^{-7}) + (263 \times 10^{-7}\, t)$$

and that from the 48-hr-fasted rats is:

$$G(t) = (-159 \times 10^{-6}) + (198 \times 10^{-7}\, t)$$

Both regressions fit the experimental data well (Fig. 2) and are highly significant ($P < 0.001$ for both fed and 48-hr-fasted rats). The slopes of the lines were not statistically different ($t = 0.613$).

More than 85% of the radioactive glycerol taken up by the tissue was converted to glyceride-glycerol and CO_2. These data are summarized in Figs. 3 and 4. The formation of both ^{14}C-labeled glyceride-glycerol (GG) and $^{14}CO_2$ as a function of time in fed and 48-hr-fasted rats may be adjusted to very significant linear regressions ($P < 0.001$ in all cases). The slopes of the regressions of $GG(t)$ in fed and 48-hr-fasted rats were not statistically different (Fig. 3), while those of the $CO_2(t)$ were smaller in the fasted than in the fed rats ($P < 0.01$) (Fig. 4). The corresponding regressions from fed rats were:

$$GG(t) = (-480 \times 10^{-7}) + (209 \times 10^{-7}\, t)$$

$$CO_2(t) = (-541 \times 10^{-7}) + (415 \times 10^{-8}\, t)$$

and those from 48-hr-fasted rats were:

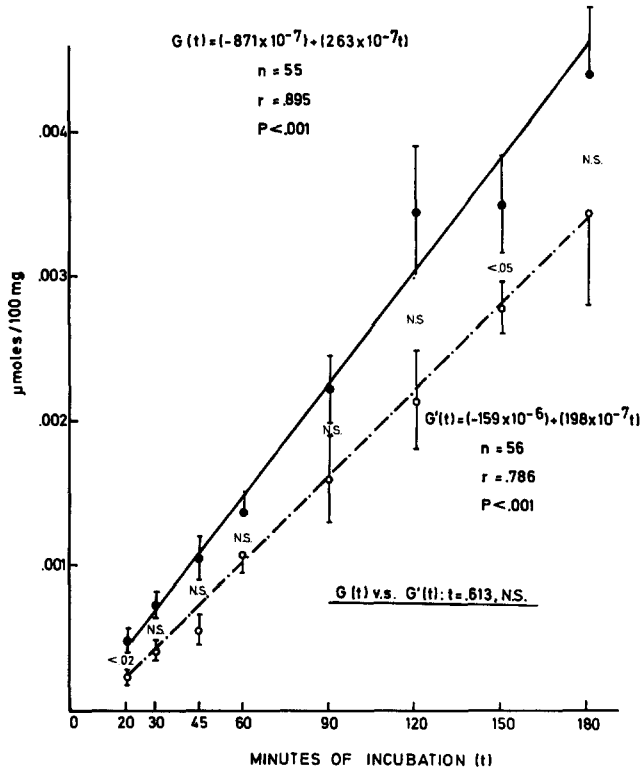

FIG. 2. Radioactive glycerol (*G*) taken up by epididymal fat pads from fed (●) and 48-hr-fasted (○) rats; incubations were in the presence of [1-^{14}C]glycerol (16.3 µM). Each value is the mean ± SEM of six to eight rats and has been calculated as µmoles as a function of the specific activity of the labeled glycerol in the medium that had been incubated without tissue (standard). The numbers between the points are the *P* values that correspond to the differences between fed and 48-hr fasted groups at a given time (N.S., not significant, i.e., $P > 0.05$). The regressions and the coefficients of correlation (*r*) obtained from all the data of each group are shown near each line. Other details are given in the text.

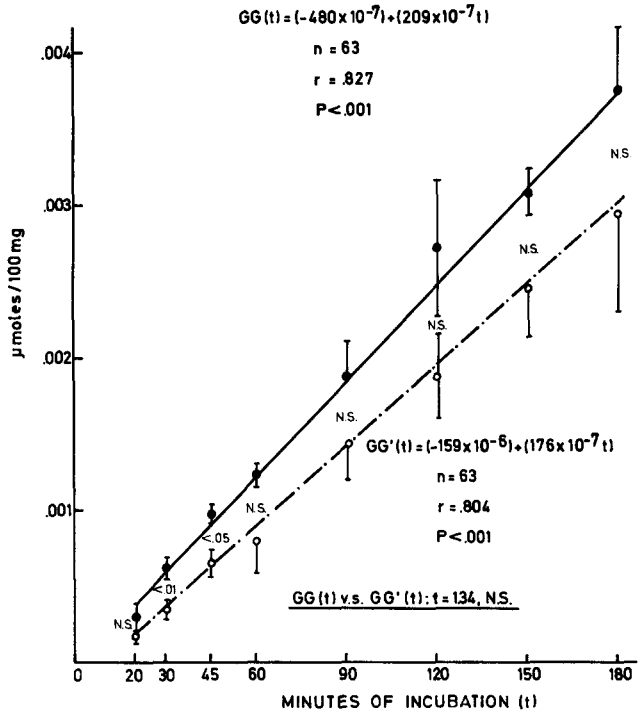

FIG. 3. Formation of ^{14}C-labeled glyceride-glycerol (*GG*) by epididymal fat pads from fed (●) and 48-hr-fasted (○) rats. Each value is the mean ± SEM of six to eight rats and has been calculated as µmoles as a function of the specific activity of the appropriate counting standard. The numbers between the points are the *P* values that correspond to the differences between fed and 48-hr-fasted groups at a given time (N.S., not significant, i.e., $P > 0.05$). The regressions and coefficients of correlation (*r*) obtained from all the data of each group are shown near each line. Other details are given in the text.

$$GG(t) = (-159 \times 10^{-6}) + (176 \times 10^{-7}\, t)$$

$$CO_2(t) = (-366 \times 10^{-7}) + (234 \times 10^{-8}\, t)$$

While the [1-^{14}C]glycerol of the medium is being taken up and used by the tissue, glycerol is coming out into the medium, as stated above. Thus, there is continuous dilution of the radioactive glycerol in the medium, and its specific radioactivity decreases progressively. This means that the radioactivity measurements underestimate the actual amount of glycerol that has been utilized by the tissue; thus, corrections must be made to obtain the actual rates of release and uptake of glycerol by the tissue.

Radioactive glycerol coming from the tissue into the medium

Experiments were carried out to determine whether or not the radioactive glycerol taken up and utilized by the tissue returns to the medium. After a preincubation of either 60 or 120 min with [1-^{14}C]glycerol, the tissues from fed and 48-hr-fasted rats were washed and incubated in cold medium for 120 and 60 min, respectively (i.e., tissues preincubated for 60 min were incubated for 120 min and vice versa). At the end of the incubation, the radioactivity in the medium never exceeded 2.93% of the total [1-^{14}C]glycerol taken up by the tissue during the preincubation period. This almost negligible recycling of the labeled glycerol is not surprising in view of the fact that the radioactive glycerol taken up by the tissue is diluted by a very large pool of nonradioactive glycerides (8) and thus does not return to the medium during the period of incubation.

Calculation of the rates of glycerol release into the medium and uptake of [1-^{14}C]glycerol by the tissue

If the tissue compartment is termed *2* and the medium compartment *1*, v_{21} represents the rate of glycerol coming from the tissue to the medium and v_{12} the rate of glycerol utilization by the tissue (i.e., medium to tissue). In like manner, V_{12} will be the rate at which radioactive glycerol goes from the medium into the tissue and V_{21} the rate at which ^{14}C-labeled glycerol goes from the tissue

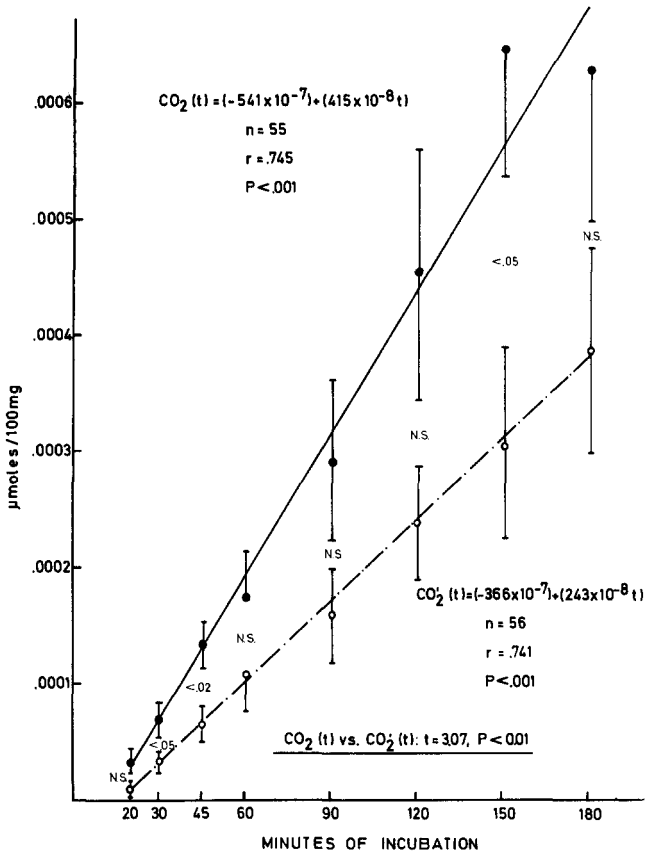

FIG. 4. Formation of $^{14}CO_2$ by epididymal fat pads from fed (●) and 48-hr-fasted (○) rats. Each value is the mean ± SEM of six to eight rats and has been calculated as µmoles as a function of the specific activity of the appropriate counting standard. The numbers between the points are the P values that correspond to the differences between fed and 48-hr-fasted groups at a given time (N.S., not significant, i.e., $P > 0.05$). The regressions and coefficients of correlation (r) obtained from all the data of each group are shown near each line. Other details are given in the text.

into the medium after being taken up by the tissue. The rate of net appearance of glycerol in the medium, expressed in µmoles/min/100 mg of tissue, will be $\frac{dg(t)}{dt}$, and so, from Eq. 1:

$$\frac{dg(t)}{dt} = b + 2at$$

It is evident that $v_{21} - v_{12} = \frac{dg(t)}{dt}$, and thus:

$$v_{21} - v_{12} = b + 2at \qquad \text{Eq. 3}$$

On the other hand, the rate of net uptake of radioactive glycerol by the tissue (µmoles/min/100 mg of tissue) will be $\frac{dG(t)}{dt}$, and so, from Eq. 2:

$$\frac{dG(t)}{dt} = B$$

It is also evident that $V_{12} - V_{21} = \frac{dG(t)}{dt}$, and so:

$$V_{12} - V_{21} = B \qquad \text{Eq. 4}$$

At a given time, the ratio V_{12}/v_{12} is equal to the ratio of the amounts of radioactive and unlabeled glycerol in the medium. The amount of radioactive glycerol in the medium is the difference between the initial [1-^{14}C]-glycerol (M) in the medium (constant) and the amount taken up by the tissue at each time ($G[t]$); therefore:

$$\frac{V_{12}}{v_{12}} = \frac{M - G(t)}{g(t)} \qquad \text{Eq. 5}$$

As we have seen above, a very small percentage of the radioactive glycerol taken up by the tissue does return to the medium during the interval of time of the experiment; thus, we can consider

$$V_{21} = 0 \qquad \text{Eq. 6}$$

Equations 3, 4, 5, and 6 form a system with four unknown rates. The development of this system may be carried out using the constants obtained in the regressions $g(t)$ (Eq. 1) and $G(t)$ (Eq. 2). When the regressions are those corresponding to the data of fed animals, the velocities are:

$$V_{12} = 263 \times 10^{-7}$$

$$v_{12}(t) = \frac{(421 \times 10^{-2}) + (266 \times 10^{-3}\, t) - (681 \times 10^{-6}\, t^2)}{(543 \times 10^{3}) - 263\, t}$$

and

$$v_{21}(t) = \frac{553 - (281 \times 10^{-2}\, t) + (681 \times 10^{-6}\, t^2)}{(543 \times 10^{3}) - 263\, t}$$

And when the regressions correspond to the data of 48-hr-fasted rats, the velocities are:

$$V_{12} = 198 \times 10^{-7}$$

$$v_{12}(t) = \frac{(127 \times 10^{-1}) + (337 \times 10^{-3}\, t) - (121 \times 10^{-5}\, t^2)}{(544 \times 10^{3}) - 198\, t}$$

$$v_{21}(t) = \frac{923 - (665 \times 10^{-2}\, t) + (121 \times 10^{-5}\, t^2)}{(544 \times 10^{3}) - 198\, t}$$

The values of v_{21} for fed and 48-hr-fasted rats are graphically represented in Fig. 5; they are the real velocities of release of glycerol from the tissue. In other words, if our assumption of the nonrecycling of radioactive glycerol from the tissue to the medium is correct and there is no other source of free glycerol except by

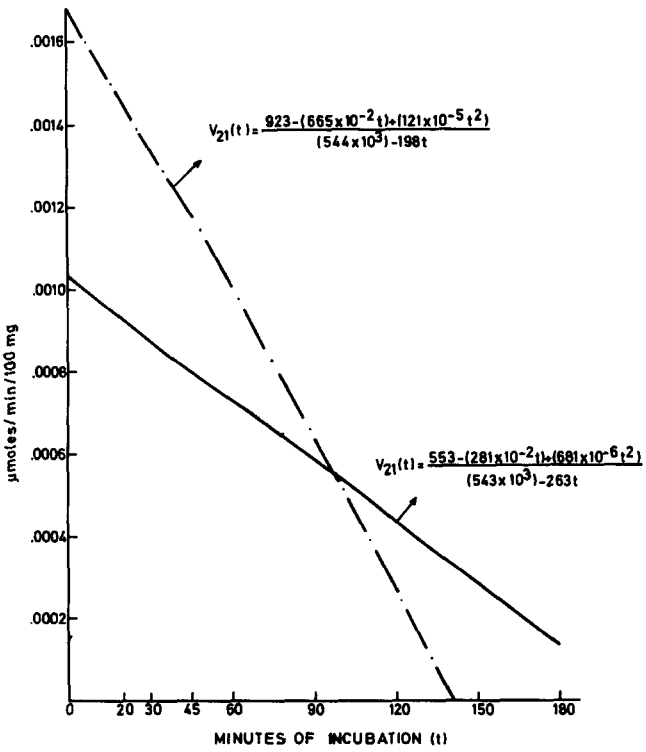

FIG. 5. Rates of glycerol release (v_{21}) into the medium by epididymal fat pads from fed (solid line) and 48-hr-fasted (broken line) rats. The values have been calculated by determining the amount of glycerol in the medium and correcting it by the labeled glycerol that has been taken up by the tissue at each time. More details are given in the Results and Discussion section.

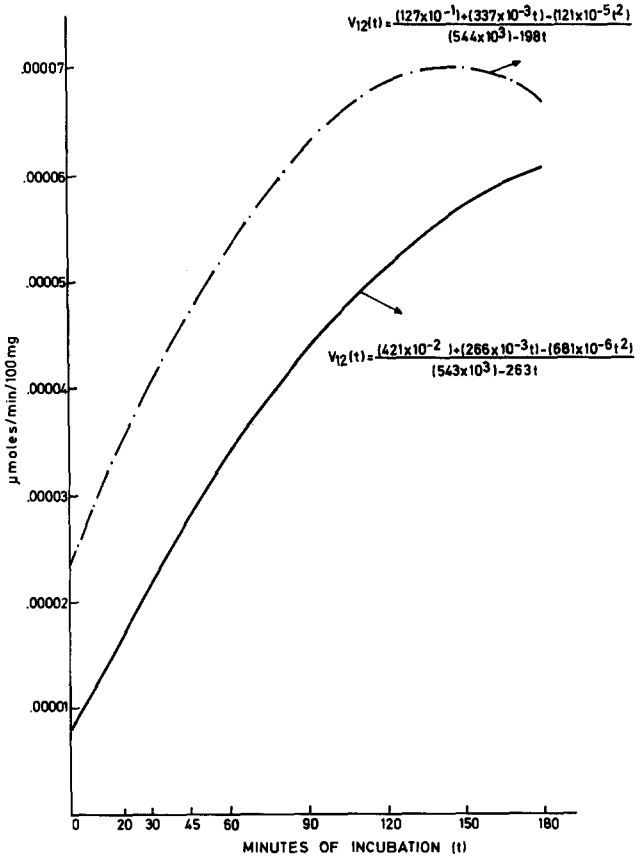

FIG. 6. Rates of glycerol uptake in vitro (v_{12}) by epididymal fat pads from fed (solid line) and 48-hr-fasted (broken line) rats. The values have been calculated by determining the uptake of labeled glycerol by the tissue and correcting it by the glycerol that enters the medium and continuously dilutes the radioactive substrate. More details are given in the Results and Discussion section.

hydrolysis of triglycerides,[1] the values of v_{21} are the *true rates of lipolysis* as a function of time. It can be seen that v_{21} decreases hyperbolically as a function of time (Fig. 5). At time 0, the rate of lipolysis is 66.9% higher in the 48-hr-fasted rats than in the fed ones. As the incubation proceeds, this rate decreases more drastically in the 48-hr-fasted rats than in the fed ones. This could be due to the inhibition of lipolysis by free fatty acids that accumulate more rapidly and to a greater extent in adipocytes from fasted animals (9). Actually, in our experimental situation this intracellular accumulation should be very high because albumin was not added to the medium. Thus, the free fatty acids not oxidized or reesterified during the incubation would accumulate in the tissue.

The values of v_{12} for fed and 48-hr-fasted rats are graphically shown in Fig. 6; they represent the real rate of glycerol uptake by the tissue during the time of incubation. It can be seen that in both experimental situations v_{12} increases hyperbolically as a function of time and that the values observed in the 48-hr-fasted rats are higher than those of the fed rats at all the times studied.

[1] Herrera, E. Unpublished results.

Rates of glyceride-glycerol and CO_2 synthesis from glycerol

Of the glycerol taken up by the tissue, we are especially interested in the amount converted to CO_2; this will give us an index of the total oxidation of glycerol and the amount that is utilized for the formation of glyceride-glycerol, which will give the true rate of esterification.

Using the regressions of release of glycerol from the tissue to the medium (Eq. 1) to determine $\frac{dg(t)}{dt}$ and the regressions of the formation of radioactive glyceride-glycerol (GG) and $^{14}CO_2$ (CO_2) from [1-^{14}C]glycerol, and following the same deductions as above, we can calculate the rates of utilization of glycerol for the synthesis of glyceride-glycerol ($v_{12}[GG]$) and for the formation of CO_2 ($v_{12}[CO_2]$). In fed animals these values are:

$$v_{12}(GG) = \frac{(335 \times 10^{-2}) + (212 \times 10^{-3}\, t) - (543 \times 10^{-6}\, t^2)}{(543 \times 10^3) - 209\, t}$$

$$v_{12}(CO_2) = \frac{(664 \times 10^{-3}) + (419 \times 10^{-4}\, t) - (108 \times 10^{-6}\, t^2)}{(543 \times 10^{3}) - (415 \times 10^{-1}\, t)}$$

And in 48-hr-fasted rats:

$$v_{12}(GG) = \frac{(113 \times 10^{-1}) + (295 \times 10^{-3}\, t) - (108 \times 10^{-5}\, t^2)}{(544 \times 10^{3}) - 176\, t}$$

$$v_{12}(CO_2) = \frac{(150 \times 10^{-2}) + (394 \times 10^{-4}\, t) - (144 \times 10^{-6}\, t^2)}{(543 \times 10^{3}) - (235 \times 10^{-1}\, t)}$$

Values of $v_{12}(GG)$ are represented in Fig. 7 as a function of time. They are the rates at which glycerol is utilized to form glyceride-glycerol and thus represent the *true rates of esterification* of glycerol, which are hyperbolic and increase with time and are higher in fasted than in fed animals.

As the rate of release of glycerol (lipolysis) decreases with time, esterification increases, and, eventually, lipolysis is slower than esterification. At that time, any glycerol that arises from the hydrolysis of glycerides is immediately reesterified in the adipocyte. This time is shorter in fasted than in fed animals due to the higher rates of lipid breakdown and glycerol utilization in fasted animals. This balance of lipid turnover will be upset in vivo by the continuous removal of the free fatty acids from the adipocyte. At all times lipid breakdown will exceed synthesis, at least when exogenous and/or endogenous sources of fatty acids are available, as in the case of the fed animals. However, our results also give an index of the in vivo situation because, as we have seen, they can be extrapolated to zero time of incubation and the values at this time will be very close to the rates of lipolysis and esterification in vivo.

The data of $v_{12}(CO_2)$ give us the rates of glycerol utilization for the synthesis of CO_2; they are represented in Fig. 8. It may be seen that the rate of synthesis of CO_2 from glycerol seems to be the same for both fed and 48-hr-fasted animals, and it increases with time as more glycerol is taken up by the tissue and is available for complete oxidation. It is interesting to note here that $v_{12}(CO_2)$ for fed and 48-hr-fasted rats is the same despite the fact that the slope of the formation of $^{14}CO_2$ from [1-^{14}C]glycerol (Fig. 4) was significantly lower in fasted than in fed animals. This is a clear example where this

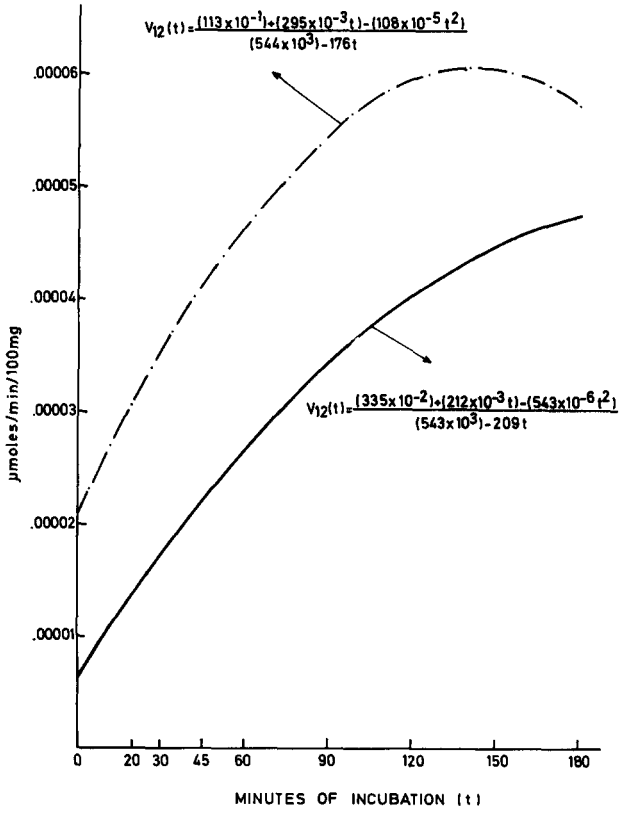

FIG. 7. Rates of glyceride-glycerol synthesis (v_{12}) from glycerol (esterification) by epididymal fat pads from fed (solid line) and 48-hr-fasted (broken line) rats. The values have been calculated by determining the amount of labeled glycerol converted to ^{14}C-labeled glyceride-glycerol by the tissue and correcting it by the glycerol that enters the medium and continuously dilutes the radioactive substrate. More details are given in the Results and Discussion section.

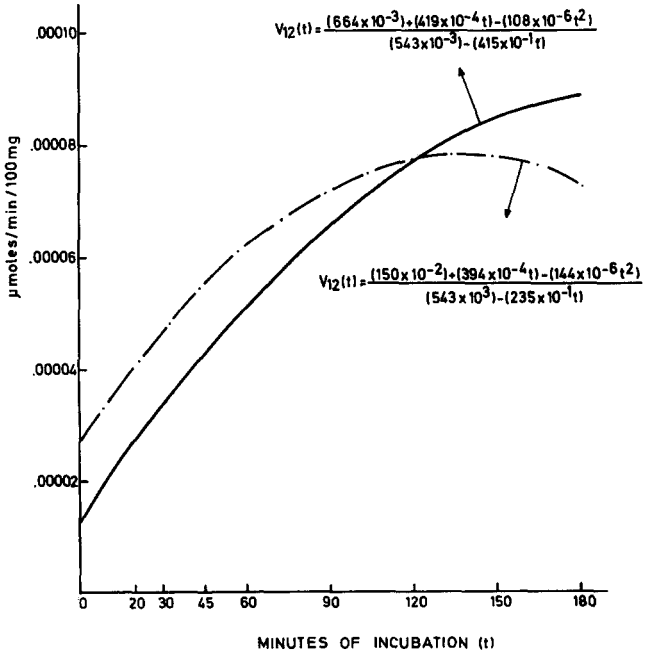

FIG. 8. Velocities of CO_2 synthesis (v_{12}) from glycerol by epididymal fat pads from fed (solid line) and 48-hr-fasted (broken line) rats. The values have been calculated by determining the amount of labeled glycerol converted to $^{14}CO_2$ and correcting it by the glycerol that enters the medium and continuously dilutes the radioactive substrate. More details are given in the Results and Discussion section.

difference is compensated for by the different dilution of the substrate. The radioactive glycerol in the medium (the same for both experimental groups) is diluted more in incubation with fat pads from fasted animals by the greater amount of unlabeled glycerol coming into the media (Fig. 1). Thus, although the formation of $^{14}CO_2$ is smaller for the fasted rats, the actual rate of formation of CO_2 from glycerol is the same when the data are corrected for by the greater isotope dilution of the radioactive glycerol.

We have seen here that, contrary to the previous conclusions (10), glycerol is utilized by adipose tissue at high rates and this affects calculations of lipolysis and esterification that do not take such utilization into account. Under the assumption that the glycerol that is utilized by the tissue does not return to the incubation medium, and that there is no other source of free glycerol except from the hydrolysis of glyceride, we have determined the rates of lipolysis and glycerol esterification, based on the corrections of glycerol production by the tissue for the [1-^{14}C]glycerol that it uses to form glyceride-glycerol. It was found that both lipolysis and esterification are higher in 48-hr-fasted rats than in fed rats. The same calculations were used to determine the rate of formation of CO_2 from glycerol; this was the same for both fed and 48-hr-fasted animals. It is most likely that in adipose tissue, as in other tissues, the glycerol must undergo phosphorylation (11) before it can form CO_2 and glyceride-glycerol; thus glycerokinase may not be lacking in adipose tissue as it was previously postulated (12). The data confirm the results of Robinson and Newsholme (13), and we can conclude that glycerokinase is present in sufficient amounts to result in a glycerol utilization that is considerably higher than previously thought. The results presented here draw attention once more to the difficulties of the interpretation of in vitro experiments in which the specific activity of the labeled substrate may be altered to an unknown degree by endogenous metabolites. We think that the mathematical analysis used in this study might be useful to perform the proper correction in those situations.

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