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Effect of Albumin on Glycerol Metabolism in Rat Adipose Tissue *

E. Herrera

Cátedra de Fisiología General
Facultad de Ciencias
Universidad de Barcelona
Barcelona - 7 (Spain)

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To study the effect of the displacement of FFA on the rates of lipolysis and glycerol utilization in adipose tissue, pieces of epididymal fat pad from fed rats were incubated in Krebs-Ringer bicarbonate containing (1-^{14}C) glycerol and 0, 5 or 10 mg of purified albumin/ml. The formation of glycerol by the tissue increases linearly with the time of incubation and it is enhanced by the presence of albumin in the media. The amount of labeled glycerol captured by the tissue and converted to $^{14}CO_2$ and ^{14}C-total lipids is not affected by albumin but when the results are corrected by the dilution of the tracer with the glycerol coming from the tissue to the medium, it appears that the actual rates of utilization of glycerol are enhanced by the presence of albumin. The results have been used to calculate the rate of lipolysis which is also enhanced by albumin. Thus, albumin can modulate glycerol metabolism in adipose tissue and this effect might have some physiological implications.

On the basis of the effects on the release of glycerol and/or free fatty acids (FFA) by adipose *in vitro*, it is commonly accepted that the addition of albumin to the incubation medium produces an increase in the rate of lipolysis (1, 2, 8, 9). It was believed that this effect of albumin was produced by decreasing the concentration of FFA into the tissue as a result of its high affinity for FFA, displacing them from the tissue (9). In contradiction to this assertion, ANGEL, DESAI and HALPERIN (1), using an accurate method, have found that the endogenous concentration of FFA was not different between adipocytes incubated *in vitro* in the presence and the absence of albumin in the medium.

We have recently shown that the utilization of glycerol by adipose tissue is considerably higher than previously thought (6, 7). Thus, the possibility exists that the albumin might also affect the reutilization

* This study is dedicated to the memory of Alejandro Ayanz. Part of this work was carried out at the Departamento de Endocrinología Experimental, Instituto G. Marañón, C.S.I.C., Madrid.

of glycerol. As the knowledge of this effect could add some new light on the action of albumin on adipose tissue metabolism, in the present work we have studied its effects on the utilization of (1-^{14}C) glycerol for the calculation of the rates of lipolysis, glycerol oxidation and glycerol esterification, using the mathematical analysis already described by us (6).

Materials and Methods

Male Wistar rats, weighing 174-212 g fed a Purina chow diet *ad libitum* were used. They were killed by cervical fracture without anesthesia. Epididymal fat pads were rapidly removed, placed in Krebs Ringer bicarbonate (KRB) buffer (11) at room temperature. The tissue from each rat was excised in several pieces, blotted lightly on Whatman no. 1 paper and weighed. 4 pieces (32-66 mg), selected at randon from each of the pads were placed in 20 ml vials containing 0.5 ml of the same buffer supplemented with different amounts of bovine albumin (Sigma, fraction V) which had been treated with charcoal adsortion (3) and extensive dialysis against 0.9 % sodium chloride, to remove FFA and organic acids. In the same experiment pooled tissue pieces coming from two rats were used to fill the vials containing the different concentrations of albumin and to be incubated for different periods of times. At zero time, 0.5 ml of the buffer containing 0.5 μCi of (1-^{14}C) glycerol (15.4 mCi/mmole) was pipetted into each vial and these were covered with rubber cups, gassed for 5 min through needles with O_2—CO_2 (95:5) and incubated in a Dubnoff metabolic shaker at 37° C for different periods of time. The incubation was stopped by the addition of $HClO_4$ and the $^{14}CO_2$ was trapped in Hyamine 10X hydroxide by a gentle shaking at room temperature for 60 min, as described previously (6, 7). Lipids were extracted from the incubated tissue (4), purified and fractionated as already described (6, 7). Aliquots of the incubation media were neutralized with saturated $KHCO_3$ and after removing the $KClO_4$ formed by centrifugation in the cold, portions of the supernatants were used for determination of glycerol (5), for measurement of radioactivity and for the isolation of ^{14}C-labeled glycerol (6, 7). 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, and calculated as micromoles as a function of the specific activity of the appropriate counting standards. The mathematical analysis of the data was carried out as previously described (6). Linear regressions were determined using standard methods (10). Statistical comparisons between two groups of data were performed by Student's test. All the calculations were carried out in an Ataio electronic calculator (Compucorp, 445).

Results and Discussion

Formation of unlabeled glycerol and utilization of (1-^{14}C) glycerol. — The formation of glycerol by pieces of epididymal fat pads from fed rats *in vitro* increases with the time of incubation (Table 1). When the tissues were incubated in KRB buffer containing 5 or 10 mg/ml, the amount of glycerol formed was higher than that of the same tissues from the same rats, incubated without albumin. The effect was specially significative from 60 min of incubation. In all the conditions studied, the formation of glycerol by the tissues as a function of time, $g(t)$, may be adjusted well to linear regressions ($P < 0.001$, in all cases) of the type:

$$g(t) = a + bt \qquad \text{Eq. 1}$$

The respective regressions for each group are also shown in Table 1, where it can be seen that the slopes of the regressions

Table I. *Effect of albumin on the formation of glycerol and on the utilization of (1-^{14}C) glycerol by epididymal fat pads from fed rats incubated* in vitro.

Each value is the mean ± SEM of six to eight pair of rats and has been calculated as μmoles/100 mg of tissue, taking into account the specific activity of the tracer. The incubations were carried out in KRB medium containing (1-^{14}C) glycerol (15.4 mCi/mM). Significant differences vs. the values from the tissues incubated without albumin have been denoted by asterisks.

Additions to the medium	Minutes of Incubation (t) 0	30	60	120	180	Regressions	r and p values of the regressions
Formation of glycerol (g)							
None	.041±.007	.050±.007	.075 ±.011	.122 ±.008	.195±.023	$g(t) = 260 \times 10^{-4} + 898 \times 10^{-6}\, t$	.867 < .001
Albumin (5 mg/ml)	.067±.009	.063±.007	.108* ±.009	.177* ±.026	.283±.042	$g(t) = 337 \times 10^{-4} + 135 \times 10^{-5}\, * t$	.816 < .001
Albumin (10 mg/ml)	.052±.006	.067±.005	.112**±.015	.213**±.031	.282±.046	$g(t) = 312 \times 10^{-4} + 154 \times 10^{-5}\, ** t$	.835 < .001
Uptake of (1^{14}C) glycerol (G) × 10^5							
None		87±6	198±21	459±41	561±52	$G(t) = 854 \times 10^{-7} + 326 \times 10^{-7}\, t$	.901 < .001
Albumin (5 mg/ml)		91±8	197±22	388±48	559±59	$G(t) = 647 \times 10^{-7} + 310 \times 10^{-7}\, t$	.878 < .001
Albumin (10 mg/ml)		101±13	181±18	418±64	580±78	$G(t) = 656 \times 10^{-7} + 302 \times 10^{-7}\, t$	.802 < .001
Formation of $^{14}CO_2$ (CO_2) × 10^5							
None		11±1	40±4	89±9	112±16	$CO_2(t) = -309 \times 10^{-7} + 678 \times 10^{-8}\, t$	.846 < .001
Albumin (5 mg/ml)		12±2	37±7	76±13	94±14	$CO_2(t) = 231 \times 10^{-7} + 538 \times 10^{-8}\, t$	.784 < .001
Albumin (10 mg/ml)		15±5	36±7	70±13	90±14	$CO_2(t) = 203 \times 10^{-7} + 514 \times 10^{-8}\, t$	.758 < .001
Formation of ^{14}C-labeled glyceride-glycerol (GG) × 10^5							
None		75±5	156±24	351±34	455±46	$GG(t) = 113 \times 10^{-6} + 246 \times 10^{-7}\, t$	.874 < .001
Albumin (5 mg/ml)		79±7	159±20	330±35	446±56	$GG(t) = 656 \times 10^{-8} + 248 \times 10^{-7}\, t$	.868 < .001
Albumin (10 mg/ml)		68±7	132±17	303±52	430±68	$GG(t) = -631 \times 10^{-7} + 248 \times 10^{-7}\, t$	.797 < .001

* = P < .05, ** = P < .01 and *** = P < .001 (no asterisk means that p > .05).

of the values from tissues incubated in the presence of albumin were significantly higher than those from the tissues incubated in its absence. It is interesting to point out here the fact that the linearity in the formation of glycerol as a function of time found here is different from what we found previously (6), where the data fitted better to second-degree equations. We do not have yet an explanation for this difference, which as we will see below produces a drastic change in the calculated rates of lipolysis. Due to this inexplicable discrepancy, in three experiments, the time of incubation was prolonged longer (up to 240 min) and we found that the appearence of glycerol in the system continued increasing during all the time of incubation in the presence as well as in the absence of albumin.

The possibility existed that the difference in the formation of glycerol among the groups could be the result of a different reutilization of glycerol by the tissue and not to a difference in the actual rate of glycerol released through out the hydrolisis of glycerides (lipolysis). Therefore, we studied the utilization of (1-^{14}C) glycerol by the tissue. As shown in table 1, the uptake of labeled glycerol and its conversion to either $^{14}CO_2$ or ^{14}C-labeled total lipids were not affected by the presence of albumin in the media. In all cases, more than 92 % of the radioactivity incorporated to lipids was in the form of glyceride glycerol, thus the values of ^{14}C-labeled total lipids might be used as an index of the initial (1-^{14}C) glycerol that has been esterified. The uptake of ^{14}C-labeled glycerol as well as its conversion to $^{14}CO_2$ and ^{14}C-labeled lipids increased with the time of incubation and does not differ between the tissues incubated with or whithout albumin in the medium (Table 1). The respective values, $G(t)$, are adjusted to highly significant ($P < 0.001$) first-degree regression of the type:

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

The regressions obtained from the data from the tissues incubated with and without albumin are also shown in Table 1. No significant differences were found between the slope values (B, in eq. 2) of the data from tissues incubated without albumin and those incubated in its presence.

At first sight it might be concluded from these results that the presence of albumin in the media does no affect the utilization of glycerol by the tissue. However, consideration of the amount of glycerol formed leads to quite a different conclusion.

Calculation of the rate of glycerol utilization. — As discussed previously (6), to calculate the actual rate of utilization of glycerol by adipose tissue incubated *in vitro*, we must take into account the change in the specific activity of the tracer during the period of incubation due to its dilution by the glycerol released from the tissue into the medium.

Following the same mathematical deduction as previously (7), from Eq. 1, the rate of net appearance of glycerol in the medium might be calculated:

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

This value corresponds to the difference between the rate of glycerol coming from the tissue into the medium through out lipolysis (V_L) minus that of glycerol utilized by the tissue (V_U):

$$V_L - V_U = b \qquad \text{Eq. 3}$$

By the same reasoning, from Eq. 2 might be calculated the rate of net uptake of radioactive glycerol by the tissue:

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

Here again, this value corresponds to the difference between the rate of radioactive glycerol going into the tissue from the medium (V_G) minus the rate of radioactive glycerol coming back from the tissue into the medium. As we have previously demonstrated that this last value might be considered zero (6), V_G will be:

$$V_G = B \qquad \text{Eq. 4}$$

Equations 3 and 4 might be linked with a third one, where the ratio V_G/V_U is equal to the ratio of the amounts of radioactive and unlabeled glycerol in the medium at a given time:

$$\frac{V_G}{V_U} = \frac{M - G(t)}{g(t)} \qquad \text{Eq. 5}$$

where M is a constant which corresponds to the initial (1-^{14}C) glycerol in the medium.

Using the regressions obtained from the experimental data (Table 1), and applying them to equations 3, 4 and 5, we can calculate the true rates of the utilization of glycerol (V_U), as well as those of glycerol released by the tissue (lipolysis) (V_L). The rate of uptake of glycerol by the tissues increases hyperbolically with the time of incubation (Fig. 1). While the respective lines flow together at the origin (zero time) (Fig. 1), after 180 min of incubation the rates of glycerol being taken up by the tissues incubated in the presence of 5 and 10 mg/ml of albumin are 40.5 and 51.6 % respectively higher than the basal values. As on other occasions (6, 7), practically all the glycerol taken up by the tissue might be accounted for that converted to CO_2 and lipids (in the form of glyceride glycerol). The rate of synthesis of both parameters from glycerol is also augmented by the presence of albumin in the medium (Figs. 2 and 3), and this is specially evident in the rates of synthesis of lipids (Fig. 3) where the observed values after

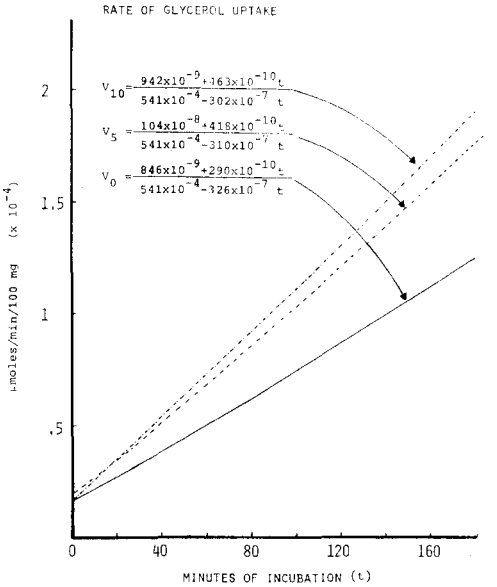

Fig. 1. *Effect of albumin on the rate of glycerol uptake* in vitro *(V) by epididymal fat pads from fed rat.*
Concentration of albumin in the medium: ——— = 0; - - - = 5 mg/ml; ····· = 10 mg/ml. The values have been calculated using the data from Table 1, as described in the text.

180 min of incubation for the tissues incubated with 5 and 10 mg of albumin/ml were 48.2 and 60.2 % respectively higher than in those incubated in its absence. These results demonstrate once more that, at least in the absence of glucose, part of the glycerol released to the medium by adipose tissue incubated *in vitro* is actually utilized by the tissue for its oxidation to CO_2 and its reesterification. The latter effect is especially enhanced in the presence of albumin in the medium probably by providing enough glycero phosphate for the esterification of the excess of endogenous FFA that have been liberated directly into the adipocytes or those recaptured by the tissue after being released into the medium. Actually, it has been shown that the FFA bound to the albumin

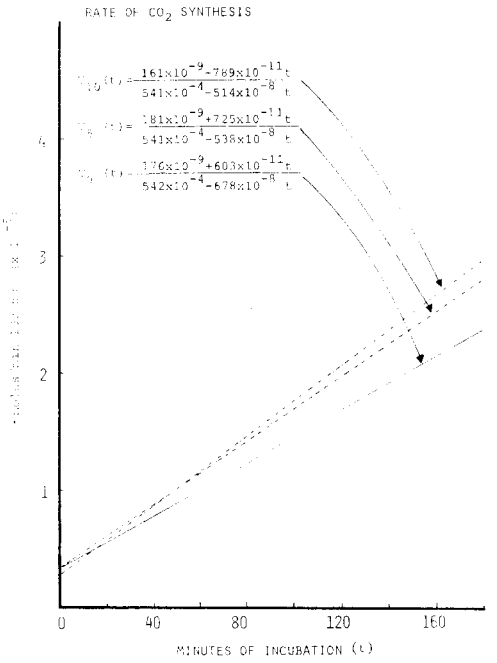

Fig. 2. *Effect of albumin on the rate of CO_2 synthesis from glycerol (V) by epididymal fat pads from fed rats.*
Concentration of albumin in the medium: ——— = 0; - - - = 5 mg/ml; ····· = 10 mg/ml. The values have been calculated using the data from Table 1, as described in the text.

in the medium may return to the adipocytes (9). Thus, the enhanced reutilization of glycerol for its phosphorilation and its further conversion to glyceride glycerol produced by the albumin could contribute to the maintainance of the unchanged concentration of FFA into the tissue even in the absence of glucose in the medium (1).

Calculation of the rate of glycerol released into the medium. — Knowing the rate of glycerol that is being utilized by the tissue (V_U), we can calculate from equation 3 the rate of glycerol coming from the tissue into the medium (lipolysis) (V_L):

$$V_L = b + V_U \qquad \text{Eq. 6}$$

These values might be calculated from the data shown in Table 1. Before going into the analysis of the effects of albumin on the values of V_L, we must analyse the equation 6, as it differs from that obtained when the data of the formation of glycerol are adjusted to second-degree regressions (6). If second-degree adjustement is used for $g(t)$, of the type:

$$g(t) = a + bt + ct^2,$$

we will have that

$$V_L - V_U = b + 2ct$$

instead of equation 3, and thus, V_L would be equal to

$$V_L = b + 2ct + V_U$$

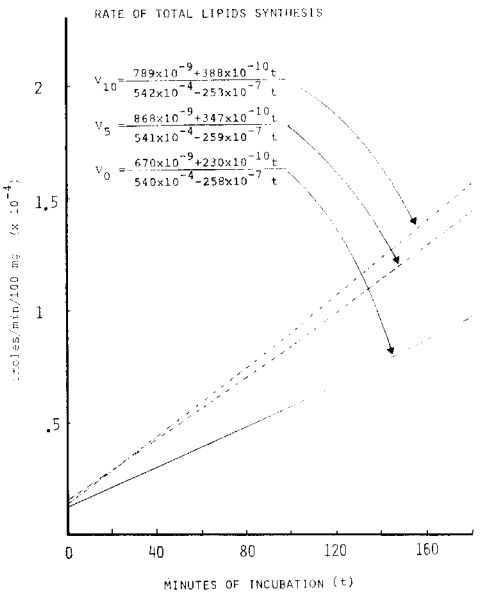

Fig. 3. *Effect of albumin on the rate of total lipids synthesis from glycerol (V) by epididymal fat pads from fed rats.*
Concentration of albumin in the medium: ——— = 0; - - - = 5 mg/ml; ····· = 10 mg/ml. The values have been calculated using the data from Table 1, as indicated in the text.

As the values of b and V_U are always positive, the sign of V_L will depend on c in such a way that when c is greater or equal to zero, V_L will be always positive but when c is smaller than zero, V_L might be either positive or negative. Contrary to before, in the present study the data of $g(t)$ adjusted better to first-degree equation (i.e. doing $c = 0$) and thus the rate of lipolysis increased with the time of incubation in a hyperbolic way (Fig. 4). We do not have yet an explanation for this discrepancy and we hope that our current investigations would give some light on this point. The presence of 5 or 10 mg/ml of albumin in the media produces an increase in the rate of lipolysis of the tissue which is of the order of 55 and 71 % higher respectively in relation with the values obtained with the tissues incubated without albumin (Fig. 4). This finding confirms previous reports where the effect of albumin on the rate of lipolysis was determined without taking into account the amount of glycerol being taken up by the tissue during the period of incubation (1, 2, 8, 9).

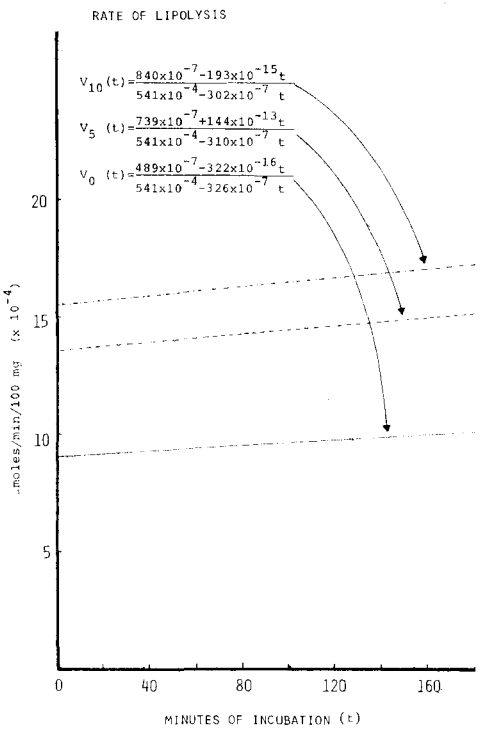

Fig. 4. *Effect of albumin on the rate of lipolysis (V) by epididymal fat pads from fed rats.*

Concentration of albumin in the medium: ——— = 0; - - - = 5 mg/ml; ····· = 10 mg/ml. The values have been calculated using the data from Table 1, as indicated in the text.

Although the primary action of albumin on adipose tissue metabolism remains to be elucidated, the possibility exist that in physiological conditions the albumin used as carrier of FFA through out the blood might act at the same time as a sensitive modulator of glycerol metabolism in adipose tissue and therefore facilitating the deposition of glycerides more than its hydrolysis or viceversa, depending on several factors such as the molecular ratio of FFA/albumin, their mutual affinities, the concentration of glycerol and ATP available, etc. The interactions of the lipolytic and antilipolytic hormones on these factors remain also to be elucidated and are under current study in our laboratory.

Resumen

Para estudiar el efecto del desplazamiento de ácidos grasos en tejido adiposo sobre las velocidades de lipolisis y utilización de glicerol, se incubaron trozos de epidídimo graso procedentes de ratas alimentadas en Krebs-Ringer bicarbonato conteniendo (1-^{14}C) glicerol y albúmina purificada, en concentraciones de 0, 5 ó 10 mg/ml. La formación de glicerol por el tejido aumenta de forma lineal con el tiempo de incubación, y es aumentada por la presencia de albúmina en el medio. La cantidad de glicerol radioactivo capturado por el tejido y convertido a $^{14}CO_2$ y lípidos totales-^{14}C, no es afectada por la albúmina. Sin embargo, cuando estos resultados se corrigen por la dilución del trazador con el glicerol procedente del tejido, se observa que la albúmina aumenta las velocidades reales de utili-

zación del glicerol. Se han utilizado estos resultados para calcular la velocidad de lipolisis, la cual es aumentada por la albúmina. Así pues, la albúmina puede modular el metabolismo del glicerol en tejido adiposo, y este efecto puede que tenga implicaciones fisiológicas.

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