

## Effects of Glycerol and Glucose on the Kinetics of Glycerol Utilization by Adipose Tissue in the Rat \*

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To study the kinetics of glycerol utilization by adipose tissue *in vitro* as function of the concentrations of both glycerol and glucose in the incubation media, pieces of epididymal fat pad from fed rats were incubated for different times in Krebs Ringer bicarbonate supplemented with 1-<sup>14</sup>C-glycerol and purified albumin. An increase in the concentration of glycerol in the medium produces a decrease in the formation of <sup>14</sup>CO<sub>2</sub> and <sup>14</sup>C-lipids from 1-<sup>14</sup>C-glycerol. When the decrease in the specific activity of the tracer is considered to calculate the respective velocities, it turns out that glycerol actually enhances the rate of synthesis of both CO<sub>2</sub> and glyceride glycerol. Glucose enhances the rate of synthesis of CO<sub>2</sub> and fatty acids from glycerol but decreases the rate of glyceride glycerol synthesis from the same substrate. While the K<sub>m</sub> of the glycerol effect is much lower than the physiological concentrations of glycerol the K<sub>a</sub> and K<sub>i</sub> of the glucose effects are above or close to its concentration in blood. The results are discussed in terms of the competitive effects of glucose and glycerol for the synthesis of α-glycerophosphate and the necessity of glucose for lipogenesis from glycerol in adipose tissue.

It was previously shown that many factors may affect the rate of glycerol utili-

zation by adipose tissue *in vitro*, including dietary status (5), insulin and epinephrine (6), thyroid hormones (10), metabolic inhibitors (3) and different drugs (7). Glucose is the main metabolic fuel in adipose tissue as well as the principal substrate for lipogenesis. In connection with this, we have recently observed that glucose enhances the rate of glycerol utilization by adipose tissue and affects the effect of both epinephrine and insulin on this para-

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meter (3, 6). In the present work we have studied the kinetics of glycerol utilization by adipose tissue *in vitro* as function of the concentrations of both glycerol and glucose in the incubation media.

### Materials and Methods

Pieces of epididymal fat pad from fed Wistar rats sacrificed by cervical fracture were distributed at random in vials (17-29 mg/vial) containing 0.5 ml of Krebs Ringer bicarbonate, pH 7.4 (KRB) (12), supplemented with 10 mg of defatted albumin and different concentrations of glycerol or glucose. At 0 time, 0.5  $\mu$ Ci of 1- $^{14}$ C-glycerol (32 mCi/mM) diluted in 0.5 ml of KRB were added to each vial and incubated for different times. Incubations were carried out under  $O_2/CO_2$  (95:5) and samples were processed as

previously described (5). The mathematical analysis of the data was carried out as previously described (4). Linear regressions were determined using standard methods (11). All the calculations were carried out in an Atao Electronic calculator (Compucorp 445). In every experimental condition, a minimum of four experiments were carried out and the data shown correspond to the data of one representative experiment in each case.

### Results

The effect of different glycerol concentrations in the medium of pieces of epididymal fat pad incubated *in vitro* for 30, 60, 120 and 180 min on the formation of  $^{14}CO_2$  and  $^{14}C$ -lipids from 1- $^{14}C$ -glycerol is shown in figure 1. The percentage of radioactivity converted to either para-

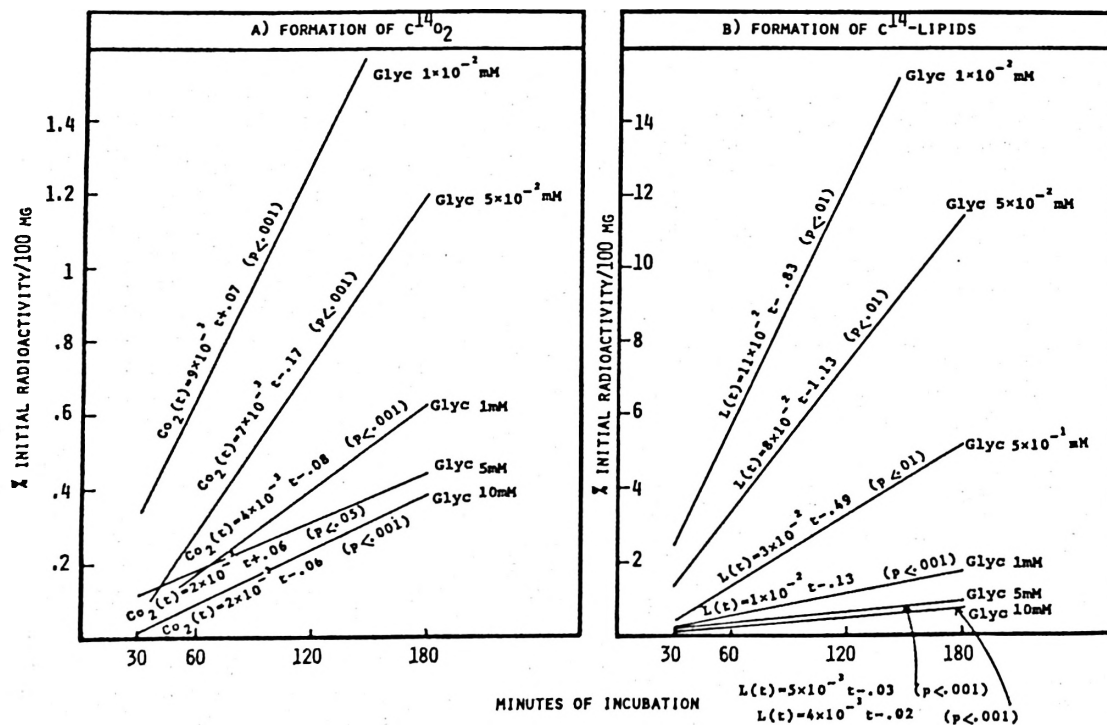


Fig. 1. Effect of different glycerol (Glyc) concentrations in the medium of pieces of epididymal fat pad from fed rats incubated *in vitro* for different times ( $t$ ) on the formation of  $^{14}CO_2$  (fig. 1A) and  $^{14}C$ -lipids ( $L$ ) (fig. 1B) from 1- $^{14}C$ -glycerol.

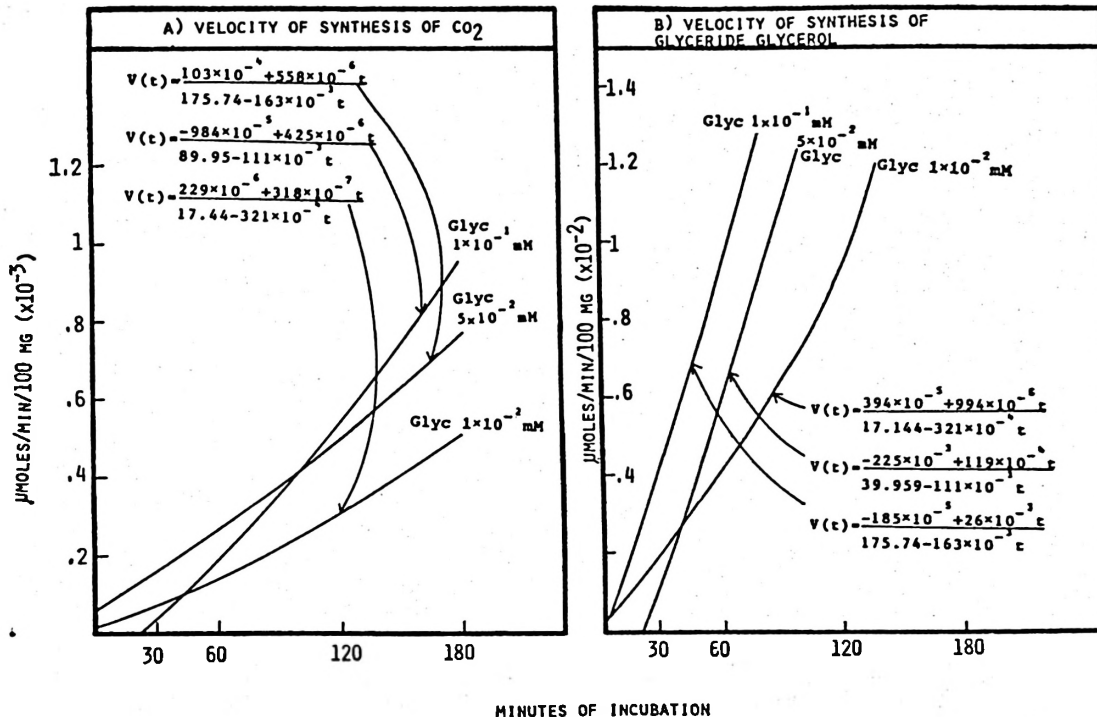


Fig. 2. Effect of different glycerol (Glyc) concentrations in the medium of pieces of epididymal fat pad pieces from fed rats incubated in vitro for different times ( $t$ ) on the velocities ( $v$ ) of CO<sub>2</sub> (fig. 2A) and glyceride glycerol (fig. 2B) synthesis from glycerol. The values have been corrected by the dilution of the substrate with glycerol going from the tissue into the medium via lipolysis.

meter increases lineary with the time of incubation and the slopes of the correspondent lines are smaller as the concentration of cold glycerol in the medium increases. Although not shown, practically all the radioactivity in the lipids is in the form of glyceride glycerol (85.6-96.7 per cent of the radioactivity in lipids was in the form of glyceride glycerol in all the situations). As the specific activity of the 1-<sup>14</sup>C-glycerol has been diluted with the cold glycerol in the medium, we have to take into account this factor to obtain the actual rates of glycerol utilization by the tissue. These calculations were carried out following the method already described (4). These calculations were only done with the values of the tissues

incubated in the presence of 10, 50, and 100  $\mu$ M glycerol, as above of these concentrations, the amount of glycerol in the medium at the end of the incubation was smaller than that at time 0. In other words, above the concentration of 100  $\mu$ M glycerol in the medium, the utilization of glycerol by the tissues exceded its production via lipolysis unabling us to make the proper corrections. Using these calculations, the rates of glycerol utilization for synthesis of CO<sub>2</sub> and glyceride glycerol were determined and their values as function of time of incubations are summarized in figure 2. The increase in the concentration of glycerol in the medium produced an increase in the rates of its conversion to both CO<sub>2</sub> (fig. 2A)

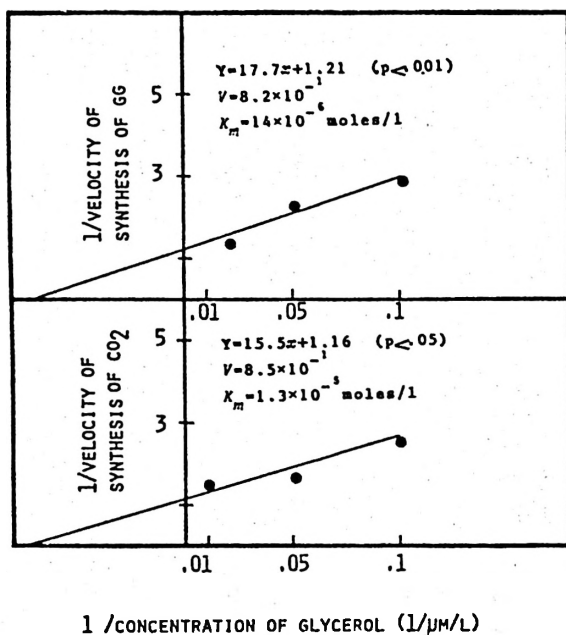


Fig. 3. Lineweaver-Burk plot of the reciprocal of the rates of  $\text{CO}_2$  and glyceride glycerol (GG) synthesis from glycerol by epididymal fat pad pieces from fed rats after 180 min of *in vitro* incubation.

and glyceride glycerol (fig. 2B). Using the Lineweaver-Burk plot of the reciprocal of the rates after 180 min of incubation against the reciprocal of the glycerol concentrations, the  $V_{\max}$  and  $K_m$  values were determined (fig. 3). These constants are very similar for both  $\text{CO}_2$  and glyceride glycerol formation, ( $V_{\max} = 0.82\text{--}0.85 \mu\text{moles/min/100 mg}$  and  $K_m = 13\text{--}14 \mu\text{M}$ ).

Using a similar protocol to that described above the effect of glucose in the medium on the rates of glycerol utilization by the tissues incubated *in vitro* was determined. The data have been corrected already by the changes in the specific activity of the tracer ( $1\text{-}^{14}\text{C}$ -glycerol) during the different times of incubation. Glucose enhances the rate of  $\text{CO}_2$  formation from glycerol and the effect is proportional to the concentration of glucose in the medium (fig. 4A). The Dixon and

Webb plot (2) with the values observed after 180 min of incubation (fig. 4B), allow to determine the  $K_a$  of glucose for the formation of  $\text{CO}_2$  from glycerol, which is 18.8 mM. The synthesis of fatty acids from glycerol is very much dependent on the presence of glucose in the medium (fig. 5). In the absence of glucose there is no synthesis of fatty acids from glycerol and in the presence of 1 mM glucose it is practically inappreciable, while it is very much stimulated with concentrations of glucose of 5 mM and above (fig. 5). It seems that there is a threshold point for glucose in the synthesis of fatty acids from glycerol from which the effect of glucose is maximal and not dependent on its concentration. Contrary to the stimulating effects of glucose on  $\text{CO}_2$  and fatty acid synthesis from glycerol, glucose decreases the rate of synthesis of glyceride glycerol from glycerol (fig. 6A). The effect is very much dependent on the glucose concentration and when the Dixon plot is drawn with the values observed after 180 min of incubation (fig. 6B) the  $K_i$  of glucose for the synthesis of glyceride glycerol from glycerol appears to be 6.5 mM.

Glucose not only affects the rate of glycerol utilization by adipose tissue, but it has a clear effect on the rate of lipolysis. The rate of lipolysis of pieces of adipose tissue incubated in the presence of different doses of glucose were calculated as previously described (4) taking into account the amount of glycerol that is being taken up by the tissue at each time of incubation. The values are summarized in figure 7A, where it may be seen that glucose increases the rate of lipolysis. Here again the effect is very much dependent of glucose concentration and the  $K_a$  observed with the Dixon and Webb plot is 8.4 mM (fig. 7B).

## Discussion

In the present study it is seen that the utilization of glycerol by adipose tissue

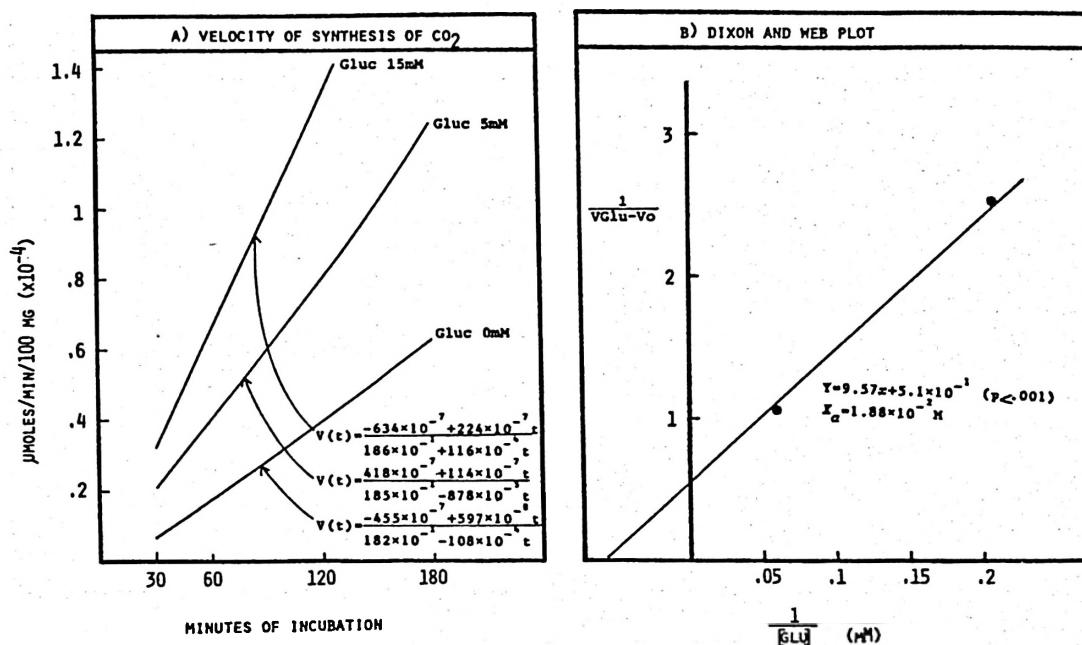


Fig. 4. Effect of glucose (Gluc) on the velocities of CO<sub>2</sub> synthesis from glycerol by pieces of epididymal fat pad from fed rats incubated *in vitro*.

Fig. 4A, corresponds to the effect after different time (t) of incubation and Fig. 4B, to the Dixon and Webb plot of the values observed after 180 min of incubation,  $V_{\text{Glu}}$  being the velocities in the presence of glucose and  $V_0$  the velocities in its absence.

*in vitro* increases with the concentration of glycerol in the incubation medium. This is true only when the labelled glycerol is corrected by the decrease of the dilution of the tracer with the cold glycerol in the medium. The  $K_m$  for glycerol utilization versus its concentration is around  $13.5 \mu\text{M}$ . This value is four to five times lower than the normal blood glycerol levels in the rat (9), which allows to suggest that small changes in the concentration of glycerol *in vivo* does not limit its utilization by the tissue. The concentration of glycerol in adipose tissue is however quite low [ $0.01 \mu\text{moles}/\text{mg}$  protein (8)] and the possibility exists that changes in the intracellular glycerol concentration could affect the reutilization of the endogenous glycerol.

Glucose enhances lipolysis as well as the utilization of glycerol for the synthesis of fatty acids and its complete oxidation

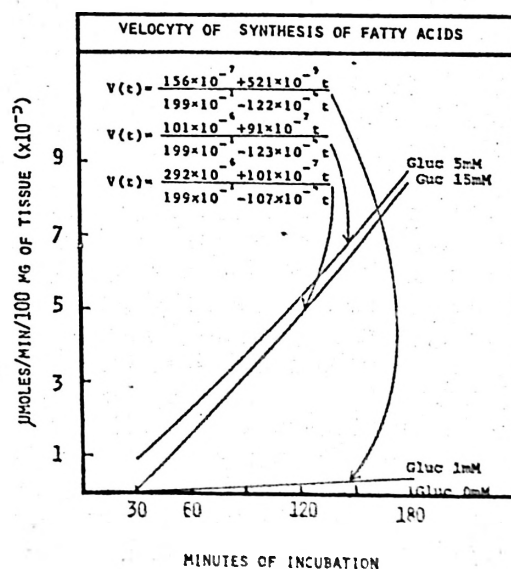


Fig. 5. Effect of glucose on the velocity (v) of synthesis of fatty acids from glycerol by pieces of epididymal fat pad incubated *in vitro* for different times (t).

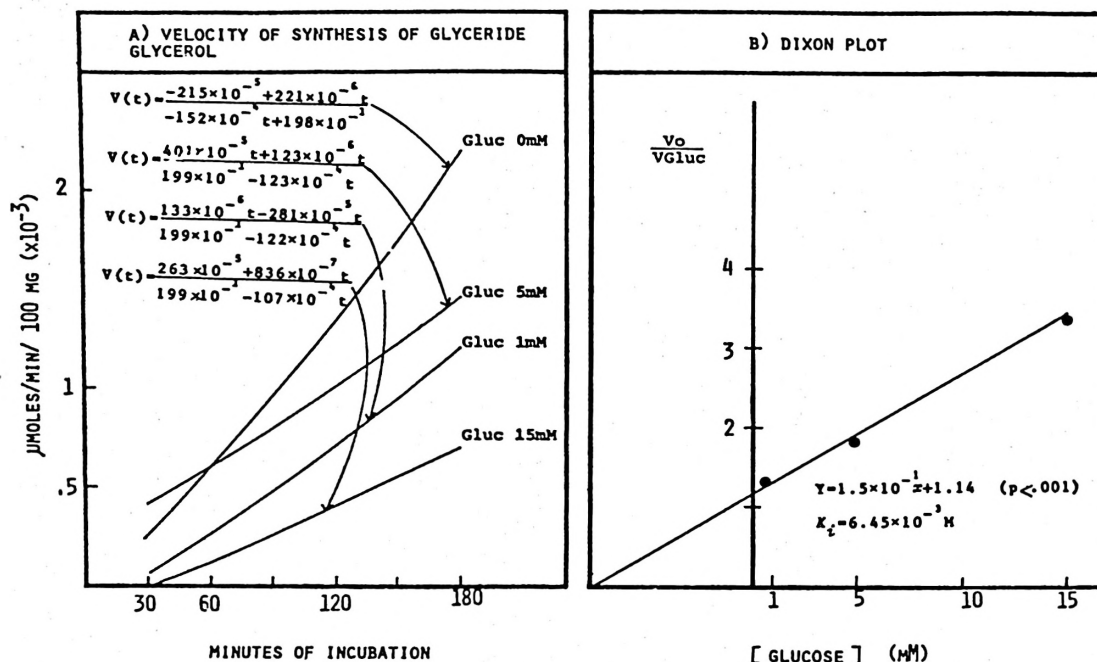


Fig. 6. Effect of glucose on the velocity ( $v$ ) of synthesis of glyceride glycerol from glycerol by pieces of epididymal fat pad incubated in vitro.

Fig. 6A, corresponds to the effect after different times ( $t$ ) of incubation and Fig. 6B, to the Dixon plot of the values observed after 180 min of incubation,  $V_{\text{Gluc}}$  being the velocities in the presence of glucose and  $V_0$  the velocities in its absence.

to  $\text{CO}_2$ . On the other hand, glucose inhibits the synthesis of glyceride glycerol from glycerol. These mixture of effects could be explained by two mechanisms: a) As glucose is the main metabolic fuel for adipose tissue, glucose can increase the availability of ATP for both the activation of lipolysis and the phosphorylation of glycerol for its further metabolization. Actually, both of these pathways are dependent of ATP (1). b) Glucose can be used for synthesizing  $\alpha$ -glycerophosphate and thus, it could compete with glycerol for the esterification of fatty acids in the synthesis of glyceride glycerol. These results allow to suggest that both mechanisms are taking place at the same time in what the effect of glucose on glycerol metabolism is concerned. Actually, the lipolytic effect of glucose through out an

enhancement in the availability of ATP is further augmented by facilitating the esterification of fatty acids with the  $\alpha$ -glycerophosphate formed from glycolysis and that from the direct phosphorylation of glycerol. An increase in the esterification of fatty acids as a result of the presence of glucose has been well documented (8) and the decrease in the intracellular pool of free fatty acids can be contributing further to the lipolytic effect of glucose.

Despite of the competitive relationship between glucose and glycerol for the synthesis of  $\alpha$ -glycerophosphate, glucose enhances the rate of glycerol utilization. This effect could be explained by an augmented availability of ATP for the phosphorylation of glycerol when glucose is present in the medium. Actually, metabolic inhibitors such as 2-deoxy-D-glucose

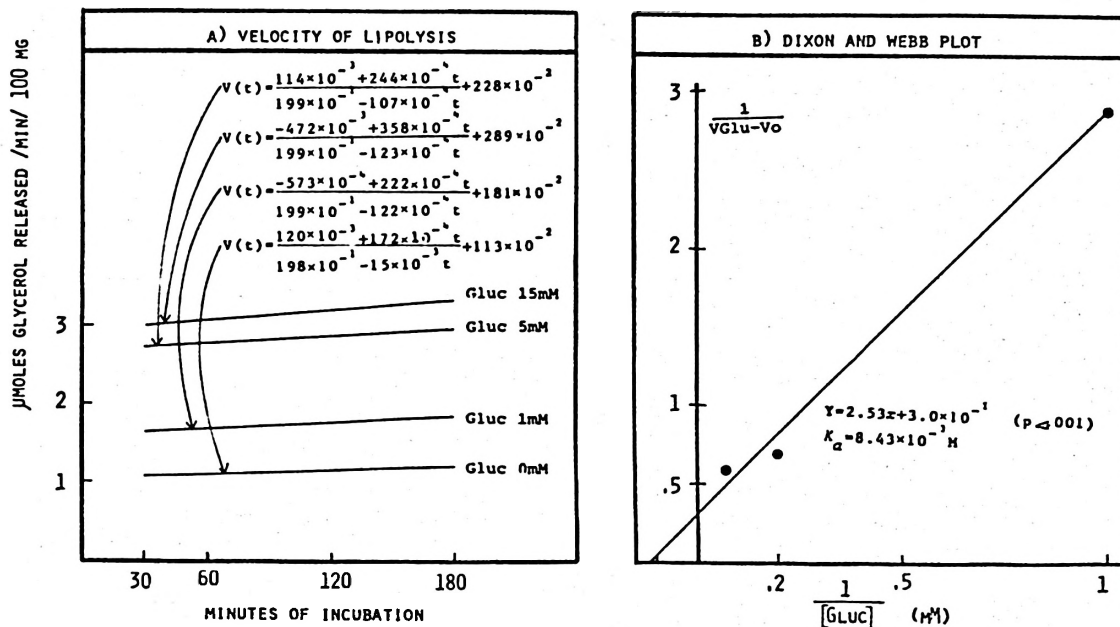


Fig. 7. Effect of glucose on the velocity ( $v$ ) of lipolysis by pieces of epididymal fat pad incubated in vitro.

Fig. 7A, corresponds to the effect after different times ( $t$ ) of incubation and Fig. 7B, to the Dixon and Webb plot of the values observed after 180 min of incubation.

and oligomycin produce the opposite effect on glycerol utilization in adipose tissue than that observed here with glucose (3). It is interesting to point out that the  $K_a$  of glucose for the formation of  $\text{CO}_2$  from glycerol ranges above the physiological concentrations of glucose in blood and allows to suggest that changes in the endogenous glucose levels could affect the glycerol utilization in adipose tissue. With the results of the effect of glucose on the synthesis of fatty acids from glycerol it is not possible to do this kind of reasoning as the pathway seems to have a threshold for glucose concentration in the medium. Actually, no fatty acid synthesis from glucose are observed without glucose in the medium and very low effect is observed with 1 mM glucose. However, 5 mM glucose produces an intense increment in the rate of fatty acid synthesis and this is not further augmented with

concentrations of 15 mM. This different response to glucose in what fatty acid synthesis is concerned can be due to the fact that glucose is not only acting on lipogenesis by giving ATP for the phosphorylation of glycerol but also by allowing enough NADPH formation throughout the hexose monophosphate shunt.

Glucose inhibits the synthesis of glyceride glycerol from glycerol and this effect is not surprising as glucose competes with glycerol for the formation of  $\alpha$ -glycerophosphate for its further esterification. The possibility exists that in the experimental conditions used here, the  $\alpha$ -glycerophosphate coming from glucose is more available for its esterification with fatty acids than that coming from glycerol, although this is being phosphorylated for its further metabolism to fatty acids and  $\text{CO}_2$  synthesis. Further experimental support is necessary to com-

pletely understand this interaction between glucose and glycerol metabolism in adipose tissue, but here again the effect of glucose is observed close to physiological concentrations ( $K_i = 6.5$  mM) which suggest that *in vivo* changes of glucose levels could be directly affecting the whole metabolism of glycerol in adipose tissue.

### Resumen

Con la finalidad de estudiar la cinética de la utilización del glicerol por el tejido adiposo *in vitro*, en función de las concentraciones de glicerol y glucosa en el medio de incubación, se incubaron trozos de epidídimo graso de rata en Krebs Ringer bicarbonato, suplementado con glicerol-1- $C^{14}$  y albúmina purificada. Las incubaciones se realizaron a distintos tiempos. Al aumentar la concentración de glicerol en el medio se produce una disminución de la cantidad de  $^{14}CO_2$  y lípidos- $C^{14}$  formados a partir del glicerol-1- $C^{14}$ . Teniendo en cuenta la disminución de la actividad específica del trazador para calcular las correspondientes velocidades, resulta que realmente el glicerol produce un aumento de la síntesis de  $CO_2$  y glicerol de glicéridos. La glucosa estimula la síntesis de  $CO_2$  y ácidos grasos a partir del glicerol, pero inhibe la velocidad de síntesis de glicerol de glicéridos a partir del mismo sustrato. Mientras que la  $K_m$  del efecto del glicerol se encuentra muy por debajo de las concentraciones fisiológicas del glicerol, las  $K_a$  y  $K_i$  de los efectos de la glucosa están próximas a los valores de glucosa en sangre. Los resultados se discuten

con relación a los efectos competitivos del glicerol y la glucosa para la síntesis de  $\alpha$ -glicero-fosfato y a la necesidad de glucosa para la lipogénesis a partir de glicerol en tejido adiposo.

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