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The Effect of Glucose, Insulin and Adrenaline on Glycerol Metabolism *in vitro* in Rat Adipose Tissue

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The uptake and utilization of [1-^{14}C]glycerol was determined in pieces of rat epididymal fat-pads incubated in Krebs–Ringer bicarbonate buffer containing albumin. Insulin (200μunits/ml), adrenaline (epinephrine; 0.5μg/ml) and glucose (0, 5, 15 and 20mM) were added to the medium. Changes in the specific radioactivity of the tracer during the incubation were taken into account in calculating the rate of glycerol utilization. Adrenaline decreased glycerol uptake, whereas insulin plus adrenaline increased it. The rate of incorporation of glycerol into glycerides was decreased by adrenaline and insulin, singly or together. Insulin increased the rate of formation of CO_2 and fatty acids from glycerol. The formation of CO_2 and fatty acids was further enhanced by insulin plus adrenaline. The decrease in glycerol uptake induced by adrenaline, the decrease in incorporation of glycerol into glycerides induced by insulin and insulin plus adrenaline and the synthesis of fatty acids were dependent on the presence of glucose in the medium. Thus insulin and adrenaline act on glycerol utilization in adipose tissue and some of their effects are mediated by action on glucose metabolism, but others are independent of this.

The phosphorylation and utilization of glycerol depends on glycerol kinase (EC 2.7.1.30), an enzyme once thought to be absent from adipose tissue (Wieland & Suyter, 1957), but later shown to be present (Robinson & Newsholme, 1967). We demonstrated that adipose tissue *in vitro* is able to utilize glycerol (Herrera & Lamas, 1970) and that its rate varies with starvation (Herrera & Lamas, 1970; Herrera & Ayanz, 1972), the thyroid status (Montoya & Herrera, 1974) and the presence of albumin in the incubation medium (Herrera, 1973). The present study was carried out to determine whether factors known to affect the utilization of glucose and the release of glycerol by adipose tissue also affect the utilization of glycerol. We investigated the effect of glucose, insulin and adrenaline (epinephrine) on the utilization of glycerol in adipose tissue.

Part of this study has been published as an abstract (Herrera & Domínguez, 1973).

Materials and Methods

Male Wistar rats weighing 188 ± 9g, fed on Purina rat chow *ad libitum*, were used. They were killed by cervical fracture without anaesthesia and four pieces of epididymal fat-pad (29.2 ± 1.7mg) from each rat were placed in 20ml vials containing 0.5ml of Krebs–Ringer bicarbonate buffer, pH7.4 (Umbreit *et al.*, 1964), supplemented with bovine serum albumin (20mg/ml) purified by the method of Chen (1967). Fresh solutions of [1-^{14}C]glycerol (20μM, 0.5μCi), in addition to glucose, insulin (Normal Novo Insulin; Novo Industri A/S, Copenhagen, Denmark; 200 μunits/ml), adrenaline (adrenaline bitartrate; Sigma Chemical Co., St. Louis, MO, U.S.A.; 0.5μg/ml) or insulin plus adrenaline in 0.5ml of the buffer solution lacking albumin were pipetted into the vials. Then they were sealed, gassed for 5min with O_2+CO_2 (95:5) and incubated at 37°C in a Dubnoff metabolic shaker at 100 cycles/min. The incubation was stopped by addition of $HClO_4$, and CO_2 was trapped in Hyamine 10-X hydroxide by gentle shaking at room temperature for 60min (Herrera & Lamas, 1970; Herrera & Ayanz, 1972). The medium was processed (Herrera & Ayanz, 1972) and glycerol was determined enzymically (Garland & Randle, 1962). Samples of medium were chromatographed in the upper phase of butan-1-ol/water/methanol/90.7% (w/v) formic acid (320:320:80:1, by vol.) and the spots were identified by radioautography by comparison with radioactive standards. In all the experimental conditions the radioactivity remaining in the medium was practically all in glycerol (99.2 ± 0.4%). The uptake of [^{14}C]glycerol by the tissues was calculated from the amount of radioactivity converted into lipids and CO_2, as this value was better grouped and more reproducible than the net amount of [^{14}C]-glycerol that disappeared from the medium and gives a more precise index of the [^{14}C]glycerol metabolized by the tissue. Lipids were extracted from the incubated

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tissue (Folch *et al.*, 1957) and purified as previously described (Herrera & Ayanz, 1972). Portions of total lipid extracts were saponified in 1M-KOH in 95% (v/v) ethanol for 2h at 100°C. Non-saponifiable lipids were extracted with heptane, and fatty acids were extracted by a second wash with heptane after acidification with 1M-H_2SO_4. Radioactivity in the glyceride glycerol was calculated from the difference between radioactivities in the total lipids and in the heptane extracts. To validate this procedure, recovery experiments were carried out with non-radioactive lipid extracts from adipose tissue supplemented with glycerol tri[1-^{14}C]palmitate. Although less than 0.2% of the radioactivity was recovered in the first heptane extract after saponification, 99.4 ± 0.5% was recovered in the heptane layer after acidification.

Analysis of the data was carried out by using an Ataio electronic calculator (Compucorp 445) as previously described (Herrera & Ayanz, 1972; Herrera, 1973). Radioactivity measurements were expressed as percentages of the total [1-^{14}C]glycerol added to each incubation vessel, related to the initial wet weight of the tissue. When the data were used to calculate the rates of glycerol utilization and/or production, the radioactivity measurements were expressed in μmol by using the specific radioactivity of glycerol in the media incubated in the absence of tissue.

Results

The utilization of [1-^{14}C]glycerol and glycerol formation observed in epididymal fat-pads incubated for 180min in medium containing various concentrations of glucose are given in Table 1. The conversion of labelled glycerol into glyceride glycerol decreased as the concentration of glucose in the medium was increased. In contrast, the formation of both ^{14}C-labelled fatty acids and $^{14}CO_2$ from labelled glycerol increased significantly as the concentration of glucose in the medium was increased. The final concentration of glycerol in the incubation medium was also significantly increased as the initial concentration of glucose was increased.

The effect of insulin and adrenaline, alone and together, on the [1-^{14}C]glycerol utilization and glycerol formation by epididymal fat-pads is shown in Table 2. In the absence of glucose, the uptake of labelled glycerol was not affected by insulin, whereas it was significantly decreased by adrenaline. This effect of adrenaline was smaller when insulin was also present in the medium. Glycerol uptake in the control group was significantly decreased by the addition of 5mM-glucose to the incubation medium. The effects of insulin and adrenaline on glycerol uptake in tissue incubated with glucose were essentially the same as those observed when glucose was omitted.

The formation of glyceride glycerol and CO_2 in the absence of glucose was not changed by insulin, whereas adrenaline significantly decreased the formation of these two metabolites. The decrease in the formation of glyceride glycerol and CO_2 was less in tissue incubated with both insulin and adrenaline in the medium. In the presence of glucose, glyceride glycerol formation decreased, whereas CO_2 production increased significantly in the control group. Insulin increased the formation of CO_2 whereas the formation of both glyceride glycerol and CO_2 was significantly decreased by adrenaline. Here again this effect was smaller when both insulin and adrenaline were present in the incubation medium.

In the absence of glucose, the formation of fatty acids was practically negligible in all the groups. This

Table 1. *Effect of glucose on the utilization of* [1-^{14}C]*glycerol and the formation of glycerol by rat epididymal fat-pads incubated in vitro*

Pieces of epididymal fat-pad were incubated for 180min in Krebs–Ringer bicarbonate medium containing albumin (10 mg/ml), [1-^{14}C]glycerol (0.5μCi/ml, 10μM) and different concentrations of glucose. The data from radioactivity measurements are expressed as percentages of the initial radioactivity in the medium/100mg wet wt. of tissue. The statistical differences between each value and that of the group without addition of glucose are shown by the P values (N.S., not significant, i.e. $P > 0.05$).

Concn. of glucose (mM)	^{14}C uptake (%/100mg)	[^{14}C]Glyceride glycerol (%/100mg)	$^{14}CO_2$ (%/100mg)	^{14}C-labelled fatty acids (%/100mg)	Glycerol (μmol/100mg)
0	15.9 ± 2.4	13.1 ± 2.4	2.54 ± 0.71	0.084 ± 0.036	0.283 ± 0.04
1	15.6 ± 1.5	11.0 ± 1.8	3.64 ± 0.73	0.97 ± 0.17	0.407 ± 0.080
	N.S.	N.S.	N.S.	$P < 0.001$	N.S.
5	14.7 ± 1.7	8.82 ± 1.61	4.70 ± 0.41	1.88 ± 0.41	0.482 ± 0.074
	N.S.	N.S.	$P < 0.05$	$P < 0.01$	$P < 0.05$
15	14.2 ± 1.9	7.86 ± 2.12	4.73 ± 0.62	2.57 ± 0.57	0.481 ± 0.054
	N.S.	N.S.	$P < 0.05$	$P < 0.01$	$P < 0.01$
20	11.5 ± 3.3	5.00 ± 1.39	5.66 ± 0.72	2.02 ± 0.69	0.508 ± 0.04
	N.S.	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$

Table 2. *Effect of insulin and adrenaline on the utilization of* [1-^{14}C]*glycerol and the production of glycerol by rat epididymal fat-pads incubated in vitro in the absence and the presence of glucose*

Pieces of epididymal fat-pad were incubated for 180min in Krebs–Ringer bicarbonate medium containing albumin (10 mg/ml), [1-^{14}C]glycerol (0.5μCi/ml, 10μM) and insulin (200μunits/ml), adrenaline (0.5μg/ml) and/or glucose (5mM). The data from radioactivity measurements are expressed as percentages of initial radioactivity/100mg wet wt. of tissue. The statistical differences between each value and that of the basal (no addition) are shown by the *P* values. Asterisks correspond to the difference between each group and the same group without addition of glucose (N.S. or no asterisks, not significant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Additions	[^{14}C]Glycerol uptake (%/100mg)	[^{14}C]Glyceride glycerol (%/100mg)	$^{14}CO_2$ (%/100mg)	^{14}C-labelled fatty acids (%/100mg)	Formation of glycerol (μmol/100mg)
No glucose in the medium					
Control	22.5 ± 2.7	18.9 ± 2.9	2.60 ± 0.45	0.365 ± 0.160	0.337 ± 0.046
Insulin	26.0 ± 2.5	23.4 ± 2.4	4.48 ± 0.99	0.432 ± 0.166	0.349 ± 0.051
	N.S.	N.S.	N.S.	N.S.	N.S.
Adrenaline	7.10 ± 2.36	3.99 ± 1.03	1.36 ± 0.28	0.109 ± 0.020	1.08 ± 0.16
	$P < 0.001$	$P < 0.001$	$P < 0.05$	N.S.	$P < 0.001$
Insulin + adrenaline	12.7 ± 2.3	8.68 ± 2.00	2.44 ± 0.61	0.390 ± 0.270	0.796 ± 0.130
	$P < 0.02$	$P < 0.02$	N.S.	N.S.	$P < 0.005$
5mM-Glucose in the medium					
Control	15.4 ± 1.9*	10.7 ± 1.9*	4.42 ± 0.55*	1.86 ± 0.57*	0.567 ± 0.082*
Insulin	21.6 ± 2.8	7.36 ± 1.65***	8.62 ± 1.33***	5.06 ± 1.33***	0.431 ± 0.043
	N.S.	N.S.	$P < 0.02$	$P < 0.05$	N.S.
Adrenaline	3.88 ± 0.60*	2.15 ± 0.42	1.38 ± 0.24	0.321 ± 0.70**	2.15 ± 0.27**
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.02$	$P < 0.001$
Insulin + adrenaline	5.80 ± 0.87**	2.50 ± 0.47**	2.03 ± 0.42**	1.39 ± 0.41**	2.29 ± 0.36***
	$P < 0.001$	$P < 0.001$	$P < 0.005$	N.S.	$P < 0.001$

parameter was significantly increased in the control group by the addition of glucose to the incubation medium, and the effect was greater in the presence of insulin and smaller in the presence of adrenaline.

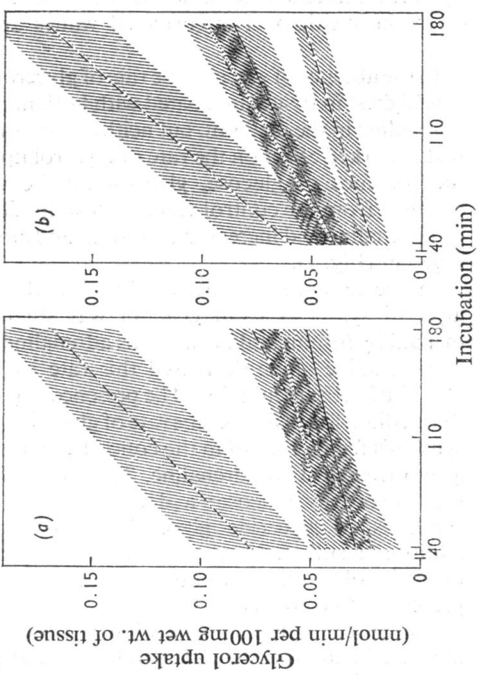
In the absence of glucose, glycerol formation was low. It was increased significantly by the addition of glucose to the incubation medium and it was significantly increased by adrenaline, whether or not glucose or insulin was present in the incubation medium.

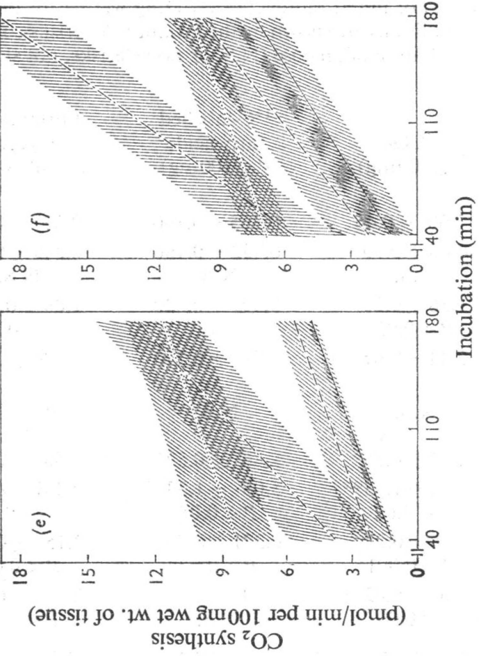
To obtain an accurate picture of the rate of glycerol utilization in the groups, the specific radioactivity of the substrate had to be taken into account. It was necessary to correct for the dilution of labelled glycerol that took place as changes occurred in the amount of unlabelled glycerol in the medium. Therefore the uptake of glycerol and the synthesis of glyceride glycerol, CO_2 and fatty acids in epididymal fat-pads after 40, 80, 120 and 180min of incubation with or without 5mM-glucose in the medium was determined. It was found that the utilization of [1-^{14}C]glycerol by the tissue and the appearance of unlabelled glycerol in the medium increased linearly with time. Thus the data were used to calculate the true rate of glycerol utilization and glycerol release as previously described (Herrera, 1973). Briefly, the calculation takes into account the change in the specific radioactivity of the [1-^{14}C]glycerol caused by the linear increase in the amount of unlabelled glycerol in the medium. The corrected values appear in Fig. 1.

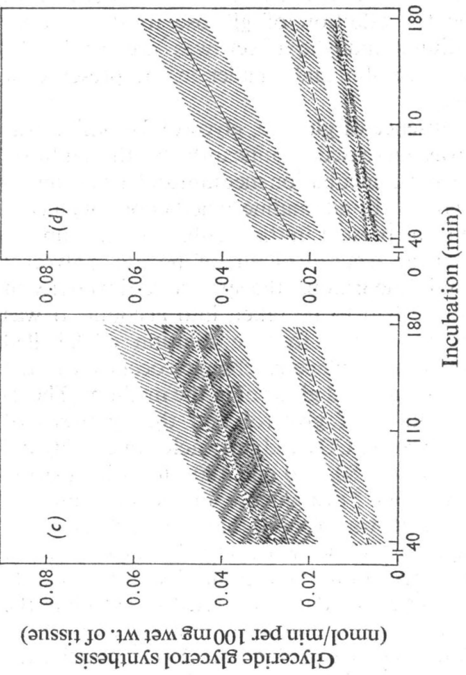
In the absence of glucose, the rate of glycerol uptake was highest in tissues incubated with both insulin and adrenaline in the medium, but neither hormone alone had a marked effect on the rate of glycerol uptake. In the presence of glucose, glycerol uptake was unchanged from the control value by insulin, decreased by adrenaline and increased by insulin and adrenaline together (Fig. 1).

In the absence of glucose, the rate of glyceride glycerol synthesis from glycerol was slightly augmented by insulin and insulin plus adrenaline, whereas adrenaline alone decreased this rate by approx. 50% during the incubation. The presence of glucose in the medium failed to alter the rate of glyceride glycerol synthesis from glycerol in the control group or in the group treated with adrenaline. The effect of insulin was altered by glucose: a decrease in the rate of synthesis of glyceride glycerol was observed. In addition, insulin failed to alter the decrease in glyceride glycerol synthesis produced by adrenaline in the presence of glucose (Fig. 1).

Compared with media lacking glucose, a slight increase in the rate of CO_2 synthesis from glycerol occurred in the control group in the presence of glucose, whereas the response to the hormones


Glycerol uptake
(nmol/min per 100mg wet wt. of tissue)
(a)
(b)
0.15
0.10
0.05
0
40
110
180
Incubation (min)


CO_2 synthesis
(pmol/min per 100mg wet wt. of tissue)
(e)
(f)
18
15
12
9
6
3
0
40
110
180
Incubation (min)


Glyceride glycerol synthesis
(nmol/min per 100mg wet wt. of tissue)
(c)
(d)
0.08
0.06
0.04
0.02
0
40
110
180
Incubation (min)

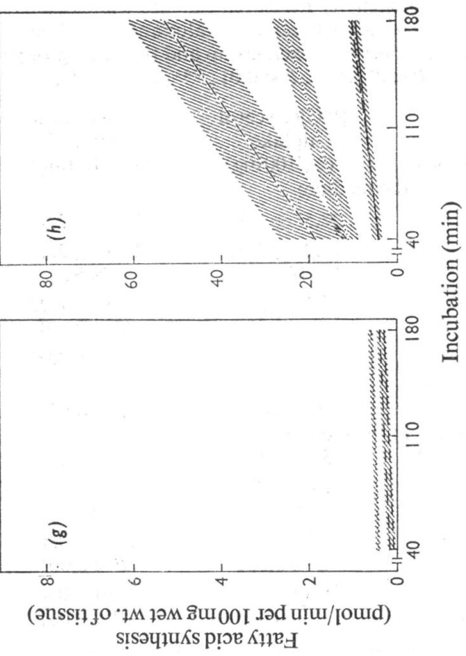

Fatty acid synthesis
(pmol/min per 100mg wet wt. of tissue)
(g)
(h)
8
6
4
2
0
80
60
40
20
0
40
110
180
Incubation (min)

Fig. 1. *Effect of insulin, adrenaline and glucose on the rate of glycerol uptake and glyceride glycerol, CO_2 and fatty acid synthesis from glycerol in the rat epididymal fat-pads incubated in vitro*

Pieces of epididymal fat-pad were incubated for 40, 80, 120 and 180min in Krebs–Ringer bicarbonate buffer containing albumin (10mg/ml) and [1-^{14}C]glycerol (0.5μCi/ml, 10μM) in the absence or the presence of 5mM-glucose. The media were supplemented with insulin (200μunits/ml) (····), adrenaline (0.5μg/ml) (– – – –) or adrenaline plus insulin (–·–·). Control samples (——) were not supplemented with hormones. The rate of glycerol utilization has been calculated by determining the amount of labelled glycerol converted into CO_2 plus total lipids (uptake), glyceride glycerol, CO_2 or fatty acids by the tissue as function of time and correcting it by the glycerol that enters the medium and continuously dilutes the radioactive substrate (Herrera, 1973). The shadowed areas represent the error values of each regression line calculated as function of the mean ± S.E. of the values of glycerol in the medium of four experiments. The respective mean regression of each curve is:

Rate of glycerol uptake

(*a*) Without glucose

Insulin + adrenaline:

$$V=\frac{130\times10^{-4}t+152\times10^{-2}}{246\times10^{-1}-919\times10^{-5}t}$$

Adrenaline:

$$V=\frac{850\times10^{-5}t+285\times10^{-3}}{242\times10^{-1}-267\times10^{-5}t}$$

Insulin:

$$V=\frac{251\times10^{-5}t+953\times10^{-3}}{241\times10^{-1}-155\times10^{-4}t}$$

Control:

$$V=\frac{300\times10^{-5}t+622\times10^{-3}}{241\times10^{-1}-877\times10^{-5}t}$$

(*b*) With glucose

Insulin + adrenaline:

$$V=\frac{174\times10^{-4}t+931\times10^{-3}}{241\times10^{-1}-289\times10^{-5}t}$$

Insulin:

$$V=\frac{790\times10^{-5}t+737\times10^{-3}}{244\times10^{-1}-143\times10^{-4}t}$$

Control:

$$V=\frac{674\times10^{-5}t+742\times10^{-3}}{241\times10^{-1}-795\times10^{-5}t}$$

Adrenaline:

$$V=\frac{481\times10^{-5}t+395\times10^{-3}}{240\times10^{-1}-125\times10^{-5}t}$$

Rate of glyceride glycerol synthesis

(*c*) Without glucose

Insulin + adrenaline:

$$V=\frac{130\times10^{-4}t+152\times10^{-2}}{239\times10^{-1}-919\times10^{-5}t}$$

Insulin:

$$V=\frac{251\times10^{-5}t+953\times10^{-3}}{240\times10^{-5}+113\times10^{-4}t}$$

Control:

$$V=\frac{300\times10^{-5}t+622\times10^{-3}}{241\times10^{-1}-737\times10^{-5}t}$$

Adrenaline:

$$V=\frac{850\times10^{-5}t+269\times10^{-3}}{292\times10^{-1}-820\times10^{-5}t}$$

(*d*) With glucose

Control:

$$V=\frac{679\times10^{-5}t+742\times10^{-3}}{241\times10^{-1}-795\times10^{-5}t}$$

Adrenaline:

$$V=\frac{481\times10^{-5}t+395\times10^{-3}}{241\times10^{-5}-577\times10^{-6}t}$$

Insulin + adrenaline:

$$V=\frac{174\times10^{-4}t+931\times10^{-3}}{240\times10^{-1}-247\times10^{-6}t}$$

Insulin:

$$V=\frac{790\times10^{-5}t+737\times10^{-3}}{244\times10^{-1}-218\times10^{-5}t}$$

Rate of CO_2 synthesis

(*e*) Without glucose

Insulin + adrenaline:

$$V=\frac{271\times10^{-4}t+902\times10^{-3}}{275\times10^{-1}-791\times10^{-5}t}$$

Insulin:

$$V=\frac{276\times10^{-5}t+982\times10^{-3}}{276\times10^{-1}-196\times10^{-5}t}$$

Adrenaline:

$$V=\frac{118\times10^{-4}t+754\times10^{-3}}{275\times10^{-1}-128\times10^{-6}t}$$

Control:

$$V=\frac{313\times10^{-4}t+612\times10^{-3}}{275\times10^{-1}-364\times10^{-6}t}$$

(*f*) With glucose

Insulin + adrenaline:

$$V=\frac{419\times10^{-4}t+135\times10^{-2}}{275\times10^{-1}-217\times10^{-6}t}$$

Insulin:

$$V=\frac{765\times10^{-5}t+360\times10^{-2}}{276\times10^{-1}+160\times10^{-5}t}$$

Adrenaline:

$$V=\frac{351\times10^{-4}t+485\times10^{-3}}{275\times10^{-1}-111\times10^{-6}t}$$

Control:

$$V=\frac{368\times10^{-4}t-243\times10^{-4}}{275\times10^{-1}-377\times10^{-6}t}$$

Rate of fatty acid synthesis

(*g*) Without glucose

Insulin:

$$V=\frac{251\times10^{-5}t+953\times10^{-3}}{242\times10^{-1}-155\times10^{-6}t}$$

Adrenaline:

$$V=\frac{850\times10^{-5}t+285\times10^{-3}}{242\times10^{-1}-214\times10^{-7}t}$$

Insulin + adrenaline:

$$V=\frac{130\times10^{-4}t+152\times10^{-2}}{242\times10^{-1}-209\times10^{-7}t}$$

Control:

$$V=\frac{300\times10^{-5}t+622\times10^{-3}}{242\times10^{-1}-77\times10^{-7}t}$$

(*h*) With glucose

Insulin + adrenaline:

$$V=\frac{174\times10^{-4}t+931\times10^{-3}}{243\times10^{-1}-901\times10^{-6}t}$$

Insulin:

$$V=\frac{790\times10^{-5}t+737\times10^{-3}}{243\times10^{-1}-400\times10^{-6}t}$$

Adrenaline:

$$V=\frac{481\times10^{-5}t+395\times10^{-3}}{242\times10^{-1}-239\times10^{-6}t}$$

Control:

$$V=\frac{674\times10^{-5}t+742\times10^{-3}}{242\times10^{-1}-903\times10^{-6}t}$$

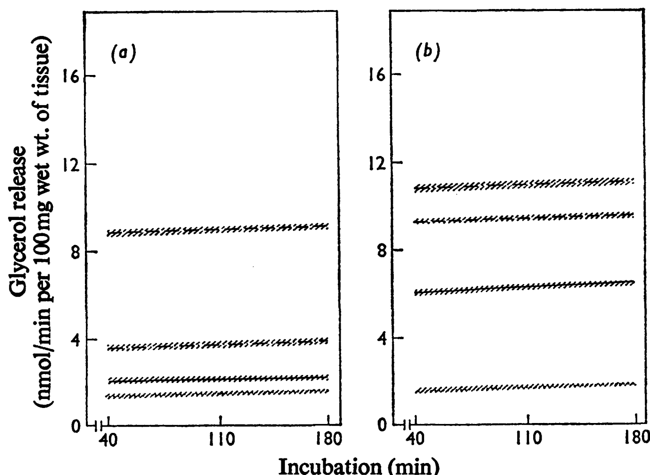


Fig. 2. *Effect of insulin, adrenaline and glucose on the rate of glycerol release into the medium (lipolysis) by rat epididymal fat-pads incubated in vitro*

Pieces of epididymal fat-pad were incubated for 40, 80, 120 and 180min in Krebs–Ringer bicarbonate buffer containing albumin (10mg/ml) and [1-^{14}C]glycerol (0.5μCi/ml, 10μM) in the absence or the presence of 5mM-glucose. The media were supplemented with insulin (200μunits/ml) (· · · ·), adrenaline (0.5μg/ml) (– – – –) or adrenaline plus insulin (–·–·). Control samples (——) were not supplemented with hormones. The rate of glycerol release has been calculated by determining the amount of glycerol in the medium and correcting it by the labelled glycerol that has been taken up by the tissue at each time (Herrera, 1973). The shadowed areas represent the error values of each regression line calculated as function of the mean ± S.E. of the values of glycerol in the medium of four experiments. The respective mean regression of each curve is:

Rate of glycerol release

(*a*) Without glucose

Adrenaline:

$$V=\frac{297\times10^{-3}t-538\times10^{-3}}{169\times10^{-2}-337\times10^{-5}t}+881\times10^{-2}$$

Insulin + adrenaline:

$$V=\frac{178\times10^{-4}t+214\times10^{-2}}{169\times10^{-1}-510\times10^{-5}t}+350\times10^{-3}$$

Control:

$$V=\frac{133\times10^{-4}t+463\times10^{-3}t}{168\times10^{-1}-669\times10^{-5}t}+199\times10^{-2}$$

Insulin:

$$V=\frac{192\times10^{-4}t+627\times10^{-3}}{168\times10^{-1}-153\times10^{-4}t}+125\times10^{-2}$$

(*b*) With glucose

Insulin + adrenaline:

$$V=\frac{209\times10^{-5}t+281\times10^{-3}}{168\times10^{-1}-266\times10^{-5}t}+107\times10^{-1}$$

Adrenaline:

$$V=\frac{229\times10^{-4}t-101\times10^{-2}}{168\times10^{-1}-248\times10^{-5}t}+925\times10^{-2}$$

Control:

$$V=\frac{392\times10^{-4}t-408\times10^{-3}}{167\times10^{-1}-625\times10^{-5}t}+602\times10^{-2}$$

Insulin:

$$V=\frac{180\times10^{-4}t+195\times10^{-5}}{163\times10^{-1}-119\times10^{-4}t}+150\times10^{-2}$$

remained unchanged. In the absence or in the presence of glucose, adrenaline produced a slight increase, whereas both insulin and insulin plus adrenaline produced a pronounced increase in CO_2 synthesis. This increase was particularly apparent after prolonged incubation of the tissues in the presence of both hormones (Fig. 1).

The rate of fatty acid synthesis was highly dependent on the presence of glucose in the medium. In its absence the formation of fatty acid was negligible. In the presence of glucose, the rate of synthesis of fatty acid was enhanced by insulin and insulin plus adrenaline, but unchanged by adrenaline alone (Fig. 1).

The rate of glycerol release was calculated as previously described (Herrera, 1973) to provide a measure of the rate of lipolysis in the tissue (Fig. 2). The rate of glycerol release was almost constant in all groups during the incubation period. In the absence of glucose, insulin failed to decrease substantially the rate of lipolysis, whereas adrenaline markedly increased it. However, insulin decreased the lipolytic effect of adrenaline.

In the presence of glucose, the rate of lipolysis in the control group was increased. Insulin had an anti-lipolytic effect in the presence of glucose, whereas the lipolytic effect of adrenaline was unchanged. The presence of both insulin and adrenaline in the medium produced a marked increase in the rate of lipolysis in the tissue.

Discussion

Our work demonstrates that glycerol utilization by adipose tissue incubated *in vitro* is greater than previously thought (Wieland & Suyter, 1957). The presence of glucose in the medium affected the utilization of glycerol by increasing the rate of synthesis of fatty acid and CO_2 without altering that of glyceride glycerol. Our findings appear to conflict with those of Hubbard *et al.* (1970), who reported a decrease in glyceride glycerol synthesis from [2-^{14}C]-glycerol in the presence of glucose. However, they used isolated fat-cells, supplemented the medium with high concentrations of glycerol and failed to correct for the formation of unlabelled glycerol. We also observed a decrease in glyceride glycerol formation from [1-^{14}C]glycerol in the presence of glucose, but it was eliminated when the decrease in the specific radioactivity of the tracer caused by the formation of non-radioactive glycerol during incubation was taken into account.

Our data fail to support the notion that glucose competes with glycerol in the formation of glycerol phosphate. Instead, they suggest that the action of glucose is mediated by facilitating the supply of ATP needed for the phosphorylation of glycerol and also by providing NADPH for lipogenesis. Alternatively the effect of glucose on glycerol metabolism in adipose tissue may represent a lipolytic action. The enhanced rate of release of glycerol by adipose tissue in the presence of glucose may enhance the availability of glycerol sufficiently to increase its utilization. In this way, the amount of glycerol in the medium and the rate of utilization of glycerol are directly related (Domínguez & Herrera, 1976*b*).

In the presence of adrenaline, a direct relationship between the concentration of glycerol in the medium and its utilization rate was not found. Adrenaline increased the rate of lipolysis but decreased the uptake of glycerol by tissue incubated in the presence of glucose. This inhibitory effect of adrenaline on the uptake of glycerol by adipose tissue may be due to competition between glucose and glycerol in the formation of glycerol phosphate, as the effect of adrenaline in enhancing the uptake and metabolism of glucose and its conversion into glyceride glycerol is well known (Leboeuf *et al.*, 1959; Rodbell, 1964). That the inhibitory effect of adrenaline on the uptake of glycerol is mediated by an action on glucose metabolism is supported by the fact that this effect failed to occur in the absence of glucose. In addition to its action on the metabolism of glucose, adrenaline alters glycerol metabolism in adipose tissue by inhibiting the synthesis of glyceride glycerol without affecting the formation of CO_2.

Insulin slightly enhanced the uptake of glycerol in adipose tissue, whereas glycerol metabolism was markedly altered. In contrast with the effect of adrenaline, the marked decrease in the formation of glyceride glycerol from glycerol produced by insulin was dependent on the presence of glucose. That the effect of insulin depends on glucose metabolism is supported by the diverse effects of insulin plus adrenaline in the presence and absence of glucose. In the presence of glucose, insulin potentiated the inhibitory effect of adrenaline, whereas it decreased the effect of adrenaline in the absence of glucose. The effects of insulin plus adrenaline in inhibiting the formation of glyceride glycerol from glycerol is consistent with the enhanced synthesis of glyceride glycerol from glucose observed in these situations (Lynn *et al.*, 1960; Cahill *et al.*, 1960; Flatt & Ball, 1964; Rodbell, 1964).

Insulin stimulated the synthesis of fatty acids from glycerol. The lipogenic action of insulin may be dependent on its effect on glucose metabolism (Flatt & Ball, 1964; Cahill *et al.*, 1960) and the net production of ATP and NADPH. The lipogenic effect of insulin is augmented by adrenaline by facilitating glucose utilization via the glycolytic and hexose monophosphate pathways (Flatt & Ball, 1964). This agrees with the marked effect of adrenaline and insulin on fatty acid synthesis from glycerol in the presence of glucose, as in this condition a greater proportion of glucose is used to provide the coenzymes for lipogenesis.

In contrast with the effects of insulin on glyceride glycerol and fatty acid synthesis, it enhanced the formation of CO_2 from glycerol independently of its action on glucose metabolism. Evidently, once glycerol is phosphorylated and converted into a glycolytic intermediate, such as dihydroxyacetone phosphate, insulin can enhance its total oxidation to CO_2. This is probably due to the insulin-induced enhancement of CO_2 formation from the oxidative decarboxylation of pyruvate, as is the case for CO_2 formed from [6-^{14}C]glucose (Flatt & Ball, 1964).

Glycerol uptake and the formation of CO_2 were maximal in the presence of both insulin and adrenaline. In the presence of glucose, the effect of these two hormones could be mediated by an increase in availability of ATP formed through an action on glucose metabolism and by an increase in glycerol formed by the lipolytic action of the hormones on the tissue. In the absence of glucose, the effect of these two hormones is difficult to explain. Perhaps it is mediated by the activation of glycerokinase by insulin and an augmented rate of fatty acid esterification.

This may decrease the endogenous pool of free fatty acids and increase the amount of ATP available for the phosphorylation of glycerol (Jeanrenaud, 1971). The relationship between the availability of ATP and the rate of glycerol utilization is supported by our findings on the effects of 2-deoxy-D-glucose, oligomycin and theophylline, which inhibit the ability of adipose tissue to metabolize glycerol (Domínguez & Herrera, 1976*a*).

We must emphasize here that compared with glucose metabolism or the rate of glycerol production, the utilization of glycerol by adipose tissue is very small and unlikely to be of any physiological significance in normal conditions. However, it could have some importance when the concentration of glycerol in both blood and adipose tissue is high, as occurs in the newborn (Novák *et al.*, 1968; Hahn & Greenberg, 1968), or in situations of hyperinsulinaemia, as occurs in obesity, where the activity of glycerol kinase in the tissue is augmented (Lochaya *et al.*, 1963; Koschinsky *et al.*, 1970, 1971; Martin & Lamprey, 1975).

Although further work is required to understand the mechanisms of action of insulin and adrenaline on the utilization and metabolism of glycerol, we believe that their effects must be taken into account in order to understand the metabolism of adipose tissue. This is specially true for the lipolytic action of these hormones, since we have shown in the present study that the rate of glycerol release by the tissue is not always related to the rate of its reutilization.

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References

Cahill, G. F., Jr., Leboeuf, B. & Renold, A. E. (1960) *Am. J. Clin. Nutr.* **8**, 733–739

Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173–181

Domínguez, M. C. & Herrera, E. (1976*a*) *Horm. Metab. Res.* **8**, 33–37

Domínguez, M. C. & Herrera, E. (1976*b*) *Rev. Esp. Fisiol.* in the press

Flatt, J. P. & Ball, E. G. (1964) *J. Biol. Chem.* **239**, 675–685

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509

Garland, P. B. & Randle, P. J. (1962) *Nature (London)* **196**, 987–988

Hahn, P. & Greenberg, R. (1968) *Life Sci.* **7**, 187–190

Herrera, E. (1973) *Rev. Esp. Fisiol.* **29**, 155–162

Herrera, E. & Ayanz, A. (1972) *J. Lipid Res.* **13**, 802–809

Herrera, E. & Domínguez, M. C. (1973) *Excerpta Med. Int. Congr. Ser.* **280**, 100

Herrera, E. & Lamas, L. (1970) *Biochem. J.* **120**, 433–434

Hubbard, R. W., Voorheis, H. P. & Therriault, D. G. (1970) *Lipids* **5**, 114–120

Jeanrenaud, B. (1971) *Diabetologia* **7**, 209–222

Koschinsky, Th., Gries, F. A. & Herberg, L. (1970) *Horm. Metab. Res.* **2**, 185–186

Koschinsky, Th., Gries, F. A. & Herberg, L. (1971) *Diabetologia* **7**, 316–322

Leboeuf, B., Flinn, R. B. & Cahill, G. F., Jr. (1959) *Proc. Soc. Exp. Biol. Med.* **102**, 527–529

Lochaya, S., Hamilton, J. C. & Mayer, J. (1963) *Nature (London)* **197**, 182–183

Lynn, W. S., MacLeod, R. M. & Brown, R. H. (1960) *J. Biol. Chem.* **235**, 1904–1911

Martin, R. J. & Lamprey, P. M. (1975) *Proc. Soc. Exp. Biol. Med.* **149**, 35–39

Montoya, E. & Herrera, E. (1974) *Hormone Res.* **5**, 129–140

Novák, M., Hahn, P. & Melichar, V. (1968) *Biol. Neonat.* **12**, 287–291

Robinson, J. & Newsholme, E. A. (1967) *Biochem. J.* **104**, 2C–4C

Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380

Umbreit, W. W., Burris, R. H. & Stauffer, S. F. (1964) *Manometric Techniques*, 4th edn., p. 132, Burgess Publishing Co., Minneapolis

Wieland, O. & Suyter, S. (1957) *Biochem. Z.* **329**, 320–331