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Requests for reprints should be addressed to: Dr. Judith S. Stern, Dept. Nutrition, Univ. California, Davis, Calif. 95616 (USA)

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## Effects of 2-Deoxy-D-Glucose, Oligomycin and Theophylline on In Vitro Glycerol Metabolism in Rat Adipose Tissue: Response to Insulin and Epinephrine\*

M. Carmen Domínguez and E. Herrera

Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Barcelona, Spain

### Summary

The effects of 2-deoxy-D-glucose (2DG), oligomycin and theophylline on the *in vitro* production and metabolism of glycerol and its response to insulin and epinephrine were studied in epididymal fat pads from fed rats. 2-DG failed to affect basal or epinephrine stimulated glycerol production but it decreased the uptake of 1-<sup>14</sup>C-glycerol by the tissue and its conversion to glyceride-glycerol. Oligomycin also failed to affect the basal production of glycerol but it inhibited the effect of epinephrine on this parameter as well as the uptake and utilization of 1-<sup>14</sup>C-glycerol. Theophylline enhanced the production of glycerol by the tissue and this effect was not further augmented by epinephrine. Theophylline also inhibited the uptake and utilization of 1-<sup>14</sup>C-glycerol; the most pronounced effect of theophylline was observed in the formation of <sup>14</sup>C-fatty acids from 1-<sup>14</sup>C-glycerol in the presence of glucose. Insulin, but not epinephrine, decreased the inhibitory effect of theophylline on glycerol utilization. It is concluded that these compounds affect more intensely the ability of adipose tissue to metabolize glycerol than to release it through lipolysis. The pathway for glycerol utilization in adipose tissue appears to be more sensitive to changes in the availability of ATP than the mechanisms responsible for the release of glycerol from the tissue.

**Key-Words:** Adipose Tissue – Glycerol Utilization – Metabolic Inhibitors – Lipolysis

### Introduction

In preliminary experiments, we have observed that the ability of adipose tissue to metabolize glycerol *in vitro* is influenced by insulin and epinephrine (Herrera and Domínguez 1973). Some of the effects of these hormones require the presence of glucose, which could serve to supply a source of ATP for the phosphorylation of glycerol. This suggests that some aspects of glycerol metabolism in adipose tissue might

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depend on the availability of ATP. One aspect of the present experiments was to determine whether glycerol metabolism is affected by metabolic inhibitors. Thus, 2-deoxy-D-glucose and oligomycin were added to the incubation media and their effects on the production and utilization of glycerol in adipose tissue *in vitro* were noted.

Another aspect of the present study was to investigate whether the elevation in intracellular cyclic AMP produced by epinephrine (Fain 1973) could be responsible for the effects of this hormone on glycerol metabolism in adipose tissue. Theophylline was used for this purpose, because it inhibits cyclic AMP phosphodiesterase (Sutherland and Rall 1958) in addition to being a metabolic inhibitor for adipose tissue (Bray 1966, Blecher 1967, Kupiecki 1973, Jarrett, Steiner, Smith and Kipnis 1972). Thus, the effects of theophylline on *in vitro* glycerol metabolism in adipose tissue were determined and compared to the effects of epinephrine.

### Material and Methods

Male Wistar rats weighing 179 ± 8 g and fed Purina rat chow *ad libitum* were used. Pieces of epididymal fat pads (22 ± 3 mg) were taken immediately after sacrifice by cervical fracture, and incubated for 180 min at 37°C in Krebs Ringer bicarbonate buffer pH 7.4 (Umbreit, Burris and Stauffer 1964), containing 0.5 µCi of 1-<sup>14</sup>C-glycerol (10 µM) and 10 mg/ml of purified bovine albumin (Chen 1967). The incubation procedure has been described in detail previously (Herrera and Ayanz 1972). Incubations were carried out in the presence or in the absence of 5 mM 2-deoxy-D-glucose (Sigma Co.), 0.5 µg/ml oligomycin (Sigma Co.), 1 mM theophylline (Sigma Co.), 200 µU/ml pork monocomponent insulin ("Actrapid", from Novo Industries) and 2.8 µM epinephrine bitartrate (Sigma Co.). The media were processed as previously described (Herrera and Ayanz 1972) and the enzymatic determinations of glycerol were carried out (Garland and Randle 1962). Lipids were extracted from the incubated tissue (Folch, Lees and Sloane-Stanley 1957), purified and fractionated as previously described (Herrera and Lamas 1970, Herrera and Ayanz 1972).

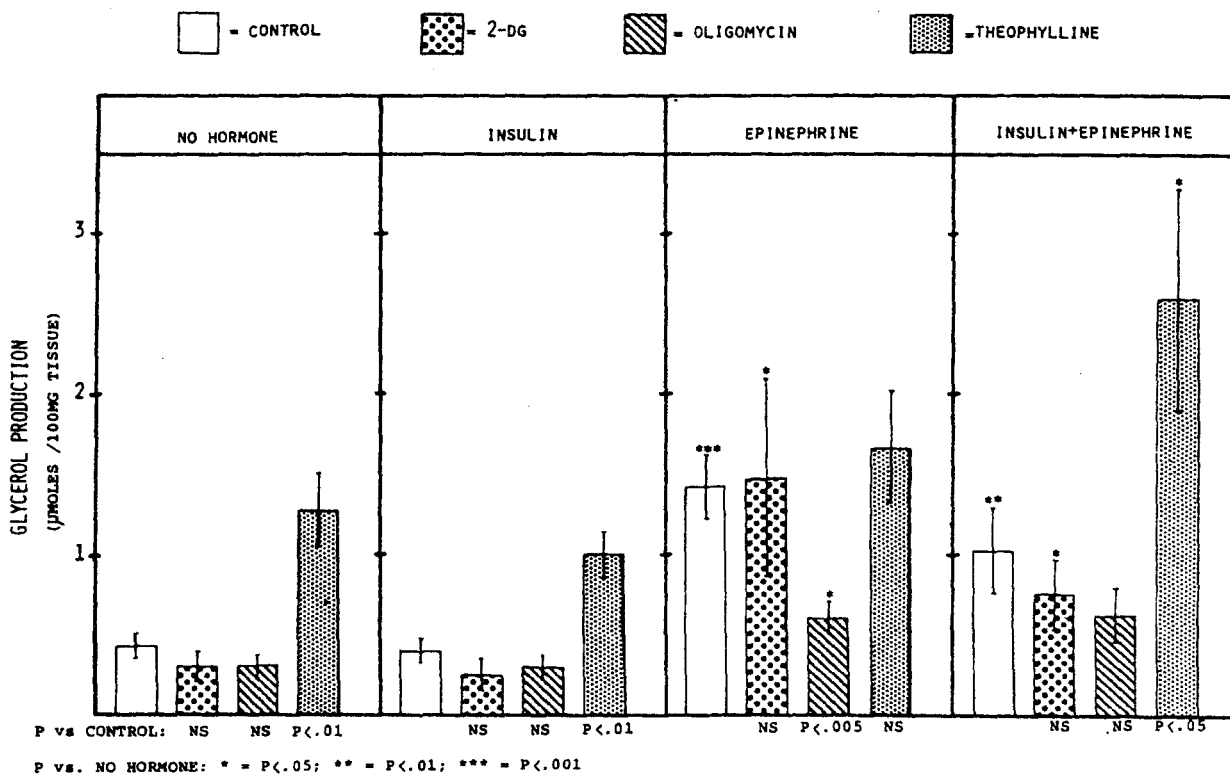


Fig. 1. The effects of 2-DG (5 mM), oligomycin (0.5 µg/ml), theophylline (1 mM), insulin (200 µU/ml) and epinephrine (2.8 µM) on the production of glycerol by pieces of epididymal fat pads incubated "in vitro" in the absence of glucose. Values are means ± SEM of 7 animals/group. p values of the differences vs. the controls are shown at the bottom of the bars (NS = not significant, i.e.  $p > 0.05$ ) and significant differences vs. the tissues incubated in the absence of hormone are denoted by asterisks.

## Results

The effects of 2-deoxy-D-glucose (2-DG), oligomycin, theophylline, insulin and epinephrine on the production of glycerol by adipose tissue pieces incubated in the absence of glucose appear in Figure 1. Basal glycerol production (No-Hormone-Control) was unaltered by 2-DG and oligomycin, while theophylline increased it significantly. Insulin failed to affect the production of glycerol compared to the No-Hormone-Control values. Neither 2-DG nor oligomycin affect the production of glycerol in the presence of insulin. Theophylline significantly increased glycerol production in the presence of insulin as well as when no hormone was present in the incubation medium (No-Hormone-Theophylline). Epinephrine stimulated glycerol production significantly compared to the basal level. Oligomycin significantly blocked the increased production of glycerol in the presence of epinephrine. Both 2-DG and theophylline failed to affect the increase in glycerol release produced by epinephrine. Glycerol production was significantly increased from basal levels when both insulin and epinephrine were present in the incubation medium. 2-DG and oligomycin failed to affect glycerol production under these conditions, while theophylline significantly increased it.

Figure 2 shows the effects of the compounds on  $1\text{-}^{14}\text{C}$ -

glycerol uptake. It was significantly decreased by 2-DG, oligomycin and theophylline when compared to the basal level (No-Hormone-Control). Insulin failed to influence significantly the uptake of glycerol, while 2-DG and oligomycin, but not theophylline, significantly inhibited glycerol uptake in the presence of insulin. Epinephrine produced a significant decline in glycerol uptake. Oligomycin and theophylline, but not 2-DG, caused a further significant reduction in glycerol uptake in the presence of epinephrine. The uptake of glycerol was also reduced significantly from basal levels when both insulin and epinephrine were present in the medium. As was the case when epinephrine was present alone, oligomycin and theophylline, but not 2-DG, significantly reduced glycerol uptake in the presence of both hormones.

Table 1 presents the effects of the substances on the *in vitro* metabolism of glycerol in adipose tissue. The formation of labelled  $\text{CO}_2$ , total lipids and glyceride-glycerol from  $1\text{-}^{14}\text{C}$ -glycerol was studied using an incubation medium lacking glucose, while the formation of  $^{14}\text{C}$ -fatty acids was determined in the presence of 5 mM glucose in the medium, as this metabolite is required to obtain a measurable synthesis of fatty acids from glycerol in adipose tissue (Dominguez and Herrera, unpublished observations). The inhibition

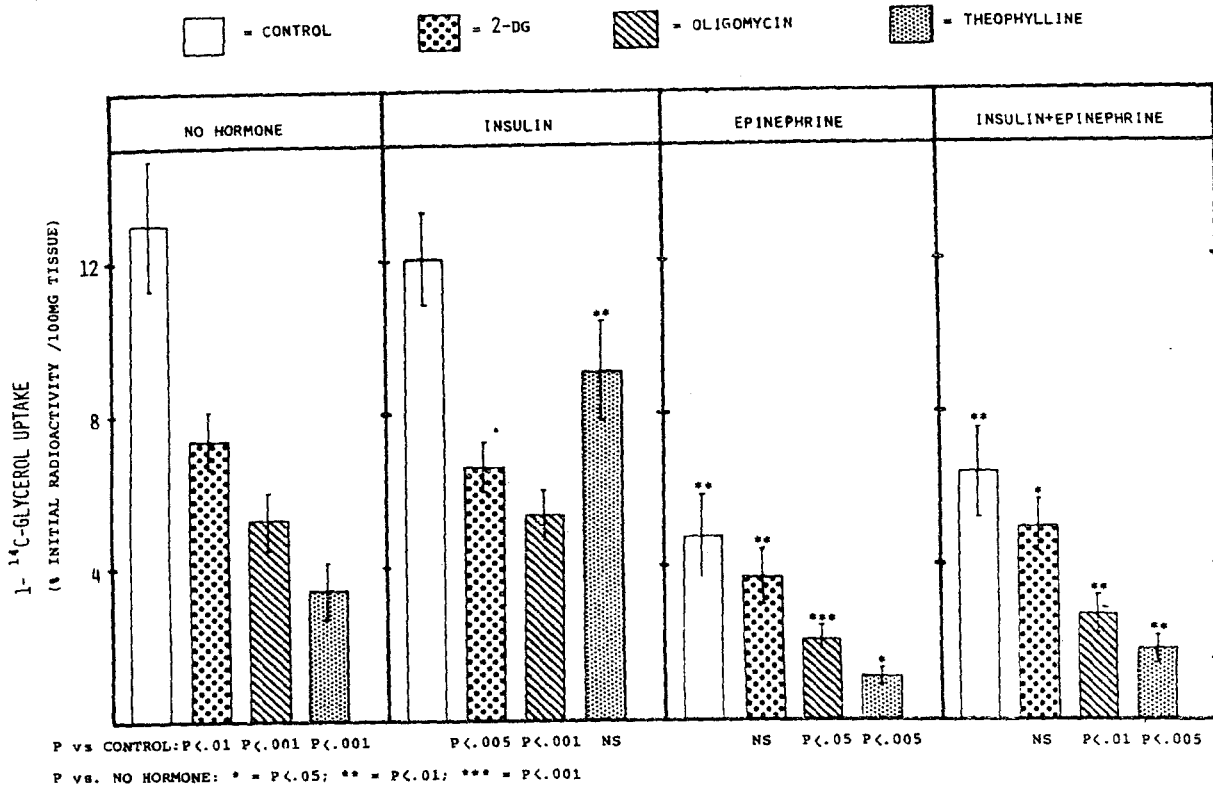


Fig. 2. The effects of 2-DG (5 mM), oligomycin (0.5  $\mu$ g/ml), theophylline (1 mM), insulin (200  $\mu$ U/ml) and epinephrine (2.8  $\mu$ M) on the uptake of labelled glycerol by pieces of epididymal fat pads incubated "in vitro" in the presence of 1- $^{14}$ C-glycerol. No glucose was present in the media. Values are means  $\pm$  SEM of 7 animals/group. p values of the differences vs. the controls are shown at the bottom of the bars (NS = not significant, i.e.  $p > 0.05$ ) and significant differences vs. the tissues incubated in the absence of hormone are denoted by asterisks.

of glycerol uptake by 2-DG under basal conditions and in the presence of insulin was associated with a significant reduction in the formation of total lipids and glyceride-glycerol. The conversion of 1- $^{14}$ C-glycerol to  $^{14}$ CO $_2$  and fatty acids was not significantly altered by 2-DG. A decrease in the formation of  $^{14}$ CO $_2$ ,  $^{14}$ C-total lipids and  $^{14}$ C-fatty acids was observed under all experimental conditions in the presence of oligomycin. Theophylline reduced significantly the formation of all these parameters of glycerol metabolism, except when the incubation medium contained only insulin. Under these conditions the formation of  $^{14}$ C-total lipid and  $^{14}$ C-fatty acids was lowered significantly by theophylline. The formation of  $^{14}$ CO $_2$  and  $^{14}$ C-glyceride-glycerol was however unaffected by theophylline in adipose tissue incubated in the presence of insulin. It is to be noted that insulin alone failed to affect significantly the formation of labelled CO $_2$ , total lipids, glyceride-glycerol and fatty acids in the tissue. Epinephrine, like theophylline, reduced significantly the formation of  $^{14}$ C-total lipids,  $^{14}$ C-glyceride-glycerol and  $^{14}$ C-fatty acids. However, the effects of theophylline on these parameters were more pronounced than the epinephrine's. Unlike theophylline, epinephrine failed to affect  $^{14}$ CO $_2$  formation significantly. Finally, the effects of insulin

and epinephrine together on glycerol metabolism were essentially the same ones as when the incubation medium contained only epinephrine.

#### Discussion

Taking glycerol production as a measure of "in vitro" lipolysis the present results show that neither the basal lipolytic activity of pieces of epididymal fat pads nor its enhancement by epinephrine were affected by 5 mM 2-DG in the medium. However, this concentration of 2-DG was sufficient to decrease both glycerol uptake and the conversion of glycerol to glyceride-glycerol through a pathway that requires glycerokinase-catalyzed phosphorylation (Robinson and Newsholme 1967, Antony, White and Landau 1969). 2-DG reduced the cellular content of ATP due to the consumption of ATP by the hexokinase-catalyzed production of 2-deoxy-D-glucose-6-phosphate (Wick, Drury, Nakada and Wolfe 1957, McComb and Yushok 1964, Hollenberg and Patten 1970). It appears therefore, that alterations in ATP levels produce a greater effect on the utilization of glycerol than on the hormone-sensitive lipase. This conclusion is also supported by our results with oligomycin. This compound is an inhibitor of oxidative phosphorylation that causes a marked decrease in ATP levels in adi-

Table 1. The effects of 2-DG oligomycin, theophylline, insulin and epinephrine on the formation of labelled CO<sub>2</sub>, total lipids, glyceride-glycerol, and fatty acids from 1-<sup>14</sup>C glycerol in adipose tissue from rats incubated "in vitro" in the absence or the presence of glucose.

Treatment	Incubation in the absence of glucose			Incubation in the presence of 5 mM glucose
	Formation of <sup>14</sup> CO <sub>2</sub> + (%/100 mg)	Formation of <sup>14</sup> C-total lipids (%/100 mg)	Formation of <sup>14</sup> C-glyceride-glycerol (%/100 mg)	Formation of <sup>14</sup> C-fatty acids (%/100 mg)
<b>No hormone</b>				
Control	2.60 ± 0.49	10.33 ± 1.30	9.63 ± 1.62	2.49 ± 0.61
2-DG	2.60 ± 0.49	4.74 ± 0.52	4.48 ± 0.67	1.55 ± 0.44
p <sup>++</sup>	N.S.	p < .01	p < .01	N.S.
Oligomycin	0.99 ± 0.38	4.06 ± 0.53	3.53 ± 0.77	1.03 ± 0.15
p	p < .05	p < .01	p < .01	p < .05
Theophylline	1.31 ± 0.32	2.14 ± 0.41	1.79 ± 0.44	0.10 ± 0.03
p	p < .05	p < .001	p < .001	p < .005
<b>Insulin</b>				
Control	2.69 ± 0.60	9.37 ± 0.94	8.07 ± 1.39	2.51 ± 0.55
2-DG	2.54 ± 0.52	4.56 ± 0.58	3.67 ± 0.77	3.10 ± 0.42*
p	N.S.	p < .001	p < .01	N.S.
Oligomycin	1.72 ± 0.39	3.62 ± 0.48	3.10 ± 0.60	1.40 ± 0.27
p	N.S.	p < .001	p < .01	p < .05
Theophylline	1.0 ± 0.16	6.05 ± 1.27**	5.65 ± 1.37**	0.79 ± 0.23**
p	N.S.	p < .05	N.S.	p < .01
<b>Epinephrine</b>				
Control	1.58 ± 0.20	3.97 ± 0.71**	3.59 ± 0.80**	0.47 ± 0.14**
2-DG	1.56 ± 0.40	2.03 ± 0.46**	1.87 ± 0.50**	0.49 ± 0.15*
p	N.S.	p < .05	p < .05	N.S.
Oligomycin	0.74 ± 0.18	1.34 ± 0.21***	1.15 ± 0.27**	0.28 ± 0.12**
p	p < .05	p < .01	p < .01	N.S.
Theophylline	0.53 ± 0.16*	0.58 ± 0.12**	0.54 ± 0.12**	0.11 ± 0.05
p	p < .01	p < .001	p < .01	p < .05
<b>Insulin + Epinephrine</b>				
Control	2.05 ± 0.50	4.38 ± 0.79**	3.60 ± 1.0**	0.97 ± 0.25
2-DG	1.75 ± 0.38	3.18 ± 0.63*	3.12 ± 0.63	0.68 ± 0.29
p	N.S.	N.S.	N.S.	N.S.
Oligomycin	1.0 ± 0.36	1.68 ± 0.33**	1.20 ± 0.30**	0.31 ± 0.05**
p	N.S.	p < .01	p < .05	p < .05
Theophylline	0.93 ± 0.27	0.85 ± 0.23**	0.53 ± 0.06**	0.13 ± 0.03
p	p < .01	p < .001	p < .01	p < .05

(+) Mean ± S.E. of 7 rats/group; %/100 mg = per cent of initial medium radioactivity/100 mg tissue

(++) p-values correspond to the differences of each group vs. the controls (NS = not significant, i.e. p > .05) = p values vs. "No hormone", \* = p < .05, \*\* = p < .01, \*\*\* = p < .001.

pose tissue (Hepp, Challoner and Williams 1968, Hollenberg and Patten 1970). It did not affect basal production of glycerol by adipose tissue. But oligomycin, unlike 2-DG, inhibited the lipolytic effect of epinephrine. It also had a more marked inhibitor effect than 2-DG on the metabolism of glycerol to CO<sub>2</sub> and fatty acids.

Theophylline markedly increased the production of glycerol by adipose tissue and the addition of epinephrine to the medium failed to enhance the production of glycerol above the level obtained in the presence of theophylline alone. This observation confirms the findings of others (Kuo and DeRenzo 1969, Moskowitz and Fain 1970, Fain 1973). The inhibitory effect of theophylline was greater than that of epinephrine on the uptake of labelled glycerol and its conversion to CO<sub>2</sub> and glyceride-glycerol in adipose tissue. The intense effect of theophylline on these

parameters is also observed when the dilution of labelled glycerol by the cold glycerol produced during incubation is taken into account (Herrera and Ayanz 1972, Herrera 1973, Montoya and Herrera 1974, Herrera and Domínguez 1973). The most pronounced effect of theophylline on glycerol metabolism was the inhibition of fatty acid synthesis in the presence of glucose. The action of theophylline on this parameter was much greater than the effect of epinephrine. An elevation in intracellular cyclic AMP levels can hardly account for the more intense inhibition of fatty acid synthesis by theophylline because cyclic AMP levels are elevated more by epinephrine than by theophylline (Moskowitz and Fain 1970, Fain 1973). It also is unlikely that the intense inhibitory action of theophylline on fatty acid synthesis from glycerol is due to an effect on the concentration of lipogenic enzymes, because insulin

partially prevents the action of theophylline on glycerol utilization (Table 1) but does not influence the inhibitory effect of theophylline on protein synthesis (Jarrett, Steiner, Smith and Kipnis 1972). Theophylline is known to decrease the oxidation of glucose via the pentose shunt pathway (Kupiecki 1973). This raises the possibility that the inhibitory action of theophylline on fatty acid synthesis from glycerol might arise from a reduction in the availability of the reduced nucleotides necessary for lipogenesis. Further experimental support is required to sustain this possibility.

In summary, the effects of 2-DG, oligomycin, and theophylline on adipose metabolism and on its response to insulin and epinephrine suggest that the utilization of glycerol is more sensitive to changes in the availability of energy in the tissue than the mechanisms responsible for its release via lipolysis. It must be pointed out, however, that other factors such as compartmentation, substrate composition, intracellular redox potential and intracellular accumulation of free fatty acids might be expected to play a significant role in the modulation of adipose tissue glycerol metabolism in response to these substances.

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Requests for reprints should be addressed to: Dr. Emilio Herrera, Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Barcelona-7 (Spain)