Biochemical Pharmacology, Vol. 27, pp. 1577–1579.

0006-2952/78/0601-1577/$02.00/0

IMPORTANCE OF THE ESTERIFICATION PROCESS IN ADIPOSE TISSUE METABOLISM AS EVIDENCED BY CYCLOHEXIMIDE

J. ADOLFO GARCÍA-SÁINZ, ENRIQUE PIÑA and VICTORIA CHAGOYA DE SÁNCHEZ

Departamento de Biología Experimental, Instituto de Biología, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, México 20, D.F.

(*Received* 10 *August* 1977; *accepted* 12 *October* 1977)

Abstract—The addition *in vitro* of cycloheximide (1 μg/ml) produces an increase in the incorporation of isotopic glucose or lactate into total lipids of epididymal fat pads obtained from fed rats. This enhancement in the incorporation of glucose proceeds via the glyceride-glycerol moiety. The synthesis of fatty acids is slightly decreased by the antibiotic which affects neither the uptake of glucose nor its oxidation. The regulation of the esterification process in adipose tissue is discussed.

Aside from the inhibitory effect of cycloheximide (CHM) on protein synthesis, intraperitoneal administration to fasted rats produced a 10-fold increase in the incorporation of [^{14}C]glucose into the lipids of epididymal fat pads, mainly through an increase in esterification of free fatty acids (FFA) [1]. By contrast, a small increase, without statistical significance, in the incorporation of glucose into lipids was found when the antibiotic was injected into fed rats [1]. It was rationalized that, in the pads from fasted animals, CHM might increase a metabolic process normally stimulated by food intake. The physiological consequences of food intake play an important role in the metabolism of adipose tissue as evidenced by lower rates of glucose uptake [2] and FFA esterification [3] observed in fasting rats compared to fed ones. Recently, we reported that CHM, added *in vitro* to epididymal fat pads from fasted rats, increases 2-fold the uptake, oxidation and incorporation of glucose into lipids, concomitently with an increase in esterification of FFA [4]. Since these parameters are increased by feeding, we have studied whether the antibiotic, added *in vitro* to epididymal fat pads from fed rats, elicits further stimulation, in an effort to elucidate the prime pathway affected by CHM.

MATERIALS AND METHODS

Cycloheximide, bovine serum albumin (fraction V), and glycerokinase were obtained from Sigma Chemical Co. (St. Louis, MO). D[U-^{14}C]glucose (200 mCi/m-mole) and L[U-^{14}C]lactic acid as the sodium salt (60 mCi/m-mole) were purchased from International Chemical and Nuclear Corp. (Cleveland, OH). Hexokinase, glucose 6-phosphate dehydrogenase, and alpha-glycerophosphate dehydrogenase were obtained from Boehringer & Soehne (Mannheim).

The experiments were performed using male Wistar rats weighing between 120 and 170 g. The animals were fed *ad lib.* with Purina rat chow; they were killed by decapitation and then exsanguinated. The epididymal fat pads were removed, rinsed in 0.85% NaCl and incubated in a Dubnoff metabolic shaker at 37° for 2 hr in a 25-ml Erlenmeyer stoppered flask. In addition to the tissue, each flask contained 3 ml of Krebs–Ringer bicarbonate buffer adjusted to pH 7.3 and supplemented with 150 mg of bovine serum albumin and 11.1 mM glucose. Some variability in the results was observed when different batches of albumin were used. To avoid this problem, albumin was systematically purified according to the procedure of Chen [5]. When radioactive glucose was used, it was added to a final concentration of 1 μCi/11.1 μmoles. Where indicated, glucose was substituted for lactate at a concentration of 1 mM, and 0.4 μCi [^{14}C]lactate was added to each flask. The incubation medium was heated to 37° and gassed with 5% CO_2–95% O_2 before the donor animals were killed; the flasks were flushed with the same gas mixture before sealing. Usually one of the two pads obtained from each animal served as the control while the other pad was in medium which contained CHM at a dose of 1 μg/ml.

Lipids were extracted according to Folch *et al.* [6]. In some experiments the lipids from the extracts were saponified and the distribution between glyceride-glycerol and fatty acids of radioactive carbon from glucose or lactate was studied by the method of Kornacker and Ball [7], with minor modifications [1]. When lipolysis and esterification were studied, one pad from each rat was used to determine the initial concentration of glycerol and FFA. The net changes in glycerol and FFA are presented. Free fatty acids were determined according to Dole and Meinertz [8] and glycerol was determined according to Wieland [9]. Glucose uptake was measured by the disappearance of the hexose from the medium according to the method of Slein [10]. Oxidation of [^{14}C]glucose was measured by its conversion to $^{14}CO_2$, using the method of Del Boca and Flatt [11]. Other experimental details have been reported elsewhere [1, 4].

RESULTS AND DISCUSSION

The incorporation of [^{14}C]glucose into lipids of epididymal fat pads from fed rats was found to be increased 50 per cent by cycloheximide. The drug

Table 1. Effects of cycloheximide on glucose metabolism*

	Control (μmoles/g)	Cycloheximide (μmoles/g)	P
Uptake (μmoles/g wet wt)	9.42 ± 1.07 (6)	11.61 ± 1.15 (6)	< 0.2
Incorporation into lipids (μmoles/g lipids)	1.55 ± 0.18 (7)	2.32 ± 0.18 (7)	< 0.02
Oxidation (μmoles/g lipids)	3.21 ± 0.39 (5)	3.09 ± 0.36 (6)	< 0.9

* The results are expressed as the mean ± S.E.M., with the number of observations in parentheses.

also produced a statistically insignificant increase in the uptake of glucose without promoting its oxidation (Table 1). To explore the metabolic pathway modified by CHM, the lipid extracts were fractionated and it was found that the antibiotic produced a marked shift in the distribution of the label from the fatty acid moiety to the glyceride–glycerol moiety (Table 2), showing a pattern of distribution similar to that found in fasted animals [4]. By combining the data from Tables 1 and 2 it is possible to calculate the amount of glucose incorporated into fatty acids or glyceride–glycerol. In control pads ≈ 0.85 μmole was incorporated into fatty acids and ≈ 0.64 μmole was channeled toward the synthesis of glycerophosphate. In pads incubated with CHM, ≈ 0.59 and ≈ 1.66 μmoles glucose/g of lipids were incorporated into fatty acids and glyceride–glycerol respectively. Therefore, the antibiotic stimulated the incorporation of glucose into lipids by ≈ 0.77 μmole/g of lipids through the synthesis of the glyceride–glycerol moiety of the neutral fats (1.02 μmoles/g of lipids) and decreased its incorporation into the fatty acids (0.26 μmole/g of lipids). The action of CHM on the esterification of FFA, reported in Table 3, is a consequence of the previous findings. The antibiotic stimulated this pathway 47 per cent; this is quantitatively identical to the CHM-mediated stimulation in the incorporation of [^{14}C]glucose into lipids (Table 1). The decrease in the incorporation of glucose into fatty acids is in agreement with the paper by McNamara *et al.* [12], who reported that CHM causes a dramatic decrease in the conversion of isotopic acetate to fatty acids in rat liver homogenates.

The increase in glucose uptake produced by the addition *in vitro* of CHM to epididymal fat pads from fasted rats [4] seemed to explain most of its actions on glucose and lipid metabolism. In pads obtained from fed rats, the slight stimulation of the uptake of glucose appears to be more a consequence of satisfying the demands of metabolites to increase the production of glycerophosphate than a direct action of the antibiotic on the uptake of the hexose. CHM enhanced the uptake of glucose 23 per cent

Table 2. Relative distribution of radioactive carbon from glucose in different fractions of lipid extracts of epididymal fat pads*

Fraction	Control (%)	Cycloheximide (%)	P
Non-saponifiable lipids	3.53 ± 0.55 (4)	3.04 ± 0.13 (4)	< 0.5
Fatty acids	55.13 ± 3.70 (4)	25.53 ± 1.12 (4)	< 0.001
Glyceride–glycerol	41.08 ± 3.77 (4)	71.43 ± 1.73 (4)	< 0.001

* Specifications are as in Table 1. Per cent values are given on the basis of the results presented in Table 1, i.e. 1.55 μmoles glucose/g incorporated into lipids for the controls, and 2.32 μmoles/g for the experiments with cycloheximide.

Table 3. Effects of cycloheximide on lipolysis and free fatty acid esterification*

Net change	Control	Cycloheximide	P
Glycerol (μmoles/g)	4.16 ± 0.63 (5)	5.49 ± 0.61 (5)	< 0.2
FFA (μEq/g)	0.58 ± 0.16 (5)	−1.00 ± 0.26 (5)	< 0.001
Rate of esterification (μEq/g)	11.90	17.47	—

* Specifications are as in Table 1.

Table 4. Effect of cycloheximide on isotopic lactate incorporation into lipids and relative distribution of the label*

	Control	Cycloheximide	P
	(cpm/mg of lipids)		
Incorporation into lipids	45.27 ± 3.26 (4)	65.77 ± 7.05 (4)	< 0.05
Distribution	(per cent)		
Non-saponifiable lipids	3.73 ± 1.38 (4)	2.35 ± 0.61 (4)	< 0.4
Fatty acids	40.10 ± 3.54 (4)	25.79 ± 2.06 (4)	< 0.02
Glyceride-glycerol	56.18 ± 4.59 (4)	71.86 ± 1.93 (4)	< 0.05

* Specifications are as in Table 1. Per cent values are given on the basis of the incorporation of lactate into lipids presented in the first line of the table.

(Table 1) and although this increase is more than enough to account for the 50 per cent stimulation of the incorporation of glucose into lipids (Table 1), this must take place specifically via glycerophosphate (Table 2) to subsequently raise the esterification of FFA (Table 3).

The results of Table 4 give support to this point. The antibiotic increased the incorporation of lactate into lipids, and the distribution of the label showed a shift to the glyceride-glycerol moiety. Furthermore, the magnitude of the stimulation (45 per cent) was quite similar to that observed with glucose (Table 1). The enhancement produced by CHM in the incorporation of glucose or lactate into lipids is of special significance since these metabolites are incorporated into lipids either through glycerogenesis (Tables 1 and 2) or glyceroneogenesis (Table 4) in order to satisfy the demands of the esterification process. These results indicate that the prime action of CHM should be localized on the esterification pathway.

The CHM-mediated increase in glucose uptake and oxidation observed in tissues from fasted animals [4] might be a consequence of the stimulation of the esterification process by the antibiotic. The lack of effect on these parameters in tissue from fed rats (Table 1) may be due to the fact that they are already stimulated by the food intake, and probably by the same mechanism that is a result of the antibiotic action in tissues from fasted animals, so that no additive action is observed. The results of this paper focus on the importance of the esterification process as a point of regulation of adipose tissue metabolism.

Acknowledgement—The authors wish to thank Dr. Antonio Peña for the careful revision of the manuscript.

REFERENCES

1. J. A. García-Sáinz, A. G. Amador, E. Piña and V. Chagoya de Sánchez, *Biochem. Pharmac.* **24**, 891 (1975).
2. M. Vaughan, *J. Lipid Res.* **2**, 293 (1961).
3. B. Shapiro, in *Handbook of Physiology, Adipose Tissue* (Eds A. E. Renold and G. F. Cahill, Jr.), Sect. 5, p. 217. American Physiological Society, Washington, D.C. (1965).
4. J. A. García-Sáinz, E. Piña and V. Chagoya de Sánchez, *J. Lipid Res.* **18**, 93 (1977).
5. R. F. Chen, *J. biol. Chem.* **242**, 173 (1967).
6. J. Folch, M. Lees and G. H. Sloane Stanley, *J. biol. Chem.* **226**, 497 (1957).
7. M. S. Kornacker and E. G. Ball, *J. biol. Chem.* **243**, 1638 (1968).
8. V. P. Dole and H. Meinertz, *J. biol. Chem.* **235**, 2595 (1960).
9. O. Wieland, in *Methods in Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 211. Academic Press, NY (1965).
10. M. W. Slein, in *Methods in Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 117. Academic Press, NY (1965).
11. J. Del Boca and J. P. Flatt, *Eur. J. Biochem.* **11**, 127 (1969).
12. D. J. McNamara, F. W. Quackenbush and V. M. Rodwell, *Lipids* **5**, 146 (1970).