

# Inhibition of free fatty acid mobilization by colchicine

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**Abstract** Segments of epididymal adipose tissue from normal male rats were incubated with micromolar concentrations of colchicine for different periods of time up to 4 hr, and the mobilization of free fatty acids (FFA) was measured during a subsequent reincubation. Although pretreatment with colchicine did not alter basal unstimulated FFA release, mobilization of FFA in the presence of epinephrine or theophylline was reduced. However, neither lipolysis, as judged by glycerol production, nor cyclic AMP accumulation was impaired under the same conditions. To assess the possibility that colchicine might limit production of fatty acids by accelerating the entry and metabolism of glucose into adipocytes, the metabolism of glucose by adipose tissue was studied. Pretreatment with colchicine did not affect uptake of glucose nor its oxidation to CO<sub>2</sub>, although colchicine-treated tissues did have slightly more [<sup>14</sup>C]glucose incorporated into the glyceride moiety of triglyceride. When adipose tissues pretreated with colchicine were incubated in an albumin-free medium, no reduction in FFA production by colchicine was observed. Because no FFA release occurs in albumin-free media, this experiment suggests that colchicine-induced inhibition of FFA mobilization results from impaired extrusion of FFA from adipose cells.

**Supplementary key words** lipolysis · glucose oxidation · adipose tissue · microtubules

In adipose tissue, triglycerides are constantly being hydrolyzed to free fatty acids (FFA) and glycerol and reformed from FFA and  $\alpha$ -glycerophosphate. Although the cellular mechanisms underlying the hydrolysis and resynthesis of triglycerides are well understood (1), the mechanisms by which FFA are released from adipocytes into the circulation remain obscure. It has been tacitly assumed that the egress of FFA from adipose tissue occurs by passive diffusion consequent to their formation and accumulation within adipocytes (2). This view has been called into question, however, by recent experiments suggesting that the extrusion of FFA from segments of adipose tissue (3) and from isolated adipocytes (4) requires the expenditure of energy. The studies reported herein indicate that the mobilization of FFA from adipose tissue is susceptible to inhibition by micromolar concentrations of the antimitotic drug colchicine. In view of the well-documented action of

colchicine blocking numerous secretory processes (5–7), it is suggested that colchicine inhibits the extrusion of FFA from adipocytes.

## MATERIALS AND METHODS

Normal male rats (140–200 g), derived from the Sprague-Dawley strain and purchased from Zivic-Miller Lab Inc., Allison Park, Pa., were fasted overnight and killed by cervical dislocation. The epididymal fat pads were rapidly excised, and the thin distal portion of each fat body was divided into 8–10 segments, each weighing between 50 and 150 mg. Tissue segments were randomly distributed among 25-ml Erlenmeyer flasks containing 4.0 ml of Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, 40 mg of bovine serum albumin per ml (Sigma, fraction V), 1 mg of glucose per ml, and various concentrations of colchicine (Calbiochem, lot 800585) and incubated at 37°C under an atmosphere of 95% O<sub>2</sub> and 5% CO<sub>2</sub> for up to 4 hr. After this preincubation, the tissues were reincubated in fresh vials containing 2.0 ml of KRB enriched with 40 mg/ml bovine serum albumin and 1 mg/ml glucose but no colchicine for up to 1 hr. FFA mobilization was stimulated by the addition of epinephrine (Parke-Davis) or theophylline (Sigma) to some vials. The amount of glycerol released into the medium was measured enzymatically (8) and served as an index of lipolysis. The FFA content of the tissue and incubation media was determined separately by the method of Dole (9). Studies on glucose metabolism in adipose tissue were performed as previously described (10).

For the experiments in which the cyclic adenosine 3',5'-monophosphate (cyclic AMP) content of adipose tissue segments was measured, segments of adipose tissue were frozen on dry ice and homogenized in 10 vol of 50% acetic acid and centrifuged at 3000 rpm for 15 min. Duplicate 100- $\mu$ l and 200- $\mu$ l aliquots of the supernate were

A preliminary report of this investigation has appeared (*Federation Proc.* 31: 351, 1972).

Abbreviations: FFA, free fatty acids; cyclic AMP, cyclic adenosine 3',5'-monophosphate; KRB, Krebs-Ringer bicarbonate buffer.

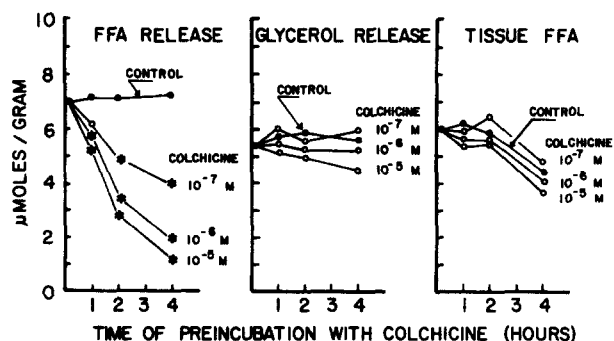


Fig. 1. Effects of pretreatment with colchicine on epinephrine-activated lipolysis. Segments of epididymal adipose tissue were preincubated for various periods of time, indicated on the abscissa, in KRB containing different concentrations of colchicine, indicated next to each line. Tissues were then transferred to fresh KRB containing 0.05  $\mu\text{g/ml}$  epinephrine and 40  $\text{mg/ml}$  albumin but no colchicine and reincubated for 1 hr. Each point represents the mean of 15 observations. Basal rates, in the absence of epinephrine, at 0, 1, 2, and 4 hr of preincubation were: 1.8, 1.5–1.8, 1.4–1.8, and 0.5–1.1  $\mu\text{moles/g-hr}$  for FFA release; 1.1, 0.8–1.2, 0.4–0.6, and 0.3–0.6  $\mu\text{moles/g-hr}$  for glycerol release; and 3.4, 2.1–2.9, 1.8–2.7, and 1.8–2.6  $\mu\text{moles/g}$  for tissue FFA content. Points designated with an asterisk are significantly different from control (no colchicine),  $P < 0.05$ .

dried in  $10 \times 75$  mm glass tubes at 80–85°C overnight to volatilize all traces of acetic acid and water. Assays for cyclic AMP were run in these tubes using the method of Gilman (11).

## RESULTS

Inhibition of epinephrine-accelerated FFA release as a function of both the concentration of colchicine present during preincubation and the duration of preincubation is shown in Fig. 1. Mobilization of FFA in response to epinephrine was not impaired by preincubation of adipose tissue segments in the absence of colchicine for up to 4 hr. However, exposure of tissue segments to as little as  $10^{-7}$  M colchicine during 2 hr of preincubation significantly reduced FFA production in response to epinephrine. Higher concentrations of colchicine or longer periods of exposure to colchicine produced a greater inhibition of

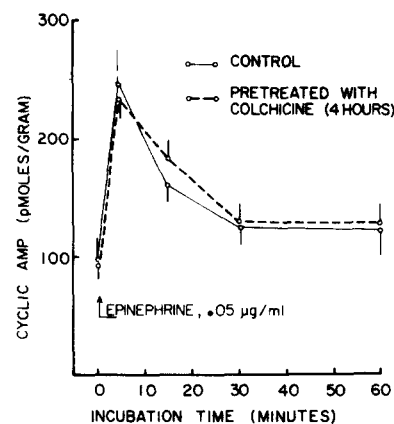


Fig. 2. Failure of colchicine to affect cyclic AMP levels in response to epinephrine. Segments of epididymal adipose tissue were preincubated for 4 hr in KRB containing  $5 \times 10^{-6}$  M colchicine or no colchicine. Tissues were then transferred to fresh KRB containing 0.05  $\mu\text{g/ml}$  epinephrine and 40  $\text{mg/ml}$  albumin but no colchicine and reincubated for 5, 15, 30, or 60 min. Each point represents the mean  $\pm$  SE of eight observations.

FFA release. In contrast, epinephrine-enhanced glycerol release, which is indicative of the rate of formation of FFA within adipocytes, was not significantly altered by preincubation with colchicine even for 4 hr. Tissue FFA levels tended to decline as a result of preincubation alone, but this decline was not altered by colchicine. In other experiments, the accumulation of cyclic AMP in adipose tissues exposed to epinephrine was not altered by preincubation with colchicine for 4 hr (Fig. 2).

Table 1 shows the effects of pretreatment of adipose tissue segments for 2 hr with colchicine on FFA and glycerol release accelerated by exposure of the tissue to theophylline. As was seen when epinephrine was used, tissues pretreated with colchicine released significantly less FFA in response to theophylline than did control tissues. In contrast, lipolysis, as judged by glycerol release, was not impaired by pretreatment with colchicine and was, in fact, slightly but significantly accelerated by  $10^{-6}$  M colchicine. Tissue FFA levels and basal lipolysis were unaffected by pretreatment with colchicine.

TABLE 1. Effects of pretreatment of adipose tissue with colchicine for 2 hr on basal and theophylline-activated lipolysis

Colchicine	Glycerol Release		FFA Release		Tissue FFA	
	Basal	Theophylline	Basal	Theophylline	Basal	Theophylline
<i>M</i>	$\mu\text{moles/g-hr}$		$\mu\text{moles/g-hr}$		$\mu\text{moles/g}$	
0	0.8 $\pm$ 0.1	6.0 $\pm$ 0.5	0.2 $\pm$ 4	13.1 $\pm$ 1.2	2.1 $\pm$ 0.8	10.1 $\pm$ 1.0
$10^{-7}$	0.7 $\pm$ 0.2	6.1 $\pm$ 0.4	0.2 $\pm$ 0.1	12.0 $\pm$ 0.8	1.9 $\pm$ 0.4	9.9 $\pm$ 0.4
$10^{-6}$	0.8 $\pm$ 0.1	7.6 $\pm$ 0.2 <sup>a</sup>	0 $\pm$ 0.3	10.1 $\pm$ 0.4 <sup>a</sup>	1.8 $\pm$ 0.3	9.1 $\pm$ 0.7
$10^{-5}$	0.7 $\pm$ 0.1	7.4 $\pm$ 0.8	-0.4 $\pm$ 0.4	8.9 $\pm$ 0.4 <sup>a</sup>	1.7 $\pm$ 0.5	9.0 $\pm$ 0.6
$10^{-4}$	0.7 $\pm$ 0.3	6.9 $\pm$ 0.5	0.1 $\pm$ 0.3	7.2 $\pm$ 0.3 <sup>a</sup>	1.8 $\pm$ 0.6	8.9 $\pm$ 1.2

Segments of epididymal adipose tissue were incubated for 2 hr in KRB containing different concentrations of colchicine and reincubated for 1 hr in KRB containing 0.30  $\text{mg/ml}$  theophylline. Each value represents the mean  $\pm$  SE of 10 observations.

<sup>a</sup> Significantly different from control (no colchicine),  $P < 0.05$ .

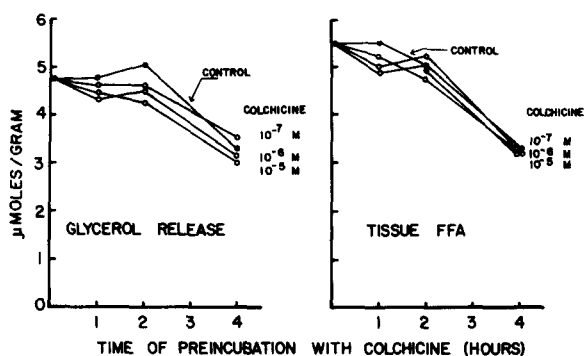


Fig. 3. Effects of pretreatment with colchicine on epinephrine-activated lipolysis in the absence of albumin. Segments of epididymal adipose tissue were preincubated for various periods of time, indicated on the abscissa, in KRB containing different concentrations of colchicine, indicated next to each line. Tissues were then transferred to fresh KRB containing 0.05  $\mu\text{g/ml}$  epinephrine but no colchicine or albumin and reincubated for 1 hr. Each point represents the mean of 10 observations. Basal rates, in the absence of epinephrine, at 0, 1, 2, and 4 hrs of preincubation were: 1.9, 1.1–1.5, 0.5–1.1, and 0.6–1.0  $\mu\text{moles/g-hr}$  for glycerol release and 4.2, 2.1–3.1, 2.0–2.8, and 1.5–2.5  $\mu\text{moles/g}$  for tissue FFA content.

Inhibition of FFA release in the absence of concomitant decreases in cyclic AMP levels or glycerol production suggested that colchicine may be promoting uptake and metabolism of glucose by adipose tissue, thereby increasing fatty acid reesterification and limiting release of FFA. To test this possibility, experiments were performed to monitor the uptake and metabolism of glucose in adipose tissue.

The data in Table 2 show the effects of pretreatment with colchicine ( $10^{-5}$  M) for 2 and 4 hr on the uptake and metabolism of glucose by segments of adipose tissue incubated in the presence or absence of epinephrine (0.05  $\mu\text{g/ml}$ ). As has been reported by others (12), epinephrine increased the oxidation of glucose by adipose tissue. Although treatment of adipose tissue with  $10^{-5}$  M colchicine significantly reduced epinephrine-accelerated mobilization of FFA (Fig. 1), neither the uptake of glucose nor its oxidation to  $\text{CO}_2$ , in either the presence or absence of epinephrine, was affected by treatment for 2 or 4 hr with colchicine. Tissue pretreated with colchicine did have slightly

more [ $^{14}\text{C}$ ]glucose incorporated into the glycerol moiety of the triglyceride than untreated tissue, but this was not statistically significant ( $0.05 < P < 0.1$ ). Similarly, pretreatment with  $10^{-5}$  or  $10^{-4}$  M colchicine for 4 hr did not increase the accumulation of the sugar D-xylose by adipose tissue (not shown). D-Xylose was selected because it has been reported to share a common transport mechanism with glucose in many tissues (13). Because colchicine pretreatment did not cause an overall stimulation of glucose metabolism in adipose tissue (Table 2) or promote increased xylose accumulation, the possibility that colchicine limits the release of FFA from adipose tissue by greatly augmenting uptake of glucose seems remote.

Alternatively, colchicine may inhibit the extrusion of FFA from adipose tissue cells. To evaluate this possibility, adipose tissue segments were pretreated with colchicine for 1, 2, or 4 hr as previously described and reincubated for 1 hr in media containing no albumin (Fig. 3). Colchicine treatment did not alter epinephrine-activated lipolysis. In the absence of albumin, when FFA cannot leave the tissues, activation of reesterification by colchicine should lead to decreased accumulation of FFA within those tissues pretreated with colchicine. The data presented in Fig. 3 show that this did not occur; FFA levels in tissues pretreated with colchicine and in untreated control tissues were identical. These data demonstrate that the inhibition of FFA production in response to epinephrine caused by prior exposure to colchicine is contingent upon the ability of FFA to leave the tissues and strongly suggest that colchicine is acting to interfere with the release of FFA from the adipocytes.

## DISCUSSION

The salient observation presented herein is that treatment of adipose tissue with micromolar concentrations of colchicine inhibits epinephrine- and theophylline-accelerated FFA mobilization. In contrast, neither lipolysis, as judged by glycerol production, nor accumulation of cyclic

TABLE 2. Effects of colchicine pretreatment on glucose uptake and metabolism in adipose tissue

Colchicine	Hours of Preincubation	Glucose Uptake		Conversion of [ $^{14}\text{C}$ ]Glucose to:					
				$\text{CO}_2$		Glyceride Glycerol		Glyceride Fatty Acids	
		Basal	Epi. <sup>a</sup>	Basal	Epi. <sup>a</sup>	Basal	Epi. <sup>a</sup>	Basal	Epi. <sup>a</sup>
<i>M</i>		$\mu\text{moles/g-hr}$		<i>cpm/mg/hr of reincubation</i>					
0	2	11.1 $\pm$ 2.1 <sup>b</sup>	11.7 $\pm$ 2.5	48.1 $\pm$ 3.9	74.5 $\pm$ 4.7	28.1 $\pm$ 1.8	40.2 $\pm$ 3.1	21.5 $\pm$ 2.4	12.4 $\pm$ 1.1
$10^{-5}$	2	11.8 $\pm$ 2.5	11.9 $\pm$ 2.9	50.9 $\pm$ 4.1	69.9 $\pm$ 4.2	28.9 $\pm$ 4.1	48.1 $\pm$ 2.1	20.1 $\pm$ 1.8	9.2 $\pm$ 1.4
0	4	14.2 $\pm$ 1.7	17.1 $\pm$ 4.1	49.3 $\pm$ 2.9	75.1 $\pm$ 4.1	24.1 $\pm$ 2.1	41.5 $\pm$ 3.2	22.4 $\pm$ 1.8	9.1 $\pm$ 0.9
$10^{-5}$	4	13.9 $\pm$ 3.8	16.8 $\pm$ 4.4	53.5 $\pm$ 5.1	75.8 $\pm$ 5.0	26.8 $\pm$ 2.7	52.8 $\pm$ 2.9	19.1 $\pm$ 2.4	7.6 $\pm$ 1.3

Segments of epididymal adipose tissue were incubated for 2 or 4 hr in the presence or absence of colchicine ( $10^{-5}$  M). Tissues were then reincubated for 1 hr in media containing 1 mg/ml [ $^{14}\text{C}$ ]glucose (0.20  $\mu\text{Ci/ml}$ ) but no colchicine.

<sup>a</sup> Epi., epinephrine, 0.05  $\mu\text{g/ml}$ .

<sup>b</sup> Each value represents the mean  $\pm$  SE of 16 observations.

AMP within the tissues was altered by prior exposure to colchicine. The finding that lipolysis is not reduced by treatment with colchicine indicates that the intracellular generation of fatty acids from stored triglyceride is not reduced after exposure to the antimetabolic drug. Because fatty acids generated by lipolysis in the presence of epinephrine or theophylline were neither released into the medium nor accumulated within the tissues, it follows that reesterification of FFA must be greater in those tissues exposed to colchicine and reincubated in albumin-containing medium. FFA not released from tissues nor retained within tissues must have been reesterified. This raises the question as to the action of colchicine in simultaneously curtailing release of fatty acids into the medium and promoting fatty acid reesterification. Two possibilities were considered. Colchicine may promote glucose uptake by the adipocytes and thereby provide more  $\alpha$ -glycerophosphate for reesterification, an action analogous to the action of insulin on adipose tissue. Such an action of colchicine might be anticipated. It is well known that many agents that at high concentrations damage adipocytes stimulate cellular glucose metabolism when present at lower concentrations (1, 14). Hence, colchicine could, by simply altering the conformation of a region of the membrane of the adipose cell, directly promote glucose uptake and metabolism. However, direct measurement of sugar transport, glucose uptake, and glucose metabolism in adipose tissues treated with colchicine failed to support this possibility (Table 2). Although tissues pretreated with colchicine did incorporate slightly more [ $^{14}$ C]glucose into glyceride glycerol than untreated tissues, this effect was not statistically significant and did not appear to result from an overall stimulation of glucose metabolism. In addition, Soifer, Braun, and Hechter (15) have reported that pretreatment of adipocytes with  $5 \times 10^{-5}$  M colchicine for 45 min failed to alter basal glucose metabolism. Finally, and most importantly, the action of colchicine promoting fatty acid reesterification was not seen when tissues were reincubated in media containing no albumin (Fig. 3), although the rates of fatty acid reesterification in tissues incubated in the presence or absence of albumin were similar. These observations argue against the possibility that the increased fatty acid esterification in tissues pretreated with colchicine is secondary to stimulation of glucose uptake.

The second possible explanation for colchicine-induced inhibition of fatty acid mobilization is that colchicine retards the release of FFA from the cells. Fatty acids not released into the medium are reesterified within the adipose tissue cells and hence fail to accumulate in increased amounts. In support of this possibility is the finding that inhibition of fatty acid production by colchicine is seen only when albumin is present in the incubation medium, thereby permitting FFA release.

If this interpretation of the data is correct, it raises the question of the mechanism of action of colchicine in inhib-

iting the extrusion of FFA from adipose cells. Because colchicine was inhibitory at the same low concentrations at which it interferes with microtubule-dependent processes in other systems, and because the time-concentration characteristic of inhibition of FFA release is similar to what has been reported in other microtubule-dependent systems (5-7, 16, 17), it is reasonable to suggest that inhibition of FFA release by colchicine is due to dissolution of cytoplasmic microtubules, which have been identified in electron micrographs of adipocytes (15, 18). Such an action of colchicine, if verified, suggests a heretofore unrecognized dependence of FFA release on this cytoplasmic organelle and provides additional evidence in support of the view (3, 4) that the release of FFA from adipose tissue cells does not occur by simple passive diffusion.

Definitive interpretation of the data presented herein is made difficult by the lack of knowledge regarding the specific cellular pathways taken by fatty acids moving from the triglyceride droplet to the extracellular medium. Micropinocytotic vesicles are frequently observed in adipocytes undergoing lipid mobilization (19-21), and some investigators have suggested that they participate in the process of lipid mobilization (20, 21). Cushman (21) has reported that serum albumin is carried into adipocytes by pinocytosis and that this process is accelerated by agents that promote lipid mobilization. Since albumin is a fatty acid carrier, it was suggested (21) that fatty acid transport into and/or out of adipocytes is mediated by pinocytosis. Colchicine-induced inhibition of fatty acid mobilization may be a consequence of an action of the antimetabolic drug to retard intracellular transport of FFA-containing pinocytotic vesicles. Arguing against this possibility, however, is the failure of investigators to detect FFA-containing vesicles in homogenates of adipose tissue (22, 23). Angel, Desai, and Halperin (24) have proposed that reversible binding of free fatty acids to mitochondria and microsomal membranes may participate in mobilization of fatty acids from adipocytes. An action of colchicine preventing the interaction between FFA and intracellular membranes could underlie the observed depression of lipid mobilization caused by the drug.

Soifer et al. (15) have reported that treatment of adipocytes with  $5 \times 10^{-5}$  M colchicine for 45 min inhibited insulin-stimulated lipogenesis and glycogen synthesis from glucose and proposed that these anabolic actions of insulin are dependent upon cytoplasmic microtubules. Although microtubules in adipocytes may well be required for diverse metabolic activities, several non-microtubule-dependent actions of colchicine have been described (25-27), and caution is indicated before microtubules are implicated in cell processes solely because those processes are disrupted by colchicine. **■**

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