Biochemical Pharmacology, Vol. 26, pp. 1783–1789. Pergamon Press, 1977. Printed in Great Britain.

EFFECT OF PYRUVATE ON THYROID HORMONE-INDUCED LIPOLYSIS IN RAT ADIPOSE TISSUE *IN VITRO*

ATSUSHI ICHIKAWA, TOSHITADA NOTSU, HISASHI MATSUMOTO, TOSHIKO IMAMURA and KENKICHI TOMITA

Department of Health Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto, Japan 606

(*Received* 27 *July* 1976; *accepted* 14 *January* 1977)

Abstract—Pyruvate markedly enhanced lipolysis induced by triiodo-L-thyronine (L-T_3) or methylxanthines in fat cells from fasted rats, but not from fed rats. In contrast, pyruvate only slightly increased lipolysis which had been submaximally elevated by epinephrine, adrenocorticotropic hormone or thyroid-stimulating hormone, and did not affect the lipolytic effect of lutenizing hormone, growth hormone plus dexamethasone or hydrocortisone. Insulin and prostaglandin E_1 blocked this pyruvate effect on T_3-induced lipolysis. In the presence of 5 mM pyruvate, a significant lipolysis was observed with 0.001 mM L-T_3; a near maximal effect was seen with L-T_3 at 0.1 mM. Conversely, lipolysis induced by 0.1 mM L-T_3 was clearly augmented with 0.01 mM pyruvate; a maximal effect was seen with pyruvate at 5 mM. Pyruvate itself had no effect on basal lipolysis, but promptly stimulated T_3-induced lipolysis after its addition. Furthermore, pyruvate (0.01 to 5 mM) did not alter the ratios of fatty acid/glycerol released during lipolysis induced by 0.1 mM L-T_3. Pyruvate also markedly augmented T_3-induced formation of cAMP in fat cells from fasted rats. This accumulation of cAMP was blocked by AMP, a potent inhibitor of lipolysis induced by T_3 plus pyruvate.

Lipolysis induced by epinephrine and other lipolytic hormones in adipose tissue *in vitro* is greatly affected by the thyroid state of the animal; it is enhanced by administration *in vivo* of thyroid hormones, and reduced by pharmacological or surgical thyroidectomy [1–10]. In addition to this permissive effect [10], a direct effect of triiodo-L-thyronine (L-T_3) on lipolysis in isolated fat cells has been reported [9, 11], although doses of L-T_3 required were much higher than those for administration *in vivo*.

Hormone-induced lipolysis *in vitro* is also known to be enhanced in the presence of glucose, either alone or in combination with insulin [12–17], probably by providing α-glycerophosphate for the re-esterification of free fatty acids (FFA) [13–18], and/or by supplying energy for the activation of the lipase system [17].

We examined the effects of glucose and its metabolites on thyroid hormone-induced lipolysis and adenyl cyclase systems in isolated rat adipocytes, since adenosine 3′:5′-monophosphate (cAMP) has been proven to mediate the lipolytic actions of various hormones [19–22]. The present report describes the marked stimulatory effect of pyruvate on T_3-induced lipolysis and on the accumulation of cAMP in fat cells.

EXPERIMENTAL PROCEDURE

Animals. Adipose tissue donors were normal male rats of the Wistar strain, weighing 150–190 g, and allowed free access to regular laboratory chow (CE-2 pellet, Nihon Clea, Tokyo, Japan) and tap water. In some experiments, animals were fasted for 48 hr, or fasted for 48 hr and then refed for 24 hr prior to killing. Surgical thyroidectomy was performed 3 weeks before the experiments as described previously [9].

Incubation of fat cells. Adipocytes were isolated from epididymal fat pads of rats according to Rodbell [23]. Cells (50–60 mg of the original tissue) were suspended in 1 ml Krebs–Ringer phosphate buffer (KRP, pH 7.4) containing one half the recommended amount of $CaCl_2$ [13] and 3% bovine serum albumin (BSA, Armour Fraction V), and incubated at 37° for up to 1 hr with gentle shaking both in the presence and in the absence of compounds to be tested.

Assays for FFA, triglycerides, glycerol and protein. FFA was extracted from aliquots (1 ml) of the incubation mixture with 0.5 ml of Dole's solution [24] followed by 0.6 ml heptane and 1 ml water. Assays for FFA [25], triglycerides (TG) [26] and glycerol [27, 28] were performed as described. The amount of FFA or glycerol released was expressed as microequivalents (μEq) palmitic acid of FFA or μmoles glycerol/m-mole of TG of g of adipose tissue protein/hr.

For the assay of protein, the fat cell suspension (1 ml) was acidified with 5% (final concentration) $HClO_4$, and then extracted twice with a mixture of ether (5 ml) and ethanol (3 ml) to remove fat before the method of Lowry *et al.* [29] was applied.

Assays for adenyl cyclase, cAMP and ATP. Adenyl cyclase activity was determined by measuring [^{14}C]-cAMP formed in adipocytes which had been previously incubated with [8-^{14}C]adenine according to Kuo and De Renzo [30]. Fat pads (15 g) were incubated with [8-^{14}C]adenine (25 μCi, 52.6 mCi/m-mole, New England Nuclear Corp.) and collagenase (12 mg, Boehringer and Sigma) in KRP at 37° for 1 hr. Dispersed labeled cells, into which about 7 per cent of

Table 1. Effect of glucose on T_3-induced FFA release in fat cells of rats*

Rat	FFA released (μEq/g protein/hr)			
	None	+ Glucose	+ T_3	+ Glucose + T_3
Fed	15.2 ± 0.8	7.8 ± 0.8	18.7 ± 1.3	17.4 ± 0.8
Fasted	39.8 ± 2.4	41.3 ± 1.4	89.6 ± 5.4	188.5 ± 4.4
Fasted–refed	24.9 ± 3.9	28.8 ± 2.4	39.1 ± 4.8	40.0 ± 3.9

* Fat cells isolated from about 50 mg of fat pads were incubated for 60 min at 37° in 1 ml of KRP with or without 0.1 ml L-T_3 and 5 mM glucose. Values are the mean ± S. E. of five incubations.

the added [8-^{14}C]adenine was incorporated, were washed four times with KRP containing 0.01 mM cold adenine, and used for the assay. [^{14}C]cAMP formed was isolated by column chromatography on Dowex 50 (H^+ form) followed by treatment with $ZnSO_4$–$Ba(OH)_2$ according to Krishna *et al.* [13].

cAMP content was assayed by the radioimmunoassay method of Okabayashi *et al.* [32]. Aliquots (0.2 ml) of the incubation mixture were mixed with 0.1 M acetate buffer (pH 6.2) (0.05 ml), heated for 3 min at 100°, and then centrifuged at 1000 *g* for 20 min. Aliquots (25 μl) of the supernatant fluid were mixed with 1% BSA (10 μl), [^{3}H]cAMP (10 μl, 0.03 μCi/pmole, Amersham-Searle), the rabbit antibody serum for human serum albumin conjugated with $O^{2'}$-succinyl cAMP (kindly supplied by Dr. T. Okabayashi, Shionogi & Co., Ltd. Osaka, Japan), 0.05 M acetate buffer (pH 6.2) (40 μl) and water (5 μl). After incubation of the mixture at 0° for 60 min, aliquots (75 μl) were poured onto a Millipore filter (0.45 μm pore, 2.5 cm diameter) previously soaked in 0.05 mM acetate buffer (pH 6.2), and the radioactivity of [^{3}H]cAMP-antibody retained on the filter was counted in 10 ml of Bray's scintillant.

ATP content was estimated by the lucifereine–luciferase method of Bihler and Jeanrenaud [33].

Sepharose-bound and succinylaminoethyl Sepharose-bount T_3. L-T_3 was covalently attached to Sepharose 4B (Pharmacia Fine Chemicals) by adapting the method of Pensky and Marshall [34] for the preparation of Sepharose-bound L-thyroxine. The amount of L-T_3 bound was determined by preparing Sepharose-bound L-T_3 with a trace amount of [^{131}I]L-T_3 (Dainabot RI Research, Tokyo, Japan). L-T_3 was also attached to succinylaminoethyl Sepharose 4B, prepared according to Cuatrecasas [35], by the method of Berman and Yound [36].

Chemicals. Growth hormone (GH), thyroid-stimulating hormone (TSH), lutenizing hormone (LH), insulin, ACTH and thyroid hormone analogues were obtained from Sigma, and cAMP and N^6,$O^{2'}$-dibutyryl cAMP (Bt_2cAMP) from Seishin Pharmaceutical Co., Tokyo, Japan. Prostaglandin E_1 (PGE_1) was a gift from Ono Pharmaceutical Co., Osaka, Japan. Other chemicals and nucleotides of reagent grade were obtained commercially.

RESULTS

Effects of glucose and related compounds on T_3-induced lipolysis in fat cells. As shown in Table 1, fat cells obtained from fasted rats were markedly more responsive to 0.1 mM L-T_3 than those from fed or fasted–refed animals [9], resulting in a more than 2-fold stimulation of FFA release. This T_3-induced lipolysis was further augmented by adding glucose to the mixture in which the fat cells from fasted rats were incubated, whereas glucose itself did not affect the basal lipolysis in each case. Therefore, we used adipocytes from rats fasted for 48 hr in most of the following experiments, unless otherwise stated.

Among various related compounds (5 mM) tested, only pyruvate was much more effective than glucose in augmenting T_3 (0.1 mM)-induced lipolysis in fat cells: FFA released (μEq/m-mole of TG/hr), control, 9.2 ± 0.4; T_3, 25.1 ± 0.7; T_3 + glucose, 37.8 ± 1.2; and T_3 + pyruvate, 62.4 ± 0.4. α-Keto-glutarate and oxaloacetate were both about equally effective as glucose, while other intermediates in glycolysis and Krebs cycle, acetate and coenzyme A were either less effective or completely ineffective in stimulating T_3-induced lipolysis.

Effects of pyruvate on lipolysis induced by T_3 analogues and various lipolytic hormones. The synergistic effect of pyruvate was also observed when lipolysis was induced by 3,5-diiodo-L-thyronine or L-thyroxine, although these analogues were only about 50 per cent as effective as L-T_3. No significant pyruvate effect on lipolysis was observed with other T_3 analogues tested (L-thyronine, triiodo-D-thyronine, thyroacetic acid and its iodinated derivatives), which were ineffective in inducing FFA release as well as in enhancing the epinephrine-induced lipolysis in fat cells [9].

In contrast to its marked stimulatory effect on T_3-induced lipolysis, pyruvate only slightly enhanced

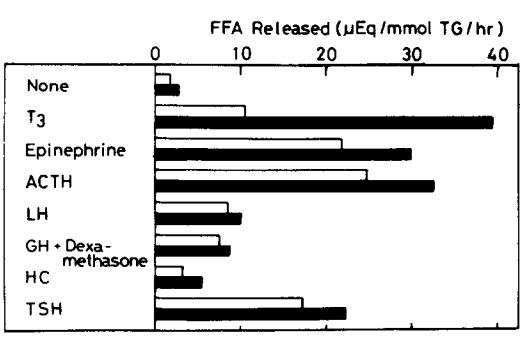

Fig. 1. Effect of pyruvate on FFA release from fat cells induced by various lipolytic hormones. Fat cells isolated from 65 mg of fat pads of rats fasted for 48 hr were incubated for 60 min at 37° in 1 ml of an incubation mixture containing each hormone, with (closed bars) or without (open bars) 5 mM pyruvate. The concentrations of hormones were: L-T_3, 0.1 mM; epinephrine, 0.55 μM; ACTH, 20 mU/ml; LH, 50 mU/ml; GH, 40 μg/ml; dexamethasone, 2.5 μM; hydrocortisone, 0.28 μM; and TSH, 0.2 mU/ml.

Table 2. Effect of pyruvate on T_3-induced FFA release from fat cells of rats*

Rat	FFA released (μEq/g protein/hr)			
	None	+ Pyruvate	+ T_3	+ Pyruvate + T_3
Fed	8.2 ± 1.5	10.2 ± 1.3	23.5 ± 2.7	25.1 ± 2.7
Fasted	15.1 ± 1.2	20.2 ± 1.7	75.5 ± 5.6	235.4 ± 12.3
Fasted–refed	11.3 ± 0.8	19.8 ± 1.3	35.3 ± 3.6	58.2 ± 8.9

* Fat cells isolated from about 60 mg of fat pads of fed, fasted and fasted–refed rats were incubated for 60 min at 37° in 1 ml of an incubation mixture containing 0.1 mM L-T_3 with or without 5 mM pyruvate. Values are the mean ± S. E. of three incubations.

FFA release which had been submaximally stimulated by epinephrine, ACTH or TSH (Fig. 1). FFA release induced by LH [21, 37], GH plus dexamethasone [4] or hydrocortisone [38] was not affected by the addition of pyruvate. In all cases, the release of glycerol was parallel to that of FFA.

Effect of feeding conditions and thyroid state of rats on lipolysis induced by T_3 and pyruvate. As in the case of the glucose effect, pyruvate showed a significant augmentative effect on T_3-induced lipolysis in fat cells of rats fasted for 24 hr, the maximum effect being obtained in the cells from animals fasted for 48–60 hr. This pyruvate effect was suppressed in cells from fasted–refed rats, and almost abolished in those from fed rats (Table 2).

The stimulatory effect of pyruvate on T_3-induced lipolysis was very much reduced, but still observed in fat cells from thyroidectomized rats fasted for 48 hr. This stimulatory effect was not observed in cells from fed animals. Injections of L-T_3 (200 μg s.c./day/rat, for 3 days) given to normal and thyroidectomized, fed rats resulted in the enhancement of T_3- or T_3 plus pyruvate-induced lipolysis in fat cells to levels comparable to those of fasted animals (Fig. 2).

However, pyruvate alone did not stimulate lipolysis in fat cells from T_3-treated, fed rats. Therefore, the potentiating effect of pyruvate in fat cells from T_3-injected, thyroidectomized rats does not appear to be due solely to the increased cellular concentration of L-T_3.

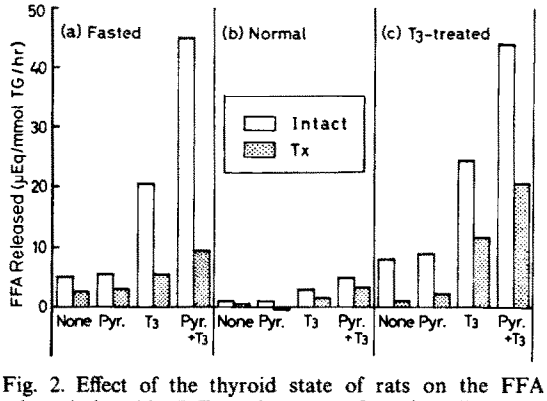

Fig. 2. Effect of the thyroid state of rats on the FFA release induced by L-T_3 and pyruvate from fat cells. Intact (open bars) and thyroidectomized (Tx, dotted bars) rats were maintained on laboratory chow until used. Key: (a) rats fasted for 48 hr, (b) fed rats, and (c) fed rats injected s.c. with L-T_3 (200 μg dissolved in 0.2 ml of 0.001 N NaOH/100 g of body weight, once a day for 3 days before killing). Fat cells were isolated from 60 mg of fat pads of intact rats or from 120 mg of those of thyroidectomized rats, and incubated for 60 min at 37° in 1 ml of an incubation mixture containing 5 mM pyruvate, 0.1 mM L-T_3, or both. Values are the mean of three incubations.

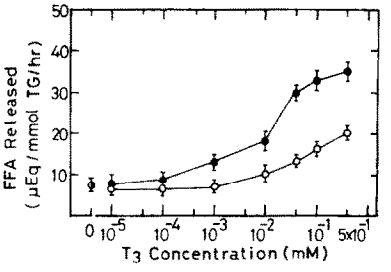

Fig. 3. Effect of concentration of L-T_3 on FFA release from fat cells, with or without pyruvate. Fat cells isolated from 54 mg of fat pads of rats fasted for 48 hr were incubated for 60 min at 37° in 1 ml of the mixture containing increasing concentrations of L-T_3 with (●) or without (○) 5 mM pyruvate. Each point represents the mean ± S. E. of three incubations. Statistical analysis: (with pyruvate) 0 to 0.0001 mM L-T_3, not significant; 0.001 mM L-T_3, $P < 0.05$; and 0.01 to 0.5 mM L-T_3, $P < 0.01$; (without pyruvate) 0 to 0.001 mM L-T_3, not significant; and 0.01 to 0.5 mM L-T_3, $P < 0.05$.

Dose relationship between T_3 and pyruvate on lipolysis. In the presence of 5 mM pyruvate, a significant FFA release was observed with 0.001 mM L-T_3, while, for the elevation of basal lipolysis, 0.01 mM L-T_3 was required. FFA release in the presence of pyruvate was further enhanced by increasing concentrations of L-T_3, and almost maximal at 0.1 mM L-T_3 (Fig. 3).

Conversely, in the presence of 0.1 mM L-T_3, the enhancing effect of pyruvate on FFA release was observed at a concentration level of 0.01 mM, maximal release being observed at a concentration from 0.2 to 5 mM, and then gradually decreasing with higher concentration (Fig. 4). The release of glycerol induced by L-T_3 was similarly enhanced in the presence of pyruvate. The ratios of FFA/glycerol released by 0.1 mM L-T_3 plus pyruvate were 2.05 to 3.0 within a wide range of pyruvate concentrations (from 0 to 20 mM).

Time course of effect of pyruvate and T_3 on lipolysis. As shown in Fig. 5, in the presence of both L-T_3 and pyruvate, FFA release from fat cells steadily increased with time. Lipolysis induced by preincubation with L-T_3 alone, for 10 min at 37°, was enhanced almost immediately after the addition of pyruvate, and increased linearly for at least 60 min. Preincuba-

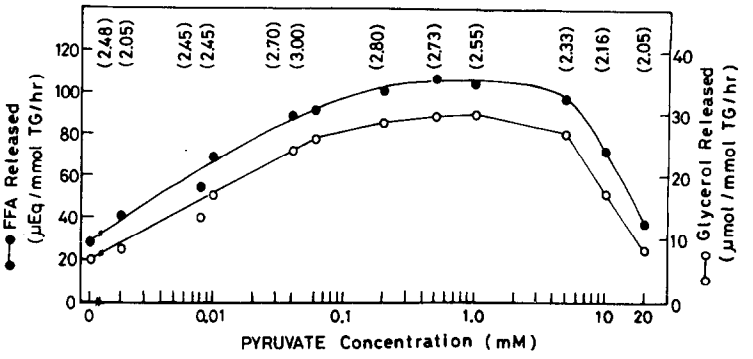

Fig. 4. Effect of concentration of pyruvate on T_3-induced lipolysis. Fat cells from about 60 mg of fat pads of rats fasted for 48 hr were incubated for 60 min at 37° in 1 ml of the mixture containing 0.1 mM L-T_3 and increasing concentrations of pyruvate. Each point represents the mean of four incubations. Values in parentheses are the ratios of FFA (μEq)/glycerol (μmoles) released.

tion of fat cells with pyruvate alone for 10 min at 37° was without effect. However, the addition of L-T_3 to pyruvate-treated cells quickly elevated FFA release without any noticeable lag period, and this release increased linearly with time, as in the former case.

Effect of pyruvate on methylxanthine- and cAMP-induced lipolysis. Pyruvate also acted synergistically with methylxanthines, inducers of lipolysis in adipocytes [20] and inhibitors for phosphodiesterase [39], in stimulating FFA release from fat cells. As shown in Fig. 6, FFA release, due to either theophylline or caffeine, was further elevated by pyruvate. In contrast, FFA release which was slightly stimulated by cAMP [20] or Bt_2cAMP, was not further augmented by pyruvate.

In additon, insulin [40, 41] and PGE_1 [42], suppressors of the accumulation of cAMP due to catecholamine in fat cells, also inhibited lipolysis induced by T_3, with or without pyruvate.

Effect of nucleotides. Since Dole [43] reported inhibitory effects of nucleic acid metabolites on epinephrine-induced lipolysis, effects of nucleotides and their related compounds on FFA release induced by T_3, with or without pyruvate, were examined. None of the compounds tested affected basal and T_3-induced lipolysis, and only AMP and adenosine showed potent inhibitory effects on FFA release induced by T_3 plus pyruvate. Inhibition by AMP was exhibited immediately after its addition to the reaction mixture at any time during lipolysis induced by T_3 plus pyruvate, and FFA release was completely suppressed for at least 40 min (Fig. 7).

A significant inhibition (50 per cent) of lipolysis induced by T_3 plus pyruvate was observed at a concentration of AMP as low as 0.005 mM, and a near maximal inhibition was seen with 0.05 mM AMP (Fig. 8A). Furthermore, inhibition by AMP was rather specific for the action of T_3 plus pyruvate. Although AMP at a higher concentration (1 mM) slightly inhibited theophylline-induced lypolysis, it did not affect FFA release stimulated by epinephrine or Bt_2cAMP.

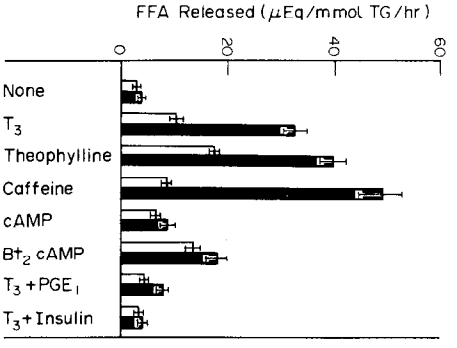

Fig. 6. Effect of pyruvate on lipolysis induced by methylxanthines, cAMP and Bt_2cAMP, and effect of PGE_1 and insulin on T_3 plus pyruvate-induced lipolysis. Fat cells from about 60 mg of fat pads of rats fasted for 48 hr were incubated for 60 min at 37° in 1 ml of an incubation mixture containing each reagent, with (closed bars) or without (open bars) 5 mM pyruvate. Concentrations of the reagents used were: L-T_3, 0.1 mM; theophylline, 1 mM; caffeine, 1 mM; cAMP, 5 mM; Bt_2cAMP, 0.2 mM; PGE_1, 5 μg/ml; and insulin, 0.2 mU/ml. Each point represents the mean of three experiments.

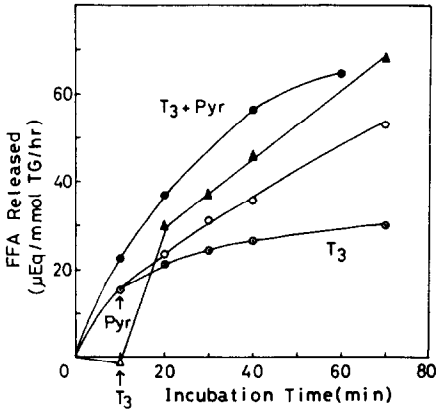

Fig. 5. Time course of effect of L-T_3 and pyruvate on lipolysis. Fat cells from about 60 mg of fat pads of rats fasted for 48 hr were incubated for 60 min at 37° in 1 ml of an incubation mixture containing 5 mM pyruvate (△), 0.1 mM L-T_3 (○), or both (●). After 10 min of pre-incubation, pyruvate (5 mM) was added to the T_3-tube (○), and L-T_3 (0.1 mM) to the pyruvate tube (▲). Each point represents the mean of three incubations.

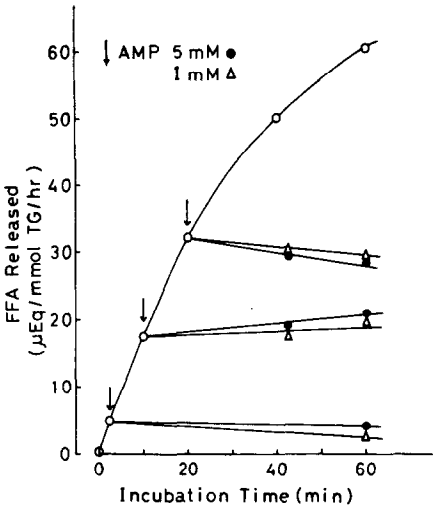

Fig. 7. AMP inhibition of lipolysis induced by T_3 plus pyruvate. Fat cells (60 mg of fat pads) of rats fasted for 48 hr were incubated in 1 ml of an incubation mixutre containing 0.1 mM L-T_3 and 5 mM pyruvate as described in the text. At the time indicated (↓), 1 mM (△) or 5 mM (●) AMP was added and incubation was continued. Each point represents the mean of four samples.

In contrast, inhibition by adenosine occurred at higher concentrations than that of AMP. Furthermore, adenosine at 1 mM significantly inhibited lipolysis induced by epinephrine or Bt_2cAMP (Fig. 8B).

Effect of T_3 and pyruvate on ATP content and cAMP formation in fat cells. Food deprivation for 2 days resulted in a marked reduction of ATP content in fat cells of rats (Table 3). The addition *in vitro* of L-T_3 further reduced ATP content in cells from fasted rats, but not in those from fed rats. The addition of pyruvate partially restored ATP levels which had been reduced by T_3.

As shown in Fig. 9, on exposure to L-T_3, the formation of [^{14}C]cAMP in fat cells, previously labeled with [8-^{14}C]adenine, was elevated 2.5-fold over the basal level within 20 min, and plateaued thereafter. The formation of cAMP was rapidly and markedly augmented by the addition of pyruvate, resulting in a 9-fold increase within 20 min, and declined slowly thereafter, while lipolysis was stimulated and proceeded linearly for at least 60 min (Fig. 5). Pyruvate in the presence of theophylline did not affect the formation of [^{14}C]cAMP, although theophylline stimulated lipolysis (Fig. 6).

The accumulation of [^{14}C]cAMP in labeled cells stimulated by T_3 plus pyruvate coincided well with the increase in the total content of cAMP (Fig. 10). AMP or adenosine, which inhibited lipolysis induced by T_3 plus pyruvate, also markedly reduced cAMP content which had been elevated by these agents.

Effect of Sepharose-bound L-T_3 on lipolysis. As shown in Table 4, both Sepharose 4B-bound and succinylaminoethyl Sepharose 4B-bound L-T_3 were as effective as L-T_3 in inducing lipolysis in fat cells, but their effects were not augmented by the addition of pyruvate. Sepharose itself did not affect FFA release induced by L-T_3 with or without pyruvate.

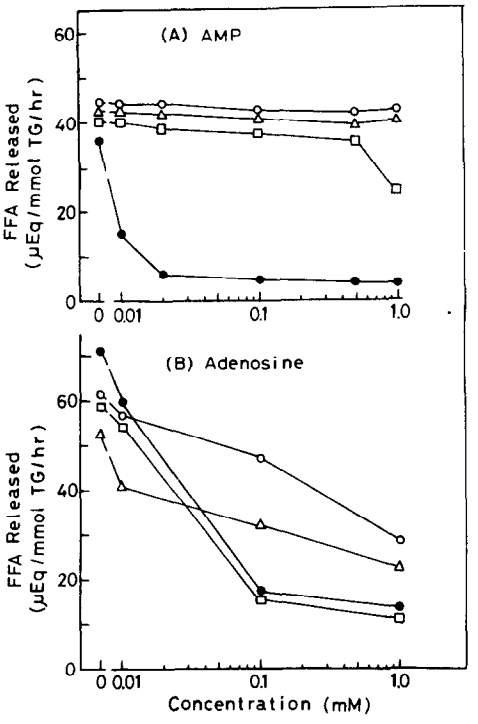

Fig. 8. Effect of AMP (A) and adenosine (B) on lipolysis induced by epinephrine, theophylline, Bt_2cAMP, and T_3 plus pyruvate. Fat cells (45 mg of fat pads of rats fasted for 48 hr) were incubated for 60 min at 37° in 1 ml of a mixture containing 1 μM epinephrine (○), 5 mM theophylline (□), 0.2 mM Bt_2cAMP (△), or 0.1 mM L-T_3 plus 5 mM pyruvate (●), in the presence of increasing concentrations of AMP (A) or adenosine (B). Values are the mean of four samples.

Table 3. Effect of T_3 and pyruvate on ATP content of fat cells*

Addition	ATP content (nmoles/g dried fat cells)	
	Fed rats	Fasted rats
None	63.5 ± 2.9	20.3 ± 1.1
Pyruvate (5 μM)	70.9 ± 3.5	23.9 ± 1.2
L-T_3 (0.1 mM)	64.2 ± 3.9	10.1 ± 0.5
L-T_3 (5 mM) + pyruvate (0.1 mM)	71.2 ± 3.7	15.6 ± 1.2

* Fat cells were incubated for 60 min at 37° in 1 ml of an incubation mixture, and ATP content was determined as described in the text. Aliquots of fat cell suspension were dried to constant weight. Values are the mean ± S.E. of six incubations.

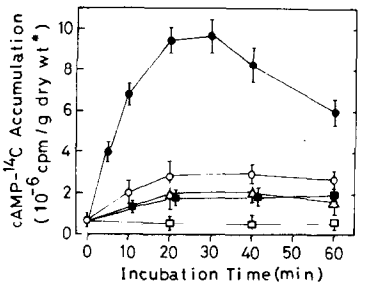

Fig. 9. Effect of T_3 plus pyruvate on cAMP accumulation in fat cells. Fat cells (about 250 mg of fat pads of rats fasted for 48 hr) prelabeled with [8-^{14}C]adenine were incubated at 37° for up to 60 min in 1 ml of the basal medium (□), containing 5 mM theophylline (■), 5 mM pyruvate (△), 0.1 mM L-T_3 (○), or both pyruvate and L-T_3 (●). Values are the mean ± S. E. of three experiments. The asterisk (*) indicates that aliquots of fat cell suspensions were dried to constant weight.

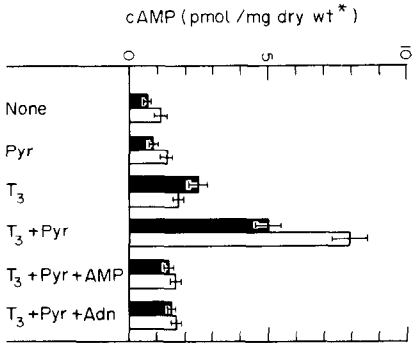

Fig. 10. Effect of T_3 and/or pyruvate on cAMP content in fat cells in the presence or absence of AMP or adenosine. Fat cells [150 mg of fat pads of fed rats (open bars) or of rats fasted for 48 hr (closed bars)] were incubated at 37° for 20 min in 0.25 ml of a medium containing 0.1 mM L-T_3 and/or 5 mM pyruvate (Pyr), with or without 0.8 mM AMP and with or without 0.8 mM adenosine (Adn). The total content of cAMP (cellular and in the medium) was determined as described in the text. Values are the mean ± S. E. of three experiments. The asterisk (*) indicates that aliquots of fat cell suspensions were dried to constant weight.

DISCUSSION

The present study shows that pyruvate synergistically and fairly specifically augmented lipolysis induced by L-T_3 in isolated adipocytes. As in the case of the effect of L-T_3 on epinephrine-induced lipolysis [9], this pyruvate effect on T_3-induced lipolysis was more clearly demonstrated in fat cells prepared from fasted rats than in those from fed or fasted–refed animals. Since the pyruvate effect was greately suppressed by insulin, the decreased availability of insulin to adipose tissue by fasting [44, 45] might be favorable to their synergistic effect.

Reshef *et al.* [46] reported that the enhancement of FFA esterification induced by 25 mM pyruvate in adipose tissue from fasted rats was accompanied with the elevation of glyceroneogenesis and phosphoenolpyruvate carboxykinase activity, and that no stimulatory effect was observed with 0.25 mM pyruvate. Since the stimulation of T_3-induced lipolysis was detectable with 0.01 mM pyruvate, and also since the ratios of FFA/glycerol released in T_3-induced lipolysis were not significantly altered by the addition of pyruvate, the possibility exists that pyruvate functions other than as the donor of α-glycerophosphate for the esterification of FFA [13–17] as postulated for glucose.

Pyruvate not only enhanced the lipolytic response of fat cells to L-T_3, but also elevated T_3-induced formation of cAMP in fat cells. These pyruvate effects were abolished by PGE_1 and insulin, inhibitors of cAMP formation by various lipolytic stimuli [21, 22, 40–42], or by AMP and adenosine, suppressors of lipolysis [47–49] and adenyl cyclase activation in adipocytes [50]. In this respect, pyruvate may function as an effector for adenyl cyclase in fat cells as in the case of the specific activation of the enzyme from *Brevibacterium liquefacience* by pyruvate [51]. However, this remains to be determined, since pyruvate itself showed no effect on either cAMP accumulation or FFA release induced by other lipolytic hormones. If L-T_3 and pyruvate act directly on the adenyl cyclase which is presumably present on cellular membranes, it is still inexplicable why pyruvate did not augment lipolysis induced by Sepharose-bound L-T_3.

In contrast to the requirement of a high concentration (0.1 mM) of L-T_3 for a direct inhibition of cAMP phosphodiesterase [9, 11, 48], FFA release was

Table 4. Effect of Sepharose-bound L-T_3 on lipolysis*

Addition	FFA released (μEq/m-mole TG/hr)
None	3.2 ± 0.2
Pyruvate (5 mM)	2.2 ± 0.1
L-T_3 (0.1 mM)	18.5 ± 0.4
+ pyruvate (5 mM)	49.3 ± 1.1
Sepharose 4B-bound L-T_3 (0.1 mM)	14.9 ± 0.3
+ pyruvate (5 mM)	12.1 ± 0.3
Succinylaminoethyl Sepharose 4B-bound L-T_3 (0.1 mM)	13.2 ± 0.4
+ pyruvate (5 mM)	14.7 ± 0.3

* Fat cells (about 57 mg of fat pads) were incubated for 60 min at 37° in 1 ml of KRP containing 0.1 mM L-T_3, Sepharose 4B-bound L-T_3 or succinylaminoethyl Sepharose 4B-bound L-T_3 and/or 5 mM pyruvate. Values are the mean ± S. E. of three experiments.

clearly elevated by 0.001 mM L-T_3 in the presence of pyruvate. However, it is not clear yet whether pyruvate is involved in the modulation of this enzyme by thyroid hormones, especially a low K_m particulate phosphodiesterase in fat cells, the activity of which was reported to be elevated in hypothyroidism [11, 48, 49].

In summary, the present studies have indicated that the addition of pyruvate results in an increased lipolytic response of fat cells to L-T_3; pyruvate appears to augment T_3-induced formation of cAMP. The physiological significance of the synergistic effect of pyruvate on T_3-induced lipolysis in fat mobilization *in vivo* or in hyperlipemia associated with hyperthyroidism [52] and high carbohydrate intake [53] in human subjects remains to be determined.

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