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Regulation of fat mobilization in adipose tissue of dairy cows in the period around parturition

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Summary

Fat mobilization was studied in biopsy samples of subcutaneous adipose tissue obtained from dairy cows in late pregnancy and in early lactation.

During the whole period around parturition noradrenalin stimulates the release of free fatty acids (FFA) and glycerol from adipose tissue by increasing the rate of lipolysis.

The rates of release of FFA and glycerol from adipose tissue samples of cows in early lactation are higher than those from tissue samples of the same animals before parturition. The increased fat mobilization after parturition is caused both by an increased rate of lipolysis and by an almost complete disappearance of the re-esterification of fatty acids in the adipose tissue. The increased release of FFA from the adipose tissue after parturition is paralleled by an increased concentration of FFA in the blood.

Glucose normally inhibits fat mobilization by stimulating the re-esterification of fatty acids, but this effect is not observed in the first weeks after parturition. Addition of insulin does not influence the effects of glucose on fat mobilization.

The over-all regulation of fat mobilization in the adipose tissue of dairy cows in the period around parturition is discussed.

Introduction

The concentration of long-chain free (non-esterified) fatty acids (FFA) in the blood of dairy cows is increased during the first weeks after calving, whereas the glucose concentration is decreased as compared with the normal level before parturition (Radloff et al., 1966; Schultz, 1968; Mulder, 1971a, 1971b). The turnover time of

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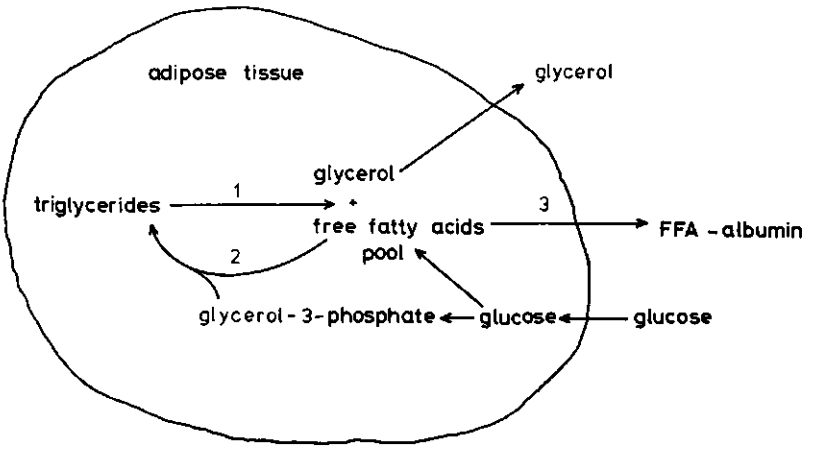

Fig. 1. Lipolysis (reaction 1), re-esterification (reaction 2) and FFA-release (reaction 3) in adipose tissue in relation to the presence of glucose and albumin.

the FFA in the blood remains almost unchanged (Jackson et al., 1968), indicating that the rate of release of fatty acids into the blood stream as well as the rate of uptake of fatty acids from the blood stream must be increased.

The FFA in the blood originate from the adipose tissue in which the fat reserves of the animal are stored as triglycerides (triacylglycerols). These triglycerides are hydrolysed by a hormone-sensitive lipase to glycerol and FFA in a process called lipolysis (Fig. 1, reaction 1). Since the enzyme glycerol kinase is absent from bovine adipose tissue (Metz, 1973), the glycerol cannot be metabolized and must be released into the circulation. Therefore, glycerol release from the adipose tissue is a true measure of lipolysis. The small pool of tissue-associated free fatty acids (TAFA) is either re-esterified to form triglycerides (Fig. 1, reaction 2) or is released into the blood, where it is bound to serum albumin (Fig. 1, reaction 3). The release of FFA from the adipose tissue is called fat mobilization. The term 'fatty acid production' is used to indicate the difference between lipolysis and re-esterification; it equals the sum of the FFA release and the increase of the TAFA pool.

From the foregoing it may be concluded that the rate of release of FFA from the adipose tissue of dairy cows is increased in the first weeks after calving. It was the purpose of the present investigation to find out whether alterations in lipolysis or in re-esterification are responsible for this increased mobilization of fat. The results presented in this paper show that the increased fat mobilization after parturition is caused both by an increased rate of lipolysis and by an almost complete absence of re-esterification in the adipose tissue during that period. It was found that glucose, which normally inhibits fat mobilization by stimulating the re-esterification process, has no such effect in adipose tissue from cows in early lactation.

Methods and materials

Animals and management
Our studies were performed with dairy cows of the Dutch-Friesian and MRY breeds in late pregnancy or early lactation. The animals were fed silage or hay and 6-8 kg of concentrates daily, so that the need for protein was satisfied both before and after parturition whereas the supply of energy was abundant before parturition and provided for 70-90 % of the need thereafter.

Technique of sampling of blood and adipose tissue
Samples of blood and subcutaneous adipose tissue were taken from the cows at regular intervals from about a month before until a month after parturition. Blood from the vena jugularis was collected in cooled glass tubes 1-2 h after the morning feeding of the animals. The samples were kept at 0 °C and rapidly transported to the laboratory. Heparin plasma was prepared immediately and stored at —18 °C until further analysis.

Adipose tissue samples were taken from the flank region (fossa paralumbalis), 2-4 h after feeding. Using the method of paravertebral anaesthesia, it was possible to anaesthetize the flank region without directly contaminating the adipose tissue with the anaesthetic (prilocain). An incision of 10-15 cm length was made in the skin and 15-25 g of adipose tissue were removed. The wound was sutured afterwards.

Handling of the adipose tissue in vitro
Immediately after removal from the animal the tissue samples were put in a physiological saline solution of 37 °C and rapidly transported to the laboratory. There they were freed as much as possible from vascular and connective tissue, cut into pieces of 10-30 mg and preincubated for 15 min in a Krebs-Ringer bicarbonate buffer (pH 7.35) with 5 % bovine serum albumin. For the experimental incubations the tissue pieces were filtered, blotted, and added in portions of 300-500 mg to polythene vials with screw caps containing 10 ml of fresh buffer of the same composition as that used for the preincubation. All incubations were carried out in a shaking water bath (100 oscillations/min) at 37 °C. After a 25-min incubation, a sample of the medium was taken for zero-time analyses and, if necessary, hormones and glucose were added as aqueous solutions. The incubations were stopped 120 minutes later by putting the vials in ice. The tussue pieces were filtered from the media and the latter were stored at —18 °C until further analysis. In some experiments, the tissue pieces were thoroughly rinsed with a physiological saline solution, blotted, and homogenized in 10 ml of Dole's extraction mixture (Dole, 1956) in order to determine the amount of TAFA. The procedures for determining the amount of TAFA have been described previously (Metz et al., 1973).

Quantitative determination of FFA in blood samples and in the incubation media was carried out by the titration method of Dole (1956). Glycerol was measured according to Eggstein & Kreutz (1966) with a test set of Boehringer, Mannheim.

Chemicals
Bovine serum albumin (Pentex, Fraction V, fatty acid-poor) was obtained from Fluka; crystalline bovine insulin and glucose were from BDH Chemicals and noradrenalin was from Sigma.

Results

Effect of noradrenalin on lipolysis in bovine and in rat adipose tissue
Lipolysis in biopsy samples of bovine subcutaneous adipose tissue is greatly stimulated by noradrenalin at all stages of pregnancy and lactation of the donor animal. The shape of the dose-effect curve is similar to that observed with rat adipose tissue (Fig. 2), although lipolysis in the latter tissue tends to be more sensitive to noradrenalin. In the bovine tissue a maximal stimulation is brought about by approx. 50 μM noradrenalin.

Fat mobilization in the period around parturition
The release of FFA and glycerol from adipose tissue samples of cows in late pregnancy and in early lactation is shown in Fig. 3 and 4. The samples were taken from

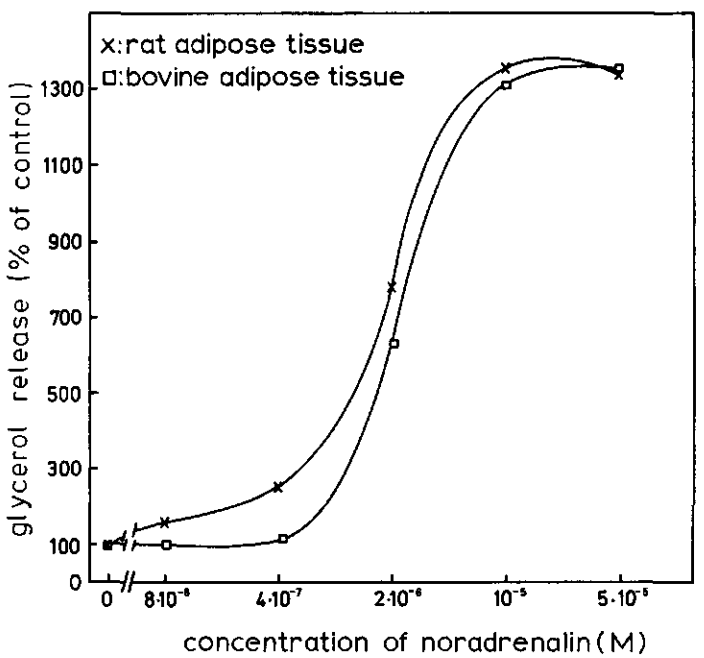

Fig. 2. The effect of noradrenalin on lipolysis in bovine subcutaneous adipose tissue (□) and in the epididymal fat pad of the rat (×). The adipose tissue was taken from a cow in late pregnancy (17 days before parturition). Fat pads were removed from three adult male rats and treated exactly like the bovine adipose tissue samples. Each point represents the average of triplicate incubations.

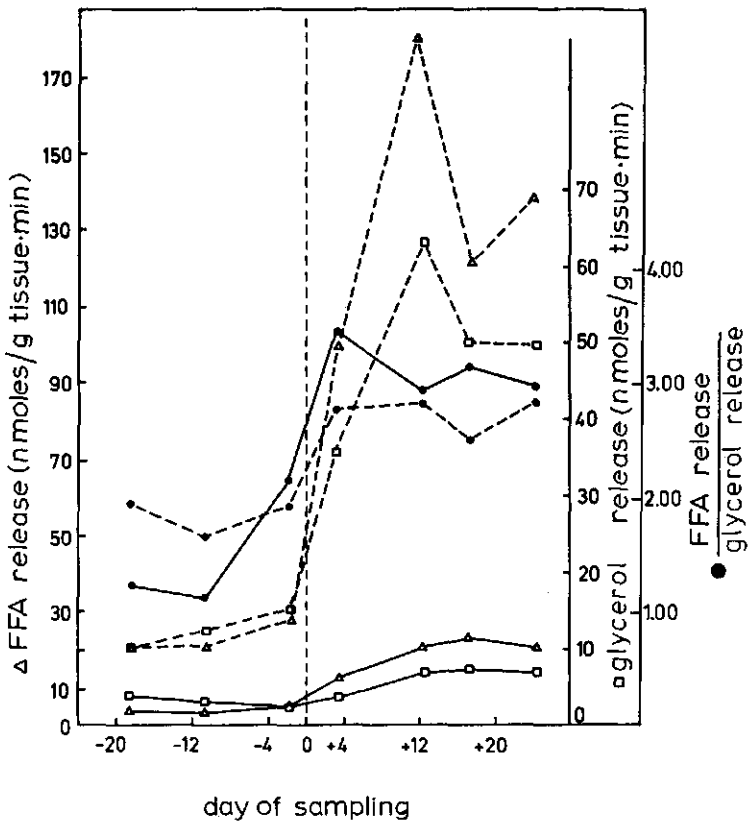

Fig. 3. The release of FFA (△) and glycerol (□) and the ratio between FFA and glycerol release (●) from adipose tissue of a cow in the period around parturition. Each point represents the average of quadruplicate incubations. ——— = without noradrenalin; - - - - = with noradrenalin ($2.5 \times 10^{-5} M$).

eight cows; on each cow 3-7 biopsies were performed. Fig. 3 shows the data of one animal; in Fig. 4 the results obtained with all animals are presented.

The rate of release of FFA and glycerol from the adipose tissue is much higher after parturition of the donor animal than before it. This is true for the basal processes (in the absence of noradrenalin) as well as for the noradrenalin stimulated release of FFA and glycerol.

The stimulatory effect of noradrenalin is increased after parturition. Moreover, after parturition the release of FFA is increased more than that of glycerol, resulting in an increased ratio of FFA release over glycerol release. In the absence of noradrenalin this ratio increases from about 1 before parturition to above 3 thereafter. In the presence of noradrenalin the ratio is about 2 before parturition and it increases to about 2.8 after parturition.

The increased FFA release from adipose tissue samples in vitro, observed after

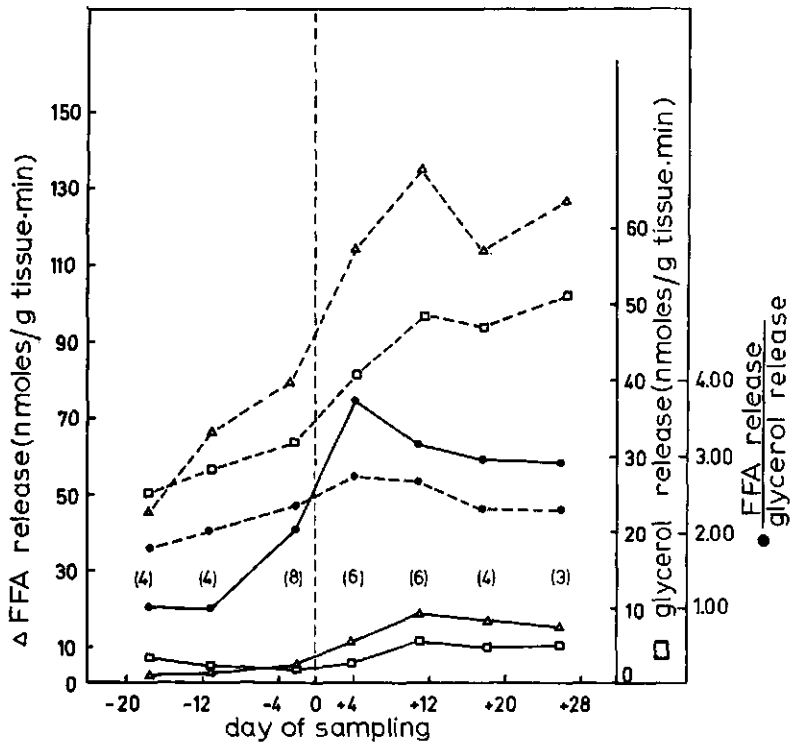

Fig. 4. The release of FFA (△) and glycerol (□) and the ratio between FFA and glycerol release (●) from adipose tissue of 8 cows in the period around parturition. From each cow tissue samples were taken both before and after parturition. The experimental period is divided in 7 phases: 28-15, 14-8 and 7-1 days before parturition and 1-7, 8-14, 15-21 and 22-28 days after parturition. The results of all experiments within each phase were combined and the averages are shown in the figure. The number of experiments within each phase is given within parentheses in the figure. Each experiment included triplicate or quadruplicate incubations. ——— = without noradrenalin; - - - - = with 2.5×10^{-5} *M* noradrenalin.

parturition, coincides with a large increase in the concentration of FFA in the blood plasma of the donor animals in vivo (Fig. 5).

Effects of glucose on fat mobilization

Concentrations of glucose which normally occur in plasma (3.5 m*M*) inhibit the basal release of FFA from adipose tissue in vitro. This inhibitory effect of glucose is not observed, however, with adipose tissue samples obtained from cows in early lactation. As shown in Table 1, the effects of glucose on the production of FFA are almost identical to those on the release of FFA.

In the presence of noradrenalin (Table 2) the situation is more complicated. Again, no inhibition of FFA production by glucose is observed in early lactation.

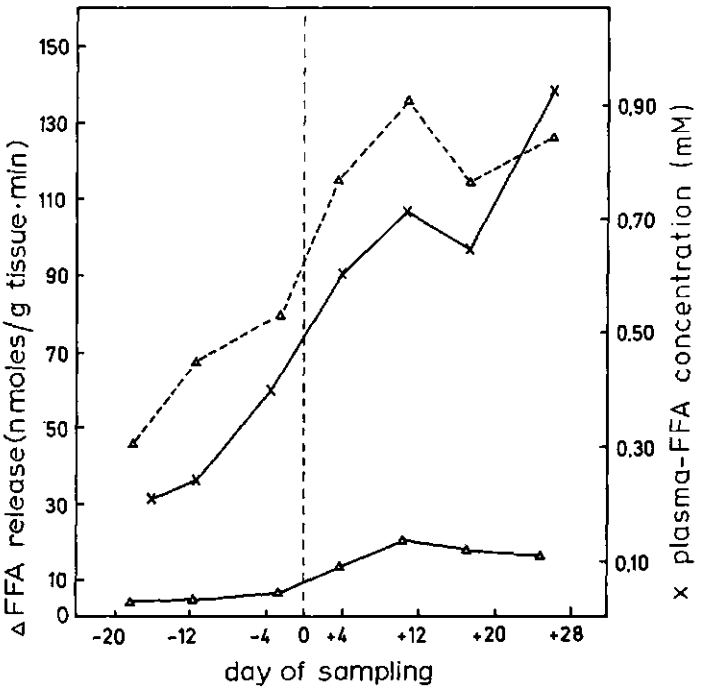

Fig. 5. The release of FFA from adipose tissue in vitro (△) and the FFA concentration in the blood plasma in vivo (×) of 8 cows in the period around parturition. Samples of blood and adipose tissue were taken on the same day from the same animal. The data on FFA release are taken from Fig. 4 (——— = without noradrenalin; - - - - = with 2.5×10^{-5} *M* noradrenalin).

On the other hand, if the donor animal is not in early lactation, glucose may either inhibit or stimulate the production of FFA.

Addition of insulin to the medium did not affect the production and the release of FFA. This lack of an effect of insulin was found both in the absence and in the presence of glucose and/or optimal concentrations of noradrenalin (Tables 1 and 2).

The basal and the noradrenalin-stimulated rates of lipolysis, as measured by the release of glycerol, are not significantly influenced by the addition of glucose (not shown).

Discussion

Stimulation of lipolysis by noradrenalin

Lipolysis in bovine adipose tissue samples is stimulated manifold by noradrenalin (Fig. 2). This effect has previously been observed in adipose tissue from men, rats, dogs and other mammalian species, but it is absent in adipose tissue from rabbits (Rudman et al., 1963). Adrenalin, which also interacts with adrenergic receptors

Table 1. Effects of glucose (3.5 m*M*) and insulin (1 mU/ml) on the release and the production of FFA in the absence of noradrenalin. Each figure represents the average of quadruplicate incubations.

Cow	Day[1]	FFA release (nmoles per g tissue per min)				FFA production (nmoles per g tissue per min)			
		no insulin		with insulin		no insulin		with insulin	
		control	with glucose	control	with glucose	control	with glucose	control	with glucose
A	+351	1.3	— 2.6**			— 2.8	— 7.0**		
B	— 9	6.4	2.0**						
	— 2	4.4	2.7*						
	+ 3	10.4	9.6						
	+ 11	30.2	32.7			26.8	28.1		
C	+ 6	12.3	13.7	10.4	12.3	7.4	9.9	6.1	8.2
D	+ 27	12.8	14.2	10.7	11.5	11.6	13.4	10.2	11.2
	+119	10.7	6.4**	12.2	8.4**	10.7	6.0**	12.2	8.0**

[1] Day of sampling with respect to day of parturition.

* $P \leqslant 0.05$ } significance of glucose effect according to Student's t-test.
** $P \leqslant 0.01$ }

Table 2. Effects of glucose (3.5 m*M*) and insulin (1 mU/ml) on the release and on the production of FFA in de presence of 2.5×10^{-5} *M* noradrenalin. Each figure represents the average of quadruplicate incubations.

Cow	Day[1]	FFA release (nmoles per g tissue per min)				FFA production (nmoles per g tissue per min)			
		no insulin		with insulin		no insulin		with insulin	
		control	with glucose	control	with glucose	control	with glucose	control	with glucose
A	+351	121.5	107.0			137.4	118.1		
B	— 9	92.5	105.5						
	— 2	60.3	56.9						
	+ 3	78.7	93.7						
	+ 11	124.3	128.7			135.4	139.8		
E	— 16	87.8	82.0	102.9	93.3	99.1	92.8	114.8	99.1
	— 4	45.7	41.1	40.0	38.0	57.3	49.3	48.0	44.2
	+ 18	111.2	116.5	111.0	113.6	124.7	127.4	123.4	124.2

[1] Day of sampling with respect to day of parturition.

in the fat cell membrane, has also been found to stimulate lipolysis in bovine adipose tissue (Thornton et al., 1972; Yang & Baldwin, 1973).

The stimulation of lipolysis by catecholamines in rat adipose tissue has been shown to be mediated by cyclic adenosine-3', 5'-monophosphate (cAMP) according to the second messenger concept developed by Sutherland et al. (1965). The rate-limiting enzyme of lipolysis is activated with increasing intracellular levels of cAMP, which is formed from ATP by the action of adenylate cyclase, located in the cell membrane. Catecholamines stimulate the activity of adenylate cyclase. On the other hand, cAMP is continously broken down by phosphodiesterase.

The following arguments lead us to believe that the same mechanism is operating in bovine fat cells:

1. The shape of the dose-effect curves is very similar in bovine and rat adipose tissue;
2. The dibutyryl derivative of cAMP stimulates lipolysis in bovine adipose tissue in vitro (Metz, 1973);
3. Theophylline, an inhibitor of phosphodiesterase, was also found to stimulate lipolysis in adipose tissue of the cow (Metz, 1973).

Lipolysis, FFA release and re-esterification in the period around parturition

Lipolysis in bovine adipose tissue samples is largely increased after parturition of the donor animal (Fig. 3 and 4). Since lipolysis is measured as the release of glycerol per gram fat, part of the observed increase is undoubtedly a consequence of the increased number of fat cells per gram adipose tissue, due to the reduced fat reserves in a period of negative energy balance. However, the increase in lipolysis is so big that for the largest part it must be caused by an increased lipolytic activity per fat cell.

The mechanism underlying this increased lipolytic activity is not established. Brodie et al. (1969) observed that in fasting rats the increase in lipolysis was accompanied by an increased amount of adenylate cyclase in the adipose tissue. Our results, obtained with adipose tissue in vitro, clearly demonstrate that the increased lipolytic activity after parturition is an intrinsic property of the adipose tissue and is not produced by short-term exogenous factors like altered concentrations of circulating lipolytic hormones.

The increased fat mobilization (Fig. 1, reaction 3) after parturition does not only result from an increased lipolysis (Fig. 1, reaction 1) but also from a diminished re-esterification (Fig. 1, reaction 2). This may be concluded from the increased ratio between FFA release and glycerol release in early lactation (Fig. 3 and 4). This ratio gives an indication of the relative rates of lipolysis and re-esterification. In the absence of re-esterification, FFA and glycerol are produced in a ratio of 3 : 1. With increasing rates of re-esterification the ratio will be lowered.

In order to calculate the rate of re-esterification, it is not enough to measure the rates of release of FFA and glycerol. Changes in the pool size of TAFA should also be known. In a number of experiments (see e.g. Tables 1 and 2) we have, therefore, determined the amount of TAFA before and after the incubation. From the results of these experiments it can be calculated that in adipose tissue samples obtained

before parturition, in the absence of noradrenalin on the average more than two molecules of FFA re-esterify per mole of triglyceride hydrolysed, whereas after parturition all the reaction products of lipolysis are released from the fat cell, i.e. after parturition no re-esterification of fatty acids occurs.

Effects of noradrenalin before and after parturition

Before parturition, addition of noradrenalin results in an increased ratio of FFA release and glycerol release; after parturition this ratio is decreased by noradrenalin (Fig. 4). The former effect can be explained by the observations that lipolysis is stimulated by noradrenalin, whereas the rate of re-esterification is not or only slightly affected. The effect of noradrenalin after parturition is more difficult to understand, since both in the absence and in the presence of noradrenalin the rate of re-esterification is negligible. Changes in the ratio of FFA release and glycerol release must, therefore, be brought about by changes in the pool size of TAFA. Such changes could indeed be observed. In the absence of noradrenalin, TAFA always decreased during incubation; in 19 experiments the average decrease of TAFA represented 35 % of the released FFA. In incubations with noradrenalin, TAFA always increased; in 18 experiments the average increase of TAFA amounted to 14 % of the released FFA.

The effect of glucose on fat mobilization

Except during early lactation, glucose inhibits fat mobilization in vitro. This inhibitory effect of glucose fully agrees with the reciprocal relationship between the concentrations of FFA and glucose in the blood which has been observed in clinically normal and ketotic cows both before and after parturition (Adler & Wertheimer, 1962; Mulder, 1971a, 1971b; Schultz, 1968; Schwalm & Schultz, 1976). It is generally accepted that glucose inhibits fat mobilization in vivo and in vitro by stimulating the re-esterification process through the formation of glycerol-3-phosphate (Fig. 1) (Patterson, 1964; Bartos et al., 1968). Triglyceride synthesis in bovine adipose tissue occurs mainly via the glycerol-3-phosphate pathway (Benson & Emery, 1971).

During the first weeks of lactation the inhibitory effect of glucose on fat mobilization is not observed (Table 1). This is in full agreement with the complete disappearance of the re-esterifying activity of the adipose tissue, observed during that period.

If ketotic cows in early lactation are given an intravenous dose of glucose, the FFA concentration in the blood is lowered (Kronfeld, 1965; Erfle et al., 1971). The seeming discrepancy between this effect of glucose in vivo and our results in vitro can be explained if we assume that the effect of glucose, observed in vivo, is not a direct effect on fat mobilization in the adipose tissue. Undoubtedly, many factors are involved in the regulation of the FFA concentration in the plasma and one or more of these factors may be affected by the glucose level in the plasma.

Effect of insulin on fat mobilization

From the results presented in Tables 1 and 2 it is clear that, at least in our experi-

REGULATION OF FAT MOBILIZATION IN COW'S ADIPOSE TISSUE

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