

## EFFECTS OF VOLATILE FATTY ACIDS, KETONE BODIES, GLUCOSE, AND INSULIN ON LIPOLYSIS IN BOVINE ADIPOSE TISSUE

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### 1. Introduction

Our interest in the aetiology of ketosis in cattle recently led us to investigate possible metabolic control mechanisms of fat mobilization in bovine adipose tissue. Acetic, propionic and butyric acid are the major sources of metabolic energy made available to the adult ruminant by digestion and absorption. No studies have been published on the effects of these volatile fatty acids on the rate of lipolysis in adipose tissue either from monogastric mammals or from ruminants.

It has been reported that in non-ruminants ketone bodies inhibit lipolysis *in vitro* [1–3] as well as *in vivo* [4, 5], but this phenomenon has not been investigated in ruminants. Glucose is reported to inhibit the release of fatty acids from adipose tissue in non-ruminants *in vivo* as well as *in vitro*, probably by stimulating the re-esterification of these fatty acids [6–8]. Similar results have been obtained with goat [9] and sheep [10] *in vivo*. Insulin enhances this inhibitory effect of glucose; moreover, insulin by itself inhibits lipolysis *in vitro*, at least in some animal species [11].

In this paper it will be shown that butyrate and  $\beta$ -hydroxybutyrate inhibit lipolysis in bovine subcutaneous adipose tissue *in vitro*. This inhibition was observed for basal lipolysis as well as for lipolysis stimulated by noradrenalin. Acetate, propionate, acetoacetate, glucose and insulin have no effect on these processes.

### 2. Methods and materials

Biopsies of subcutaneous adipose tissue of dairy cows in late pregnancy or early lactation were taken

from the flank region after paravertebral anaesthesia with prilocaine. Using this method of anaesthesia, contamination of the biopsy sample with the anaesthetic could be avoided.

Immediately after removal from the animal, the adipose tissue sample was put in a physiological saline solution at 37°, transported to the laboratory in a few minutes, freed as much as possible from vascular and connective tissue, cut into pieces of 10–30 mg and kept for 15 min in a Krebs–Ringer bicarbonate buffer pH 7.35 with 5% bovine serum albumin. The tissue was then blotted and portions of 300–500 mg were added to polythene vials containing 10 ml of the same medium. After about 25 min, samples of 5 ml of the medium were withdrawn for zero-time analysis and final additions were made.

All incubations were carried out in a shaking water bath (100 oscillations per min) at 37°. After 120 min the incubations were stopped by putting the vials in ice. The tissue was filtered from the medium and the media were stored at –18° until further analysis.

Non-esterified fatty acids were measured according to Trout's [12] modification of Dole's titration method [13]. Glycerol was determined according to Eggstein and Kreutz [14] with a test set of Boehringer, Mannheim.

Bovine serum albumin (fatty acid poor) was obtained from Fluka; crystalline bovine insulin and ethylacetoacetate were from BDH Chemicals Ltd; noradrenalin (L-arterenol-HCl) and DL- $\beta$ -hydroxybutyrate (sodium salt) were from Sigma Chemical Company. Lithium-acetoacetate was prepared from ethylacetoacetate according to Hall's procedure [15].

### 3. Results

Large differences in basal and in noradrenalin-stimulated lipolytic activity were observed between individual biopsies. Therefore, in the figures and the table the results are given of several experiments. From these results it is clear, however, that the effects of the various additions can be observed at all levels of activity.

The effects of addition to the incubation medium of acetate, propionate, butyrate, acetoacetate and  $\beta$ -hydroxybutyrate on the release of fatty acids and glycerol from adipose tissue samples in the presence of noradrenalin are shown in fig. 1. Whereas acetate, propionate and acetoacetate do not significantly affect lipolysis under these conditions, butyrate and  $\beta$ -hydroxybutyrate strongly inhibit this process. In the presence of these inhibitory metabolites the release of

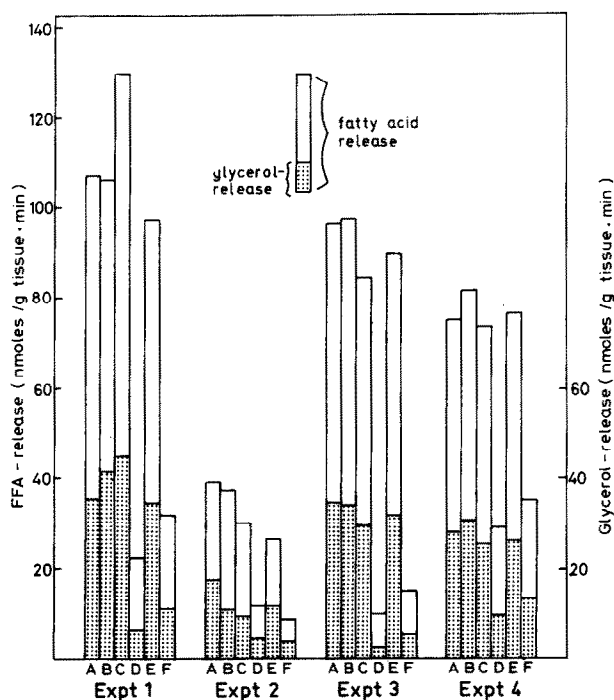


Fig. 1. Effects of volatile fatty acids and of ketone bodies on lipolysis in bovine subcutaneous adipose tissue in the presence of noradrenalin (5  $\mu$ g/ml). A: Control; B: with 10 mM Na-acetate; C: with 10 mM Na-propionate; D: with 10 mM Na-butyrates; E: with 10 mM Li-acetoacetate; and F: with 10 mM Na-DL- $\beta$ -hydroxybutyrate. All data shown were the means of triplicate experiments.

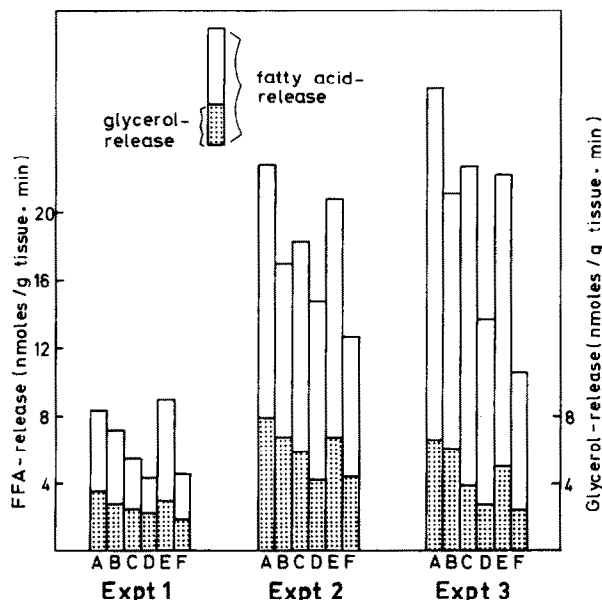


Fig. 2. Effects of volatile fatty acids and of ketone bodies on basal lipolysis in bovine subcutaneous adipose tissue. For explanation see fig. 1.

fatty acids and glycerol is decreased to the same extent.

In fig. 2 the same experiment is shown in the absence of noradrenalin. Again, only butyrate and  $\beta$ -hydroxybutyrate significantly influence the rate of lipolysis.

As shown in table 1, glucose and insulin do not significantly affect fatty acid release from bovine adipose tissue, both in the absence and in the presence of noradrenalin.

### 4. Discussion

The concentration of the volatile fatty acids in bovine blood varies with the physiological state of the animal. In ketosis acetate levels usually rise 100–400% above normal [16]. As shown in figs. 1 and 2, these high levels of acetate do not influence lipolysis in bovine subcutaneous adipose tissue *in vitro*. On the other hand, Abramson and Arky [17] observed a lower level of free fatty acids in human blood after infusion of acetate, and they concluded from this observation that acetate has an antilipolytic effect. It

Table 1  
Effects of glucose and insulin on fatty acid release from bovine subcutaneous adipose tissue.

Experiment	Noradrenalin (5 µg/ml)	Fatty acid release (nmoles/g tissue·min)			
		control	with glucose (3.5 mM)	with insulin (1 mU/ml)	with glucose and insulin
1	+	87.8	82.0	102.9	93.3
2	+	45.7	41.1	40.0	38.0
3	+	111.2	116.5	111.0	113.6
4	-	12.3	13.7	10.4	12.3
5	-	12.8	14.2	10.7	11.5

The values given are means of quadruplicate experiments.

is possible, however, that the lipolytic process in fat depots other than the subcutaneous adipose tissue is sensitive to inhibition by acetate, the main precursor for lipogenesis in the fat cells of ruminants. It has been suggested that propionate, a glucogenic substance, may play an important role in the regulation of lipolysis in ruminants [18]. Our data do not support such a direct effect of propionate on lipolysis.

Butyrate inhibited lipolysis in our experiments. From the fact that the inhibition was observed in the absence as well as in the presence of noradrenalin, it may be concluded that the site of action of butyrate is not, or not only, the adrenergic receptor site of the fat cell.

The same can be concluded for  $\beta$ -hydroxybutyrate which is quantitatively the most important ketone body in dairy cattle in ketosis. Like butyrate, it lowered basal lipolysis by 45–60% and noradrenalin-stimulated lipolysis by 55–85%. On the other hand, acetoacetate had no inhibitory effect in our experiments, which is in contrast to findings with rat epididymal fat pieces [1] and isolated fat cells [2] in which acetoacetate as well as  $\beta$ -hydroxybutyrate inhibited lipolysis.

Concerning the mechanism of inhibition of lipolysis by ketone bodies in adipose tissue, Nakano and Ishii [2] suggest an interaction of ketone bodies either with the action of cyclic AMP or with the hormone-sensitive lipase activating system beyond it. Hotta et al. [3] conclude from their experiments with rat epididymal fat pads that the inhibition of lipolysis by  $\beta$ -hydroxybutyrate occurs through an inhibitory effect on the formation of cyclic AMP. The mechanism of inhibition of lipolysis by  $\beta$ -hydroxybutyrate in our system is momentarily under investigation.

The strong inhibitory effect of  $\beta$ -hydroxybutyrate, at a concentration which is only 2–4 times higher than the plasma concentration in ketotic cows, suggests an important mechanism of feedback control, in which a product derived from excessive breakdown of fatty acids participates in the regulation of fatty acid mobilization.

The lack of an effect of glucose (table 1) on the release of fatty acids from bovine adipose tissue *in vitro* is rather surprising and in disagreement with the generally accepted idea that glucose inhibits fatty acid release by stimulating the re-esterification of free fatty acids in the fat cell. According to this idea, insulin should enhance the effects of glucose on re-esterification and fatty acid release [19] but this effect, too, was lacking in our experiments.

It should be noted, however, that our biopsies were taken from animals in late pregnancy and early lactation which were mobilizing fat at a high rate. The lack of a measurable effect of glucose may indicate that the activity of the enzymes of the re-esterification pathway is very low during a period of rapid fat mobilization. We found strong evidence for such a lowered activity in studies on basal lipolysis (unpublished). Vaughan [6] also observed that in epididymal fat pads of rats the rate of re-esterification decreased with respect to the rate of lipolysis, when the rats were fasted for 42 hr. In human adipose tissue Galton and Wilson [20] found a lowered capacity for re-esterification after fasting and they suggested that this was caused by a lower activity of the enzyme glycerolphosphate acyltransferase (Acyl-CoA: L-glycerol-3-phosphate-*O*-acyltransferase, EC 2.3.1.15).

Insulin affected neither basal nor noradrenalin-stimulated lipolysis in our experiments. The latter observation seems to be in contrast to reports in the literature for rats [21] and for men [22]. However, we used rather high concentrations of noradrenalin (5  $\mu\text{g/ml}$ ); under these conditions the sensitivity to insulin is relatively low. Jungas and Ball [21] observed that lipolysis in rat fat pads became less sensitive to inhibition by insulin at a concentration of 1 mU/ml – the same concentration that we used – when the concentration of adrenalin was increased. Kuo [23] observed the same for noradrenalin with isolated rat fat cells. The latter author also found no effect of insulin on basal lipolysis, as in our experiments. However, Burns and Langley [22] observed a reduction of basal lipolysis by insulin in human fat cells. This effect of insulin could only be demonstrated when the rate of the basal lipolysis was high.

It remains possible, however, that the lack of an effect of insulin is caused by an insensitivity of the tissue, as mentioned for chickens by Langslow and Hales [24].

It is attractive to speculate on a possible relationship between the lack of an effect of glucose and the strong inhibitory effect of butyrate observed in our experiments. It might well be that in ruminants the volatile fatty acids, being the main source of metabolic energy, have taken over the regulatory function of glucose, which is the main metabolic regulator of fatty acid mobilization in adipose tissue of non-ruminants.

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