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Hormonal Influence on Fat Synthesis in Cattle

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Fat synthesis was enhanced by acylation-stimulating protein in cultured bovine tissue. This supports the idea that external fat or marbling could be modified by manipulating this hormone in cattle.

Summary

The ability of adenosine, insulin and human acylation-stimulating protein to modify fat synthesis was determined using cultures of fat tissue from steers. Adenosine did not influence fat synthesis. However, acylation stimulating protein and insulin promoted fat synthesis. These observations, coupled with knowledge of how fat synthesis is regulated in other species, justify investigation of whether cattle synthesize acylation-stimulating protein, and how this synthesis is regulated. An understanding of how acylation-stimulating protein production and action is regulated should expose potential places for intervention to manipulate fat synthesis in cattle.

Introduction

Marbling is a major reason behind feeding grain to cattle. This intramuscular fat improves the eating qualities of beef. A problem associated with feeding cattle to increase marbling, however, is the parallel fat deposition in other parts of the carcass. The synthesis of fat in these other depots consumes significant feed energy, although this fat is of little value to beef customers. Beef producers could increase both efficiency and product quality by using a management tool which shifts fat synthesis to intramuscular depots. Development of this tool will require a better understanding of how fat synthesis is regulated in cattle.

Although the hormones controlling fat synthesis in cattle have not been identified, several hormones have been shown to influence fat synthesis in human and mouse cells. Adenosine, a molecule secreted by adipocytes, can stimulate fat synthesis in mice. Another hormone stimulating fat synthesis in both humans and mice is acylation-stimulating protein (ASP). ASP is also secreted by adipocytes. The objective of these experiments was to determine if adenosine and ASP can stimulate fat synthesis in cattle.

Procedure

Tissue culture of bovine adipose explants

Cultures of fat tissue obtained from steers were used to test effects of adenosine and ASP. This technique provides a rapid (less than six hours) analysis of fat synthesis and does not require injecting animals with radioactive materials. For each experiment, fresh tissue obtained by biopsy was incubated at body temperature in a solution which provided all essential nutrients, was pH buffered and oxygenated. Synthesis of fat was measured by monitoring the incorporation of ^{14}C-labeled substrates into triacylglycerol. In cattle, fat is synthesized mainly from acetate, but also from fatty acids like palmitate or oleate. Both acetate and palmitate were used in these experiments.

Experiment 1: Effect of adenosine

The hypothesis was that adenosine would increase synthesis of fat in cultured tissue. Since adipose tissue contains some adenosine, an enzyme (adenosine deaminase) was used to

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destroy the pre-existing adenosine. This allowed comparison between untreated and treated cultures. Two additional treatments provided a test of added adenosine. Additional cultures were treated with low and high doses of the adenosine analog, PIA (N6-phenylisopropyl adenosine), which resists degradation by adenosine deaminase.

Experiment 2: Effect of adenosine

Experiment 2 was similar to experiment 1 except the dose range for PIA was greater. The treatments were: 1) no hormone; 2) 0.75 U/ml adenosine deaminase; 3) 0.75 U/ml adenosine deaminase plus 10 nM PIA; 4) 0.75 U/ml adenosine deaminase plus 100 nM PIA; and 5) 0.75 U/ml adenosine deaminase plus 1,000 nM PIA.

Experiment 3: Effect of acylation stimulating protein (ASP)

The second hormone suspected to increase synthesis of fat in cattle is ASP. To test the effect of ASP on fat synthesis in cattle, cultures were designed with increasing concentrations of ASP and some cultures with added insulin, a glucose uptake-promoting hormone. It is possible that ASP only increases fat synthesis if insulin is present. On the other hand, an effect of ASP may be more pronounced if insulin is absent. The treatments were: 1) no hormone; 2) 0.5 μM ASP; 3) 5 μM ASP; 4) 10 nM insulin; 5) 0.5 μM ASP plus 10 nM insulin; and 6) 5 μM ASP plus 10 nM insulin.

Experiment 4: Acylation stimulating protein dose curve

In experiment 4, a wider range of ASP doses was evaluated: 1) no hormone; 2) 0.01 μM ASP; 3) 0.1 μM ASP; 4) 1 μM ASP; 5) 5 μM ASP; and 6) 10 μM ASP.

Experiment 5: Acylation stimulating protein dose curve with and without insulin

Treatments were: 1) no hormone; 2) 0.01 μM ASP; 3) 0.1 μM ASP; 4) 1 μM ASP; 5) 5 μM ASP; 6) 10 μM ASP; 7) 1 nM Insulin; 8) 0.01 μM ASP plus 1 nM insulin; 9) 1 μM ASP plus 1 nM Insulin; and 10) 10 μM ASP plus 1 nM insulin.

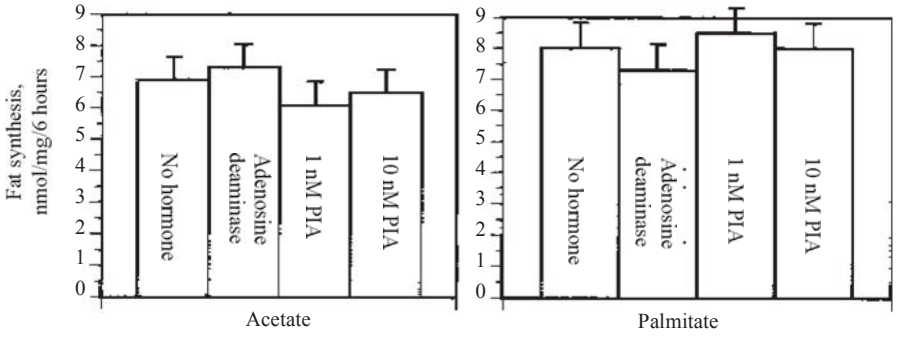

Figure 1. (Experiment 1). Synthesis of fat by cultured bovine adipose tissue as influenced by removal of adenosine (deaminase) and addition of an adenosine analog (PIA). No effect of treatment was detected.

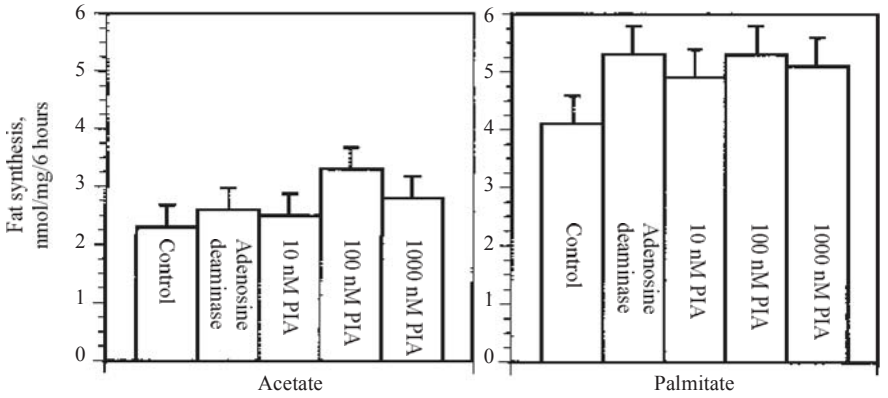

Figure 2. (Experiment 2). Effect of adenosine on synthesis of fat from acetate (left) and palmitate (right). No effect of treatment was detected.

Lipid extraction and quantification of triacylglycerol synthesis.

Following incubation, tissues were extracted in 2 ml of chloroform:methanol (2:1, v/v), and washed with both 1 M KCl-0.15N HCl, and 0.38 M sodium carbonate. Incorporation of radioactivity was determined in a beta counter.

Triacylglycerol was distinguished from total fat by use of thin layer chromatography. Fat synthesis was calculated from radioactivity incorporated and expressed as nmol acetate (or palmitate)/ mg tissue / six hours. Data were analyzed by ANOVA with main effects of animal and treatment. The animal x treatment interaction was used in tests of treatment significance.

Results

Fat synthesis in cattle occurs in two general steps. First, fatty acids are synthesized. Second, fatty acids are linked to glycerol phosphate in a process called esterification. In animals consuming high-fat diets, such as humans or mice, fatty acid synthesis is less than in cattle. Incorporation of ^{14}C-acetate estimates fatty acid synthesis plus esterification while incorporation of ^{14}C-palmitate estimates esterification independent of fatty acid synthesis.

The effect of adenosine on triacylglycerol synthesis in bovine adipose tissue.

Neither fatty acid synthesis nor fatty acid esterification were affected by addition of PIA (an adenosine analog) or adenosine deaminase (an enzyme which

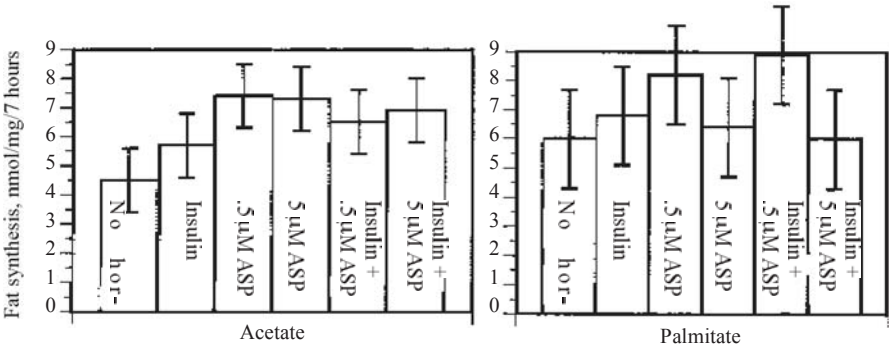

Figure 3. (Experiment 3). Effects of ASP and insulin on fat synthesis from acetate (left) or palmitate (right). The effect of ASP approached significance (P=.11) but because of the high coefficient of variation, this experiment did not provide a strong test.

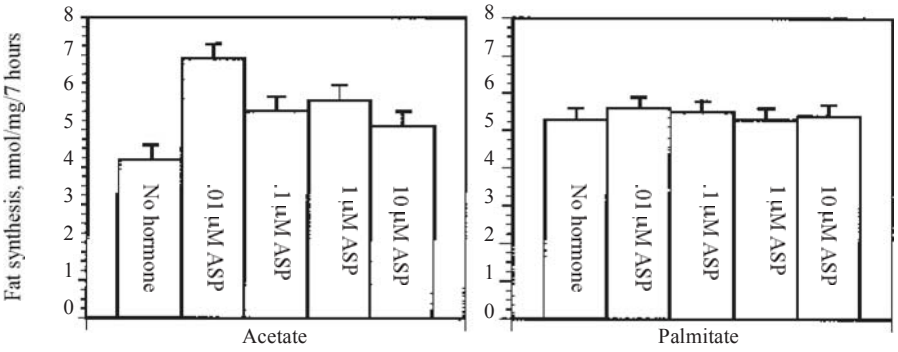

Figure 4. (Experiment 4). Effect of varying concentration of ASP on fat synthesis in absence of insulin. Acetate incorporation (left graph) was increased (P<.05) by ASP but all doses caused similar response. ASP did not influence palmitate incorporation (right graph).

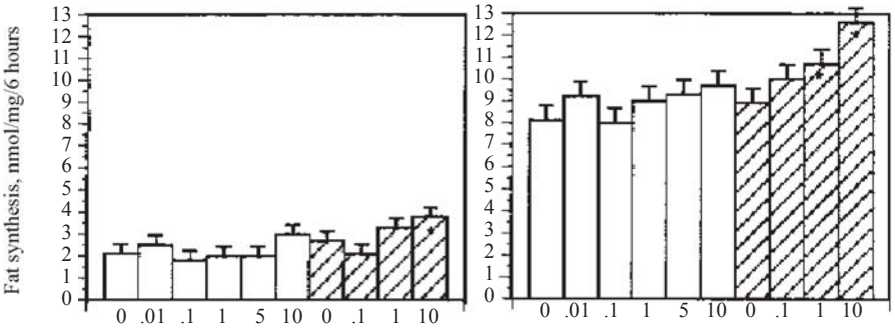

Figure 5. (Experiment 5). Interaction of ASP with insulin in stimulation of fat synthesis from acetate (left) and palmitate (right). Hatched bars represent cultures treated with 10 nM insulin. ASP treatment (μM) is indicated below bars. Incorporation of both acetate and palmitate was influenced by treatment (P<.05) and the significant ASP X Insulin interaction indicates that insulin is required for ASP to stimulate fat synthesis. *P<.10.

destroys adenosine) to cultures of adipose tissue (Figures 1 and 2). Adenosine apparently does not have an effect on fatty acid synthesis or esterification of fatty acids in cattle.

The effect of acylation stimulating protein on triacylglycerol synthesis in bovine adipose tissue.

The second hormone evaluated was ASP. Initially, it appeared ASP stimulates fat synthesis (Figure 3). In this experiment, however, the degree of variation in fat synthesis was large and treatment effects were not significant (P = .11). As shown in Figure 4, ASP did increase the use of acetate for fatty acid and triacylglycerol synthesis. However the synthesis of fat from palmitate (a fatty acid) was not affected by ASP. This indicates ASP may have a greater effect on enzymes of fatty acid synthesis than on esterification enzymes. This would be logical, as most fat synthesis in cattle is from acetate and dietary fat intake is low. Finally, the interaction of insulin and ASP in regard to fat synthesis was evaluated (Figure 5). Fat synthesis was enhanced by increasing doses of ASP only when cultures also contained insulin. Our interpretation is that while both insulin and ASP promote fat synthesis, each hormone influences different parts of the pathway.

In conclusion, human ASP appears capable of promoting fat synthesis in bovine tissue. Our prediction is that bovine ASP is at least as potent. Based on information from a cDNA clone of bovine ASP, we know this molecule differs from human at several amino acid positions. We are developing a method for purification of bovine ASP. In the future we hope to determine whether intramuscular fat cells utilize ASP differently than fat cells in other fat depots. Perhaps this can lead to a method for promoting marbling while reducing total carcass fat.

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