HORMONAL RESPONSE OF LIPOLYSIS IN RUMINANTS OF DIFFERENT BIOLOGICAL TYPES

by

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ABSTRACT

The rate of lipolysis as measured by glycerol release and as influenced by hormonal treatment was compared using subcutaneous adipose tissue from cattle of varying frame size. Cattle were assigned to frame size according to the USDA feeder grade standards. They were fat biopsied at three different times on a finishing diet (166, 194 and 214 days). An isolated fat cell method was utilized to determine rate of lipolysis as measured by glycerol release. Epinephrine, insulin and a combination of both were used as the hormone treatments. Incubation periods were 60 and 120 minutes in length. Rate of lipolysis was compared to the carcass composition of the various frame size cattle.

The epinephrine treatment caused the largest ($P<.05$) release of glycerol regardless of frame size. Insulin was established as an antilipolytic agent because of the observed antagonistic action on epinephrine and its influence on rate of lipolysis.

The rate of lipolysis was greater ($P<.05$) in cattle of the large frame size (LF) as compared to medium (MF) and small frame (SF) cattle. Comparison by time on feed demonstrated that there was minimal effect on lipolysis as time on feed increased.

The chemical carcass composition data revealed that LF cattle had the lowest ($P<.05$) percent lipid with the MF and SF following, respectively. A comparison of days on feed within frame size demonstrated that SF animals deposited lipid at a greater rate than the other two groups.

1. INTRODUCTION

The process of fattening (i.e. deposition of lipid in adipose tissue) is a normal occurrence in growth and development of meat producing animals. The rate at which this process takes place is determined by the net rate of two key metabolic functions. They are: (1) the removal of lipid and lipid precursors from the bloodstream during times of sufficient caloric intake, and (2) the subsequent release of fatty acids and glycerol during periods of caloric deficiency. This latter function is an extremely important metabolic phenomenon for the maintenance of life during periods of energy deprivation and stress. During periods of prolonged fasting or heavy muscular work, up to 50% of the total CO_2 produced by mammalian species is derived by the oxidation of fatty acids (Havel et al., 1963).

The rate of lipid mobilization varies in response to physiological and pathological changes. These changes can be in the form of nutritional regimens or endocrinological stimulus which can affect the interaction between fat and carbohydrate metabolism. Endocrine regulation has received much attention in the nonruminant, and the

mechanisms involved are well elucidated. This is not the case with ruminant animals.

The ruminant possesses an abundance of energy in adipose tissue. Deposition of fat may or may not be desirable depending on the quantity deposited and its location. Intramuscular fat contributes to meat quality while a minimum amount of subcutaneous fat on carcasses reduces moisture evaporation and shrinkage during chilling. However, excessive amounts of subcutaneous fat must be trimmed during processing. Perirenal and visceral fat must be removed at slaughter or trimmed after chilling, and significant amounts of intermuscular fat must be removed at the retail market. It is estimated that four billion pounds of excess fat is produced annually as an unneeded commodity (Allen et al., 1976). An understanding of the utilization of adipose sites may result in the development of methods to allow the animal to utilize this source of energy more efficiently. Theoretically, if the animal could mobilize its vast adipose deposits more efficiently, it would not have to rely as heavily on the diet it receives to meet its total energy requirement. Thus, making it possible to reduce the amount of feed to bring the animal to the desired slaughter condition by an increase in efficiency of gain.

The objectives of the study were: (a) to measure the effects of lipolytic and antilipolytic hormone on animals of varying biological types, and (b) to determine the effect of the time on a finishing diet on lipolytic activity.

2. REVIEW OF THE LITERATURE

Adipose tissue is a very dynamic and active tissue which comprises a large portion of the ruminant's body. From values given in body composition analysis and caloric values, one can calculate that a ruminant at market weight has up to 80% of its total body calories in the form of fat (Reid et al., 1955; Bauman and Davis, 1974). This indicates that there is a tremendous amount of stored energy available to the animal; however, it is of little value to the livestock retailer. There is very little known about the control of lipid metabolism and the regulation of lipolysis in the ruminant.

In lipolysis, the effect on the adipose cell is a hydrolysis of triglycerides to form free fatty acids (FFA) and glycerol. As the concentration of these lipid precursors increases in the adipose cells, they begin to diffuse out into the bloodstream in sufficiently high concentrations that they are readily detectable. The turnover rate of plasma FFA is very rapid, their half-life being approximately two minutes (Fredrickson and Gordon, 1958). The reason for this rapid movement into the bloodstream is due to the mechanism by which they are able to pass through the cell membrane. Rate of passage is directly related to the

concentration because there is no barrier at the membrane level; consequently, the uptake of FFA in most cells is directly related to their concentration in the extracellular fluid and plasma (Armstrong et al., 1961). This principle is a great determining factor on the use of fatty acids as a form of energy. For example, skeletal muscle (Eaton and Steinberg, 1961) and liver (Fine and Williams, 1960; Harken et al., 1969) are directly dependent upon the concentration of fatty acid for the utilization of these metabolites. Direct diffusion across the membrane of the cell is a privilege of fatty acid and ketone bodies which is not shared by other important metabolites such as glucose (Hasseblatt, 1971).

2.1 Hormone Sensitive Lipase

It was first observed that lipolytic activity was stimulated in homogenate of fat epididymal adipose tissue by epinephrine (Hollenberg et al., 1961). This is activated by a variety of biologically similar compounds including: norepinephrine, ATCH, glucagon, TSH, insulin and prostaglandin. However, the stimulation does not occur in equal amounts between different species (Rodbell, 1964; Prigge and Grande, 1971) with the ruminant being 10 to 60 fold less than rat adipose tissue.

The rapid hydrolysis of adipose tissue triglycerides, which results in FFA mobilization, is triggered by

the activation of hormone sensitive lipase (HSL) (Vaughn et al., 1964; Strand et al., 1964). This enzyme plays a key role in the lipolytic activity as shown in Figure 1. The enzyme cleaves the first ester bond from triglycerides which is the rate limiting step in the metabolic process of lipolysis (Khoo et al., 1972).

HSL has been investigated in homogenates and partially purified preparations, but the activation by hormonal stimulation is greatest in intact cells. Since activation of lipolysis by epinephrine is not inhibited by puromysin (Fain, 1963) enzyme activation rather then enzyme induction is believed to occur.

Hormone sensitive lipase, purified 100 fold from rat adipose tissue, was activated 50 to 100% by incubation in a medium containing: AMP, ATP-Mg^{2+} and a protein kinase preparation from rabbit muscle. Protein kinase played a key role in the activation along with cyclic AMP and ATP. In addition, in the presence of ATP, activation was accompanied by the transfer of radioactivity to the lipase. The time course for phosphorylation closely paralleled that for lipase activation (Huttunen et al., 1970).

2.2 Protein Kinase

It has been demonstrated that protein kinase plays a physiological regulatory role in the activation of hormone sensitive lipase (Soderling et al., 1973).

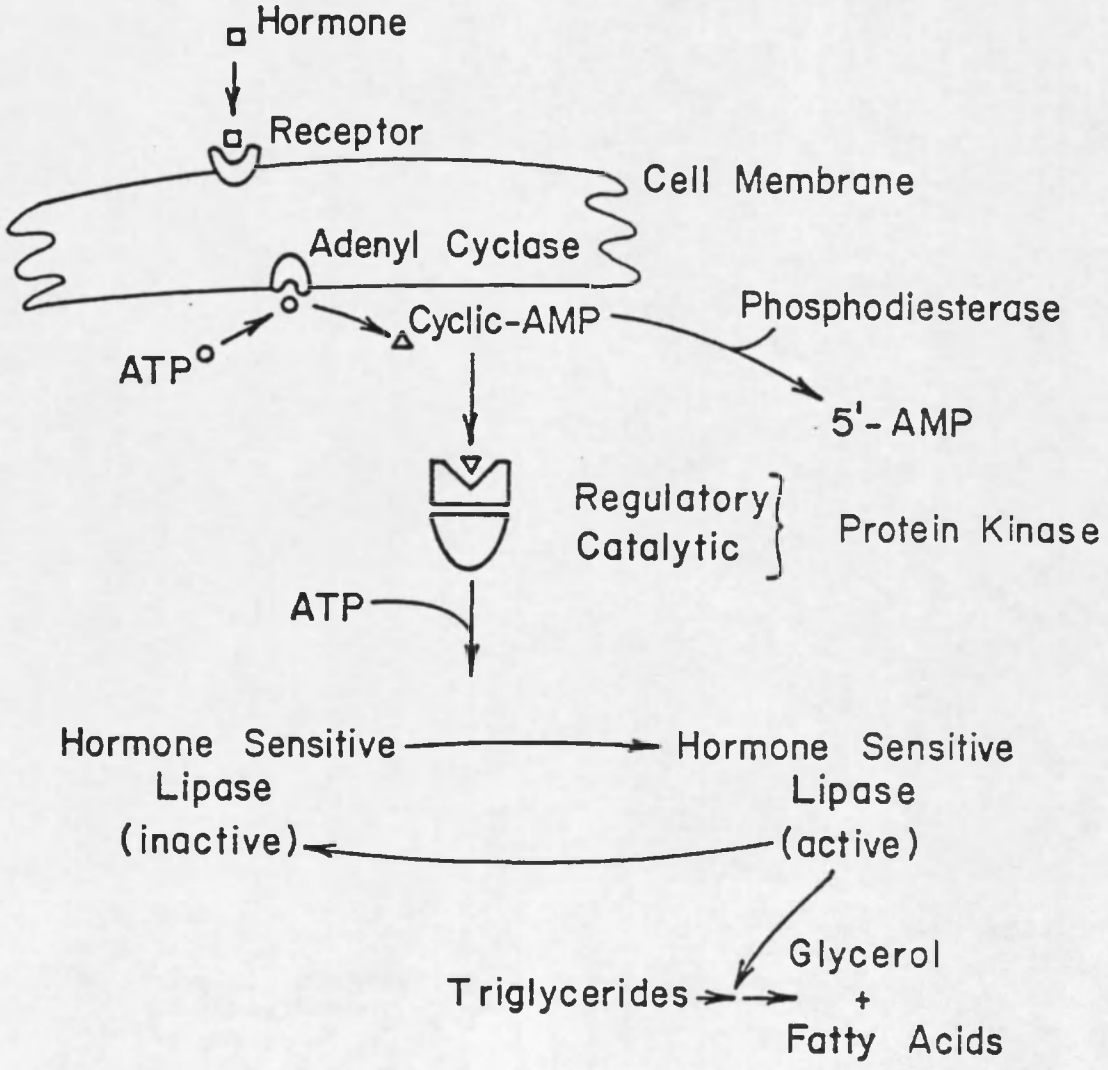

Figure 1. Action of catecholamine and peptide hormones on the adipocyte membrane receptors and lipolytic function.

Incubation of rat epididymal fat pads with epinephrine showed that there was an increase of cyclic AMP dependent protein kinase activity. Activation was associated with the transformation of the inactive holo enzyme to the catalytic form. The process was elucidated through the assay by the resolution of these two forms of protein kinase on sephadex G-100 columns. The role of conversion of the enzyme from inactive to active form was similar to the increased concentration of cyclic AMP caused by the introduction of epinephrine. Other lipolytic hormones have been shown to produce the same effect (activation of protein kinase). This data would seem to agree with the theory that hormones with lipolytic activities modify the cyclic AMP dependent protein kinase by changing the concentration of cyclic AMP.

2.3 Role of Cyclic AMP and Lipolysis

Work with the mechanism of action of sympathomimetic amine and glucagon led to the discovery of a cyclic adenine ribonucleotide in mammalian tissue (Rall et al., 1957; Rall et al., 1958; Sutherland et al., 1968.) This cyclic nucleotide was later identified as adenosine 3', 5'-monophosphate (cyclic AMP). Cyclic AMP was first implicated as a mediator of the lipolytic response of the rat epididymal fat pads by Butcher et al. (1965) working with epinephrine. Work done by Robison et al. (1968),

Sutherland et al. (1968), and Hardman et al. (1971) has established cyclic AMP as an intracellular second messenger mediating many of the physiological responses induced by numerous hormones.

The levels of cyclic AMP at any given space in time have been linked to the activity of two enzymes. The formation of cyclic AMP has been shown to be catalyzed by the enzyme adenyl cyclase, the substrate being adenosine triphosphate (ATP) (Sutherland et al., 1962). Magnesium ion has been found to be a cofactor in the catalysis of this reaction. Cyclic AMP and inorganic pyrophosphate (PPi) are products in this reaction (Rall et al., 1962).

The second enzyme involved in the regulation of cyclic AMP is a specific cyclic 3', 5'-nucleotide monophosphate phosphodiesterase (Butcher et al., 1962; Kupiecki, 1973). This enzyme acts in the reduction of cyclic AMP by hydrolysis to the inactive form 5'-AMP. Both adenyl cyclase and phosphodiesterase appear to be membrane bound and the cyclase is believed to be located on the plasma membrane.

It is now well understood that adenyl cyclase and the product it produces (cyclic AMP) play a key role in the effect of catabolic hormones on numerous metabolic processes (Sutherland et al., 1965). Butcher and Sutherland (1967) have found that catabolic hormones, such as glucagon

and epinephrine, increase the concentration of intracellular cyclic AMP in the adipose cell and other cell types in the rat.

The idea of a second messenger system, which was proposed by Robison et al. (1967), came out of the above evidence. The system is comprised of the hormone being the first messenger, stimulating an intermediate enzyme (adenyl cyclase) on the membrane of the cell of a specific target tissues, which causes a change in the concentration of a second messenger (cyclic AMP) within the cell. Adenyl cyclase can be affected by different hormones thus acting as a discriminator for metabolic function. By this mechanism there is a cascade effect through the enzyme system causing an amplification in the ultimate response of the cell. Each cell type has its own enzymatic profile which affects the levels of cyclic AMP, given any type of stimulus. Also, the effect of cyclic AMP on metabolic activities is dependent on the sensitivity of that system to the nucleotide.

Hormonal control of enzymes in living systems must have some type of mechanism in which these effects, by the hormone, can be stopped. In the formation of cyclic AMP the method by which the cell reduces the concentration of active cyclic AMP is by the enzyme cyclic 3', 5'-nucleotide monophosphate phosphodiesterase (phosphodiesterase).

The mechanism of this inactivity is through hydrolysis of cyclic AMP to an inactive form. This enzyme adds a second point at which lipolysis can be regulated besides that of the adenyl cyclase enzyme. Hormones and other compounds have been demonstrated to cause an increase or decrease in the rate at which lipolysis occurs. Vaughn and Steinberg (1963) reported that caffeine inhibited the reaction of phosphodiesterase, thus increasing levels of cyclic AMP. Insulin, which has been known to decrease lipolytic activity, has been implicated in increased levels of phosphodiesterase (Senft et al., 1968). However, other research has shown that this might not be the cause (Blecher et al., 1968) instead there is a decrease in the activity of adenyl cyclase.

2.4 Receptor Sites on Cell Membrane

For many years research has been conducted to determine the mechanism by which catecholamines stimulate response in target tissues. Investigations by Clark (1933) led to the postulation that the initial interaction takes place at a specific site on the cell membrane termed the "receptor". The response was found to be in direct proportion to the number of receptors occupied by the catecholamine (Williams and Lefkowitz, 1978).

Work by Ahlquist (1948) showed that there were possibly two types of cell membranes located on the cell

membrane. These he termed alpha and beta, based on their function upon the target tissue.

Work with radioactive ligand binding studies was performed to quantify the number of sites upon a cell membrane (Williams et al., 1978). Binding sites were studied in rat tissue membranes from adipose (Jarett et al., 1974), liver (Tomasi et al., 1970; Marinetti et al., 1978) and heart tissue (Lefkowitz et al., 1971).

Studies have indicated that physiological changes can be regulated by agents that change the adrenergic response (Williams et al., 1978). Researchers have shown that different stimuli or inhibitory factors have either reduced or increased the number of receptor sites on the cell membrane (Mickey et al., 1975). This was shown not to be a complete loss of the receptor site, but a change in conformation, thus preventing it from binding with agents that would otherwise bind (Mickey et al., 1975).

Chemical agents are not the only things that have proven to cause a change in response. The influence of growth, cell size and age have an effect on the response that can be detected (Giudicelli and Pecquery, 1978).

2.5 Endocrine Activation and Modulation of Lipolysis

Several chemical substances have been shown to have an effect on the rate at which lipolysis takes place in

the body. Most of these chemical substances are hormones that play an important role in maintaining homeostasis. These hormones are not secreted at uniform rates. Some of them, such as the adrenal hormones, are secreted in a diurnal rhythm. Others, like the gonadotrophins and sex steroids in the female, are secreted in cylces which correspond to physiological events such as ovulation, menstruation, pregnancy or lactation. Still others are excreted depending on the diet and intake (insulin and aldosterone). Hormones have a definite influence on adipose tissue. Epinephrine and norepinephrine additions stimulate the release of glycerol and free fatty acids from ruminant adipose tissue slices or isolated fat cells (Sidhu and Emery, 1972 and 1973). The response has been shown to be about 10 to 60 fold less than that of rat adipose tissue (Rodbell, 1964; Prigge and Grande, 1971). The mechanism of this reaction has been shown to be these catecholamines reacting with the beta-adrenergic receptors (Williams et al., 1978).

Insulin, which causes a marked inhibition of lipolysis in several nonruminant species, has a less dramatic effect on ruminant adipose tissue. Investigation by Yang and Baldwin (1973) using adipocytes isolated from lactating dairy cows demonstrated that insulin had no significant effect on basal lipolytic rates. They do, however,

show that insulin has an antagonistic effect when used in conjunction with epinephrine. Glucagon has been shown to increase lipolysis at a rapid rate in rat tissue (Hagen, 1961). However, increases in rates of lipolysis were not evident in market lambs and steers (Etherton et al., 1975).

Hormones such as ACTH (White and Engel, 1958), TSH (Freinkel, 1961), LH (Butcher et al., 1968), serotonin (Bieck et al., 1966), vasopressin (Vaughn, 1964) and growth hormone (Raben and Hallenberg, 1959) have all been shown to produce an increase in the rate at which lipolysis takes place in rat epididimyal tissue. Whereas, prostaglandin (Steinberg et al., 1964), nicotinic acid (Carlson, 1963) and beta-adrenergic blockers (Mayer, 1961) have been shown to reduce lipolytic activity. There have been several other chemical compounds that have to be evaluated for their possible effects on lipolysis. Theophylline has been shown to increase the rate at which lipolysis occurs (Etherton et al., 1974). In the same study, theophylline was shown to have a synergistic effect with epinephrine to increase the rate 1½ times above that of epinephrine stimulated treatment alone. The mechanism for this response is that methyl xanthines, of which theophylline is the most potent, have been shown to be an inhibitor at the cyclic 3', 5'-nucleotide. The effects of these hormones with

regard to ruminant adipose tissue has not been investigated thoroughly.

2.6 Ruminant Adipose Function in Differing Physiological and Nutritional States

Adipocyte lipolysis in the beef animal involves both subcutaneous and internal fat depots. These depots are mobilized during different physiological stresses on the animals. The following is a review of lipid mobilization in beef cattle and factors, other than endocrine influences, which affect it.

2.6.1 Nutrition

Nutritional changes that occur during the growth of beef cattle have a marked effect on the way in which the adipose tissue responds to these changes. During times of nutritional inadequacy, lipolytic rates are increased to maintain an adequate supply of metabolizable energy. These responses are not as great as those found in nonruminants. As previously described, the endocrine responses in lipid synthesis and mobilization in ruminants are much smaller in magnitude than found in nonruminants. It seems likely that this is related to the availability of nutrients and the physiology of ruminant animals. The large food reservoir in the rumen results in ruminant animals absorbing a rather constant and continuous supply of nutrients relative to non-ruminant species (Bauman, 1976). Certainly the tissues of

ruminant animals do not experience the dramatic diurnal variations in metabolite availability typically occurring in nonruminants. As a consequence, ruminant animals would generally have a much longer period to adapt at a tissue level to many physiological situations.

However, partial restriction of nutrients has had some effect on lipolysis. Pothoven et al. (1975) found that beef cattle on a restricted diet had a reduced rate of lipolytic activity compared to beef cattle fed ad libitium. It was interesting to note that with this reduced rate in lipolysis it compensated for the reduced feed intake. This would result in similar amounts of fat being deposited in both treatments.

The composition of the diet has been shown to effect the rate of lipolysis. Baldwin et al. (1973) and Benson (1972) demonstrated that dairy cattle on a high concentrate, low roughage diet had a reduced rate of lipolysis compared to animals on a control diet. There was also an increase in the lipogenic capacity, thus the propensity for the animal to accumulate a large amount of fat during the finishing stage in the feedlot.

2.6.2 Pregnancy

Pregnancy presents a great stress on the animal and causes several events to take place so the animal can cope with this additional stress. Pregnancy and lactation have

been of prime importance to the dairy farmer. However, information gained from studies involving these stress conditions may also be of benefit to the cattle producer.

Early pregnancy is characterized by increased storage of lipid in adipose depots, while lipid mobilization predominates during late pregnancy and lactation. This increase results in order to meet caloric requirements of the animal (Knopp et al., 1973; Biezenski, 1974). The same results have been reported in the ewe by Noble et al. (1971). Blood lipid patterns and adipose lipogenic and lipolytic enzyme pattern changes have been recorded during pregnancy in nonruminants and are useful in comparison to beef cattle. Presumably during late gestation lipolysis is caused by the hormonal changes associated with parturition (Otway et al., 1968; Knopp et al., 1970; Knopp et al., 1973).

2.6.3 Lactation

Lactation creates another demand on the energy supplies available to the bovine. Lipid mobilization in early lactation is accompanied by depletion of triglycerides in adipose cells (Elias et al., 1973). This rise in lipid mobilization and a drop in blood lipids with increasing levels of milk production, presumably reflect increased removal of body lipids by the mammary gland (Baldwin et al., 1976).

The stress of early lactation in the dairy cow and her ability to mobilize body fat to meet caloric requirements involves both endocrine and enzymatic systems. These metabolic and enzymatic changes at the onset of lactation have been measured in adipose slices (Baldwin et al., 1973). However, lipogenic enzyme patterns indicate only negligible change in activities when measured during lactation (Baldwin et al., 1973).

2.6.4 Age

The aging process has been shown to effect the rate of mobilization of lipid from adipose cells. Aging is a complex, yet obscure, phenomenon in which various metabolic alterations are known to occur. These alterations are hard to define because the aging process and increase in fat deposition occur simultaneously.

These changes have been studied in some detail in rats. Manganiello and Vaughan (1972) observed a gradual decline in the response of fat cells to glucagon with age. Miller and Allen (1973) demonstrated this same decrease with age when catecholamines were used as the simulating agent. Another interesting observation made by Miller and Allen (1973) was the effect of insulin on catecholamine mediated lipolysis. Insulin acts as an antagonist to catecholamine, and it has a greater effect on younger animals. Many other researchers have demonstrated that

there is a decrease in lipolysis with age (Benjamin et al., 1967; Hartman et al., 1971; Hubbard and Matthew, 1971; Nakano et al., 1971).

The cause of this decrease has also received attention. Several researchers (Zinder and Shapiro, 1971; Hartman et al., 1971) contend that the decline in lipolysis observed during growth and fat accumulation is a result of fat cell enlargement, with fewer cells in a given mass of tissue, rather than a decline in lipolytic activity of the individual cells. However, Miller and Allen (1973) suggest in their research that as cells get older, they reflect an altered cellular activity. Their study was based on cellular protein concentration which does change with age.

Giudicelli and Pecquery (1978) demonstrated that the altered cellular activity could be attributed to a change in the number of beta-adrenergic receptors contained on the cell membrane. Studies have also been performed with genetically obese mice which showed as they aged there was an impaired response of adenyl cyclase to stimulation by epinephrine. The investigators have suggested that an inhibition may occur in the coupling between the hormone receptor and the catalytic site of the cyclase in these obese animals (Laudat and Pairault, 1975).

The effect of aging on lipolysis is not very well defined because of the limited amount of research done on the subject. Pothoven et al. (1975) suggest that there is some correlation in the loss of lipolytic activity in stimulated adipose cells. They also mention that basal lipolysis did not show this decrease. The lack of information on the subject demonstrates the need for further research into this area.

2.7 Biological Types - Its Effect on Lipolysis and Carcass Composition

Meat animal researchers have traditionally fed cattle to a common chronological age, slaughter weight, days on feed, or expected quality grade before sending them to slaughter. Brungardt (1972) has shown that when animals are fed to an expected quality grade, there are large differences in the carcass composition. Since these great differences are present there needs to be a method by which proper slaughter time can be selected. Guenther (1974) stated if indices of potential growth rate and maturity could be developed and used during a beef cattle's life a considerable savings in feed and management costs could be realized. Blaxter (1968) demonstrated that the best direct relationship of carcass composition among differing animals is physiological maturity. With the introduction of the exotic breeds, the producer has been given a new genetic

resource. With these new resources there have been significant changes in growth rate, carcass composition, and biological size of the animal (Cole et al., 1964; Hedrick et al., 1970; Adams et al., 1973; Koch et al., 1976; Smith et al., 1976).

Animals of the traditional British breeds, such as, Hereford and Angus, have been noted for their small and medium frame size. Whereas the exotic breeds, such as Charolais, Limousin and some dairy breeds have been noted to be larger in mature size than the British breeds. (Cole et al., 1964, Adams et al., 1973; Peacock et al., 1979).

The efficiency of these animals also has been shown to vary compared to the traditional breeds. The exotic breeds were found to be slightly more efficient than the traditional breeds during post-weaning growth (Smith et al., 1976). There was also a significant difference in the composition of different biological type animals when slaughtered at the same weight. The smaller type cattle had a higher fat percentage than the larger type cattle. This was the exact opposite when compaing the protein and moisture (Cole et al., 1964; Koch et al., 1976; Koch et al., 1979). It was also demonstrated that the smaller animals had a thicker than average backfat thickness than did its

larger frame counterpart in the same situation (Cole et al., 1964; Koch et al., 1976).

The relationship of frame size to the rate of lipid mobilization has not been elucidated at this point, but some of the points mentioned might have some bearing on how a specific frame size animal will mobilize it's lipid stores. Such things as efficiency and utilization of available energy could have some effect on lipolysis. The difference in maturity would also be a factor in the rate of lipolysis in animals varying in biological types.

3. MATERIALS AND METHODS

3.1 Experimental Animals

One hundred and forty four steers of mixed breeding averaging 227 kg were scored for frame size, based on the proposed U.S. Feeder Grade Standards. These cattle were involved in the W-145 Regional Beef Marketing Project. Approximately 48 steers were assigned to each of the frame size categories of small (SF), medium (MF) and large (LF). Each frame size was divided into two groups and fed as replicates. They were group fed a ration of: 49% concentrate, 40% roughage, 8% molasses blend and 4% supplement for 7 days. They were then fed: 64% concentrate, 25% roughage, 7% molasses blend and 4% supplement for 14 days. They were finished on a 79% concentrate, 10% roughage, 6% molasses blend and 5% supplement diet. The supplement contained non-protein nitrogen (NPN), calcium, potassium, phosphorus, magnesium, sodium chloride, vitamin A and rumensin.

Six cattle were randomly selected from the group of 144 cattle, to be used in the lipolytic study. These cattle were selected to have two in each frame size classification. The six steers were fat biopsied after 166 and

194 days on feed. The third fat sample was collected after 214 days on feed directly from the animals during the slaughter procedure.

3.2 Beef Cattle Adipose Tissue Biopsy and Cell Isolation

The tail-head area was selected as the site for the fat biopsies. This area was selected for the following reasons. First, it is an area in which an ample amount of adipose tissue could be removed and second, the adipose tissue there is representative of lipolytic activity of all the subcutaneous fat of the animal. The area was prepared for biopsy by clipping and washing the area of the incision with betadine, followed by an injection of 5 ml of 2% lidocaine hydrochloride solution which served as a local anesthetic.

A skin incision approximately 8 cm long was made and approximately four grams of adipose tissue was removed and placed directly in a 25 ml Erlenmeyer flask with 10 ml of albumin buffer, pH 7.4. The albumin buffer was prepared fresh for each sample and contained 128 mM NaCl, 1.4 mM $CaCl_2$, 1.4 mM $MgSO_4$, 5.2 mM KCl, 10 mM Na_2PHO_4 and 3% bovine serum albumin (Fraction V powder: Sigma Chemical Co., St. Louis, Missouri). Fresh buffer was utilized to minimize the possibility of bacterial contamination due to the high concentration of albumin.

The buffer and tissue was maintained at a constant temperature of 37C, this was to prevent any loss of activity of the adipose tissue due to temperature changes. Crude collagenase, 2.5 mg/ml (Clostridium Histolyticuum; Worthington Chemical Co., Freehold, N.J.), was added to the buffer and tissue samples to free the individual fat cells. This method of isolating adipose cells was similar to methods of Rodbell (1964) and Yang and Baldwin (1973). The suspension of albumin buffer, containing adipose tissue was incubated in a shaker water bath, 60 cycles per minute, at 37C for 50 minutes. The digestion was terminated by filtering the cells through one layer of cheesecloth followed by centrifugation in an I.E.C. clinical centrifuge at approximately 1000 rpms for 20 to 30 seconds. Cells were washed three times with warm buffer, then they were resuspended in albumin buffer (37C). The washings removed the collagenase enzyme and the other extra material such as blood and residual amounts of lidocaine that may have been present which may interfere with subsequent reactions.

Washed cells were then transferred to separate incubation tubes (12 x 75 mm glass tubes) using a 1 cc syringe fitted with a short piece of surgical tubing (12 cm), this made transfer and allocation into the incubation tubes easier. The volume of isolated cells (approximately 50,000 cells/ml) and albumin buffer in each tube

was 950 µl. The fat cells were counted from aliquots taken from the cell suspension which had been diluted with warm buffer (37C). A 5 µl aliquot of dilute suspension containing approximately 10 to 30 fat cells was placed on a hemocytometer (AO Instrument Co., Buffalo, New York) and counted several times using a light microscope.

The lipolytic studies began by dispensing 50 µl of L-epinephrine bitartrate and 50 µl of insulin in the respective tubes. In the treatment in which both hormones were used, 50 µl of L-epinephrine and 50 µl of insulin were combined in their respective tubes. The final concentrations of these hormones in the incubation tube was 10 µM/ml for epinephrine and 10^{-3} µIU for insulin.

The tubes with the adipose cells, buffer and hormone were incubated in a metabolic shaker water bath at 37C for 30 cycles per minute. The incubations were carried on a timed basis of 60 and 120 minute treatments. The incubation was terminated at the end of each time period by dispensing 50 µl of 20% trichloroacetic acid (TCA) in the reaction tubes. The use of TCA also removed the protein in the incubation media to prevent it from interfering with the analytical procedures used in the glycerol analysis. To account for glycerol that might have been released during tissue handling a baseline value was established. To accomplish this an incubation tube, containing media

with cells, was treated with TCA before all other incubations were started. This baseline value was determined for each incubation period which was subtracted from each value obtained from the treatment tubes. All glycerol samples were then frozen and stored at -20C until assay procedures were performed.

3.3 Glycerol Analysis

The incubation tubes were removed from the freezer and allowed to thaw. Approximately 20 µl of 4 N NaOH was used to neutralize the incubation media. The pH of the media was measured with the use of a Perkin-Elmer Metrion IV pH Meter (Coleman Instrument Division). The tubes were neutralized to approximately a pH of 7.4.

With the use of a Glycerol Reagent Kit (Calbiochem Corp., La Jolla, California), the amount of glycerol was quantitated. The method of quantitation was by the following oxidation reduction reactions.

1. Glycerol + ATP $\xrightarrow[\text{Kinase}]{\text{Glycerol}}$ alpha-Glycerol Phosphate + ATP
2. ADP + Phosphoenol Pyruvate $\xrightarrow[\text{Kinase}]{\text{Pyruvate}}$ Pyruvate + ATP
3. Pyruvate + NADH $\xrightarrow[\text{Dehydrogenase}]{\text{Lactate}}$ Lactate + NAD

The decrease in concentration of NADH was measured spectrophotometrically (Gilford Instrument Lab Inc., Oberlin, Ohio) at 340 nm.

Glycerol measurements are considered the best indicator of lipolytic activity in adipose tissue. Their lack of the enzyme, glycerol kinase, prevents the re-incorporation of glycerol into the cells, so it simply diffuses out into the surrounding medium.

Glycerol enzymatic incubations were conducted in 250 µl microtiter plates (Dynatech Laboratories, Inc., Alexandria, Virginia) at 30C. Standard glycerol curves were prepared using incubation media treated in the same manner as the medium plus fat cell glycerol. The data are expressed in $\mu M/10^6$ cells/time of incubation. The incubation treatments were run in duplicate and the spectrophotometric determination was read in triplicate.

3.4 Chemical Composition of Carcasses

The animals were slaughtered in one of 3 groups. Each group contained 3 subgroups of 16 cattle representing each of the 3 different frame sizes. The slaughter schedule corresponded to the 3 biopsy dates, on each biopsy date a group of 48 cattle was slaughtered.

The animals used in the lipolytic studies were not sacrificed until the last slaughter date. Average composition of their pen mates, who had been slaughtered on the first 2 biopsy dates, was used to determine chemical composition.

Hot carcass weights were obtained at the time of slaughter. Carcasses were allowed to chill for 24 hours then the left sides were ribbed between the 12th and 13th ribs. Quality and yield grade factors were determined following standard procedures. Rib wholesale cuts from the left side were removed from the carcasses and shipped to The University of Arizona Meats Laboratory. The ribs were chilled for 12 hours after receipt.

Each rib was separated into bone and soft tissue. The weight of the bones and soft tissue for each rib wholesale cut was recorded. The soft tissue was ground through a 1.3 cm chopper and mixed thoroughly. A random .5 kg sample of ground soft tissue from each rib was homogenized in a Hobart food cutter (model 10814) until a homogenous mixture was obtained. All samples were placed in evacuated cryovac bags then placed in a large polyethylene bag and stored at -25C until chemical analyses were conducted.

3.5 Chemical Analysis

Chemical analyses were conducted to determine percent lipid, protein and moisture of the wholesale rib cuts on a boneless basis. All analyses were performed using duplicate determination and reruns were made when the values did not agree within 1% of the duplicate determination.

Crude protein was determined according to AOAC (1965). Samples were also analyzed for total extractable lipid and total moisture content by chloroform-methanol extraction following the modified procedure of Ostrander and Dugan (1961) as outlined by Wooten et al. (1979). The procedure provided a means by which the total lipid and moisture content could be determined as separate steps of the same procedure.

3.6 Prediction of Whole Carcass Composition

Using chemical composition data of the rib and carcass cooler traits, total carcass composition on a boneless basis was determined by using prediction equations which were developed at The University of Arizona as a part of the W-145 Regional Beef Marketing Project. The equations were developed from stepwise regression using data collected from 263 carcasses (unpublished data from Regional Beef Marketing Project, University of Arizona). The equations are as follows:

Percent Lipid =

19.5225 + (0.2793 x Rib Percent Lipid) + (1.3467 x Yield Grade) + (1.0362 x kg of Kidney Fat) - (0.4770 x Rib Percent Protein) - (0.0172 x kg of Hot Carcass Weight) + (0.2313 x Marbling).

Percent Protein =

26.3963 + (0.165 x Rib Percent Protein) - (0.2299 x Yield Grade) - (0.158 x Rib Percent Lipid) - (0.1426 x Rib Percent Moisture) - (0.0014 x Quality Grade) - (0.4263 x Fat Thickness in cm) + (0.0094 x Ribeye Area).

Percent Moisture =

58.4128 - (0.2677 x Rib Percent Lipid) - (1.1715 x kg of Kidney Fat) + (90.0332 x Ribeye area in cm^2) + (0.3455 x Rib Percent Protein) + (0.0104 x kg of Hot Carcass Weight).

The quality grade was placed on a numerical score so that it could be incorporated into the equations. The values for quality grades are:

Standard -	525	Good +	675
Standard	550	Choice -	725
Standard +	575	Choice	750
Good -	625	Choice +	775
Good	650	Prime -	800

3.7 Fatty Acid Profile Analysis

At the time of fat biopsy, a second 5 gm sample was obtained for fatty acid analysis. This sample was placed in a test tube, capped and frozen. The lipid was extracted using the chloroform-methanol extraction procedure as previously discussed. The extracted lipids were esterified using the transesterification method described by Cramer and Marchello (1964) and modified by Wooten et al. (1979).

Purification was also performed by the method outlined by Wooten et al. (1979).

The following procedure was used in identification and quantitation of the methylated fatty acids. A Hamilton 1.0 microliter syringe was used for the introduction of the sample into the gas-liquid chromatograph. Each sample injected ranged from 0.1 to 0.3 microliters. The esters were chromatographically separated using a Beckman GC 72-5 instrument equipped with a flame ionization detector. A 1.83 m (3.2 mm O.D.) coiled stainless steel column packed with 100-120 mesh chromosorb w (HP) and coated with 15% of diethylene glycol succinate was used for the fatty acid separations. Instrument operating conditions were: column temperature about 170C; inlet temperature, 205C; detector line temperature; 240C; detector temperature, 260C; and carrier gas flow (nitrogen), 28 ml/minute.

Identification of the fatty acid methyl esters was accomplished by comparison of the relative retention times with those of standard solutions of known composition. The weight percent of each ester was determined by computing its proportionate amount, as measured by a disc integrator, to the total area of the chromatogram.

3.8 Statistical Treatment of Data

All data were analyzed by analysis of variance according to Nie et al. (1975). Duncan's multiple range

test and main treatment interactions were performed to compare treatment means. Significance was determined at the 5% level of probability. Interactions between main effects were analyzed by nesting frame size within time on feed and time on feed within frame size. The differences between the hormone treatments were evaluated for significance using the students T test.

4. RESULTS

4.1 Endocrine Responses to Lipolysis

Glycerol release is a good indicator of lipolysis due to the action of hormone sensitive lipase on triglycerides liberating free fatty acids (FFAs) and glycerol. The lack of an enzyme, glycerol-kinase, in adipose cells prevents glycerol reincorporation into the cell. Comparison of the hormone treatments to the control (no hormone addition) with regard to lipolysis is made in Figure 2. Values are expressed as glycerol released per 10^6 cells by time of incubation. To account for glycerol release which would have occurred during tissue handling, a baseline value was established as stated previously. This was then subtracted from each value.

Epinephrine was the most active stimulator ($P<.05$) of lipolysis (Figure 2). This is in agreement with observations by Yang and Baldwin (1973), Etherton et al. (1974), Pothoven et al. (1975), and Jaster et al. (1979) in that the use of epinephrine resulted in a one to four fold increase in the level of lipolysis over the control. The average release of glycerol for a 60 minute incubation was 1.69 $\mu M/10^6$ cells and the glycerol release for the 120 minute incubation was 3.78 $\mu M/10^6$ cells. Glycerol release

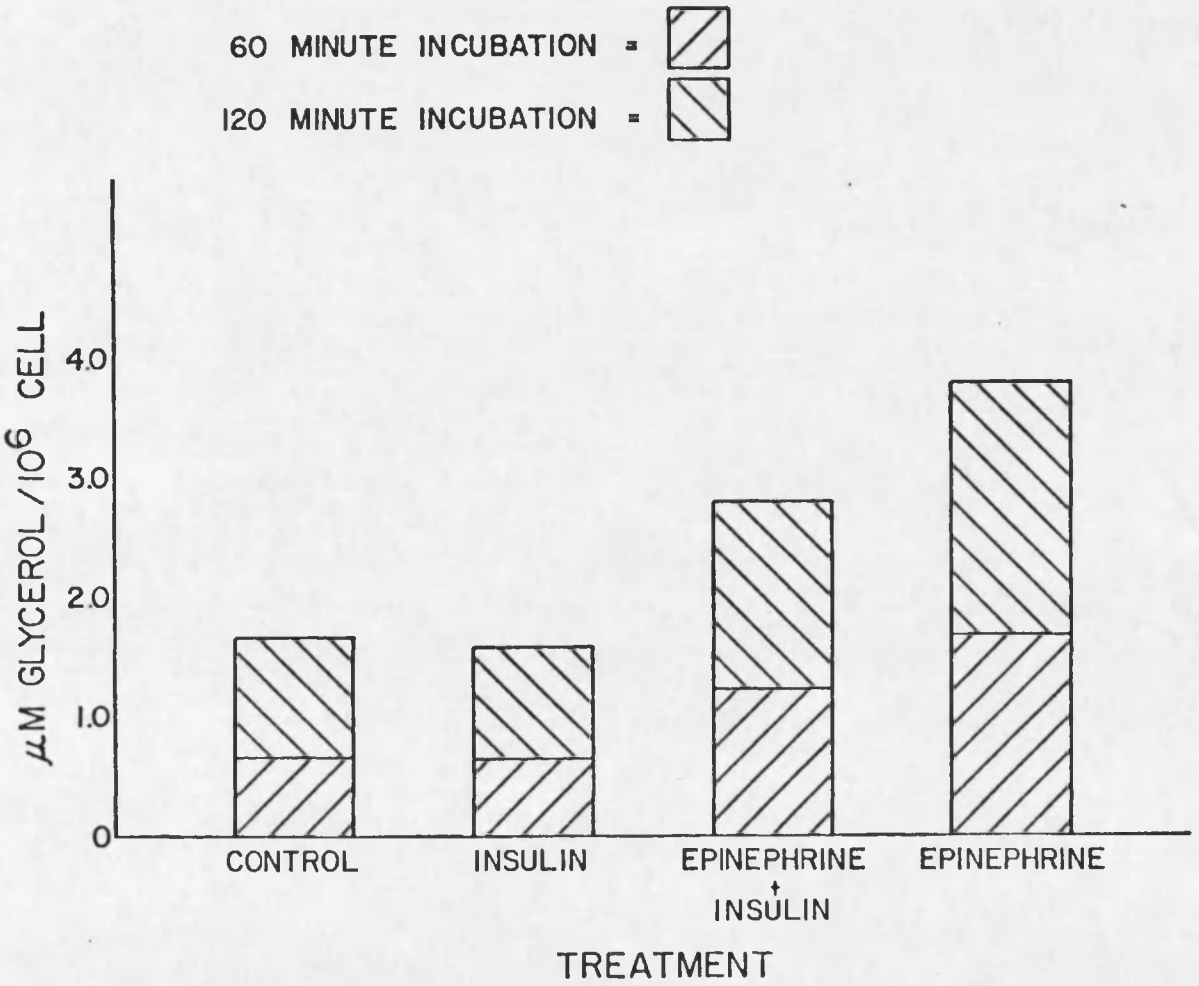

Figure 2. Means of hormone treatments for all cattle.

at basal levels were .66 μM/10^6 cells and 1.68 μM/10^6 cells for 60 and 120 minute incubations, respectively. The epinephrine stimulation also showed a linear relationship between glycerol release and time.

Insulin/induced glycerol release was not different ($P>.05$) from the control level. There was, however, a tendency for glycerol release to be slightly lower than for the control levels, particularly in the 120 minute incubation. Insulin stimulation averaged 1.58 μM/10^6 cells, whereas the control level was 1.68 μM/10^6 cells. This is in agreement with the findings of Yang et al. (1973) and Mears et al. (1974).

When epinephrine and insulin were combined, the release of glycerol by the adipose tissue was 1.21 μM/10^6 for 60 minutes and 2.79 μM/10^6 cells for 120 minutes. Thus the epinephrine + insulin treatment accounted for a 30% decrease in the amount of glycerol released when compared with only epinephrine stimulated treatment. This demonstrates that there is an antagonistic action elicited on epinephrine by insulin. The amount of antagonism was more than reported by Yang and Baldwin (1973), who observed a 10 to 25% decrease in lipolysis over that of the epinephrine stimulated tissue.

4.2 Influence of Frame Size on Lipolysis

Glycerol release in relation to different frame sizes of cattle was investigated to determine any possible significance between the three (Table 1). The basal level rates (control treatment) of lipolysis showed significant difference when comparing the SF (.61 $\mu M/10^6$ cells) and MF cattle (.63 $\mu M/10^6$ cells) to the LF cattle (.73 $\mu M/10^6$ cells) at the 60 minute incubation. This significance between each frame size was also found in the 120 minute incubation. The LF cattle (1.79 $\mu M/10^6$ cells) had the greatest release of glycerol followed by the MF cattle (1.68 $\mu M/10^6$ cells) then SF cattle (1.57 $\mu M/10^6$ cells).

The amount of glycerol released in the insulin treatment was slightly less or no different than the control values. However, there was a difference ($P<.05$) noted between frame sizes for both incubation time periods; LF cattle had the highest glycerol release (.73 $\mu M/10^6$ cells for 60 minutes and 1.66 $\mu M/10^6$ cells for 120 minutes) followed by the MF and SF cattle in that order.

LF cattle were the most responsive ($P<.05$) to the epinephrine treatment followed by the MF and SF cattle, respectively. The LF cattle demonstrated a significantly greater increase in epinephrine induced lipolysis in both incubation time periods when compared to MF cattle.

Table 1. Hormonal stimulus by frame size.[a]

	Frame Size[b]		
	Small	Medium	Large
Hormone Treatment[c]	μmoles glycerol released/(10^6 cells x hr)		
C-60	.61 ± .02[d]	.63 ± .01[d]	.73 ± .03[e]
I-60	.55 ± .01[d]	.63 ± .01[e]	.71 ± .02[f]
E+I-60	1.19 ± .02	1.19 ± .02	1.27 ± .04
E-60	1.58 ± .02[d]	1.65 ± .01[d]	1.85 ± .05[e]
C-120	1.57 ± .02[d]	1.68 ± .02[e]	1.79 ± .03[f]
I-120	1.50 ± .02[d]	1.57 ± .01[e]	1.66 ± .01[f]
E+I-120	2.65 ± .02[d]	2.68 ± .03[d]	3.04 ± .06[e]
E-120	3.34 ± .06[d]	3.66 ± .05[e]	4.34 ± .11[f]

[a]Values are means of all cattle for each frame size (± S.E.M.).

[b]Frame size determined by USDA Feeder Grade Standards.

[c]C=control, I=insulin and E=epinephrine. The incubation is given in minutes, 60=60 minutes and 120=120 minutes.

[d,e,f]Values on a given line followed by a different superscript differ significantly ($P<.05$).

Significant differences between MF and SF cattle, however, were only observed in 120 minute incubation time period.

When both insulin and epinephrine were added to the incubation media there was an antagonistic effect demonstrated for all three frame sizes when compared with the epinephrine treatment. The magnitude of this antagonistic effect of insulin on epinephrine increased with LF cattle.

When comparing hormone responses by cattle frame size, all hormones except insulin showed that differences in glycerol release became greater as time of incubation increased (Table 1). In the epinephrine treatment group, the difference in glycerol release between the MF and LF cattle showed a 4 fold increase from the 60 to 120 minute incubation period. When comparing results from the epinephrine + insulin group, the antagonistic response of insulin on epinephrine was markedly greater in the longer incubation time (Figure 3).

4.3 Influence of Time on Feed on Lipolysis

Knowledge of mechanisms by which fat is removed from fat depots and utilized in the body may be of benefit in decreasing the amount of feed used and improving the efficiency of weight gain in cattle. Table 2 shows the relation of time on feed and its effect on the release of glycerol. Although there was not statistical significance

Table 2. Hormonal stimulus by time on feed.[a]

	Time on Feed[b]		
	166 days	194 days	214 days
Hormone Treatment[c]	μmoles glycerol released/(10^6 cells x hr)		
C-60	.64 ± .02	.67 ± .02	.66 ± .04
I-60	.62 ± .04	.65 ± .03	.62 ± .03
E+I-60	1.19 ± .04	1.24 ± .03	1.21 ± .02
E-60	1.64 ± .06	1.73 ± .07	1.69 ± .07
C-120	1.66 ± .05	1.68 ± .03	1.71 ± .05
I-120	1.59 ± .02	1.58 ± .04	1.58 ± .04
E+I-120	2.83 ± .10	2.84 ± .08	2.70 ± .07
E-120	3.85 ± .23	3.86 ± .16	3.62 ± .22

[a]Values are means of all cattle for each biopsy time (±S.E.M.).

[b]Time on feed is the length of time the cattle were in the feedlot until the biopsy date.

[c]C=control, I=insulin and E=epinephrine. The incubation is given in minutes, 60=60 minutes and 120=120 minutes.

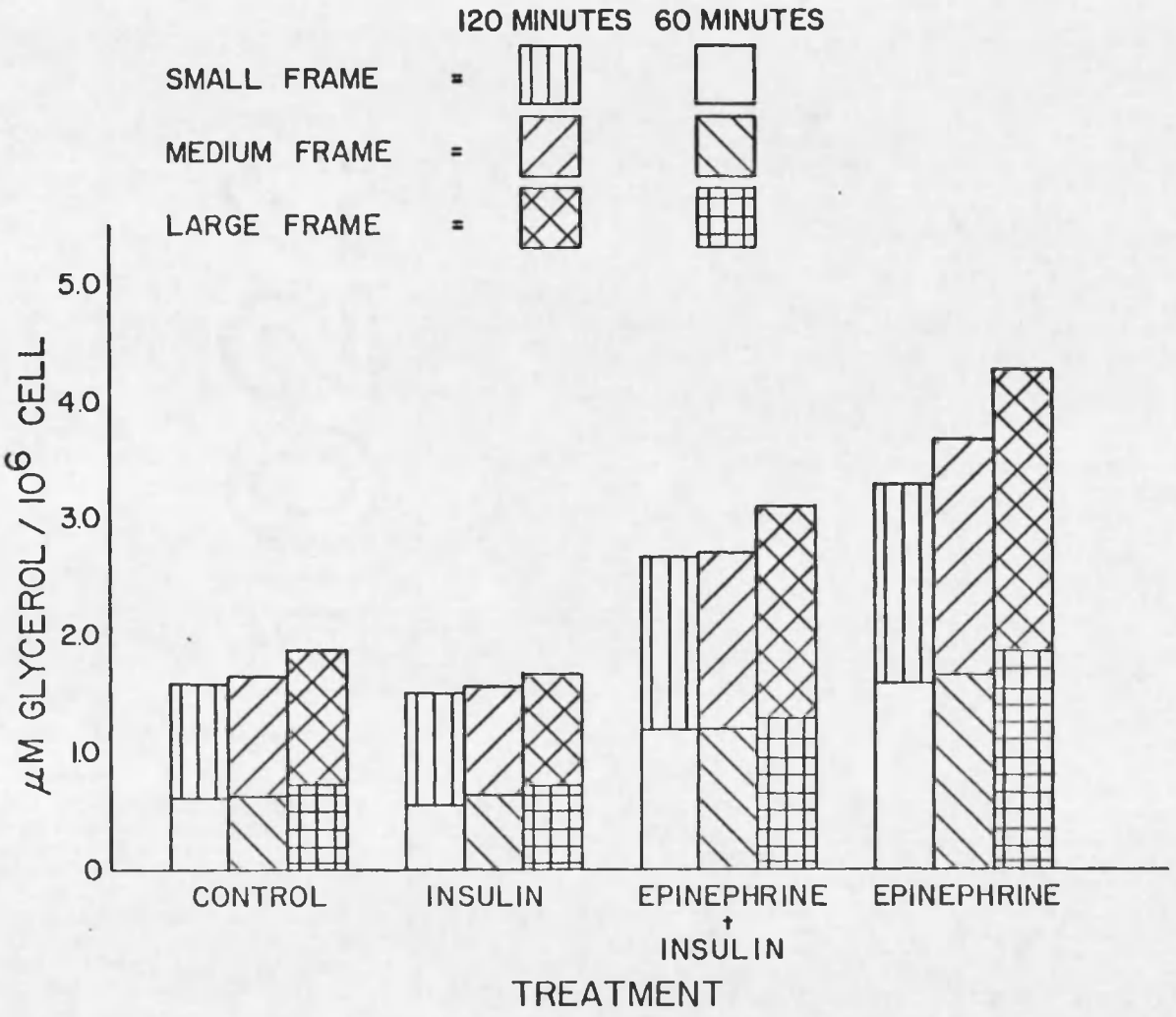

Figure 3. Means of hormone treatments of cattle within each frame size.

in the data there were trends evident which could perhaps lead to some speculation with regard to the rates of lipolysis and time on feed.

While control treatments did not demonstrate significant differences in the rates of lipolysis. There was, however, a slight increase in the rate of removal of triglyceride, with the first time period (166 days) having the lowest rate of lipolysis (.64 $\mu M/10^6$ cells for 60 minutes and 1.66 $\mu M/10^6$ cells for 120 minutes) and the third time period (214 days) having the highest rate of lipolysis (.66 $\mu M/10^6$ cells for 60 minutes and 1.71 $\mu M/10^6$ cells for 120 minutes).

The insulin treatment demonstrated little change in glycerol release with differing times on feed in either incubation period (Table 2). However, when comparing the 60 minute incubation period with 120 minute incubation period there was a 2½ fold increase in the longer time period.

The largest differences among the values recorded based on time on feed were with the epinephrine treatments. This was particularly evident for the 120 minute incubation time (Table 2). There was no difference between the 166 day period and the 194 day period. However, a drop in glycerol release at the third time period was noted (Figure 4). For the SF cattle, the difference between

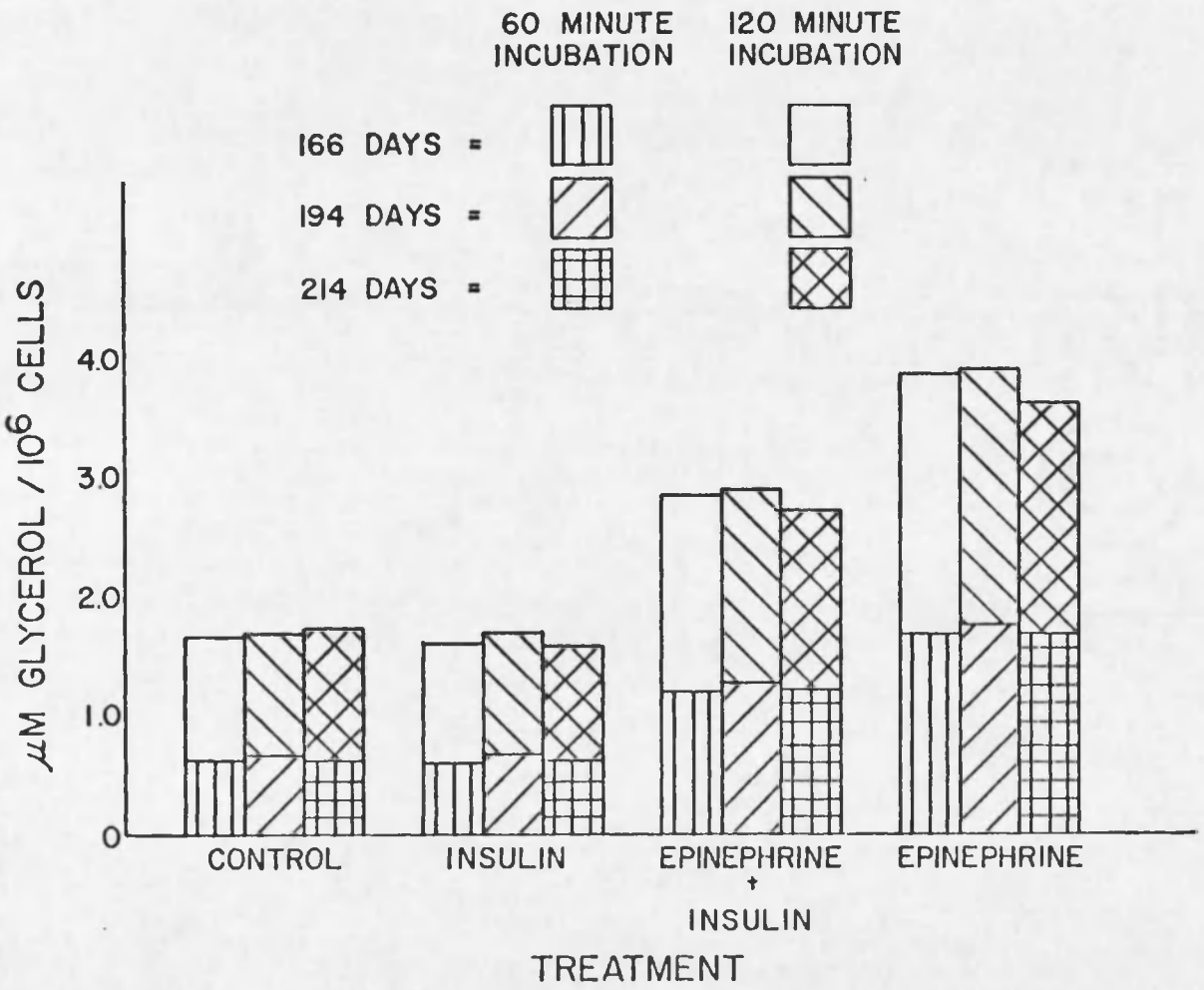

Figure 4. Means of hormone treatments for all cattle at each time on feed.

the first two time periods (3.35 μM/10^6 cells) and the third (3.10 μM/10^6 cells) were significantly different at the 120 minute incubation. This, however, was the only frame size that demonstrated a significant difference.

In the epinephrine + insulin treatment no difference ($P>.05$) was found between the means. However, for the 120 minute incubation there was a slightly lower value recorded for the third period (214 days) than the other two time periods (Table 2). This could possibly suggest that there is an increased antagonistic effect of insulin on epinephrine with a longer feeding period.

4.4 Influence of Frame Size on Chemical Composition

Table 3 gives the chemical composition of the cattle, by frame size, which is based on a boneless tissue basis. The protein content of the cattle increased with the size of the cattle, the SF cattle being the lowest (13.8%), followed by the MF (14.5%) and LF cattle (14.5%). A significant difference existed between the SF cattle and the other two frame sizes.

The SF cattle had the higher percent lipid (36.5%) with the LF cattle (34.7%) and the MF cattle (33.8%) having lower values, respectively. Statistical differences were noted between SF and MF cattle, but the LF cattle were

Table 3. Carcass composition on a boneless basis by frame size.[a]

	Frame Size[b]		
	Small	Medium	Large
Protein	13.66 ± .30[c]	14.49 ± .11[d]	14.50 ± .09[d]
Lipid	36.50 ± 1.24[d]	33.78 ± .30[c]	34.65 ± .48[cd]
Moisture	47.97 ± .72[c]	48.87 ± .40[d]	49.70 ± .34[cd]

[a]Values are the mean percents for each frame size (±S.E.M.).

[b]Frame size was determined by USDA Feeder Grade Standards.

[c,d]Values on a given line followed by a different superscript differ significantly. ($P<.05$).

not different (P>.05) from either of the other two frame sizes.

The percent moisture was lowest in the MF cattle (48.9%) which was significantly different from the SF cattle (47.9%), but was not different (P>.05) from the LF cattle (49.7%). The data was similar to that collected by Koch et al. (1976), when the cattle were at the same chronological age and fed on similar diets.

4.5 Influence of Days on Feed

The carcass composition in relation to the number of days in the feedlot is compared in Table 4. Percent protein decreased successively from the first time period through the third period. Significance was noted between the first (14.5%) and the last (13.9%) dates.

The process of fattening was noticed in the percent lipid of carcass composition. As expected the percent lipid increased. This was significantly different when comparing the first biopsy date (33.2%) and the third (36.5%). The percent moisture followed a similar pattern to that of protein, in that it decreased (P<.05) from the first date (49.8%) to the third date (47.2%).

A nested analysis was performed to determine if there was an interaction between time on feed and frame size (Table 5). The results demonstrated that each frame

Table 4. Carcass composition on a boneless basis by time on feed.[a]

	Time on Feed[b]		
	166 days	194 days	214 days
Protein	14.60 ± .37[d]	14.18 ± .08[cd]	13.88 ± .09[c]
Lipid	33.28 ± .07[c]	34.84 ± .42[cd]	36.81 ± 1.10[d]
Moisture	49.82 ± .06[c]	48.81 ± .26[cd]	47.91 ± .81[d]

[a]Values are the mean percents in each time period.

[b]Time on feed is the length of time the cattle were in the feedlot until the biopsy date.

[c,d]Values on a given line followed by a different superscript differ significantly. ($P<.05$).

Table 5. Carcass composition on a boneless basis.[a]

	Frame Size[b]								
	Small			Medium			Large		
Time on Feed (days)	166	194	214	166	194	214	166	194	214
Protein	14.32[f]	13.94[e]	12.74[d]	14.78[d]	14.32[d]	14.38[d]	14.70[d]	14.28[e]	14.51[de]
Lipid	33.50[d]	35.84[e]	40.18[f]	33.10[d]	33.57[d]	34.67[d]	33.25[d]	35.10[e]	35.59[de]
Moisture	49.75[f]	48.25[e]	45.91[d]	50.00[d]	49.60[d]	49.50[d]	49.70[d]	48.59[d]	48.32[d]

[a]Values are mean percents of all cattle at each biopsy date within frame size (±S.E.M.).

[b]Frame size was determined by USDA Feeder Grade Standards.

[c]Time on feed is the length of time the cattle were in the feedlot until the biopsy date.

[d,e,f]Values within a frame size on the same line with different superscripts differ significantly ($P<.05$)

size was different. There was an increase in the deposition of lipid; much faster in the SF cattle than was noted in the other two frame sizes. This increase was about 2% from 166 days to 214 days on feed, and about a 5% increase from 194 to 214 days. The only other sizeable increase in percent lipid was noted for the LF cattle. Lipid increased about 2% from the first time period to the third.

4.6 Fatty Acid Profile Results

The fatty acid composition of the cattle used in the study showed the major fatty acid to be oleic acid (C18:1) at 47.7%. This was followed by palmitic acid (C16:0) at 28.9% and stearic acid (C18:0) 10.0%. These three fatty acids comprised about 85% of the total fatty acid profile. Myristic (C14), myristoleic (C14:1), palmitoleic (C16:1), margaric (C17), and linoleic (C18:2) comprising the majority of the remaining 15%. Pentadecylic (C15:0) and isopalmitic (IsoC16) were also found in trace amounts. These data are in agreement with Clemens (1973) in which he reported about 80% of the fatty acid profile consisted of oleic, palmitic and stearic acids, with the remaining 20% consisting of the five fatty acids mentioned above. The only polyunsaturated fatty acid found in significant amounts was linoleic which was 1.6% of the total composition.

Fatty acid composition in relation to frame size was examined (Table 6). It was determined there was no significant difference among frame sizes. The differences between the frame sizes were small and there was no established pattern which would indicate a difference did exist.

The fatty acid composition in relation to time on feed indicated no significant difference when analyzed for major components (Table 7). However, it was noted that two of the major fatty acids, oleic and stearic, changed with time on feed. Oleic acid increased from 46.1% in the first period (166 days) to 48.6% for the 214 day period. Stearic acid showed a tendency to decrease with time on feed increasing. At 166 days on feed 11.2% was observed, whereas 9.5% was recorded for 214 days on feed.

This decrease in stearic and increase in oleic has been reported by several researchers. Link et al. (1970) found that as the cattle grew and accumulated fat in their adipose stores the percent of saturated fatty acid (stearic) decreased as the percent of monoenoic fatty acid (oleic) increased.

Table 6. Percent fatty acid of adipose tissue by frame size.[a]

	Frame Size[b]		
	Small	Medium	Large
C14	3.74 ± .16	3.66 ± .16	3.92 ± .08
C14:1	.82 ± .15	.83 ± .11	1.27 ± .08
C15	.42 ± .07	.56 ± .04	.52 ± .04
IsoC16	.04 ± .02	.07 ± .04	.07 ± .02
C16	29.71 ± .56	28.44 ± .41	28.65 ± .73
C16:1	4.46 ± .55	3.78 ± .32	5.43 ± .85
C17	1.33 ± .19	1.63 ± .25	1.22 ± .14
C18	10.70 ± 1.01	11.34 ± 1.27	9.36 ± .93
C18:1	47.57 ± 1.12	47.71 ± 1.30	47.78 ± .60
C18:2	3.40 ± 2.24	2.00 ± .12	1.78 ± .33

[a]Values are means (±S.E.M.).

[b]Frame size was determined by USDA Feeder Grade Standards.

Table 7. Percent fatty acid of adipose tissue by time on feed.[a]

	Time on Feed[b]		
	166 days	194 days	214 days
C14	3.99 ± .13	3.66 ± .07	3.67 ± .16
C14:1	.95 ± .15	.91 ± .13	1.06 ± .19
C15	.58 ± .04[d]	.49 ± .04[d]	.43 ± .07[c]
IsoC16	.12 ± .03[c]	.06 ± .02[c]	0 ± 0[d]
C16	29.81 ± .68	28.11 ± .42	28.87 ± .53
C16:1	4.40 ± .64	4.22 ± .24	5.05 ± .93
C17	1.42 ± .24	1.51 ± .18	1.23 ± .19
C18	11.22 ± 1.18	10.72 ± 1.00	9.46 ± 1.09
C18:1	46.14 ± .81	48.38 ± .82	48.54 ± 1.13
C18:2	1.37 ± .22	3.96 ± 2.14	1.85 ± .27

[a]Values are means (±S.E.M.).

[b]Time on feed is the length of time the cattle were in the feedlot until the biopsy date.

[c,d]Values on a given line followed by a different superscript differ significantly. ($P<.05$).

5. DISCUSSION

The method of cell isolation (Yang and Baldwin, 1973) utilized in the present lipolytic study gave a linear glycerol release with time. One advantage of this method is it allowed for the data to be expressed on a per cell basis instead of a wet tissue weight basis. Varying cell size does not matter with regard to the number of cells present in each incubation tube which is a factor when using the tissue slice procedure.

Attempts at relating fat cell incubation results to the live animal adipose tissue activity must be made with caution. The metabolic activity in an isolated fat cell incubation does not necessarily mean that the reaction under study occurs *in vivo* in the same manner as the *in vitro* state. This is due to other factors which are much more difficult to control in the *in vivo* state. Factors such as nutrition, age, genotype and physiological stresses have been implicated in changing the rate of lipolysis. The objective of this study was to determine if there was a change in lipolytic action due to several endocrinological, physiological and genetic changes.

In the nonruminant, epinephrine appears to be the most important physiological signal for an instantaneous

supply of energy by releasing free fatty acids and glycerol (Tepperman, 1968). Epinephrine addition to rat adipose tissue incubations resulted in a 10 to 60 fold increase in lipolysis (Rodbell, 1964; Prigge et al., 1971). These values are large in comparison to the epinephrine stimulated cattle lipolysis reported in this study (Figure 2). Addition of epinephrine to cattle adipose cells resulted in about a two fold increase in lipolysis over basal levels. This data is consistent with results reported by: Yang and Baldwin (1973), Etherton et al. (1974), Pothoven et al. (1975) and Jaster (1979). In vivo studies do not show this increase with the injection of epinephrine into the live animal. Sidhu and Emery (1971) reported even when large doses of epinephrine have been injected into cattle only small increases in plasma free fatty acid levels have been observed.

The action of epinephrine, in causing a rise in the rate of lipolysis, is the result of its ability to bind to the beta-adrenergic receptor sites on the cell membrane (Clark, 1933; Ahlquist, 1948). This causes an increase in the second messenger cyclic AMP which activates hormone sensitive lipase through a series of reaction steps as illustrated in Figure 1. The apparent difference between the lipolytic response of the ruminant versus the nonruminant might well reflect a difference in number and/or

specificity of beta-adrenergic receptors on the cell membrane. It is known that rats, and presumably other species, have individual hormone specific receptor sites (Birnbaumer and Rodbell, 1969). Alteration of the rate of lipolysis could be determined by the number of receptor sites. Indeed, the possibility exists that the ruminant could have considerably fewer receptor sites per cell than the nonruminant. This in itself could be a possible explanation for the lower epinephrine stimulated response since the potential to increase lipolysis would likely be related to the number of receptor sites. If this is true, the logical question is why are the number of receptors per cell less for the ruminant than found for the nonruminant. Bauman (1976) reported that this difference might be related to the availability of nutrients and physiology of the ruminant animal. The rumen acts as a large storage container, which results in ruminant animals absorbing a rather constant and continuous supply of nutrients relative to nonruminant species. Conversely, the nonruminant tissue experiences dramatic diurnal variations in metabolite availability, whereas, the ruminant tissue experiences this to a much lesser degree. With the consistency in energy supplies a ruminant animal would generally have a much longer period to adapt at tissue

level to many physiological situations. The nonruminant would require more rapid changes in metabolic activity to maintain homeostasis.

Insulin has been noted to be an antilipolytic agent in nonruminant tissue. It appears that this inhibition, created by insulin, is less dramatic in ruminant adipose tissue then in nonruminant tissue (Baldwin et al., 1976). Baldwin et al. (1973) reported that insulin had no significant effect on the basal lipolytic rate. The present study concurs with the findings of Baldwin et al. (1973) (Figure 2). However, there was a slightly lower mean value recorded for the insulin compared to the basal levels this, however, was not significant.

When insulin and epinephrine were combined in a treatment there was an antagonistic effect elicited between the two hormones. This was demonstrated by a 30% decrease in the release of glycerol compared to the epinephrine stimulated treatment. Similar work by Baldwin et al. (1973) supports these findings in that they observed a 10 to 25% decrease in response.

The mechanism of the antagonism of insulin on epinephrine seems to be related to the levels of the second messenger cyclic AMP. Butcher et al. (1968) reported a lowering of cyclic AMP levels by insulin in rat fat cells exposed to epinephrine. Jungas and Ball (1963) who also

noted that the rate of epinephrine stimulated response decreased with the addition of insulin, suggested that the two hormones may be acting on separate counter-balancing processes which regulate HSL activity. Epinephrine as mentioned earlier, stimulates lipolysis via interaction with the beta-adrenergic receptors thus increasing levels of cyclic AMP. Insulin's ability in reducing levels of cyclic AMP probably is caused by its function in activating 3', 5'-cyclic AMP phosphodiesterase within the cell (Senft et al., 1968). Phosphodiesterase reduces the levels of active cyclic AMP by converting it to the inactive 5' AMP form. Lower levels of cyclic AMP would have the effect of lowering levels of active HSL and thus reduce the rates of lipolysis.

Time on feed (finishing diet) and varying frame size were used in this study to determine if they had any influence on lipolytic activity. Time on feed revealed no significance on lipolysis between each time period (166 days, 194 days and 214 days). However, there was a drop in epinephrine stimulated rates at 120 minute incubations between the 194 day and 214 day periods. The drop in the stimulated rate of lipolysis could be of biological significance. Pothoven et al. (1975) noticed that as cattle grew, the rates of hormone stimulated lipolysis decreased. Whereas the basal level slightly increased. This indicates

that maturing of cattle might have an influence on the way the adipose tissue reacts to hormonal stimulus. The significance noted between the animals in Pothoven's studies might also have been realized in the present study if the period of days between tissue biopsies had been increased.

Frame size had a significant effect on the lipolytic rate. These differences possibly could be due to: efficiency (feed per unit gain), average daily gain, feed consumption or physiological age and genotype of the animal. These factors have also been noted to differ in relation to frame size (Cole et al., 1964; Hedrick et al., 1970; Adams et al., 1973; Smith et al., 1976; Koch et al., 1976). At the cellular level the ultimate cause for differences are the variation in metabolic activities within the cells. The differences noted ($P<.05$) in the lipolytic rate between cattle of varying frame sizes are probably due to these variations (Table 1).

There are a few possibilities that might explain this difference in lipolysis. These are: (1) the concentration of lipolytic enzymes, primarily HSL (rate limiting enzyme), (2) a variation in the number and/or specificity of the beta-adrenergic receptors on the cell membrane, this would alter the response to stimulus, and (3) prolonged exposure to levels of a hormone such as insulin, could

cause a lack in responsiveness to a specific hormone treatment. The possibilities above might also account for larger differences among animals (SF and LF cattle) as the incubation time increased. For example, increased numbers of receptors or increased concentration of HSL in a fat cell would show a greater difference in lipolytic activity over an extended period of time than a cell that did not have these high levels.

If the animals were managed in similar ways the genetic background would likely be the cause for the differences noted in lipolytic rates and carcass composition. The LF cattle in comparison with SF cattle would have a greater genetic potential for utilizing energy predominantly for protein deposition relative to fat deposition. The carcass composition data (Tables 3 and 5) seem to support this idea. The requirement for more energy by the LF cattle could be satisfied by two sources, they are: (1) increased feed consumption which is evident, and/or (2) the utilization of energy stores in adipose tissue. Data in Table 1 indicates that this is also the case.

Data in Table 5 indicate these differences in cattle; in that the SF cattle were depositing fat at a much faster rate than their LF and MF counterparts. This difference could be accounted for by either an increase in the rate of lipogenesis or a decrease in the rate of

lipolysis. The results in Table 2 indicated that SF cattle had a lower rate of lipolysis than the other two groups.

One of the underlying factors that may be influencing the rate of lipolysis in the different frame size cattle is the physiological maturity of the animals. The age factor has an important role in lipolytic action. Miller and Allen (1973) determined in rats that with increasing chronological age there was a decrease in lipolytic activity. This decrease they say is caused by altered cellular activity. Pothoven et al. (1975) in study, mentioned earlier, noticed a decrease in stimulated lipolytic activity in cattle as they matured. This change could also be attributed to altered cellular activity.

Studies dealing with maturing characteristics of cattle show that larger frame size animals matured at a much later chronological age than smaller size cattle (Cole et al., 1964; Adams et al., 1973; Peacock et al., 1979). Since larger frame size animals might mature later chronologically they would probably require a greater length of time in the feedlot to accumulate sufficient amounts of intramuscular fat to meet USDA grading standards for choice carcasses.

There is some evidence, obtained in this study, that would indicate that there was a difference in physiological

age between the frame size groups of cattle. Data in Table 1 demonstrate that the SF cattle had a lower stimulated lipolytic rate than the LF cattle. This could be an indication that the SF cattle may have already reached a period in their life where the stimulated lipolysis begins to decline with age. Other results may indicate that the SF cattle are older physiologically than their LF counterparts is the carcass composition data.

Hedrick (1968) reported that an increase in percent lipid and subsequent decrease in protein and moisture has been observed in the process of maturing. Table 5 revealed that the SF cattle were increasing in lipid in each consecutive time ($P<.05$) period, whereas, the LF cattle did not demonstrate this pattern. The probable cause for this pattern could be to lower the lipolytic rates of the SF cattle which, in turn, could be caused by an older physiological age.

Certainly research should continue in the area of lipolysis to more accurately extablish factors affecting it. Added knowledge of the metabolic processes of the adipose tissue may help in understanding the regulatory mechanism of lipolysis.

6. Summary

The present study was undertaken to investigate lipolysis, as influenced by hormonal treatment, in beef cattle of varying frame size. The release of glycerol was used as a measure of lipolysis. Six cattle were assigned to different frame sizes according to the USDA feeder grade standards. Subcutaneous fat biopsies were performed at three different times (166, 194 and 214 days) on cattle being fed a finishing diet.

The cells were isolated and treated with a hormone treatment and incubated for 60 and 120 minutes. Epinephrine, insulin and a combination of both were used as the hormone treatments. Lipolytic activity was compared to carcass composition of the various frame size cattle.

The epinephrine treatment produced about a two fold increase in lipolysis over control levels, regardless of frame size. The insulin treatment when compared to the control treatment, showed no significant difference. However, there was an antagonistic effect elicited by insulin upon epinephrine when the two hormones were combined. This was demonstrated when epinephrine stimulation was compared to epinephrine + insulin stimulation; there

was a 30% decrease in glycerol release in the treatment with both hormones. It is postulated that the cause for the antagonistic action of insulin on epinephrine, is due to insulin's ability to increase the concentration of active 3', 5'-cyclic AMP phosphodiesterase.

Comparison of lipolytic activity by time on feed demonstrated there was minimal effect on lipolysis as time on feed increased. However, there was a decrease of glycerol release in the epinephrine stimulated treatment, at 120 minutes between the 194 and 214 day time periods. This was not significant, but could be of some biological importance because it might give some indication that the maturing process is taking place.

Lipolytic activity was greater ($P<.05$) in the large frame (LF) cattle as compared to medium (MF) and small frame (SF) cattle. These observed differences could possibly be due to variations at the cellular level. Changes in the concentration of rate limiting enzymes, such as hormone sensitive lipase (HSL) or a difference in the specificity and/or number of receptor sites on the cell membrane could account for these cellular differences.

The chemical carcass comparison data revealed that the SF cattle were depositing fat at a much faster ($P<.05$) rate than the other two frame sizes (Table 5). The underlying factor that might cause these differences between

frame size is the physiological maturity of the animal. The carcass composition and lipolytic activity seem to indicate that the SF cattle are physiologically more mature than their LF counterparts. Continued investigations into lipolysis would increase the knowledge of lipolytic regulation in cattle.

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