Hormonal and dietary regulation of lipolysis in bovine adipose tissue

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HORMONAL AND DIETARY REGULATION OF LIPOLYSIS
IN BOVINE ADIPOSE TISSUE.
IOWA STATE UNIVERSITY, PH.D., 1979
Hormonal and dietary regulation of lipolysis in bovine adipose tissue

by

Nancy Margaret Evans DiMarco

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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Effect of exogenous prostaglandin E\textsubscript{2} on rates of lipolysis in sections of subcutaneous adipose tissue biopsied from fed and 9-day fasted Holstein steers was determined. The interaction of prostaglandin E\textsubscript{2} with exogenous effectors of lipolysis and of the adenylate cyclase-cAMP system (epinephrine, dibutyryl cAMP, and theophylline) also was measured. Rates of lipolysis were expressed as the amount of glycerol released from adipose tissue incubated for two hours. Epinephrine increased the rate of basal (nonstimulated) lipolysis by 1.9-fold. Prostaglandin E\textsubscript{2} had no effect on either basal or epinephrine-stimulated rates of lipolysis. Dibutyryl cAMP increased basal rates of lipolysis by 1.4-fold; this increase, however, was less than the stimulation observed with either epinephrine or theophylline which increased basal rates of lipolysis by more than 2-fold. Theophylline had an additive effect on epinephrine-stimulated rates of lipolysis. Dibutyryl cAMP increased theophylline-stimulated rates of lipolysis but not those of epinephrine-stimulated lipolysis. Prostaglandin E\textsubscript{2} had no effect on epinephrine-, dibutyryl cAMP-, or theophylline-stimulated rates of lipolysis. Fasting decreased the basal rate of lipolysis by 40 percent. Furthermore, rates of lipolysis in tissue incubated with PGE\textsubscript{2}, epinephrine, or PGE\textsubscript{2} plus epinephrine decreased from 30 to 50 percent because of the 9-day fast. As also shown in tissue from fed steers, PGE\textsubscript{2} did not alter basal or epinephrine-stimulated rates of lipolysis in tissue from fasted steers. These comparisons of rates of lipolysis in adipose tissue from fed and
fasted steers indicate that prostaglandin E₂ does not control the adenylate cyclase-cAMP system that regulates lipolysis in bovine adipose tissue.
INTRODUCTION

It has been stated that the amount of feed needed to produce approximately 4 billion pounds of nonconsumed fat in steers for slaughter is equivalent to nearly 1 annual corn crop of Iowa (Kauffman, 1976). Traditionally, consumers have held the view that an abundance of fat on the outside of an animal's carcass would produce the most desirable meat. Also, due to relatively cheap feed grains and their ready availability, it is economically desirable to have carcasses grade choice or prime. It seems unlikely that this practice will be changed rapidly unless one or more of the following things happen: 1) the price of feed grains in relation to other feedstuffs becomes too great, 2) the grading system of USDA quality meat undergoes a major revision, and 3) the demand by consumers for lean meat is substantially increased (Allen, 1976).

In all likelihood, it seems that the most rapid change would come about by using suggestion 3. In past years, fat was an important constituent of the American diet because of high energy requirements for most occupations. As automation has supplanted hard labor, the need for fat consumption also has decreased even though Americans still consume 40 to 45% of their calories as fat (Mattson, 1976). Although the cause and effect relationship is very clouded, many scientists are of the opinion that dietary cholesterol and dietary fat, with a greater ratio of saturated to unsaturated fats, are underlying risk factors in the development of atherosclerosis and the incidence of coronary heart disease (McGill and Mott, 1976).
Current breeding, feeding, and management practices demonstrate that the livestock industry is trying to reduce fat production in meat animals. When we consider that there are still approximately 4 billion pounds of fat being "wasted" in the United States each year, it is obvious that more and better improvements must be made in the meat industry and in human consumption of the same.

The objectives of this research were to study hormonal and dietary factors involved in regulation of lipolysis in adipose tissue of cattle. To date, much controversy surrounds this area of research, and few definite answers have arisen from research. The objectives of the studies described in this dissertation were:

1) to study the role of metabolic effectors in regulation of lipolysis in bovine adipose tissue.

2) to measure the interaction of prostaglandin E2 with other metabolic effectors.

3) to study the effects of diet and energy intake on lipolysis.

4) to measure the response of metabolic effectors during conditions of fasting and feeding.

These objectives need to be studied to understand the basic mechanism of lipolysis. The regulation of the same process may allow animal scientists to develop techniques through breeding, feeding, and management that will allow more efficient production of a highly desirable beef carcass with a greater ratio of lean to fat.
This literature review will describe the morphology and biochemistry of the fat cell and regulation of lipid metabolism in the fat cell by diet and hormones and will relate these parameters to fat deposition in the meat animal.

Characterization of Adipose Tissue

**Components of adipose tissue**

Kauffman's definition of fat states that "fat is the collection of adipose cells suspended in a layer of connective tissue matrix that are distended with cytoplasmic lipids, water, and other constituents" (1976). The other constituents are collagen fibers, fibrocytes, histocytes, mast cells, and unmyelinated nerve fibers (Wasserman, 1965). In meat animals, white fat constitutes the major type, although brown fat does exist in young and especially hibernating animals. Brown fat is characterized by its higher concentration of mitochondria and its high rate of respiration (Fain and Reed, 1970).

As the adipocyte matures, it is believed to acquire a predetermined size and volume dependent upon its anatomical location, age, species, and state of nutrition (Kauffman, 1976). In the bovine species, the largest intramuscular adipocytes are 170 μm in diameter while those from subcutaneous or perirenal depots in the same animal are larger (~200 to 220 μm in diameter) (Allen, 1976). Waters (1909) stated that bovine inter- and intramuscular adipocytes are always smaller in diameter than subcutaneous or perirenal due to mechanical pressure exerted by the
surrounding muscle mass. He concluded that this mechanical pressure prevents the expansion of adipocytes in these depots.

Mature white fat cells, also called unilocular cells, have an unusual characteristic feature which is the large central lipid droplet (Napolitano, 1965; Wasserman, 1965; Galton, 1971). The central lipid inclusion is completely surrounded by a thin rim of cytoplasm (Galton, 1971). This central lipid inclusion dwarfs the other cellular components and pushes them adjacent to the cell membrane (Napolitano, 1965; Wasserman, 1965; Galton, 1971). The lipid droplet is not, however, enclosed by a membrane. The plasma membrane surrounds the cytoplasm while the plasma membrane is enclosed further by the basement membrane. Close to the surface of the basement membrane are capillaries. In nearly all cases, white fat cells are separated by their own basement membranes and small collagen bundles which provide support (Napolitano, 1965; Galton, 1971).

Other components of adipose tissue include neural elements, which are important in regulating metabolism of adipose tissue, heparin-containing mast cells, which may play some role in assimilation of lipid brought to adipose tissue by the circulation, and the capillaries of the circulatory system, which are necessary for all aspects of fat cell metabolism (Napolitano, 1965; Wasserman, 1965; Galton, 1971).

Development of adipose tissue

Although the actual development of adipose tissue is unclear, many theories have been advanced to explain this mechanism. Currently, three hypotheses may be considered: 1) adipose cells are fibroblasts which accumulate excess lipid, 2) adipose tissue develops from lipoblasts which
differentiate in early embryonic life, or 3) adipose cells develop initially from a mesenchymal cell which differentiates into a fibroblast that, in turn, differentiates into a preadipocyte. The preadipocytes are transformed into lobular epithelial-type cells that develop into either brown adipose tissue or white adipose tissue directly (Bloom and Fawcett, 1968).

Growth of adipose tissue in early postnatal development is due to both cellular hypertrophy and hyperplasia (Hood, 1977). The age at which animals lose the capacity to acquire new adipocytes is still a matter of controversy. In cattle, by 14 months of life, hyperplasia is complete in all but intermuscular adipose tissue (Hood and Allen, 1973; Allen, 1976). The first adipocytes that form are always adjacent to blood vessels with the exceptions of perirenal and omental fat depots (Allen, 1976). Allen (1976) also has provided some insight into the problem of why in cattle the fat accretion curve does not follow the body protein accretion curve. He suggests one of two possibilities and also an unlikely third: 1) a large number of preadipocytes form during the initial proliferative phase but do not all differentiate and accumulate lipid at the same time; 2) a new population of adipocytes can be made during the fattening period by reinitiation of adipocyte hyperplasia; 3) the size of the adipocyte is without limits. Adipocyte hyperplasia is complete in subcutaneous and perirenal depots but not intermuscular adipose tissue in steers fed to approximately 470 kg live weight or about 14 months of age (Allen, 1976; Hood, 1977). Allen (1976) also has shown that in very fat cattle there exists a biphasic distribution of sizes of adipocytes that includes many small adipocytes (less than 25 μm). Further increases in diameter or
volume of adipocytes are slight in subcutaneous or intermuscular depots as they enlarge beyond some set point. It is at this stage that the biphasic diameter distribution becomes obvious as the population of small adipocytes increases. This smaller population may be due to either differentiation of preadipocytes followed by lipid filling or reinitiation of hyperplasia. Both may help explain the fat accretion curve.

Holstein steers, in general, have less dissectable subcutaneous fat from a standard 9-10-11th rib than Hereford X Angus steers of an equivalent age and live weight. Also, cellular hyperplasia of adipose tissue may be complete in the Holstein before 14 months of age. The total cell number (roughly 70 to 120 X 10^9 cells/carcass) is highly correlated to depot size in Holsteins. Both cell size and cell number are significantly smaller in Holstein steers than in Hereford X Angus steers (Hood and Allen, 1973).

The development of adipose tissue in humans is still unclear and many questions remain. Contrary to past popular opinion, the adipocyte number may not remain fixed in the adult (Ng et al., 1971; Ashwell and Garrow, 1973; Widdowson and Dauncey, 1976). Few studies have been conducted on development of adipose tissue in early life of the human. Dauncey and Gairdner (1975) showed that the adipose cell size increases steadily from 3 months before birth to 3 months after birth. The mean cell diameter increased from 40 to 90 μm during this 6-month period. Adipocyte size continues to increase to a diameter of 100 to 130 μm between the age of 6 and 9 months. By the time the infant is 1 year old, the size of the adipocyte appears to remain constant throughout childhood of normal body weight children (Hirsch and Knittle, 1970).
At certain times fat is deposited in the life of the human. During the second half of gestation, a large portion of fat is deposited in the fetus, increasing the percentage of body fat from 0.5 to 5.0% at 20 weeks of gestation and further to 16% at term. At 6 months of age, the infant has attained 26% body fat. By 3 years of age, the fat percentage has decreased to 20%. In girls, from the age of 8 years until the end of puberty, there is normally a gradual increase in subcutaneous adipose tissue. In boys, a preadolescent increase typically occurs between the ages of 10 and 12 years followed by another rise during puberty (Widdowson and Dauncey, 1976).

Knittle and Hirsch (1968) have shown that the number of fat cells can be decreased in the rat by deprivation during early growth before weaning but whether the same holds true for humans is not known. These experiments suggest that critical periods exist when nutritional influences can cause permanent changes in adipose cellularity. The newborn human infant has 20 to 25% of the adult number of fat cells and 25% of the adult amount of lipid (Hirsch, 1972). Hirsch further stated that if nutritional influences can most affect the ultimate adipocyte number when adipocytes are first appearing, then the critical periods of development in man are likely to be the last months of gestation, very early in life, and during adolescence.

Number of fat cells in adipose tissue of the domestic pig and of the Hormel mini-pig plateaus between 5 and 6 months of age; increases in adipose depots beyond this age are due to cell enlargement only (Anderson and Kauffman, 1973; Hood and Allen, 1977). Mersmann et al. (1975) have observed that at birth all adipocytes were multilocular but by day 3 most
of the multilocular cells had differentiated to the unilocular (one major, central lipid droplet) state. Until 160 days after birth, small cells were observed, giving a biphasic diameter distribution.

Adipose tissue development of four depots in the rabbit has been observed by Nougues (1975). After 70 days of age, growth of the neck depot occurred mainly by cell enlargement. The perirenal deposit also could increase in mass by this mechanism, but its increases seemed more likely to occur by maintenance of a long-term cellular multiplication which was completed by 180 days of age. Finally, increases in weight of epididymal and subcutaneous depots are caused primarily by cellular hypertrophy. At 300 days of age, differences of average adipocyte size in these depots were negligible.

In the rat, hyperplasia continues until 15 weeks of age, and afterward no increase in adipose cell number is observed (Hirsch and Han, 1969). However, in the genetically obese Zucker rat, adipocyte number increases until the 26th week of age (Hirsch and Batchelor, 1976).

Adipose cell number in the sheep increases up to about 12 months of life; the increase in quantity of adipose tissue is caused by both cellular hypertrophy and hyperplasia. After 12 months of age, there is no significant increase in fat cell number (Hood, 1977).

**Location of adipose tissue**

Mammals develop adipose tissue in specific locations which Vague and Fenasse (1965) divided into two general areas, deep-seated and subcutaneous. Perirenal, omental, and mesenteric depots comprise the deep-seated adipose tissues that are found in the abdominal and thoracic
cavities, bone marrow, head, neck, and limbs; the subcutaneous depots are found between the skin and the underlying fascia (Vague and Fenasse, 1965). Some of these mentioned depots have a greater ability to accumulate fat cells than others. The ability to accumulate more fat cells is determined by circulation; activity of endogenous enzymes; physical activity of animal, neural, and hormonal factors; and number of fat cells present (Vague and Fenasse, 1965).

The fatty acid composition of lipid in the various anatomical locations is distinct. Kauffman (1976) points out that in the 3 subcutaneous backfat layers of swine, the outer layer contains the greatest amount of unsaturated fatty acids in comparison to inner and middle subcutaneous layers. Concentration of saturated fatty acids in perirenal adipose tissue is greater than that in subcutaneous layers.

**Functions of adipose tissue**

The primary metabolic function of the fat cell of adipose tissue is storage of triglycerides. This function includes 2 important processes: 1) transport of triglyceride precursors and triglyceride catabolites across the plasma membrane; transported compounds consist mainly of entry of glucose, acetate, and fatty acids into the cell during triglyceride synthesis and exit of glycerol and free fatty acids during lipolysis, and 2) conversion of precursors into triglycerides and the enzymatic hydrolysis of stored triglycerides to glycerol and fatty acids (Galton, 1971).

The physical functions of adipose tissue include shock absorption (e.g. hands and soles of feet of humans), insulation, provided by
subcutaneous adipose tissue, and heat generation (as observed in brown fat of hibernating or young, immature animals) (Dole, 1965).

Metabolism of Adipose Tissue

Lipid metabolism, especially triglyceride hydrolysis, will be emphasized in this section, but many other processes take place in adipose tissue. The adipose cell contains all the constituents of other "typical" mammalian cells such as mitochondria, Golgi apparatus, and enzyme systems to conduct protein synthesis (Herrera and Renold, 1965), glycogen synthesis and degradation (Shafrir et al., 1965), β-oxidation of fatty acids and glycolysis (Flatt and Ball, 1965), and oxidative processes in the hexose monophosphate (HMP) pathway and tricarboxylic acid (TCA) cycle (Weber et al., 1965). This section will discuss the primary metabolic pathways of lipids that take place in fat cells of adipose tissue such as fatty acid synthesis assimilation of plasma triglycerides, triglyceride synthesis, and triglyceride hydrolysis.

Fatty acid synthesis

Species differences are the most striking feature of the process of fatty acid synthesis. In nonlactating animals, adipose tissue and liver are the principal sites of fatty acid synthesis. Ingle et al. (1972) have shown that adipose tissue is the major tissue involved in conversion of acetate into storage fats in the nonlactating ruminant. Swine are similar to cattle and sheep in the tissue site of fatty acid synthesis while humans and avian species rely upon the liver to synthesize triglycerides, using the adipose tissue as a storage organ for fats synthesized in liver.
In lactating ruminants, however, the mammary gland becomes the major site of lipogenesis (Bauman and Davis, 1974).

The major carbon precursor for lipogenesis in ruminants is acetate from rumen fermentation while the major carbon precursor for lipogenesis in nonruminants is glucose (Hanson and Ballard, 1967). A deficiency of ATP:citrate oxaloacetate lyase (E.C. 4.1.3.8), NADP-malate dehydrogenase (E.C. 1.1.1.40), and pyruvate carboxylase (pyruvate:carbon dioxide ligase (ADP), E.C. 6.4.1.1) in ruminant adipose tissue prevents utilization of glucose as a substrate for lipogenesis (Hanson and Ballard, 1967). Because ruminants absorb little glucose from the digestive tract, the inability to use glucose for synthesis of fatty acids is advantageous from a glucose homeostasis point of view.

Although the controlling factors of lipogenesis are not fully understood in ruminants, most recent research indicates that acetyl CoA carboxylase (acetyl CoA:carbon dioxide ligase (ADP), E.C. 6.4.1.2) is the key regulatory enzyme modulating fatty acid synthesis (Ingle et al., 1972). Factors that regulate the activity of this enzyme in ruminants remain to be determined but probably involve cytosolic citrate as an allosteric activator.

Assimilation of plasma triglycerides

Blood triglycerides are a second source of fatty acids available to adipose tissue for fat deposition. Triglycerides are transported in blood plasma as lipoproteins of varying composition. Blood triglycerides circulating primarily as chylomicra are formed in the intestinal mucosa following ingestion of dietary fatty acids. The endogenous sources of
blood triglycerides are the very low density lipoproteins (VLDL) which originate principally in the liver and are then transported to other tissues for storage (Robinson, 1970). The triglyceride fatty acids of both chylomicra and VLDL are removed rapidly from the blood. The half-life of chylomicron and VLDL triglyceride fatty acids is only a few minutes (Robinson, 1970). The chylomicra and VLDL are only 2 classes of the circulating lipoproteins. Also present are the low density lipoproteins (LDL), which are thought to consist of a hydrophobic core of triglycerides and cholesterol esters and surrounded by a coat of protein capable of interacting with phospholipid-cholesterol complexes. The other type of plasma lipoproteins is the high density lipoproteins (HDL) that are much smaller than the LDL and are thought to consist of pseudomolecular aggregates having a more definite quaternary structure. The liver synthesizes HDL which is responsible for the transport of cholesterol esters and phospholipids after their release from chylomicrons and VLDL. In the extracellular portion of muscle and adipose tissue, triglycerides of chylomicra and VLDL are hydrolyzed by lipoprotein lipase (diacylglycerol acylhydrolase, E.C. 3.1.1.34), which removes triglycerides from the core of these lipoproteins. They are further degraded by lecithin:cholesterol acyltransferase (E.C. 2.3.1.43), which catalyzes the transfer of a fatty acid from lecithin to cholesterol to form a cholesterol ester which may reenter the core of the particle or be absorbed by muscle or fat cells. The result of the loss of triglyceride and cholesterol esterification from chylomicra and VLDL is LDL formation (Newsholme and Start, 1973). Extracellular hydrolysis of lipoprotein triglycerides, followed by reutilization of the liberated fatty acids,
occurring in muscle and adipose tissue, is an extremely rapid process that is compatible in dealing with normal rates of entry of VLDL triglycerides into blood. This process of hydrolysis is catalyzed by lipoprotein lipase at the capillary endothelial cell surface. Adipose tissue and the body musculature are the major sites of extrahepatic removal of triglycerides in the fed animal (Robinson, 1970).

The role of lipoprotein lipase in the transport of plasma triglycerides has been illustrated by Robinson et al. (1975). Chylomicra and VLDL are thought to be sequestered by the enzyme at the lumen surface of the capillary endothelial cell in extrahepatic tissue. Hydrolysis then occurs at this site, followed by uptake of free fatty acids into the tissue.

**Characteristics of lipoprotein lipase** The enzyme responsible for regulating hydrolysis of plasma triglycerides is known as clearing factor lipase, heparin-induced lipase, or lipoprotein lipase (Robinson and Wing, 1970). It is present in adipose tissue, heart, skeletal muscle, lactating mammary gland, spleen, lung, kidney medulla, and diaphragm but not brain (Desnuelle, 1972). Liver, likewise, has a lipoprotein lipase but the characteristics used by most investigators to identify lipoprotein lipase are not apparent in liver: 1) activation by heparin, 2) activation by a protein cofactor in serum (the apolipoprotein of serum responsible for activation is apoLP-GLU, or CII; the other C peptides, CI (apoLP-SER) and CIII (apoLP-ALA), have been reported to both activate and inhibit lipoprotein lipase activity) (Ekman and Nilsson-Ehle, 1975), and 3) inhibition by sodium chloride, protamine, and pyrophosphate (LaRosa et al., 1972; Fielding and Fielding, 1976).
Lipoprotein lipase has a pH optimum of 8 to 9, a temperature optimum of 37°, and a molecular weight of 72,600 daltons; its natural substrates are chylomicra and VLDL (Fielding, 1969). Albumin is the free fatty acid acceptor for triglyceride hydrolysis by lipoprotein lipase in vivo and is generally used as the acceptor for free fatty acids in the in vitro enzyme assay (Whayne and Felts, 1972).

**Mechanism of action of lipoprotein lipase** In 1971, Robinson accurately proposed two forms of lipoprotein lipase in adipose tissue, one present in a stable state and the other in an unstable state. Schotz and Garfinkel (1972) demonstrated two forms of lipoprotein lipase that differed in size and response to heparin. They designated the two forms LPL_a and LPL_b and theorized that one might be the precursor of the other. LPL_a was stimulated by heparin whereas LPL_b was not. They hypothesized that LPL_b was synthesized in the adipose cell, converted to LPL_a, and transported to the capillary wall where it can be released by heparin into circulation. LPL_b seems to comprise a larger portion of the basal activity of the enzyme. Schotz and Garfinkel (1972) showed that an increase in the activity of at least one and possibly both species is protein synthesis-dependent. They suggested that LPL_b is probably located in the adipocyte, and its activity is dependent on new protein synthesis. Desnuelle (1972) observed that a direct interaction between lipoprotein lipase and heparin leads to release of the enzyme while at the same time stabilizing it. It has been suggested that heparin or a heparin-like substance is a component or an allosteric modifier of lipoprotein lipase (Garfinkel and Schotz, 1973). Schotz and Garfinkel (1972) further showed that the enzyme activity varies with the
nutritional state of the animal, the activity being decreased with fasting.

Lipoprotein lipase catalyzes the initial hydrolysis of triglycerides but final hydrolysis may be completed by action of monoglyceride esterase (E.C. 3.1.1.23) that is thought to be part of LPLα activity (Nilsson-Ehle et al., 1974). Lipoprotein lipase has specificity for the 1 (3) position of triglycerides (Horley and Kuksis, 1972; Paltauf and Wagner, 1976). The triglyceride is hydrolyzed to 1 (2), 2 (3) diglycerides. The 1 (2), 2 (3) diglycerides are split to 2-monoglycerides. These compounds then isomerize to the 1 (3) isomer. The isomerization step is nonenzymatic and rate limiting. The 1 (3) monoglyceride then is hydrolyzed yielding glycerol and a free fatty acid (Nilsson-Ehle et al., 1974).

Regulation of lipoprotein lipase The activity of lipoprotein lipase varies with changes in nutritional and physiological conditions (Garfinkel and Schotz, 1973). During times of dietary energy excess, the enzyme activity is high in adipose tissue but low in muscle; during energy deficit conditions, opposite tissue relations exist (Robinson et al., 1975).

The regulation of this enzyme is achieved mainly by hormonal control. Lipoprotein lipase in epididymal adipose tissue of fasted rats increases rapidly in vivo after intramuscular injections of insulin. Cycloheximide, however, abolishes lipoprotein lipase activity increases due to insulin (Garfinkel et al., 1976). The major site of regulation of lipoprotein lipase activity appears to be the secretion of the enzyme by the adipocyte because the early effect of insulin on adipose tissue is to increase the extracellular lipoprotein lipase activity.
Besides insulin, lipoprotein lipase activity is increased by glucose or dibutyryl cyclic AMP but is inhibited by epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), glucagon, or thyroid stimulating hormone (TSH). These effects are opposite to those observed with hormone-sensitive lipase, which will be discussed in a section to follow (Robinson, 1971).

The current model explaining the control of lipoprotein lipase in adipose tissue has been proposed by Robinson et al. (1975). Previously, an increase in activity of the enzyme in adipose tissue from fed rats that had been fasted probably involves an increase in the proportion of LPL_B or the lower molecular weight form (Schotz and Garfinkel, 1972; Garfinkel and Schotz, 1973). This result suggests that LPL_B is under hormonal control because this increase can be duplicated by insulin and inhibited by epinephrine (Davies et al., 1974).

Robinson et al. (1975) suggested that LPL_B exists in two forms which have similar molecular weights and have designated them b and b'. They further postulated that form b, but not b', is capable of associating with heparin or a heparin-like compound. This heparin binding potentiates a polymerization of form b to form a and commits form a to be exported from the cell. Thus, regulation of LPL activity could occur at the site of synthesis of LPL_B, the interconversion of LPL_B and LPL_B', the polymerization of LPL_B', or the transport of LPL_a to the receptor site on the capillary wall.
Triglyceride synthesis

There are two major pathways in animals for the biosynthesis of triglycerides. In the first, triglycerides are synthesized completely from their simplest components, activated fatty acids and L-glycerol-3-phosphate; free glycerol is not acylated (Marinetti, 1970). In the second, partial glycerides are reacylated (Gurr and James, 1975). The former pathway is referred to as the α-glycerol phosphate pathway while the latter is known as the monoglyceride pathway.

In the α-glycerol phosphate pathway, α-glycerol phosphate is derived from two different sources. Its normal precursor is dihydroxyacetone phosphate (DHAP), the product of the aldolase (fructose-1,6-diphosphate-D-glyceraldehyde-3-phosphate lyase, E.C. 4.1.2.13) reaction of glycolysis. Dihydroxyacetone phosphate which results from glyceraldehyde-3-phosphate isomerization is reduced by NADH to form L-glycerol-3-phosphate. This reaction occurs in the cytosol (Lehninger, 1975). It also may be formed from free glycerol in a reaction catalyzed by glycerokinase (adenosine triphosphate:glycerol phosphotransferase, E.C. 2.7.1.30) and using ATP. This reaction, however, is limited in adipose tissue due to the extremely low activity of glycerokinase (Ryall and Goldrick, 1977). Significant activity has, however, been observed in adipose tissue of rats, mice, domestic chickens, pigs, and humans (Ryall and Goldrick, 1977). In this pathway, two molecules of fatty acyl CoA are condensed with α-glycerol phosphate by α-glycerol phosphate acyltransferase (E.C. 2.3.1.15) to yield first a lysophosphatidate and then a phosphatidate. Phosphatidate then undergoes hydrolsis to form a diacylglycerol; this reaction is catalyzed by phosphatidate
phosphatase (E.C. 3.1.3.4). A third fatty acyl CoA is condensed with the
diacylglycerol to yield a triacylglycerol by the action of diacylglycerol
acyltransferase (E.C. 2.3.1.20) (Shapiro, 1965; Marinetti, 1970).

The second pathway, the monoglyceride pathway, is not considered
important in adipose tissue (Shapiro, 1965; Marinetti, 1970). Schultz
and Johnston (1971), however, reported that hamster adipose tissue was
capable of using the monoglyceride pathway for triglyceride synthesis.
They reported the greatest amount of activity in the microsomal fraction
and that, in overall ability to synthesize triglycerides in whole cells,
it was similar to the α-glycerol phosphate pathway. The physiological
significance of the monoglyceride pathway probably is limited because of
low supply of monoglycerides.

The concentrations of α-glycerol phosphate and long chain fatty acyl
CoA may be critical in determining the rate of triglyceride synthesis.
However, when additions of insulin, epinephrine, fatty acids, and acetate
were made to tissues actively synthesizing triglycerides, poor correla-
tions were found between changes in rate of triglyceride synthesis and
tissue concentrations of α-glycerol phosphate and fatty acyl CoA (Denton
and Martin, 1970). As yet, unrecognized factors regulate this pathway.

**Lipolysis**

The fourth process that alters lipid storage in adipose tissue is the
enzymatic hydrolysis of stored triglycerides or the process known as
lipolysis (Gurr and James, 1975). The major purpose of this section is to
review some of the most recent research and controversy concerning tri-
glyceride hydrolysis in adipose tissue.
Fatty acid transport cycle

The major metabolic substrates for animals during fasting are fatty acids. Even though heart and skeletal muscle oxidize endogenous fatty acids under fed conditions, during a prolonged fast the primary energy source for these tissues is provided by fatty acids mobilized from adipose tissue (Steinberg and Khoo, 1977).

Once the fatty acids are mobilized from adipose tissue, several fates are possible. The liver serves as a major site for uptake of plasma free fatty acids; they also are taken up rapidly from the blood by a number of other tissues. The rate of uptake appears to increase as the plasma FFAs:albumin molar ratio increases (Masoro, 1977). Approximately 30% of the free fatty acids are cleared from blood upon first passage through the liver of ad libitum-fed rats (Havel et al., 1970). Complete oxidation to CO₂ is the fate of 30 to 50% of the fatty acids assimilated by liver. About 30% of the absorbed fatty acids are converted to ketone bodies and oxidized elsewhere, which is a process of indirect utilization of mobilized fatty acids from adipose tissue. The remainder of the fatty acids absorbed by the liver are converted to cholesterol esters, phospholipids, and triglycerides; a major fraction of these products is used in synthesis of VLDL which is then transported out of the liver (Havel et al., 1970; Robinson, 1970). This closes the cycle as fatty acids incorporated into lipoproteins may be absorbed by adipose tissue that had liberated them. This ability to cyclically transport fatty acids into and out of adipose tissue may be an important process in fatty acid homeostasis (Steinberg, 1964).

Lipolysis is under fine metabolic control but there is little or no evidence that the rest of the fatty acid transport cycle is under any
type of regulation. The evidence for this statement is that fatty acids can be mobilized rapidly from adipose tissue and sometimes this occurs in excess which may exceed the ability of the liver to metabolize fatty acids resulting in the development of fatty liver (Steinberg and Khoo, 1977). The liver usually handles excess fatty acids by synthesizing VLDL. The rate of secretion of VLDL-triglyceride is directly correlated to the plasma free fatty acid concentration so that as the fatty acid concentration increases, so does the rate of secretion of VLDL (Masoro, 1977).

Inputs and outputs of adipose tissue

In adipose tissue metabolism is provided by uptake of acetate, amino acids, glucose, ketone bodies, and lactate as well as lipoprotein triglyceride primarily in VLDL. As discussed earlier, lipoprotein lipase facilitates uptake of VLDL-triglyceride and is distinct from the enzyme involved in lipolysis, hormone-sensitive lipase (Scow and Chernick, 1970; Masoro, 1977; Steinberg and Khoo, 1977).

Outputs from adipose tissue occur exclusively by liberation of free fatty acids and glycerol and release of CO₂ from oxidative metabolism. There is no evidence for mobilization of glycerides (Scow and Chernick, 1970). It generally is believed that hormone-sensitive lipase is the rate limiting enzyme of triglyceride hydrolysis and, therefore, the rate of mobilization is limited by the rate at which triglycerides are hydrolyzed. This is based on the finding that in many different types of adipose tissue preparations (in vivo, isolated fat cells, and tissue slices), diglycerides and monoglycerides do not accumulate when free fatty acids are mobilized. Additionally, diglyceride and monoglyceride lipase activities are 3 to 20 times greater than triglyceride lipase
activity in adipose tissue (Scow and Chernick, 1970). There is evidence that under conditions of maximum stimulation by lipolytic hormones, diglycerides have been found in adipose tissue in vivo and in perfused isolated tissue indicating that, in extreme situations, diglyceride lipase may be rate limiting (Scow and Chernick, 1970). Normally, triglycerides in adipose cells exist in a dynamic state, being constantly synthesized and hydrolyzed.

Free fatty acids are released from adipose tissue subject to substrate level regulation. Increased glucose availability causes increased free fatty acid reesterification and decreased mobilization by providing α-glycerol phosphate and fatty acyl CoA as precursors of triglyceride synthesis (Steinberg and Khoo, 1977). In vitro, mobilization of free fatty acid is influenced by a bewildering number of hormones and metabolic effectors (Figure 1). They include the catecholamines, ACTH, glucagon, TSH, growth hormone, glucocorticoids, thyroxine, insulin, prostaglandins, nicotinic acid, and methyl xanthines. It is difficult to determine which of these lipolytic stimulators are important in vivo except perhaps the catecholamines. The catecholamines act by binding a β-receptor on the surface of the adipocyte; this binding leads to increased adenylate cyclase (ATP pyrophosphatase, cyclizing, E.C. 4.6.1.1) activity that, in turn, increases intracellular cyclic AMP (cAMP) concentration resulting in the activation of hormone-sensitive lipase (Masoro, 1977). In avian species, glucagon is probably the physiologically important lipolytic hormone (Boyd et al., 1975; Malgieri et al., 1975).
Figure 1. Metabolic effectors of lipolysis
(Putcher, 1970)
effectors

Catecholamines
B-adrenergic blockers
ACTH
Glucagon
TSH
etc.
Growth hormone
Glucocorticoids
Thyroxine
Insulin
Prostaglandins
Nicotinic acid
etc.
methyl xanthines

extracellular

intracellular

ATP

ADENYL CYCLASE

Mg+2

cAMP

inactive PROTEIN KINASE

active PROTEIN KINASE

inactive LIPASE

active LIPASE

Triglyceride

FFA+diglyceride

2FFA+glycerol

PHOSPHODIESTERASE

5'AMP

Mg+2
Insulin is the principal hormone inhibiting lipolysis; inhibition is due, in part, to stimulation of glucose uptake and free fatty acid reesterification by insulin. In addition, insulin prevents activation of hormone-sensitive lipase via the cAMP mechanism (Steinberg and Khoo, 1977). It also has been proposed that insulin decreases cAMP levels by increasing the activity of phosphodiesterase but this does not seem to be the principal mode of action of insulin inhibition (Masoro, 1977). Again, lipolysis in avian species is unaffected by insulin; it may even stimulate free fatty acid mobilization (Steinberg and Khoo, 1977). Prostaglandins may act as antilipolytic agents, but their physiologic role remains unclear. They will be discussed in detail in a section to follow.

**Mechanism of lipolysis**

**Membrane receptors**

Hormones that either promote or prevent lipolysis interact first with the plasma membrane and most likely with hormone-specific receptor proteins (Siddle and Hales, 1975). It has become clear that the fat cell possesses more than one type of receptor for a given hormone. Activation of adenylate cyclase with subsequent stimulation of lipolysis by physiological concentrations of catecholamines involves β-adrenergic receptors (Robison et al., 1971). Arner and Ostman (1976) presented conclusive evidence that both α- and β-adrenergic receptors for catecholamines are present in adipose tissue of man and hamster; rat, swine, dog, and guinea pig have only β-sites. In human adipose tissue, stimulation of α-adrenergic sites inhibits lipolysis probably because there is a concomitant decrease in the production of cAMP (Reckless et al., 1976) whereas stimulation of β-receptors accelerates lipolysis due to an increase in the intracellular concentration of cAMP (Arner and
Ostman, 1976; Arner, 1976; Masoro, 1977). Arner (1976) and others (Fain and Rosenberg, 1972; Siddle and Hales, 1975), however, were unable to correlate the rate of lipolysis and tissue concentration of cAMP under basal conditions. The significance of α-adrenergic effects on adipose tissue at physiological concentrations of catecholamines remains uncertain.

Studies of insulin binding to adipocytes have produced conflicting results as to number and affinity of receptor sites and exactly how insulin mediates its effects. Crofford (1975) has theorized that formation and dissociation of the insulin-receptor complex is a slow process relative to the other steps involved in glucose transport and that receptor occupancy is the primary determinant of the overall rate of glucose metabolism in adipose tissue. He has observed a half-life of 14 minutes for the dissociation of the insulin-receptor complex at 37°. Smith et al. (1976) have shown that the long-term effect of insulin on adipose tissue is lipolytic while the short-term effect (2 hours or less) may be antilipolytic.

Metabolic studies using increasing concentrations of insulin have shown a reversal of its effects on lipolysis (Desai et al., 1973; Lambert et al., 1976). Desai et al. (1973) have shown that insulin is antilipolytic at decreased concentrations of norepinephrine and lipolytic at increased concentrations of norepinephrine. They termed these effects the bimodal effect of insulin, which supposedly correlates positively with changes in maximal intracellular concentrations of cAMP. Further suggestions are that the antilipolytic effect of insulin is mediated by inhibition of adenylate cyclase and increased activity of membrane-bound,
low $K_m$ cAMP-phosphodiesterase (3',5'-cyclic AMP phosphodiesterase, E.C. 3.1.4.17) (Desai et al., 1973; Solomon, 1975; Malbon and Fain, 1977). Kono et al. (1977) observed that ATP or some other compound metabolically related to ATP may be necessary for the actions of insulin on glucose transport and phosphodiesterase.

Cyclic AMP is still the most likely candidate, among the several possible, that may be involved in the transmission of the insulin signal from the plasma membrane to the interior of the fat cell (Haring et al., 1976), although cyclic GMP (cGMP) and Ca$^{++}$ are being considered. Fain and Butcher (1976) concluded that, although the addition of insulin to rat fat cells resulted in variable but consistent elevations of cGMP concentration, the physiological significance of cGMP in isolated white fat cells is minimal with respect to regulation of lipolysis. As far as Ca$^{++}$ is concerned, Siddle and Hales (1974) showed that with the addition of local anesthetics, the inhibitory effect observed on hormone-sensitive lipase supported their idea that changes in the Ca$^{++}$ concentration, in addition to the cAMP concentration, may be important in mediating insulin action. McDonald et al. (1976a,b,c) observed two distinct classes of binding sites on the adipocyte plasma membrane for Ca$^{++}$, high and low affinities, with affinity constants of $4.5 \times 10^4$ M$^{-1}$ and $2.0 \times 10^3$ M$^{-1}$, respectively. The high affinity site has a pH optimum of 7.0, while the low affinity site has a broad pH range optimum of 6.0 to 9.0. Also, increasing the concentration of insulin to 100 µU/ml, the capacity of the plasma membrane to bind Ca$^{++}$ also increased. They concluded that Ca$^{++}$ is the possible second messenger for insulin action. Indirect evidence supporting this has included the identification of intracellular
enzymes which are sensitive to both insulin and Ca^{++}. Calcium exists in at least two forms in cellular organelles, including ionized pools and stable or complexed pools as observed in the adipocyte plasma membrane. Insulin is capable of altering a sizable portion of the stable Ca^{++} pool into the exchangeable pool. All of the above studies have raised doubts about the mechanism by which insulin can influence the activity of cAMP and whether or not cAMP definitely is involved in antilipolytic action of insulin.

Cyclic AMP concentration

Cyclic AMP is synthesized and hydrolyzed by adenylate cyclase and low K_m cAMP-phosphodiesterase, respectively, which are both membrane-bound enzymes. It is an established fact that the catecholamines stimulate adenylate cyclase, which results in increased cAMP concentration (Robison et al., 1971). As shown, insulin is known to inhibit adenylate cyclase and stimulate phosphodiesterase (Desai et al., 1973; Solomon, 1975; Malbon and Fain, 1977).

After hormonal stimulation of isolated fat cells, increases in intracellular cAMP content are transient, while the effects of this nucleotide are sustained (Siddle and Hales, 1975; Arner, 1976; Birnbaum and Goodman, 1977). The decline in intracellular cAMP content after its initial peak may be attributed to a number of factors. Schimmel (1974) noted that following stimulation of rat epididymal adipose tissue with epinephrine, cAMP content increased to maximal values within 5 minutes. Thereafter, cAMP concentration declined reaching values which were not significantly different from prestimulatory concentrations. The anticipated rise in cAMP following a second stimulation by epinephrine was not observed. This refractoriness was observed with epinephrine and ACTH.
but not with theophylline, suggesting that the cAMP concentration increase resulted from activation of adenylate cyclase. In hypophysectomized rats, Birnbaum and Goodman (1977) observed that this spike in cAMP content after hormone stimulation may be the trigger to activate lipolysis but is not sufficient to sustain the process of triglyceride hydrolysis. They suggest that continued activation of protein kinase (E.C. 2.7.1.37) may be essential for sustained lipolysis.

The fall in cAMP content may be related to a feedback regulator of adenylate cyclase as suggested by Fain and Shepherd (1975). Addition of lipolytic agents to isolated rat fat cells resulted in the increase of free fatty acids into the medium, which blocked the stimulation of cAMP accumulation. The authors concluded that when the primary binding sites on albumin are saturated during lipolysis, the free fatty acids bound to the secondary sites are inhibitors of adenylate cyclase activity.

Fain and Wieser (1975) also showed that adenosine is an important modulator of hormone action in fat cells because it inhibits cAMP accumulation. Fredholm and Sollevi (1977) showed that adenosine is released from isolated rat fat cells and inhibits cAMP accumulation but had no significant effects on basal rate of glycerol release. In another report, Wieser and Fain (1975) showed that by adding a catabolic enzyme of adenosine, adenosine deaminase, an increase in cAMP content of incubated fat cells was noted, and the rate of lipolysis increased. They concluded, however, that the effect of adenosine on lipolysis is secondary to the inhibition of cAMP accumulation. Neither uptake of adenosine by fat cells nor its incorporation into intracellular ATP is required for inhibition of cAMP in fat cells. These observed effects, which decrease cAMP content
after an initial peak, are quite distinct from the cAMP-lowering and antilipolytic actions of insulin.

The relationship between cAMP concentration and the rate of lipolysis has never really been resolved. Boyd et al. (1975) suggested that even though cAMP is involved in the regulation of lipolysis in chick adipocytes, no simple relationship exists between total cAMP content and lipolytic rate. In the presence of epinephrine, the decline in the peak cAMP content is not sufficient to account for the rate of lipolysis (Arner, 1976; Birnbaum and Goodman, 1977). Dalton et al. (1974) did attempt to measure this relationship and found that the rate of production of intracellular cAMP from 0 to 2 minutes is related to the rate of lipolysis from 0 to 20 minutes, but that the rate of lipolysis is independent of the final concentration of cAMP attained.

Protein kinase Intracellular effects of cAMP are believed to be mediated through activation of a protein kinase (Steinberg and Khoo, 1977). The cyclic nucleotide binds to protein kinase, dissociating an inhibitory subunit from the holoenzyme which enables protein kinase to catalyze the transfer of the terminal phosphate group of ATP to a variety of proteins including hormone-sensitive lipase (Birnbaum and Goodman, 1977; Steinberg and Khoo, 1977). Other studies have shown that changes in activity of protein kinase parallel changes in cAMP concentration (Birnbaum and Goodman, 1977), and prolonged stimulation of adipose tissue with epinephrine causes elevated cAMP-dependent protein kinase activities. These data support the fact that continued activation of protein kinase may be necessary for maintenance of an accelerated rate of lipolysis (Birnbaum and Goodman, 1977). On the other hand, presumed inhibitors of
cAMP accumulation (such as adenosine) have been unable to change the activity of protein kinase in fat cell homogenates (Fain and Wieser, 1975). This leaves the understanding of how protein kinase participates in the regulation of lipolysis unclear.

**Hormone-sensitive lipase** Finally, phosphorylation of hormone-sensitive lipase by activated protein kinase from the relatively inactive form b to the active form a has only been demonstrated definitively in the rat (Siddle and Hales, 1975; Steinberg and Khoo, 1977). Activation of the enzyme is paralleled by phosphorylation (Steinberg and Khoo, 1977). Physical characteristics of hormone-sensitive lipase are: 1) a lipid-rich particle with diameter of 150 to 250 Å, 2) sedimentation coefficient of 33 to 35 S, 3) estimated molecular weight of 3 to 7 X 10^6 daltons, and 4) approximately 50% lipid by weight in particle.

It has been suggested that hormone-sensitive lipase and lipoprotein lipase are inversely related. In starvation or exposure of adipose tissue to lipolytic hormones, the activities of these two enzymes vary reciprocally. Recently, the activities of these enzymes have been resolved in chicken adipose tissue, and they have been shown to be immunochemically distinct (Khoo et al., 1976). Their ability to vary reciprocally may relate to cAMP and the ability of cAMP to alternately "switch on or off" the proper enzyme according to the nutritional situation; but, the effect of cAMP on lipoprotein lipase seems indirect and may relate to changes in free fatty acid concentration in adipose tissue during lipolysis (Steinberg and Khoo, 1977).

**Other lipase activities** Adipose tissue contains at least four lipase activities known as cholesterol esterase (E.C. 3.1.1.13) and
triglyceride, diglyceride, and monoglyceride lipases (Scow and Chernick, 1970). Rat tissue contains a large content of cholesterol esterase, and its activity is increased by cAMP-dependent protein kinase. However, diglyceride and monoglyceride lipase activities do not seem enhanced in this same manner (Steinberg and Khoo, 1977). Hormone-sensitive lipase is activated by cAMP-dependent protein kinase, but this activation rarely approaches 100%; it is more like 50%. In addition, diglyceride and monoglyceride lipase activities are 3 to 20 times greater than hormone-sensitive lipase in homogenates (Scow and Chernick, 1970). In partially purified fractions of adipose tissue incubated with ATP-Mg++ and cAMP, the maximum percentages of activation of hormone-sensitive lipase and cholesterol esterase are always much greater than diglyceride and monoglyceride lipases (Steinberg and Khoo, 1977). Recently, Khoo and Steinberg (1978) demonstrated that the acyl hydrolases were subject to reversible activation and deactivation dependent on Mg++, reflecting lipase phosphatase activity. Most importantly, this study showed clearly that diglyceride lipase is activated by cAMP-dependent protein kinase to the same extent as hormone-sensitive lipase when carried out in a low ionic strength buffer. The diglyceride lipase is reversibly deactivated by pure protein phosphatase. The authors concluded that both triglyceride and diglyceride lipase activities actually reside in a single enzyme protein.

**Regulation of lipolysis** Besides the most important regulators of lipolysis in vivo, catecholamines and insulin, many hormones and other metabolic effectors are known to influence the concentration of cAMP and thereby hormone-sensitive lipase activity (Figure 1).
Other hormones that probably exert their action in fat cells through activation of adenylate cyclase are ACTH, glucagon, and TSH (Masoro, 1977). The difference between these peptide hormones and the catecholamines are that they act on distinct receptors and their effectiveness depends on the species. There is considerable evidence that these hormones that stimulate lipolysis can be divided into two groups dependent on how effectively they activate adenylate cyclase. The first group, to which the catecholamines, ACTH, glucagon, and TSH belong, is known as the fast-acting hormones because they have a rapid effect on lipolysis that is short-lived. The other characteristics are that their lipolytic effect can be suppressed in vitro by insulin or prostaglandin E\textsubscript{1} if the lipolytic stimulus is small and that their action is greatly enhanced by the addition of methyl xanthines (Scow and Chernick, 1970; Masoro, 1977).

Little evidence for the mechanism of action of ACTH can be presented. However, Masoro (1977) points out that ACTH may be the substance directly acting on the adipocyte membrane that causes increased rate of fat mobilization during exercise.

The effect of glucagon in adipose tissue, with respect to species, is most intriguing. For example, glucagon exhibits a marked stimulatory effect in rabbits and birds, a moderate effect in rats, and a minimal effect on lipolysis in dogs and humans (Shafrir et al., 1965). Swine adipose tissue is not responsive to glucagon at any age and only marginally to ACTH; however, the response to ACTH is greatly increased by the addition of theophylline (Mersmann et al., 1976a,b). Etherton et al. (1977) indicated that glucagon had no effect on glycerol or free fatty acid release in both sheep and cattle adipose tissue. On the other hand,
the mechanism of activation of lipolysis in avian species is fundamentally the same as in all other mammalian tissues. Glucagon is the major regulator of lipolysis in chickens, whereas the catecholamines have little stimulatory effect and insulin is unable to increase glucose metabolism or inhibit lipolysis (Boyd et al., 1975; Malgieri et al., 1975).

Like ACTH and glucagon, TSH is thought to stimulate lipolysis by increasing the activity of adenylate cyclase. In rats, Goodman (1970) showed that TSH markedly potentiated the lipolytic response to epinephrine and theophylline.

The second group of lipolytic hormones, growth hormone and glucocorticoids, are called the slow-acting hormones because they are thought to act primarily through a mechanism requiring protein synthesis (Scow and Chernick, 1970). Some of their characteristics are that the effect on lipolysis has a lag time of at least one hour and lasts for several hours. Their action is prevented by either insulin administration or refeeding the animal. The lipolytic mechanism also can be blocked by inhibitors of DNA-dependent RNA synthesis (actinomycin D) or of protein synthesis (puromycin, cycloheximide), suggesting that the stimulation by these hormones is dependent on new protein synthesis. Their action also is enhanced further by methyl xanthines such as theophylline (Masoro, 1977). Goodman (1970) observed that adipose tissue from rats treated with dexamethasone showed increased glycerol and free fatty acid production in the presence of small concentrations of epinephrine. The effect of dexamethasone required a lag time in excess of 2 hours and was blocked by actinomycin D. When growth hormone also was present, the potentiation of the lipolytic process was even greater. Lamberts et al. (1975) showed
that either dexamethasone or cortisol treatment of rat epididymal fat cells enhanced epinephrine-stimulated lipolysis and increased the activity of cAMP-dependent protein kinase.

The mechanism of growth hormone is less well understood. Growth hormone has been known to have lipolytic properties, especially within one hour after addition, but it also may produce the opposite effect. Birnbaum and Goodman (1977) showed that in hypophysectomized rats, growth hormone antagonized the lipolytic action of epinephrine. Other evident factors were that responses observed with growth hormone were insulin-like and biphasic in nature, that protein synthesis inhibitors prolonged the effect, and that the tissues became refractory for several hours following administration. Naturally, these effects are much more observable in hypophysectomized than intact animals, but the overall effect of growth hormone remains unclear.

Other metabolic compounds which exert an effect on lipolysis are dibutyryl cAMP, a cAMP analog, and theophylline, a methyl xanthine. Both stimulate lipolysis in in vitro systems and are thought to act by increasing the intracellular concentration of cAMP. Theophylline reduces phosphodiesterase activity, and dibutyryl cAMP competes as a substrate for phosphodiesterase (Baum and French, 1976) or by inhibiting the enzyme (Jarett and Smith, 1974). Theophylline stimulates lipolysis in vivo as well as in vitro, while dibutyryl cAMP inhibits in vivo lipolysis (Baum and French, 1976). In vivo, theophylline increases circulating glycerol and free fatty acids while dibutyryl cAMP decreases them slightly but only the free fatty acids significantly. In vitro, theophylline and
dibutyryl cAMP both promote glycerol release and act additively with epinephrine (Baum and French, 1976; Bauman, 1976).

In summary, the regulation of lipolysis in adipose tissue of monogastric animals involves many factors. Under different physiological situations, the rapid changes in lipolytic rates that occur during stress or exercise are mediated through the sympathetic nervous system via the catecholamines. The long-term effects that take place due to dietary intake, temperature adjustment, or fasting are probably due to changes in rates of hormone secretions from the pituitary, thyroid, and adrenal glands plus the concentration of plasma insulin. The process of regulation of lipolysis is far from being understood completely and the complexities are only now beginning to emerge.

**Lipolysis in bovine adipose tissue** The control of lipid mobilization in nonruminants is believed to involve the adenylate cyclase-cAMP system in which protein kinase followed by hormone-sensitive lipase are activated. Studies in ruminant adipose tissue have been very limited, and only a few of the hormones and metabolic effectors of lipolysis have been researched. In Table 1, a summary of the compounds investigated has been made (Bauman, 1976).

The in vitro response of ruminant adipose tissue to epinephrine and norepinephrine additions is small when contrasted to a 10- to 60-fold increase in lipolysis observed in rat adipose tissue (Rodbell, 1964). Metz et al. (1973) observed that it is difficult to stimulate basal rates of lipolysis more than 2- or 3-fold probably because the free fatty acid: albumin ratio is high and may be an important modulator of the rate of lipolysis. Yang and Baldwin (1973b) demonstrated that insulin had no
Table 1. Metabolic effectors of in vitro ruminant lipolysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effect on lipolysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>Less than 1- to 4-fold increase</td>
<td>Yang and Baldwin, 1973a,b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pothoven et al., 1975</td>
</tr>
<tr>
<td>Glucagon</td>
<td>None</td>
<td>Etherton et al., 1977</td>
</tr>
<tr>
<td>Insulin</td>
<td>Small decrease</td>
<td>Yang and Baldwin, 1973b</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Less than 1- to 4-fold increase</td>
<td>Yang and Baldwin, 1973b</td>
</tr>
<tr>
<td>Theophylline</td>
<td>1- to 3-fold increase</td>
<td>Etherton et al., 1977</td>
</tr>
<tr>
<td>Theophylline + epinephrine</td>
<td>Additive of each alone</td>
<td>Etherton et al., 1977</td>
</tr>
</tbody>
</table>

Effect on basal lipolysis but diminished epinephrine-stimulated lipolysis by 10 to 25%. Glucagon and β-estradiol additions did not increase the lipolytic rate in adipose tissue from either dairy steers or sheep (Etherton et al., 1977).

In an attempt to verify that lipolysis in the ruminant involves the adenylate cyclase-cAMP system, theophylline was added in addition to epinephrine; this combination resulted in an increased lipolytic rate approximately equal to addition of effects of both hormones (Etherton, et al., 1977). Therefore, the mechanism of lipid mobilization in ruminants seems similar to that in nonruminants. Adenylate cyclase, phosphodiesterase, and protein kinase activities as well as cAMP content have never been measured in ruminant adipose tissue. This should be done to confirm or negate the proposed adenylate cyclase-cAMP system. Thus far, investigations concerning regulation of this process have not been extensive enough to allow firm conclusions to be drawn. What is
definitely known is that ruminant adipose tissue is less sensitive to metabolic regulators than is nonruminant adipose tissue.

Prostaglandins and lipolysis

A comprehensive review of prostaglandin (PG) synthesis, mechanism of action, and tissue response will not be contained in this section. Rather, only a review of mode of action of PG on metabolism in adipose tissue will be made. For pertinent reviews, the reader is referred to one of the following excellent manuscripts: Hinman (1972), Hinman and Weeks (1972), Weeks (1972), Russell et al. (1975), and Pike (1976).

Mechanism of action It seems probable that most, if not all, biological actions of PG are mediated through either cAMP or cGMP, although the physiological significance of increased cGMP content in isolated rat fat cells is minimal with respect to regulation of lipolysis (Fain and Butcher, 1976). The mechanism is thought to be initiated by a β-adrenergic site, causing adenylate cyclase to become activated. It is still unclear how this activation process occurs. Adenylate cyclase may itself be the receptor that has both an outer regulatory and inner catalytic subunit, or there may be some intermediary between the β-receptor on the outer cell membrane and adenylate cyclase on the inner surface of the cell membrane. Whatever the arrangement, adenylate cyclase does display "sidedness" because ATP is only cyclized on the inner surface (Curtis-Prior, 1976). The rate of catabolism of cAMP to 5'-AMP by phosphodiesterase is comparatively slow so that cAMP does accumulate within the fat cell.
Interaction of prostaglandin and the cAMP system

Steinberg et al. (1963, 1964) were the first researchers to observe that cAMP-mediated, hormone-stimulated lipolysis was blocked by PG. Ramwell and Shaw (1966) were the first to postulate that the physiological role of prostaglandin E (PGE) in the regulation of lipolysis is that of a negative feedback modulator. Later, Christ and Nugteren (1970), Illiano and Cuatrecasas (1971), and Dalton and Hope (1974) provided further evidence of a negative feedback regulation of hormonal activity (Figure 2).

Kuehl (1974) completed research that attempts to define the role of PG. Using the prostaglandin antagonist, 7-cxa-13 prostynoic acid, PG-induced cAMP formation is blocked in smooth muscle. Further work in their laboratory indicated that PG binding sites exist in adipose tissue. Prostaglandin E₁ and PGA₁ binding sites are found in rat adipocyte plasma membrane (Gorman and Miller, 1973). The binding of PG to their receptor sites may be one of the initial events in regulation of lipolysis. Gorman and Miller (1973) further speculate that regulation at this level would involve adenylate cyclase.

Source of polyunsaturated fatty acids for cellular biosynthesis of the PG

The precursor of PG capable of modulating cAMP action in the fat cell is still uncertain. Arachidonic acid is present and esterified in the cell membrane, and one would expect increased activity of phospholipase A (E.C. 3.1.1.4) with hormonal mediation along with increased activity of adenylate cyclase and accumulation of cAMP. This evidence, however, is not available.

Polyunsaturated fatty acids are available for PG synthesis in adipose tissue. During basal and hormone-stimulated lipolysis, PG biosynthesis...
Figure 2. Role of cAMP in regulation of PGE$_2$ synthesis in rat fat cells
(Dalton and Hope, 1974)

TG = triglyceride
PL = phospholipid
FA = fatty acid
PGE$_2$ = prostaglandin E$_2$
consists mainly of PGE\(_2\) formation (Paoletti and Puglisi, 1973). The isolated adipose cell contains prostaglandin synthase (E.C. 1.14.99.1) that is located in the microsomal fraction and converts dihomo-\(\gamma\)-linolenic acid and arachidonic acid to the PG endoperoxides (PGG\(_1\), PGH\(_1\), and PGG\(_2\), PGH\(_2\)) (Vergroesen, 1977). The endoperoxides are converted into the classical PG (E\(_1\), F\(_{1\alpha}\), E\(_2\), and F\(_{2\alpha}\)).

It has become clear in the last few years that an increase in dietary linoleic acid intake directly influences PG synthesis (Hwang et al., 1975). Furthermore, essential fatty acid-deficient rats show an increased response to lipolytic agents, and the increased response may be due to decreased formation of PGE\(_2\) (Danon et al., 1975; Nazinski et al., 1975; Hwang et al., 1975). Christ and Nugteren (1970) showed that in adipose tissue from essential fatty acid-deficient rats there is a decreased release of PGE\(_2\) and an increase in lipolysis by addition of PGE\(_2\) when compared to controls.

**Effects of PG on lipolysis** If we assume that during in vivo adipose tissue lipolysis PGE\(_2\) is released and acts in a negative feedback fashion to decrease adenylate cyclase activity and hormone-sensitive lipase activity (Figure 2), we also may assume that the output into the extracellular fluid of glycerol and free fatty acids is likewise decreased. One then may look at all the evidence that may elucidate the role of PG in this process.

In 1970, Christ and Nugteren observed that during lipolysis isolated fat cells from rat epididymis as well as fat pads from rat epididymis release both arachidonic acid and PGL\(_2\). Further, they observed that the enzyme system capable of converting essential fatty acids into PG was
present in isolated fat cells and that low concentrations of PGE$_2$ effectively inhibited lipolysis. They concluded that in essential fatty acid-deficient rats 1) a decrease in release of PGE$_2$, 2) an increase in lipolysis, and 3) inhibition of lipolysis by exogenous PGE$_2$ all are consistent with the negative feedback inhibition of adenylate cyclase.

Illiano and Cuatrecasas (1971) hypothesized that after the initial fall in cAMP content isolated fat cells became resistant to additional hormonal stimulation, and some compound is released into the media, which in fresh cells inhibits the accumulation of cAMP. They concluded their study by using indomethacin, which effectively blocks endogenous PG synthesis, and which resulted in enhancement of basal and epinephrine-stimulated rates of lipolysis in isolated rat fat cells. Their studies provide strong evidence for a physiological role of endogenously synthesized PG in the regulation of basal and hormone-stimulated lipolysis.

Fain et al. (1973) reported opposite results to those of Illiano and Cuatrecasas (1971). Because indomethacin did not affect lipolysis in isolated rat fat cells, they concluded that PG are not important feedback regulators of adenylate cyclase or lipolysis in this system. Their results point to a substance other than a PG as a primary feedback regulator of adenylate cyclase, which they believe is adenosine. Dalton and Hope (1973) also showed that indomethacin did not enhance basal or norepinephrine-stimulated release of free fatty acids or glycerol. This study also failed to support the negative feedback hypothesis for adipose tissue. However, they did observe that hormonally-stimulated PG synthesis attenuates the hormonal lipolytic response.
In a following paper, Dalton and Hope (1974) demonstrated a PG-generating system in isolated rat fat cells and showed that accumulation of PGE$_2$, but not PGE$_1$ or PGF$_{2\alpha}$, was stimulated by addition of norepinephrine, theophylline, or dibutyryl cAMP. They also observed inhibition of PG synthesis by indomethacin. Arachidonic acid was in high concentration in fat cell phospholipids. They concluded that these observations are consistent with a negative feedback regulation of hormone-stimulated lipolysis. They also hypothesized that perhaps the rate-limiting step in this process is the activation of phospholipase A. Haye et al. (1973) suggested that, because the precursor polyunsaturated fatty acids are abundant in the phospholipids, PG release is due to neosynthesis and not depletion of storage material. On the basis of that in the thyroid gland, they suggested that activity of endogenous phospholipase A is the factor regulating biosynthesis of PG.

A direct relationship between the rate of cAMP accumulation and the rate of lipolysis has not been firmly established as discussed in a prior section. Dalton et al. (1974) showed that such a relationship may exist during the initiation of lipolysis. They concluded that the mechanism of action of PGE$_2$ is mediated by the inhibition of the early rate of cAMP accumulation rather than the total production of cAMP.

In a series of two papers, Fain and Wieser (1975) explained the proposed effect of adenosine. According to their hypothesis, adenosine is an important modulator of hormone action on fat cells. Adenosine inhibits cAMP accumulation in rat fat cells and is continuously released to the medium during incubation. Adenosine deaminase (E.C. 3.5.4.4) essentially removes all cellular adenosine and increases cAMP content.
and lipolysis. In the second paper, Wieser and Fain (1975) showed that glucose oxidation, already stimulated by insulin, was enhanced by the addition of adenosine, PGE$_1$, or nicotinic acid, indicating that these agents are not increasing glucose oxidation solely as a result of an inhibition of lipolysis. Adenosine, PGE$_1$, and nicotinic acid act synergistically with insulin to stimulate glucose oxidation and inhibit lipolysis in the presence of norepinephrine. They concluded that adenosine may be necessary for insulin action and that the effects of adenosine, PGE$_1$, and nicotinic acid on glucose metabolism and lipolysis are secondary to inhibition of cAMP accumulation. They also found that neither uptake nor subsequent incorporation of adenosine by fat cells into intracellular ATP is required for inhibition of cAMP accumulation in fat cells.

Also in 1975, Fain and Shepherd hypothesized that the free fatty acids rather than PG are the heat stable and nondialyzable inhibitors of lipolysis that are released by fat cells into the medium that contains albumin. The authors suggest that when the primary binding sites on albumin are saturated with free fatty acids, the plasma membrane of the fat cells effectively competes with albumin for binding of free fatty acids. The free fatty acids bound to the membrane may alter the membrane's enzymatic activity in a specific way that adenylate cyclase activity is decreased significantly.

**Effect of other PG on lipolysis** The influence of an infusion of PGB$_1$ on circulating levels of insulin, glucose, and free fatty acids in anesthetized dogs (both in the basal state and following stimulation with intravenous glucose or norepinephrine) was not significantly different
from saline-treated controls. The authors concluded that PGB₁ has no physiological role in the modulation of glucose, insulin, or free fatty metabolism (Robertson and Guest, 1974).

In rat hepatic tissue, PGE₁ failed to stimulate cAMP or lipolysis even though other known agents (glucagon and epinephrine) activate cAMP in this same system. These data did not support a physiological role for PGE₁ in hepatic lipolysis. Although the effect of PGE₁ on gluconeogenesis, lipogenesis, ureogenesis, or amino acid transport was not investigated in this study, the author concluded that PGE₁ modulation of these processes would occur independently of changes in cAMP content (Levine, 1974).

In a study by Gorman et al. (1975), the prostaglandin endoperoxide PGH₂ inhibited basal adenylate cyclase activity to 11% of basal activity and epinephrine-stimulated activity to 30 to 35% of basal activity in fat cell ghosts. The stimulation of adenylate cyclase by ACTH, glucagon, or TSH as well as epinephrine was antagonized by PGH₂, suggesting that this endoperoxide may be an endogenous feedback regulator of hormone-stimulated lipolysis in adipose tissue. Their data also indicate that PGE₁, PGE₂, PGA₁, PGD₂, and PGE₂α do not alter epinephrine-stimulated adenylate cyclase activity. This is probably the first report which indicated that PG, particularly PGH₂, had any effect on adenylate cyclase in a broken cell preparation. The authors suggested that PGH₂ inhibits lipolysis at a point distal to the hormone receptors because PGH₂ inhibits basal as well as hormone-stimulated activity. Furthermore, the discovery that the endoperoxides might play a role in hormone-sensitive systems suggests that perhaps they represent a group of potent, short-lived
compounds that have the capacity to modulate cellular activity possibly by regulating the cyclic nucleotides.

Subsequently, Fredholm and Hamberg (1976) showed that PGH₂ inhibited norepinephrine-induced cAMP accumulation in isolated rat fat cells in a dose-dependent manner but only after conversion to PGE₂, which is biologically more active than the endoperoxide in adipose tissue. These data did not support the idea of a physiological role for PGH₂ as a modulator of lipolysis.

The elucidation of the role of PG in the control of lipolysis is still unclear and much research remains to be done in order to understand the underlying control mechanisms of this process.
MATERIALS AND METHODS

Experimental Animals

Ten Holstein steers weighing an average of 675 kg (623 to 774 kg) were used in this study. Steers were fed ad libitum a diet consisting of a grain mixture (Table 2) plus corn silage. Steers were fed this diet several months, fasted 9 days, and then refed. During fasting, steers received ad libitum salt and water only and were housed individually in unbedded tie-stalls.

Table 2. Composition of grain mixture

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentageb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracked corn</td>
<td>67.5</td>
</tr>
<tr>
<td>Soybean oil meal (44% protein)</td>
<td>25.0</td>
</tr>
<tr>
<td>Ground whole oats</td>
<td>5.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.5</td>
</tr>
<tr>
<td>Trace-mineralized saltc</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin supplementd</td>
<td>trace</td>
</tr>
</tbody>
</table>

Steers received 10 kg of grain mixture per day and ad libitum corn silage.

Percentage of as-fed grain mixture.

Components of trace-mineralized salt: 99.15% NaCl, 0.35% ZnO, 0.28% MnO, 0.175% Fe (CO₃), 0.036% CuO, 0.007% Ca (IO₄)₂, and 0.007% CoCO₃.

Supplied 2 million IU retinyl palmitate and 250,000 IU cholecalciferol per 908 kg of grain mixture.
Tissue Biopsies

Biopsies were taken from the subcutaneous adipose tissue over the longissimus dorsi muscle of the steers according to the schedule shown in Table 3. To obtain the subcutaneous adipose tissue biopsy the skin around the incision site was shaved, disinfected, and anesthetized with a 20-ml subcutaneous injection of lidocaine hydrochloride (Astra Pharmaceutical Products, Inc., Worcester, MA). A linear 9-cm incision was made approximately 5- to 6-cm laterally from the midline to expose the fat pad. Approximately 10 g of fat was removed and maintained at 37° in physiological saline until incubated.

Table 3. Biopsy schedule

<table>
<thead>
<tr>
<th>Steer number</th>
<th>Day of biopsy before fasting</th>
<th>Day of biopsy during fasting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7,4,2</td>
<td>5,8</td>
</tr>
<tr>
<td>2</td>
<td>7,5,1</td>
<td>7,8</td>
</tr>
<tr>
<td>3</td>
<td>8,5,2</td>
<td>2,5</td>
</tr>
<tr>
<td>4</td>
<td>8,4,1</td>
<td>4,7</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>6,9</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>5,8</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>4,9</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>5,8</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>6,7</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>4,7</td>
</tr>
</tbody>
</table>

aData of biopsy within each period were randomly assigned to each steer.

bDay 1 is first day of fast.
Assay of Lipolysis

A section of adipose tissue weighing approximately 50 mg was incubated in 14 x 125 mm test tubes containing 3 ml of Krebs and Henseleit-Ringer buffer, pH 7.4, with one-half the recommended amount of CaCl₂ (Laser, 1961). Reaction mixtures used to assay basal lipolysis also contained 60 mg of bovine serum albumin (fraction V, fatty acid poor, Sigma Chemical Company, St. Louis, MO) and 3 mg of glucose (Sigma Chemical Company, St. Louis, MO). Concentrations and combinations of metabolic effectors added into the incubation media are shown in Table 4.

The amount of epinephrine, dibutyryl cAMP, and theophylline that was added to each assay gave maximal lipolytic response; the amount of prostaglandin E₂ added was similar to that used by Dalton et al. (1974) which gave maximal inhibition of in vitro lipolysis. Prostaglandin E₂ was dissolved in small quantities of ethanol and diluted before use to working concentration with water.

The test tubes were gassed with 95% O₂:5% CO₂ and stoppered with rubber septa. Triplicate samples were placed in a metabolic shaker and were incubated at 1 hr at 37° with constant shaking (90 strokes per min). The reaction was stopped by placing the test tubes in crushed ice. Tissues were blotted and weighed; the media was stored at -20° until analyzed. Samples without tissue were used as controls and were treated similarly.
Table 4. Additions of different combinations of lipolytic effectors to incubation media

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Epinephrine&lt;sup&gt;b,c&lt;/sup&gt;</th>
<th>Dibutyryl cAMP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Theophylline&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Basal)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
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<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
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<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>Effectors were added to the incubation media at these concentrations: epinephrine at 1.5 μM, dibutyryl cAMP at 1.0 mM, theophylline at 500 μM, and PGE<sub>2</sub> at 140 nM.

<sup>b</sup>Obtained from Sigma Chemical Company, St. Louis, MO.

<sup>c</sup>Ascorbic acid (0.3 mg) was added with epinephrine to reduce oxidation of epinephrine.

<sup>d</sup>Generously donated by The Upjohn Company, Kalamazoo, MI.

<sup>e</sup>(-) represents no addition of effector(s) to media, and (+) represents addition of effector(s) to media.

**Analytical Procedures**

The extent of triglyceride hydrolysis was measured by analyzing the amount of glycerol released into the incubation media according to the method of Garland and Randle (1962). Number of fat cells in a known mass of adipose tissue from each biopsy of 4 steers was determined by the
method of Hirsch and Gallian (1968). Fixed cells of 25-250 μm diameter were separated by filtration through nylon screens (Small Parts Inc., Miami, FL) and were counted with a Model ZB Coulter Counter (Coulter Electronics Inc., Hialeah, FL).

Statistical Analysis

Data in Table 5 were analyzed as a randomized design in which steers represented blocks and the 16 effector combinations indicated in Table 4 represented treatments. An l.s.d. was computed to make pairwise comparisons of treatment means (Snedecor and Cochran, 1967).

Table 5. Effect of prostaglandin E₂ (PGE₂) on lipolysis in bovine adipose tissue

<table>
<thead>
<tr>
<th>Addition</th>
<th>Lipolytic rate²⁻PGE₂</th>
<th>Lipolytic rate²⁺PGE₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>790±42ᵃ</td>
<td>767±51ᵃ</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1486±143ᵇ</td>
<td>1336±152ᵇ,c</td>
</tr>
<tr>
<td>Dibutyryl cAMP</td>
<td>1065±70ᶜ</td>
<td>1218±89ᵇ</td>
</tr>
<tr>
<td>Theophylline</td>
<td>1656±170ᵇ</td>
<td>1561±139ᶜ,d</td>
</tr>
<tr>
<td>Epinephrine + dibutyryl cAMP</td>
<td>1661±87ᵇ</td>
<td>1673±108ᵈ,e</td>
</tr>
<tr>
<td>Epinephrine + theophylline</td>
<td>1928±113ᵈ</td>
<td>2190±208ᶠ</td>
</tr>
<tr>
<td>Dibutyryl cAMP + theophylline</td>
<td>1911±146ᵈ</td>
<td>1877±181ᵉ</td>
</tr>
<tr>
<td>Epinephrine + dibutyryl cAMP + theophylline</td>
<td>2070±108ᵈ</td>
<td>2260±161ᶠ</td>
</tr>
</tbody>
</table>

¹Concentrations of each effector in assay mixtures were: epinephrine at 1.5 μM, dibutyryl cAMP at 1 mM, theophylline at 500 μM, and PGE₂ at 140 nM.

²Values are means ± SEM in 3 biopsies per steer taken during the fed (prefast) period in 4 steers. Values within a column with different superscripts are significantly different (P<0.05). Values within a given line are similar (P>0.05). Average number of fat cells (25-250 μm in diameter) per gram of tissue was 1.30 X 10⁶.
Data in Table 6 were analyzed as a split plot design with the whole plot arranged in a randomized block design in which steers represented blocks and the effect of feeding or fasting represented the whole plot treatment. The subplot treatments were represented by the four effector combinations that were used. An l.s.d. was computed to make pairwise comparison of treatment means (Snedecor and Cochran, 1967).

Table 6. Effect of fasting on rates of lipolysis in bovine adipose tissue

<table>
<thead>
<tr>
<th>Addition</th>
<th>Lipolytic rate^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
</tr>
<tr>
<td></td>
<td>nmoles glycerol released/(g x hr)</td>
</tr>
<tr>
<td>None (basal)</td>
<td>1076±91^a</td>
</tr>
<tr>
<td>Prostaglandin E₂</td>
<td>1002±127^a</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1532±143^b</td>
</tr>
<tr>
<td>Prostaglandin E₂ + epinephrine</td>
<td>1396±133^b</td>
</tr>
</tbody>
</table>

1*Prostaglandin E₂ concentration in assay mixture was 140 nM, that of epinephrine was 1.5 μM.

2Values are means ± SEM for 10 steers and were calculated as follows: Three biopsies were taken from each of 4 steers and one biopsy from each of 6 steers during the fed (prefast) period. Mean rate for each of 4 steers biopsied three times was determined and then averaged with rate in single biopsy from each of the other 6 steers. Values within same column or line with different superscripts are significantly different (P<0.05).
RESULTS AND DISCUSSION

The role of prostaglandins in the control of lipolysis in bovine adipose tissue was evaluated by measuring the effect of prostaglandin E₂ (PGE₂) on in vitro lipolysis. To determine whether PGE₂ acts upon lipolysis by way of the adenylate cyclase-cAMP system, PGE₂ was added to lipolysis assay mixtures that contained different combinations of epinephrine, dibutyryl cAMP, and theophylline. Measuring rates of lipolysis in tissue from fasted steers also was used as a model system to evaluate the regulatory role of PGE₂. Prostaglandin E₂ was chosen as the prostaglandin to study because other workers (Paoletti and Puglisi, 1973) have demonstrated that PGE₂ has a greater inhibitory effect on lipolysis in adipose tissue than other prostaglandins. Glycerol release expressed as nmoles/(g of wet tissue X hr); rather than fatty acid release from incubated adipose tissue, was chosen as the measure of lipolysis because bovine adipose tissue contains 1) negligible glycerokinase activity and 2) significant capacity for reesterification of intracellular fatty acids as triglycerides (Metz et al., 1973). For ease of comparison of our data with those of others, lipolytic rates were expressed on a tissue-weight basis rather than a cell-number basis. The conversion factor relating tissue weight to cell number is indicated in Table 5. Lipolytic rates caused by different effector combinations had the same relationship to each other when expressed on either the tissue-weight basis or the cell-number basis.
PGE<sub>2</sub> Interaction with Lipolytic Effectors

The effects of epinephrine, dibutyryl cAMP, and theophylline with and without PGE<sub>2</sub> on basal lipolysis are shown in Table 5. Epinephrine significantly stimulated the rate of basal lipolysis by 1.9-fold. The magnitude of the stimulation by epinephrine compares favorably with observations of others who have shown that epinephrine stimulates basal lipolysis in adipose tissues from several different anatomical sites of ruminants from 1- to 4-fold (Metz and Van Den Bergh, 1972; Yang and Baldwin, 1973b; Pothoven et al., 1975; Bauman, 1976; Etherton et al., 1977). These data reflect the important physiological regulation of lipolysis by catecholamines in animals (Masoro, 1977). Lipolysis in bovine adipose tissue is less responsive to epinephrine stimulation than is that in rat adipose tissue which is stimulated from 10- to 60-fold by epinephrine (Prigge and Grande, 1971). Specific reasons for the species difference are unknown. Although not shown previously in ruminants, epinephrine promotes lipolysis in adipose tissue by activation of hormone-sensitive lipase by a cAMP-dependent protein kinase (Steinberg and Khoo, 1977).

Addition of dibutyryl cAMP or theophylline significantly stimulated the basal rate of lipolysis (Table 5). The increase in the lipolytic rate caused by dibutyryl cAMP addition was significantly less than the stimulation of lipolysis by addition of epinephrine or theophylline. Based upon lipolytic rates, epinephrine increases the intracellular concentration of cAMP greater than adding exogenous dibutyryl cAMP at concentrations that give maximal lipolytic rates. It is known that
the dibutyryl form of cAMP enters mammalian cells more easily and is more resistant to protein kinase low $K_m$-cAMP-phosphodiesterase (E.C. 2.7.1.37, phosphodiesterase) cleavage than is cAMP (Zapf et al., 1975; Baum and French, 1976).

Theophylline, an agonist of lipolysis which inhibits phosphodiesterase and, thus, increases the intracellular concentration of cAMP (Butcher and Sutherland, 1962), increased basal lipolysis by 2.1-fold. Because the stimulation by theophylline was similar to that by epinephrine, both agonists, although acting by different mechanisms, seem to elicit maximal rise in the concentration of intracellular cAMP which is positively correlated with free fatty acid mobilization from adipocytes (Dalton et al., 1974). No one has demonstrated a non-cAMP mechanism for stimulation of lipolysis.

Prostaglandins of the E series have been shown by many investigators to inhibit lipolysis in adipose tissue from several species by inhibition of cAMP formation (Steinberg et al., 1963; Rairwell and Shaw, 1966). The interaction of PGE$_2$ with effectors of lipolysis that are known to act through the adenylate cyclase-cAMP system was studied. Prostaglandin E$_2$, at a slightly greater than physiological concentration, had no effect on basal lipolysis (Table 5). Neither did PGE$_2$ alter epinephrine-, dibutyryl cAMP-, or theophylline-stimulated lipolysis in adipose tissue from steers.

The lack of a lipolytic response of PGE$_2$ in our study could not be attributed to degradation of PGE$_2$ because its presence in the incubation media was confirmed by mass spectrometry (Stenhagen et al., 1974). The lack of inhibition of basal and stimulated lipolysis by PGE$_2$ is consistent
with the observations of Fain et al. (1973) and Dalton and Hope (1973) who concluded that prostaglandins are not important regulators of adenylate cyclase or of lipolysis in white fat cells from rats, and the observations of Mersmann et al. (1976a,b) who showed that PGE2 does not inhibit lipolysis in isolated adipocytes from swine. In contrast, other workers (Steinberg et al., 1963; Carlson and Micheli, 1970; Christ and Nugteren, 1970; Dalton et al., 1974) reported that prostaglandin E1 inhibited catecholamine- and theophylline-stimulated lipolysis in rat adipose tissue. Therefore, additional work is required to resolve the question of whether the prostaglandins control lipolysis in adipose tissue.

To further investigate the role of the adenylate cyclase-cAMP system in control of lipolysis in bovine adipose tissue, effects of several combinations of agonists that act by way of different mechanisms to increase intracellular concentration of cAMP were measured. The addition of dibutyryl cAMP concomitantly with epinephrine increased the rate of lipolysis to about 1.6 fold above that when only dibutyryl cAMP was added to the incubation media, but not above that when only epinephrine was added. Addition of dibutyryl cAMP probably increased the intracellular concentration of butyrate, which inhibits lipolysis (Metz and Van Den Bergh, 1972), and increased the intracellular concentration of cAMP as its N6, monobutyryl-metabolite (Kaukel et al., 1972). We propose that epinephrine plus dibutyryl cAMP increases the intracellular cAMP to a greater concentration than the metabolites of dibutyryl cAMP alone. Measurement of concentrations of cAMP and metabolites of dibutyryl cAMP in bovine adipocytes is needed to support this explanation.
Theophylline increased the rates of lipolysis in tissue already stimulated by epinephrine, dibutyryl cAMP, or epinephrine plus dibutyryl cAMP to about 2.5-fold above basal rates. This synergistic effect of theophylline with epinephrine or dibutyryl cAMP also has been observed in rats, swine, and cattle by Baum and French (1976), Mersmann et al. (1976a,b), and Etherton et al. (1977), respectively. Due to the proposed mechanism of action of theophylline (Butcher and Sutherland, 1962), the intracellular concentration of cAMP probably is increased above that caused by addition of either epinephrine or dibutyryl cAMP; the additional increase in cAMP causes greater, and perhaps maximal, hormone-sensitive lipase activity.

To further test whether prostaglandins control lipolysis by regulating the adenylate cyclase-cAMP system, the effect on lipolysis by adding PGE2 to incubation media already containing several different combinations of epinephrine, dibutyryl cAMP, and theophylline was determined. Regardless of which combination of these three agonists was studied, PGE2 caused no change in the rate of lipolysis. Work by others indicates that the interaction of prostaglandins with agonists of the adenylate cyclase-cAMP system is unclear, as discussed previously. The lack of effect of PGE2 in bovine adipose tissue suggests that PGE2 does not control the intracellular concentration of cAMP, which ultimately determines the rate of lipolysis.

Effect of Fasting on Regulation of Lipolysis

The effect of PGE2, epinephrine, and PGE2 plus epinephrine on lipolysis in adipose tissue from fed and 9-day-fasted steers is shown in
Table 6. The basal rate of lipolysis was decreased by about 40% because of fasting for 9 days. Furthermore, the rates of lipolysis in tissues incubated with PGE$_2$, epinephrine, or PGE$_2$ plus epinephrine were decreased from 30 to 50% by the 9-day fast. As also shown for the tissue from fed steers (Tables 5 and 6), PGE$_2$ did not alter the basal lipolytic rate in tissue from fasted steers. Epinephrine increased the rates of basal lipolysis in tissue in both fed and fasted steers to a similar magnitude (1.4- and 1.6-fold, respectively). As with fed steers, PGE$_2$ did not alter epinephrine-stimulated lipolysis in tissue from the fasted steers. Data from different researchers on effect of fasting (and restricted feeding) on lipolysis in adipose tissue are inconsistent. Pothoven et al. (1975) showed that both basal and epinephrine-stimulated lipolytic rates in adipose tissue from steers decreased with restricted feeding. Adipose tissue obtained from obese human subjects submitted to prolonged fasting was significantly less responsive to catecholamines than was tissue from fed subjects (Arner and Ostman, 1976). A greater number of investigators, however, have shown that both basal and catecholamine-stimulated rates of lipolysis are increased in nonruminants by fasting (Robison et al., 1971; Gilbert and Galton, 1974; Klain et al., 1977; Zapf et al., 1977).

Two possible explanations for the lower in vitro rates of lipolysis in tissue from fasted steers are possible. Firstly, steers may have been more highly stressed by the biopsy procedures during the fed period than during the fasting period, causing abnormally high concentrations of endogenous catecholamines in biopsied tissue. Secondly, and probably most likely, because the extracellular free fatty acid concentration was elevated in fasted steers (Pothoven and Beitz, 1975), the intracellular
concentration of free fatty acids at time of tissue biopsy was greater (Angel et al., 1971); the increased concentration of intracellular free fatty acids caused greater inhibition of lipolysis in tissue from fasted steers than in tissue from fed steers, even in the presence of lipolytic agonists.
SUMMARY

To determine if prostaglandins play a role in the regulation of lipolysis in bovine adipose tissue, studies were conducted to estimate the extent of this regulation by observing the interaction of PGE₂ with known effectors of the adenylate cyclase-cAMP system, which controls lipolysis. Lipolytic activity of adipose tissue from both fed and fasted Holstein steers weighing approximately 675 kg was measured. Samples of adipose tissue were obtained by a biopsy technique from subcutaneous adipose tissue over the longissimus dorsi muscle of 10 steers according to a randomized schedule during fed and fasting periods. The statistically significant (P=0.05) results are summarized as follows:

1. Prostaglandin E₂ plays no role in the regulation of the basal rate of lipolysis in adipose tissue of cattle. Neither did PGE₂ alter epinephrine-, dibutyryl cAMP-, or theophylline-stimulated rates of lipolysis.

2. Epinephrine increased the rate of basal lipolysis by 1.9-fold. Also, both dibutyryl cAMP and theophylline increased the basal rate of lipolysis by 1.4-fold and 2.0-fold, respectively.

3. The basal rate of lipolysis was decreased by about 40% because of fasting for 9 days. Furthermore, the rates of lipolysis in tissue incubated with PGE₂, epinephrine, or PGE₂ plus epinephrine were decreased from 30 to 50% by the 9-day fast.

4. Prostaglandin E₂ also did not alter either the basal or stimulated rates of lipolysis during fasting.
Data presented in this dissertation showed that exogenous prostaglandin E\textsubscript{2} did not alter the rate of lipolysis in ad libitum-fed steers. Further work was indicated that would show whether prostaglandins produced endogenously exerted any control in lipolysis in bovine adipose tissue. Because endogenously produced prostaglandins may act as feedback regulators (Dalton and Hope, 1973), the effect of indomethacin, a potent inhibitor of prostaglandin biosynthesis, was measured in adipose tissue from 6 steers, 3 of which were given an ad libitum level and 3 of which were given a maintenance level of dietary energy. Steers fed ad libitum and maintenance diets were fasted and refed to provide, for study, adipose tissue with different in vivo rates of lipolysis.

The effects of indomethacin on lipolysis in ad libitum- and maintenance-fed steers during fed and fasting periods are shown in Appendix Tables 7 and 8, respectively. In fed cattle, epinephrine promoted lipolysis in both groups of steers; because of a large variance, the increase was not significant in the ad libitum-fed group. Prostaglandin E\textsubscript{2} had no effect on basal lipolysis in adipose tissue from either ad libitum- or maintenance-fed steers; moreover, PGE\textsubscript{2} did not alter epinephrine-stimulated rates in tissue from steers in either dietary group. This finding in bovine adipose tissue confirms that reported in this dissertation and by others in rat adipose tissue (Dalton and Hope, 1973; Fain et al., 1973). The results of studies on the influence of PGE\textsubscript{2} on lipolysis in adipose tissue, however, are inconsistent because many researchers have demonstrated antilipolytic activity of prostaglandins E\textsubscript{1} and E\textsubscript{2} (Steinberg et al., 1963; Carlson and Micheli, 1970; Christ and Nugteren, 1970; Dalton et al., 1974).
To determine whether endogenously produced prostaglandins do not have antilipolytic activity as does exogenously added PGE₂, the effect of indomethacin on lipolysis in bovine adipose tissue was measured. The addition of indomethacin did not change basal lipolytic rates in either ad libitum- or maintenance-fed steers in fed or fasted states (Appendix Tables 7 and 8). In preliminary dose-response studies over a concentration range of 10⁻⁷M to 10⁻⁴M, indomethacin was ineffective. Ramwell and Shaw (1966) proposed a feedback regulatory system in which hormonally-stimulated release of free fatty acids from stored triglycerides provide substrates for endogenous synthesis of prostaglandins that, in turn, decrease activity of adenylate cyclase and, therefore, of hormone-sensitive lipase. If this proposal is applicable to lipolysis in bovine adipose tissue and if indomethacin functions only as an inhibitor of prostaglandin biosynthesis, then indomethacin should promote lipolysis; this postulate assumes that intracellular prostaglandins inhibit adenylate cyclase, which control hormone-sensitive lipase (Steinberg and Khoo, 1977). In our assay system of lipolysis, addition of indomethacin concomitantly with epinephrine caused no change in rates of lipolysis in either ad libitum- or maintenance-fed steers. Neither did addition of indomethacin with PGE₂ alter the basal rate of lipolysis. Dalton and Hope (1973) and Fain et al. (1973) also reported that indomethacin did not enhance norepinephrine stimulated release of free fatty acids or of glycerol in rat epididymal adipose tissue or isolated fat cells of rats, respectively.
The statistically significant (P=0.05) results are summarized as follows:

1. Indomethacin does not influence lipolysis and confirms our proposal as well as the findings of Dalton and Hope (1973) and Fain et al. (1973).

2. Epinephrine promoted lipolysis in both groups of steers; the increase was not significant in the ad-libitum fed ones.

3. Prostaglandin E₂ did not alter basal lipolysis in adipose tissue from either ad libitum- or maintenance-fed steers during fed or fasted periods; further PGE₂ did not change epinephrine-stimulated rates in tissue from steers in either dietary groups during fed or fasted periods. Although our results do not support a regulatory role for PGE₂ in lipolysis, they do not negate the possibility of a different role for prostaglandins such as functioning as vasodilators to increase blood flow to adipose tissue.
LITERATURE CITED


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Table 7. Effect of indomethacin on lipolysis in cattle of two levels of energy intake

<table>
<thead>
<tr>
<th>Diet</th>
<th>Additive</th>
<th>- Indomethacin</th>
<th>+ Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmoles glycerol released/(g X hr)</td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>None (Basal)</td>
<td>582±141&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>326± 47&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>866±253&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>745±284&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>403± 75&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>380± 65&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Epinephrine + PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>612±146&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Maintenance</td>
<td>None (Basal)</td>
<td>595± 54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>436± 67&lt;sup&gt;d,e&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Epinephrine</td>
<td>1061±159&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>1223±252&lt;sup&gt;f&lt;/sup&gt;</td>
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<td></td>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>624±209&lt;sup&gt;a&lt;/sup&gt;</td>
<td>560±122&lt;sup&gt;d,e&lt;/sup&gt;</td>
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<td></td>
<td>Epinephrine + PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1207±151&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>1</sup>Value is mean ± SEM of 6 observations from 3 steers. Values with different superscripts within a column are significantly different (P<.05). Addition of indomethacin caused no significant difference (P>.05).
Table 8. Effect of indomethacin on lipolysis in fasted cattle

<table>
<thead>
<tr>
<th>Diet before fasting</th>
<th>Additive</th>
<th>- Indomethacin</th>
<th>+ Indomethacin</th>
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<tbody>
<tr>
<td></td>
<td>nmoles glycerol released/(g X hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>None (Basal)</td>
<td>557± 39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>534± 25&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>1108±30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1443±249&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>583± 37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>522± 73&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Epinephrine + PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1026±268&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Maintenance</td>
<td>None (Basal)</td>
<td>557±150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>491± 27&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Epinephrine</td>
<td>1479±327&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1396±212&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>608± 73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>509± 54&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Epinephrine + PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1295±127&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Value is mean ± SEM of 6 observations from 3 steers. Values with different superscripts within a column are significantly different (P<.05). Addition of indomethacin caused no significant difference (P> .05).