Effects of diet, age, fasting and refeeding on lipogenesis in bovine adipose tissue

by

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INTRODUCTION

Fat deposition in cattle has both economic as well as scientific interest. Marbling fat makes a positive contribution to the palatability characteristics of meat and generally increases the economic value because of the higher quality grade. Excessive deposition of subcutaneous, intermuscular and perirenal fat, however, does not contribute to meat quality, but results in excessive fat trim and economic losses. In 1968, retailers in the United States trimmed more than two billion pounds of fat from fed beef cattle (Hood and Allen, 1973). To put this much fat on these cattle could require between one-third and one-half billion bushels of corn. Therefore, site and quantity of fat deposition in beef cattle are of major importance to meat quality and efficiency of production.

Through improved genetic selection and nutrition, livestock producers have been able to provide leaner animals for market. Even with advanced technology, many animals still accumulate excess fat. The degree and location of fat deposition depends on the genetics, animal age and sex of the animal. The physiological processes through which these factors affect fat accumulation are unclear. The processes involved in regulation of fat deposition include fatty acid synthesis, assimilation of fatty acids from the circulation and mobilization of fatty acids from adipose.

The objective of this research was to study factors possibly involved in regulation of fatty acid synthesis. Many studies have

been conducted in recent years on the regulation of fatty acid synthesis. Even though a large amount of work has been done, the controlling factor of fatty acid synthesis still has not been pinpointed. With a process as complex as fatty acid synthesis, there probably are several factors involved many of which are dependent upon each other.

In the first study, the effect of level of energy intake on capacity for fatty acid synthesis in bovine adipose tissue was determined. In the second study, capacity for fatty acid synthesis from acetate and activities of acetyl-CoA carboxylase, acetate thiokinase, NADP-isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were measured in adipose tissue of steers fasted for 8 days and then refed for 8 weeks. Also, plasma levels of acetate and free fatty acids were determined.

REVIEW OF LITERATURE

This literature review will be restricted to the regulation of the biosynthesis of long-chain fatty acids in animals. There have been several reviews written in this research group on the general aspect of fatty acid biosynthesis, especially pertaining to ruminant adipose tissue (Al-Shathir, 1971; Pothoven, 1972; Thornton, 1973). This review will include regulation by some lipogenic enzymes (acetate thiokinase, acetyl-CoA carboxylase and fatty acid synthetase), reducing equivalent supply, hormones, substrate level, metabolic activators and inhibitors, and diet.

Acetate Thiokinase (EC 6.2.1.1, acetate: CoA ligase (AMP))

In nonruminants, glucose is the major precursor of fatty acid synthesis (Flatt, 1970; Winegrad, 1965). Glucose is catabolized in the cytoplasm to pyruvate which enters the mitochondria. Pyruvate is converted to oxaloacetate and to acetyl-CoA which combine to form citrate. Citrate enters the cytoplasm and is cleaved into oxaloacetate and acetyl-CoA. Acetyl-CoA can then be used for fatty acid synthesis in the cytoplasm (Hanson and Ballard, 1967). With this pathway acetate thiokinase is not required to form cytoplasmic acetyl-CoA.

In ruminants, in which acetate is the main precursor of fatty acids, the acetate in the cytoplasm is activated to acetyl-CoA by acetate thiokinase (Hanson and Ballard, 1967). Cytoplasmic acetyl-CoA is not obtained from glucose for two reasons. First, the level of citrate cleavage enzyme (EC 4.1.3.8, ATP: citrate oxaloacetate-lyase

(CoA-acetylating and ATP-dephosphorylating)) is so low that it can be considered insignificant in ruminant tissues (Ballard, Hanson and Kronfeld, 1969). Citrate is the principal form in which acetyl-CoA can leave the mitochondria of a mammalian cell. Secondly, tissue glucose is at a premium in the ruminant because it must be synthesized de novo, so it will be conserved (Annison et al., 1963). It is possible that acetate thiokinase may be a regulatory factor in fatty acid synthesis in the ruminant.

Steiner and Cahill (1966) found that exogenous long-chain fatty acids inhibited fatty acid synthesis in brown adipose homogenates from rats. Major site of inhibition seemed to be the acetate thiokinase reaction. Barth, Sladek and Decker (1972) showed that the activity of acetate thiokinase in liver cytoplasm and epididymal fat tissue homogenates decreased to about 50% of normal after fasting for two days. Refeeding a high carbohydrate diet for two days increased its activity 170% in liver cytoplasm and 550% in epididymal fat tissue. No change in mitochondrial acetate thiokinase in liver was observed, supporting evidence of two different enzyme proteins. In 1972, Murthy and Steiner proposed that acetate thiokinase was a possible regulatory step in lipogenesis. Their results demonstrated that acetate thiokinase was inhibited to a greater extent by the addition of exogenous oleate or by fasting than were the enzymes involved in the conversion of acetyl-CoA to long-chain fatty acids. Starvation and diabetes, which are associated with an elevated concentration of plasma free fatty acids, reduced acetate thiokinase activity. So it was proposed that, in

addition to acetyl-CoA carboxylase and fatty acid synthetase, rat liver lipogenesis may be controlled by acetate thiokinase.

Currie (1972) found a significant linear regression between changes in milk fat content and activity of acetate thiokinase throughout the lactation of a cow. If acetate polymerization into long-chain fatty acids is limiting milk fat synthesis rather than uptake of blood triglycerides, then it might be supposed that acetate thiokinase could be rate limiting for fatty acid synthesis in ruminant mammary tissue.

Others, however, disagree with the importance of acetate thiokinase as a regulatory enzyme of fatty acid synthesis. Kornacker and Lowenstein (1965) showed that acetate thiokinase activity decreased with starvation in rat liver. The decrease was gradual though, with 50% of the activity still present after 2 days of fasting. Refeeding the two-day starved rats a balanced diet caused acetate thiokinase to increase by only about 30% above normal. In contrast, citrate cleavage enzyme increased several-fold upon refeeding. Ingle et al. (1973) reported that in adipose tissue of fattening lambs acetate thiokinase activity decreased substantially during fasting, but the decrease did not occur until 48 hours after fasting. Its activity did not increase significantly with refeeding. These studies show that acetate thiokinase activity decreases with fasting, but not as rapidly as fatty acid synthesis decreases. Upon refeeding, its activity increases very slowly. Acetate thiokinase may play a role in long-term regulation of fatty acid synthesis, but its activity does not seem to respond

quickly enough for immediate or short-term regulation.

Acetyl-CoA Carboxylase (EC 6.4.1.2, acetyl CoA:CO_2 ligase)

Evidence suggests that the conversion of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase is the rate-limiting step in fatty acid synthesis (Numa, Matsuhashi and Lynen, 1961; Ganguly, 1960). Acetyl-CoA carboxylase was discovered rather recently. In 1960, Wakil and Gibson showed that the formation of malonyl-CoA from acetyl-CoA was catalyzed by acetyl-CoA carboxylase, a biotin-containing enzyme. Later that year, Ganguly suggested that it was the rate-limiting enzyme in fatty acid synthesis. In 1961, Numa et al. showed that acetyl-CoA carboxylase activity decreased along with fatty acid synthesis during fasting. Also, addition of acetyl-CoA carboxylase to the incubation media containing liver homogenates from both fasted and normal rats stimulated fatty acid synthesis. Therefore, they concluded that conversion of acetyl-CoA to malonyl-CoA is the rate-limiting step in fatty acid synthesis and that the decrease in fatty acid synthesis in fasted animals is due to a decrease in activity of this enzyme. Activity of acetyl-CoA carboxylase very closely paralleled the progressive decrease in fatty acid synthesis by ovine adipose tissue slices during starvation and the increase upon refeeding (Ingle et al., 1973).

Acetyl-CoA carboxylase can exist either as the protomeric form or as a polymeric form (Gregolin et al., 1966a; Numa, Ringelmann and Riedel, 1966). The polymeric form of the enzyme is the active form. Molecular weight of the protomeric subunit is 409,000. There is one biotin per subunit. Molecular weight of the active polymeric form is

8.3 or 4.1 million, 20 or 10 subunits (Gregolin et al., 1966b). Acetyl-CoA carboxylase is activated by di- and tricarboxylic acids, especially citrate (Gregolin et al., 1966a; Vagelos, Alberts and Martin, 1963). This activation is associated with a change in the structure and sedimentation coefficient of the enzyme. The sedimentation coefficient of unactivated acetyl-CoA carboxylase is 18.8 s, that of carboxylase activated with citrate is 43 s (Vagelos et al., 1963). Activated acetyl-CoA carboxylase has a filamentous structure which is lost upon inactivation but can be restored rapidly by a di- or tricarboxylic acid (Gregolin et al., 1966a). Apparently acetyl-CoA carboxylase is quite similar between animal species. Kleinschmidt, Moss and Lane (1969) found carboxylase from bovine adipose tissue and avian liver to have similar filamentous structures when in the active state.

Acetyl-CoA carboxylase can change very rapidly between the active polymeric form and the inactive protomeric form. Addition of the polymeric form to an assay mixture without citrate results in a decline in catalytic activity to a level less than 2% of that obtained in the presence of citrate (Moss et al., 1972). This decay seems associated with depolymerization to a less active protomeric form. Citrate can change the protomeric form to the polymeric form for the bovine carboxylase in less than 10 seconds (Moss and Lane, 1972). Position of the protomer-polymer equilibrium is an indicator of carboxylase activity. The initial rise in the rate of fatty acid synthesis observed in rat liver within 8 hours of refeeding is not due to an increase in quantity of acetyl-CoA carboxylase, but may rather be attributable to changes in

concentration of allosteric effectors such as citrate and long-chain fatty acyl-CoA derivatives. After 8 hours, the content of carboxylase begins to increase (Nishikori, Iritani and Numa, 1973).

The quantity of total enzyme present (active and inactive) has very little control over short-term regulation (less than a day) of fatty acid synthesis. Nakanishi and Numa (1970) found the half life of acetyl-CoA carboxylase to be 59 hours in a fed rat and 31 hours in a fasted rat. Majerus and Kilburn (1969), however, observed a half life of 18 hours in fasted rats. So, the quantity of enzyme present may be able to regulate the day-to-day level of fatty acid synthesis but not the more short-term regulation.

Carlson and Kim (1973) have proposed that acetyl-CoA carboxylase is regulated by phosphorylation and dephosphorylation. Carboxylase is activated by incubation with Mg^{++}; this activation is inhibited by F^{-}. Also, carboxylase is inactivated by ATP. These properties suggest that acetyl-CoA carboxylase could be regulated by a phosphorylation-dephosphorylation sequence of reactions. The carboxylase protein is phosphorylated, and the activity is decreased by an ATP-dependent kinase (Allred and Roehrig, 1973b). It is reactivated by dephosphorylation that is mediated by a Mg^{++}-dependent phosphatase.

Compounds of the tricarboxylic acid cycle stimulate the carboxylation reaction of fatty acid synthesis (Martin and Vagelos, 1962; Matsuhashi, Matsuhashi and Lynen, 1964). Citrate is the most stimulatory of the di- and tricarboxylic acids and also probably the most important. Isocitrate and malonate are also quite important as activators. Citrate

does not take part in the carboxylation reaction by providing carbon dioxide (Martin and Vagelos, 1962). Prior incubation of acetyl-CoA carboxylase with citrate alone caused an activation that could not be explained by the formation of a free, activating intermediate derived from citrate. This suggests that the enzyme itself may be activated. Possibly the biotin portion of the carboxylase enzyme, which is the active group, is involved in the activation, because carboxylation of acetyl-CoA goes through a CO_2-biotin-enzyme intermediate. Citrate activates acetyl-CoA carboxylase by increasing the V_{max} values for the substrate (Numa, Ringelmann and Lynen, 1965b).

There are several metabolites, in addition to citrate and other di- and tricarboxylic acids, that will promote the in vitro aggregation of the protomer to form the polymer of acetyl-CoA carboxylase. Inorganic phosphate, acetyl-CoA, high protein concentration and a pH of 6 to 7 promote the conversion to the polymeric form (Gregolin et al., 1968).

Carnitine has a regulatory effect on lipogenesis, especially at the carboxylation step (Marquis, Francesconi and Villee, 1968). In the absence of citrate or other carboxylase activators, fatty acid synthesis from acetate in cell-free preparations of rat liver proceeds at a very low rate. Addition of carnitine further decreases fatty acid synthesis. When a high concentration of citrate is added, carnitine stimulates fatty acid synthesis. The reason for this dual effect according to Marquis et al. (1968) is that in the absence of citrate cytoplasmic acetyl-CoA is converted to acetylcarnitine, but at high

concentration of citrate and increased carboxylase activity, acetyl-CoA is preferentially used for fatty acid synthesis. Carnitine enhancement of fatty acid synthesis may be accomplished by combining with a feedback inhibitor of acetyl-CoA carboxylase, palmitoyl-CoA, forming palmitoylcarnitine. Palmitoylcarnitine stimulates lipogenesis and acetyl-CoA carboxylase but does not affect the incorporation of malonyl-CoA into long-chain fatty acids, suggesting the site of action of long-chain acylcarnitine to be at the carboxylation step. Marquis et al. (1968) suggest that perhaps the antagonistic effect of palmitoyl-carnitine on inhibition by palmitoyl-CoA may have some physiological significance.

Acetyl-CoA carboxylase in isolated rat adipose tissue is activated by insulin (Lee, Thrall and Kim, 1973). This activation occurs in the presence of inhibitors of protein synthesis and can be observed as early as 15 minutes. So, the carboxylase is directly regulated without involving protein synthesis, but the mechanism of insulin activation is unclear.

Acetyl-CoA carboxylase is strongly inhibited by long-chain, acyl-CoA derivatives (Porter and Long, 1958; Tubbs and Garland, 1964; Numa, Bortz and Lynen, 1965a; Goodridge, 1972). This inhibition by long-chain, acyl-CoA derivatives is reversible and competitive with regard to citrate and noncompetitive with regard to acetyl-CoA, bicarbonate or ATP (Numa et al., 1965a). Inhibition increases with chain length of acyl-CoA derivatives up to stearic acid, and saturated acyl-CoA derivatives are more inhibitory than unsaturated. The citrate-induced

increase in the sedimentation coefficient of carboxylase can be abolished by the simultaneous addition of palmitoyl-CoA (Numa et al., 1965b).

There is some disagreement as to whether the CoA derivative is necessary or if the free fatty acid itself will cause inhibition. Tubbs and Garland (1964) reported that free palmitate did not cause inhibition of fatty acid synthesis when palmitoyl-CoA would. Korchak and Masoro (1964), on the other hand, found that free fatty acids did not need to be converted to the CoA derivative to be inhibitory. Because free fatty acids inhibited the conversion of acetyl-CoA to fatty acids, the suggestion that free fatty acids inhibit lipogenesis by competing for coenzyme A seems improbable. So, free fatty acids primarily inhibit the carboxylase reaction. This disagrees with the findings of Masoro (1962) who reported that free fatty acids inhibited fatty acid synthesis by competing for coenzyme A.

Recently, Goodridge (1973b) has suggested three ways by which fatty acyl-CoA derivatives can inhibit fatty acid synthesis from pyruvate. First, fatty acyl-CoA directly inhibits acetyl-CoA carboxylase. Second, fatty acyl-CoA inhibits the mitochondrial citrate carrier resulting in a lower concentration of extramitochondrial citrate for the activation of carboxylase. Simultaneously, the direct inhibition of carboxylase by fatty acyl-CoA will be increased because the inhibition by fatty acyl-CoA is competitive with citrate. Third, inhibition of the citrate carrier will result in a slower rate of production of extramitochondrial acetyl-CoA, the substrate for acetyl-CoA carboxylase. So, the effect

of relatively small changes in fatty acyl-CoA concentration will be amplified to produce very large changes in the rate of fatty acid synthesis. Bortz and Lynen (1963) proposed that the inhibition by fatty acyl-CoA derivatives was due to competition with acetyl-CoA for the active site on the enzyme.

There is some question about the validity of the inhibitions caused by fatty acyl-CoA derivatives. Palmitoyl-CoA, for example, is very nonspecific in regard to the enzymes it inhibits. Examples of enzymes that are inhibited by palmitoyl-CoA are citrate condensing enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutamic dehydrogenase, fumarase and malic dehydrogenase, in addition to acetate thiokinase, acetyl-CoA carboxylase and fatty acid synthetase (Srere, 1965; Taketa and Pogell, 1966). A likely explanation is the detergent-like properties of the long-chain acyl-CoA derivatives which produce conformational changes of the various enzymes.

Acetyl-CoA carboxylase may also be under the feedback contol of the end product malonyl-CoA (Gregolin et al., 1966b; Chang et al., 1967). Malonyl-CoA concentration in vivo may be sufficient to depress carboxylase activity. Malonyl-CoA inhibition is competitive with respect to both acetyl-CoA and citrate. The competitive relationship between malonyl-CoA and citrate is the result of an equilibrium of an active polymeric form of acetyl-CoA carboxylase promoted by citrate and a catalytically inactive protomeric form promoted by malonyl-CoA. Inhibition of carboxylase by malonyl-CoA could provide a safeguard against excessive acetyl-CoA utilization for malonyl-CoA, providing a

fine control over the committed step of fatty acid synthesis. This regulatory role of malonyl-CoA may be even more important because of implications that the inhibitory effect of fatty acyl-CoA derivatives is due to their detergent effects and, therefore, not of physiological significance (Chakrabarty and Leveille, 1969).

Cyclic AMP also is an inhibitor of acetyl-CoA carboxylase (Allred and Roehrig, 1973b). The mechanism by which cAMP inhibits carboxylase is unknown. It may act directly on the enzyme, or possibly cAMP may activate a protein kinase which leads to enzyme inactivation. As mentioned earlier, acetyl-CoA carboxylase activity may be dependent on the ratio of the active dephosphorylated form and the inactive phosphorylated form (Carlson and Kim, 1973). Conversion from the active to the inactive form by the phosphorylation-dephosphorylation reaction is cAMP dependent.

There are other factors that can cause inhibition of acetyl-CoA carboxylase. ATP-Mg^{++} will cause dissociation of carboxylase (Gregolin et al., 1966b). The carboxylated enzyme, enzyme-CO_2 complex, has a greater tendency to dissociate than the uncarboxylated enzyme. Also, pH values greater than 7.5 cause dissociation of the polymeric form (Gregolin et al., 1968).

Recent studies indicate that the activity of acetyl-CoA carboxylase may not be the only factor controlling fatty acid synthesis. Acetyl-CoA carboxylase activity seems higher in the fat cells of well-marbled cattle than in those of low-marbled cattle. The rate of lipogenesis in the fat cells of well-marbled cattle, however, was not higher than

the rate in low-marbled cattle (Chakrabarty and Romans, 1972). Chakrabarty and Romans do not believe that the difference in carboxylase activity is the sole cause of different marbling levels in cattle. Goodridge (1973a) found that when nonfed, neonatal chicks were given a single glucose injection the activities of acetyl-CoA carboxylase and fatty acid synthetase did not increase until six hours after administration, while the rate of fatty acid synthesis was stimulated by more than five-fold at 1.5 hours after injection. These results suggest that acetyl-CoA carboxylase and fatty acid synthetase are not part of an "operon" and that changes in their activities did not initiate changes in the rate of fatty acid synthesis. Goodridge proposes that the concentration of the lipogenic enzymes are regulated primarily by the concentration of intermediates in the lipogenic pathway.

Fatty Acid Synthetase

Conversion of malonyl-CoA to palmitic acid is catalyzed by the multienzyme complex, fatty acid synthetase (Bressler and Wakil, 1961). Requirements for fatty acid synthesis are acetyl-CoA, malonyl-CoA and NADPH (Brady, Mamoon and Stadtman, 1956; Martin, Horning and Vagelos, 1961). NADH cannot be substituted for NADPH. Fatty acid synthetase is located, along with acetyl-CoA carboxylase, in the cytoplasm of the cell (Gibson, Titchener and Wakil, 1958).

Chang et al. (1967) found that in livers of mice, chickens and rats the capacity of acetyl-CoA carboxylase to generate malonyl-CoA approximately equaled the capacity of fatty acid synthetase to incorporate malonyl-CoA into long-chain fatty acids. Similarity in

carboxylase and synthetase activities suggests that malonyl-CoA may play a significant role in regulating acetyl-CoA carboxylase (Chakrabarty and Leveille, 1969; Chang et al., 1967).

Free palmitic acid is the major product of the fatty acid synthetase reaction (Porter and Long, 1958). Myristyl- and palmitoyl-CoA arise via condensation of C_2 units and then are deacylated to free acids. Free acids are again esterified with reduced coenzyme A through the action of thiokinase. Palmitoyl-CoA, at levels of 0.2 mmolar or higher, causes marked inhibition of fatty acid synthesis in vitro. In addition to inhibiting acetyl-CoA carboxylase, palmitoyl-CoA inhibits fatty acid synthetase (Porter and Long, 1958). However, Dorsey and Porter (1968) concluded that palmitoyl-CoA inhibits fatty acid synthetase by virtue of its detergent nature. Sodium lauryl sulfate, a strong detergent, acts in a similar way on fatty acid synthetase. In fattening lambs, Ingle et al. (1973) found that fatty acid synthetase activity did not begin to decrease until after 48 hours of fasting, while fatty acid synthesis already had decreased to about 40% of normal. After 8 days of refeeding, the activity of fatty acid synthetase had not changed, while fatty acid synthesis had increased five-fold over the fasted level (8 days of fasting). There is little evidence that indicates fatty acid synthetase is the main regulating enzyme in fatty acid synthesis.

NADPH-Generating Enzymes

In 1964, Flatt and Ball proposed that the availability of reduced coenzymes may be rate limiting in fatty acid synthesis. They showed

that only about 50% of the required NADPH for fatty acid synthesis could be furnished by the pentose phosphate pathway. In the non-ruminant, malic enzyme (EC 1.1.1.40, L-malate: NADPH oxidoreductase) can furnish NADPH to help meet the requirement (Young, Shrago and Lardy, 1964; Wise and Ball, 1964). Malic enzyme can provide NADPH directly or through transhydrogenation from NADH. Ingle, Bauman and Garrigus (1972) have proposed that acetate can provide NADPH via NADP-isocitrate dehydrogenase (EC 1.1.1.42, threo-D_s-isocitrate: NADP oxidoreductase (decarboxylating)) in ruminant adipose tissue.

Many studies have been conducted to measure changes in NADPH-generating enzymes during the life cycle of many species and as a result of diet or fasting and refeeding. In rats (Taylor, Bailey and Bartley, 1967), pigs (Allee et al., 1971b) and cattle (Pothoven and Beitz, 1973), the NADPH-generating enzymes change along with the respective changes in fatty acid synthesis during growth. Long-term fasting causes decreases in the activities of the NADPH-generating enzymes, but not to the extent that lipogenesis decreases. This has been demonstrated in the rat (Tepperman and Tepperman, 1961; Leveille, 1966), chick (Leveille, 1966), pig (O'Hea and Leveille, 1969), sheep (Ingle et al., 1973) and cattle (Pothoven and Beitz, 1973). Short-term fasting, however, does not affect NADPH-generating enzymes significantly (Yeh and Leveille, 1970, Ingle et al., 1973). Careful studies of the relationship between fatty acid synthesis and NADPH-generating enzymes has shown that changes in NADPH-generating enzymes are the result of decreased lipogenesis and not the cause of it (Leveille, 1966; O'Hea and Leveille, 1969).

Hormones

In 1958, Winegrad and Renold reported that insulin in the presence of glucose stimulated lipogenesis from acetate. Lipogenic capacity is very low in diabetic pigs (Romsos, Leveille and Allee, 1971). Subcutaneous injections of insulin completely restored the capacity for lipogenesis in adipose tissue slices from diabetic pigs. Addition of insulin to the *in vitro* incubation medium did not restore lipogenesis. The decrease in insulin during fasting is greater than the decrease in glucose (Trenkle, 1970). After 72 hours of fasting, plasma insulin decreased to 32% of the peak value and glucose decreased to 81% of the peak value, while plasma free fatty acids increased to a maximum level of six times the minimum value after 48 hours of fasting.

Gellhorn and Benjamin (1966) correlated the depression of fatty acid synthesis in adipose tissue during fasting with a virtual cessation of synthesis of "heavy" RNA molecules. Restoration of RNA synthesis with refeeding precedes the restoration of fatty acid synthesis. In diabetes the defects in lipid synthesis are corrected by the administration of insulin, and this can be prevented by the concomitant administration of actinomycin D. So, Gellhorn and Benjamin (1966) proposed that fasting, aging and diabetes affect RNA synthesis primarily, and the enzymatic failures are secondary to this. More recently, Lee *et al.* (1973) suggest that acetyl-CoA carboxylase is directly regulated by insulin without involving protein synthesis. They found that insulin activated the enzyme in the presence of inhibitors of protein synthesis and as early as 15 minutes.

Glucagon and cAMP markedly decrease glucose oxidation and conversion into fatty acids in rat liver slices (Meikle, Klain and Hannon, 1973). Epinephrine is much less effective. Meikle et al. suggest that hepatic glucagon levels are important in regulating glucose oxidation and its conversion to fatty acids. This effect seems mediated via modification of cAMP levels in hepatic tissue. Allred and Roehrig (1973a) also agree that cAMP could serve as the messenger to regulate lipogenesis, because glucagon increases cAMP level and insulin decreases cAMP concentration. Point of action of cAMP could be acetyl-CoA carboxylase as discussed earlier (Lee et al., 1973; Allred and Roehrig, 1973b). Prostaglandins also can affect cAMP levels. The full extent of their role and mechanism of their action is still unknown.

Hypothalamus

In 1950, Kennedy proposed that the hypothalamus controlled fat deposition via regulation of caloric intake. The young rat adjusts its food intake so precisely to its energy needs that its fat stores remain almost constant (Kennedy, 1953). This suggests that the primary function of the ventromedial nucleus of the hypothalamus is to stabilize fat stores by regulating food intake. In 1968, Mu et al., however, showed that body fat content is controlled by food intake, not body fat content controlling food intake, and the body is able to store whatever energy that is consumed.

Lepkovsky and Furuta (1971) reported that forced feeding of excessive amounts of food to normal White Leghorn cockerels caused obesity. After cessation of forced feeding, the body fat content

quickly returned to normal. During forced feeding, voluntary food intake was zero. After cessation of forced feeding, food intake remained zero for 6 to 10 days and then gradually increased as the amount of fat in the adipose tissues returned to normal. The hypothalamus did not differentiate between calories from adipose tissue and those from food. Lepkovsky (1973) proposes that the hypothalamus gives a set point determining the normal amount of fat in adipose. For example, the White Leghorn cockerel regulates its food intake so that it is normally lean. So, the hypothalamus gives the control system of the adipose tissue of this breed a set point that requires very little fat. The amount of fat in the adipose tissue is regulated by *in situ* lipogenesis and lipolysis and uptake of triglycerides from the blood.

Adipose Cellularity

Adipose tissue develops by two processes, hyperplasia and hypertrophy. In the young animal hyperplasia is the main method of growth. Hypertrophy becomes more important than hyperplasia at some later stage of adipose development. This can vary with the fat depot site and species of animal. Epididymal and retroperitoneal adipose depots of rats grow exclusively by cellular enlargement after the 15th week of age, with no further change in cell number (Hirsch and Han, 1969). Carcass adipose tissue of pigs expands by a combination of adipocyte hyperplasia and hypertrophy up to 5 months of age, after which adipose expansion is by hypertrophy only (Anderson and Kauffman, 1973). Hyperplasia seems nearly complete in subcutaneous and perirenal depots

of cattle by about 8 months of age, whereas marbling fat is a late-developing depot and hyperplasia is still an active process at 14 months of age (Hood and Allen, 1973). Subcutaneous adipose tissue from animals of the leaner Holstein breed contain smaller and fewer adipose cells than that from the fatter Hereford x Angus animals.

Early nutrition may affect the development of adipose tissue. Knittle and Hirsch (1968) showed that undernutrition of rats during the first three weeks after birth followed by ad libitum feeding resulted in fewer and smaller cells in the epididymal fat pads at 20 weeks of age than in the well-fed rats. Cell number as well as cell size, however, was still increasing in undernourished rats between the 15 and 20 week measurement, whereas the adipose cells of the well-fed rats were increasing in size but not in number. Hirsch and Han (1969) reported that severe starvation during the sixth week of life of rats followed by normal feeding had no lasting effect on cell size or cell number. Prolonged food restriction beginning the 15th week reduced cell size but cell number was unaffected. It is still unknown if early nutrition affects the final adipose cell number in the adult.

Summary

Regulation of fatty acid synthesis has been well studied; however, it is still not well understood. It is well agreed upon that acetyl-CoA carboxylase is the rate-limiting enzyme. All the factors that control the activity of acetyl-CoA carboxylase are not elucidated. Hormones, such as epinephrine, glucagon and insulin, probably regulate many of the enzymes involved in fatty acid synthesis through cAMP.

Also, the hypothalamus may regulate fatty acid synthesis and deposition by controlling food intake. Very probably, there is a combination of factors that regulate fatty acid synthesis. It is improbable that a process as complicated as fatty acid synthesis could be regulated by one factor or enzyme.

SECTION 1.

EFFECT OF AGE AND LEVEL OF ENERGY ON LIPOGENESIS IN BEEF CATTLE

Introduction

Fat accumulation at any one adipose depot in beef cattle results from the interplay of three physiological processes: one, synthesis of fatty acids at the storage site; two, mobilization of storage lipid into circulation; and three, uptake of fatty acids from circulating blood lipids. We have attempted to estimate the potential of the first process, lipogenesis, as it might be influenced by age and nutrition of beef cattle.

The rate of fatty acid synthesis per unit weight of adipose tissue decreases with increasing animal weight in sheep (Ingle, Bauman and Garrigus, 1972), pigs (Allee et al., 1971b), rats (Gellhorn and Benjamin, 1966) and cattle (Pothoven and Beitz, 1973). The activities of the lipogenic enzymes expressed on an adipose cell basis increase markedly during the rapid increase in percentage of body fat between 3.5 and 5 months (Anderson and Kauffman, 1973). These enzyme activities reach a peak at 5 months of age, after which activities decrease to values approaching mature levels. Lipogenic capacity expressed on a cytosol-protein basis in adipose from steers fed a high concentrate diet decreases linearly with increasing animal weight, 278 to 528 kg (Pothoven and Beitz, 1973).

The effect of nutrition on lipid metabolism in adipose tissue has been studied extensively in nonruminants (Leveille, 1966; Yeh and Leveille, 1969; O'Hea and Leveille, 1969; Allee et al., 1971a); much

less has been conducted with ruminants. The effect of a concentrate versus hay diet on enzyme activities important for lipogenesis has been studied in bovine adipose tissue (Young, Thorp and DeLumen, 1969) and in ovine adipose tissue (Martin et al., 1973). Tissues from animals fed the concentrate diet had higher glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme and NADP-isocitrate dehydrogenase activities.

The purpose of this study was to determine if fatty acid synthesis and fat deposition could be regulated by feeding steers different levels of energy to similar slaughter weights. This was accomplished by determining the rate of lipogenesis in adipose of beef steers of different weights fed different planes of energy.

Materials and Methods

Experimental animals

Twenty-four crossbred steers were used for this study. All steers were from Hereford x Angus dams, 12 were Charolais-sired and 12 Angus-sired. Animals were slaughtered at four different weights, averaging 113, 231, 363 and 505 kg. Steers of the 113-kg group (2 Charolais- and 2 Angus-sired) nursed their dams until slaughtered. The other steers were weaned at 180 kg and group-fed a growing diet (Table 1) up to 231 kg. Two Charolais- and two Angus-sired steers were slaughtered at this weight. The remaining steers were individually fed the finishing diet shown in Table 1. Steers were paired by sire and randomly assigned to a slaughter weight group and to a nutritional treatment of ad libitum or restricted feeding. Restricted-fed steers

Table 1. Composition of diets

Growing diet	Percentage[1]
Cracked corn	48.00
Ground cobs	1.10
Soybean meal (44% protein)	5.35
Molasses, cane	4.80
Premix	0.75
Ground alfalfa hay	40.00
Finishing diet	**Percentage**
Cracked corn	80.00
Ground cobs	5.00
Soybean meal (44% protein)	8.90
Molasses, cane	4.85
Premix	1.25
Premix	**Percentage**
Soybean meal (44% protein)	85.25
Ground limestone	8.72
Salt	3.36
Vitamin A[2]	2.47
Vitamin E[3]	0.04
Aureomycin 50[4]	0.16

[1]Percentage of air-dry diet.

[2]1,034,208 IU/kg.

[3]56,700 IU/kg.

[4]American Cyanamid Co.

were fed to gain 67% the rate of ad libitum-fed steers. Eight steers (4 restricted- and 4 ad libitum-fed) were slaughtered at 363 kg; the remaining eight steers were slaughtered at 505 kg.

Adipose tissue sites

Immediately after slaughter, inner layer of subcutaneous adipose (backfat) and omental adipose tissue were sampled. Tissue samples were maintained at 37 to 39°C in physiological saline until incubated.

Measurement of lipogenesis

Incubation procedure for measuring the rate of acetate incorporation into fatty acids was the same as previously described (Pothoven and Beitz, 1973). The lipid was extracted as before and washed by the method of Folch, Lees and Sloane-Stanley (1957). Because nonsaponifiable lipids and glycerol-glyceride constitute an insignificant percentage of the total lipids synthesized from acetate, the radioactivity found in the total lipid was measured. After the fourth washing of the lipid extract, the chloroform-methanol was evaporated and the residue was dissolved in 10 ml of toluene-PPO (0.3%)-POPOP (0.017%) scintillation solution for counting.

Carcass fat content

Fat was physically separated from the muscle and bone of the chilled left side of the carcass (DeWitt, 1973).

Statistical analysis

The data were analyzed by means of analysis of variance and Student's t test (Snedecor and Cochran, 1967).

Results

Rates of acetate incorporation into long-chain fatty acids (free plus esterified) for ad libitum-fed steers are given in Table 2. Individual animals values are given in Appendix Table 1. Results of Angus-sired and Charolais-sired steers were combined, because there was no significant difference between the two when all weight groups and tissue sites were considered. Rate of acetate incorporation in inner backfat adipose tissue increased with increasing weight up to

Table 2. Incorporation of acetate into long-chain fatty acids in adipose tissue of ad libitum-fed steers at different weights

Tissue	Animal Weight (kg)			
	113	231	363	505
	------ nmoles acetate/100 mg tissue/2 hr -----			
Inner Backfat[1]	252±126[a,2]	468±137[b]	575±67[b]	222±41[a]
Omental Adipose	176± 63[b]	101± 26[a]	220±56[b]	45± 9[a]

[1]Values are means ± SEM of four steers.

[2]Mean values on a given line followed by the same superscript letter do not differ significantly ($P < 0.05$).

363 kg, then it decreased sharply for the 505-kg steers. For omental adipose, there was a decrease in the rate of acetate incorporation between the 113-kg and 231-kg steers, an increase in the rate for the 363-kg steers, and then, a decrease for the 505-kg animals.

Differences in rates of fatty acid synthesis in adipose of ad libitum-fed and restricted-fed steers are shown in Table 3. Overall, adipose of ad libitum-fed steers had a higher ($P < 0.01$) rate of acetate incorporation into fatty acids than that of restricted-fed steers. For individual tissues, backfat adipose was more responsive to feed intake than omental adipose. For the 363-kg steers, backfat adipose of ad libitum-fed steers had an acetate incorporation rate three times that of restricted-fed steers, and omental of ad libitum-fed steers had a rate twice as fast. For the 505-kg steers, the rate of acetate incorporation in backfat adipose of ad libitum-fed steers was twice the rate in the restricted-fed steers, but omental adipose had the same rate for

Table 3. Incorporation of acetate into long-chain fatty acids in adipose tissue of ad libitum-fed and restricted-fed steers

Tissue	Animal Group			
	363 kg[1]	363R kg[2]	505 kg[1]	505R kg[2]
	----- nmoles acetate/100 mg tissue/2 hr -----			
Inner Backfat[3]	575±67	172±34	202±41	119±50
Omental Adipose	220±56	113±43	45± 9	39±22

[1]Ad libitum-fed steers.

[2]Restricted-fed steers.

[3]Values are means ± SEM of four steers.

both groups. Although restricted feeding might have been expected to cause less total fat accumulation in animals of the same size, restricted-fed animals had as much fat accumulation as ad libitum-fed animals (DeWitt, 1973). Carcasses of the 363-kg ad libitum-fed and restricted-fed steers had 19% and 20% fat, respectively; those of the 505-kg steers had 32% and 31% fat, respectively.

Discussion

The rate of fatty acid synthesis per unit weight of adipose decreases with increasing fatness in rats (Taylor et al., 1967), pigs (Allee et al., 1971b), sheep (Ingle et al., 1972) and cattle (Pothoven and Beitz, 1973). This study confirmed these observations, but there are other factors that affect the rate of fatty acid synthesis in adipose tissue during animal growth. Table 2 shows the changes in the rate of fatty acid synthesis during the normal growing-finishing stages

of beef cattle. The 113-kg calves were nursing, and the rate of fatty acid synthesis was low probably because of the high fat intake. Increased levels of dietary fat decrease the rate of fatty acid synthesis. This has been demonstrated in rats Taylor et al., 1967) and pigs (Allee et al., 1971b). Functionally, a nursing calf will be similar to a nonruminant, because the milk will pass directly to the abomasum, by-passing the rumen.

The 231-kg steers were fed a growing diet from the time of weaning. Compared with the rate of fatty acid synthesis in the nursing animals, there was a large increase in the rate of fatty acid synthesis in backfat adipose tissue of the 231-kg steers. The reason for the drop in fatty acid synthesis in omental adipose of the 231-kg steers is unclear. At 363 kg, the steers had been fed a high-concentrate diet for several months. At this time, the rate of fatty acid synthesis reached its maximum. Then, there was a decrease in the rate of fatty acid synthesis in both sites of the 505-kg steers. This is partially because of increased fat cell size and fewer cells per unit weight of tissue. Capacity for fatty acid synthesis expressed on a soluble-protein basis also decreases with increasing animal weight in cattle (Pothoven and Beitz, 1973).

Restricted- and ad libitum-fed steers had equal amounts of depot fat at both 363 and 505 kg. When the capacity for fatty acid synthesis was compared, however, adipose of ad libitum-fed steers was much more active than that of restricted-fed steers at both 363 and 505 kg (Table 3). So, differences in degree of fatness cannot be explained

by capacity for fatty acid synthesis alone. The two remaining processes that influence fat accumulation, lipolysis and absorption of preformed fatty acids from the blood, may possibly explain the amount of fat deposited.

Thornton (1973) studied lipolysis in the same steers of this experiment. Adipose tissue of restricted-fed steers had lower basal and epinephrine-stimulated lipolytic activities than that of ad libitum-fed steers, especially in backfat adipose. This would explain, in part, the reason restricted-fed steers had as much fat deposited as did ad libitum-fed steers. Even though adipose tissue had a lower fatty acid synthesizing capacity, the restricted-fed steers were able to deposit as much fat because of lower lipolysis.

No attempt was made to measure the third parameter of fat accumulation, uptake of fatty acids from the blood. This process could also play an important role in determination of the amount of fat accumulated. Fat deposition in subcutaneous and perirenal adipose as assessed by lipoprotein lipase activity expressed on a cellular basis was greater in adipocytes from obese pigs than those from lean pigs (Weisenburg and Allen, 1973). In perirenal adipose tissue of sheep, lipoprotein lipase activity per unit protein decreased along with the decrease in the rate of fat deposition (Sidhu et al., 1973). In ovine subcutaneous adipose, however, lipoprotein lipase activity did not seem highly related to the rate of fat deposition.

Reesterification of free fatty acids from lipolysis also could play a role in regulation of fat deposition. A closer investigation

will have to be made to quantitate the role of hormones and other factors, such as genetics, in determining the degree of fatness. Once the control of fat deposition is determined, then progress can be made toward the regulation by nutrition or other means of desired and undesired fat depots.

SECTION 2.

CHANGES IN FATTY ACID SYNTHESIS AND LIPOGENIC ENZYMES IN ADIPOSE TISSUE FROM FASTED AND FASTED-REFED STEERS

Introduction

Fasting causes a marked reduction in the capacity for fatty acid synthesis in mammals (Tepperman and Tepperman, 1961; O'Hea and Leveille, 1969; Ingle et al., 1973; Pothoven and Beitz, 1973). Fasting also causes a large decrease in the activity of acetyl-CoA carboxylase, the rate-limiting enzyme of fatty acid synthesis (Numa et al., 1961; Ingle et al., 1973). The NADPH-generating enzymes activities, however, are not affected to the same extent as fatty acid synthesis and acetyl-CoA carboxylase (Young et al., 1964; O'Hea and Leveille, 1969; Young et al., 1969; Ingle et al., 1973; Pothoven and Beitz, 1973).

Restoration of fatty acid synthesis and activities of enzymes involved in fatty acid synthesis after fasting has been studied extensively in nonruminants and avians (Tepperman and Tepperman, 1961; Young et al., 1964; Leveille, 1969; O'Hea and Leveille, 1969; Yeh and Leveille, 1970). Few measurements in ruminants have been made on the full restoration of fatty acid synthesis after fasting. Martin et al. (1973) showed full restoration of NADPH-generating enzymes in adipose of sheep fasted 27 days after 14 days of concentrate refeeding. Ingle et al. (1973) showed only a partial restoration of fatty acid synthesis or lipogenic enzyme activity in adipose of sheep after 8 days of refeeding after an 8-day fast. Feed intake, however, after 8 days of refeeding represented only 80% of the maintenance requirement. Con-

sidering rumen fill as well as other tissues of the animal complicates experiments on the restoration of lipogenesis in ruminants.

These studies were conducted to investigate the changes in the rate of fatty acid synthesis during fasting and during refeeding until lipogenesis was fully restored. To study possible factors involved in regulation of fatty acid synthesis, several other measurements were made. Activities of NADPH-generating enzymes, acetate thiokinase and acetyl-CoA carboxylase were compared with changes in the rate of fatty acid synthesis. Plasma levels of free fatty acids and acetate were also determined.

Materials and Methods

Experimental animals

Six Brown Swiss steers weighing an average of 550 kg were used for this study. These steers were fasted for 8 days and then refed for approximately 8 weeks. Before the experiment, the steers were group-fed ad libitum the concentrate mixture shown in Table 4 plus approximately 10 kg of corn silage per day. During fasting, the steers received only water and salt and were penned in individual, unbedded pens. On the first day of refeeding the steers received 2.0 kg of grain mix (Table 4) and 2.3 kg of alfalfa-brome hay divided between two feedings. Grain was increased by 1.0 kg per day. On the fourth day, 7 kg of corn silage were added to the diet. The steers were on full feed of grain plus 7 kg of corn silage and 2.3 kg of hay after 7 days. During refeeding, the steers were individually fed, and the pens were bedded with oat straw.

Table 4. Composition of grain mix fed to Brown Swiss steers

Component	Percentage[1]
Ground shelled corn	59.0
Ground oats	20.0
Soybean oil meal (44% protein)	15.0
Molasses (cane)	2.5
Dicalcium phosphate	1.0
Limestone	1.5
Trace-mineralized salt	1.0
Vitamin supplement[2]	-
Antibiotic supplement[3]	-

[1]Percentage of air-dry diet.

[2]Supplied 5375 IU vitamin A palmitate and 990 IU vitamin D_2 per kg of grain mix.

[3]Supplied 27.5 mg aureomycin (American Cyanamid Co.) per kg of grain mix.

Tissue biopsies

Eight biopsy samples of the subcutaneous adipose tissue (backfat along the longissimus) were taken during the experiment. A control sample was obtained the day before fasting, three samples during fasting and four samples during the first 24 days of refeeding. The time schedule of taking biopsy samples was arranged so that samples would be taken on every day of fasting and on many of the days during refeeding, especially during the first two weeks. A ninth sample was obtained at slaughter approximately 8 weeks after fasting.

The area from which the tissue sample was to be removed was shaved, disinfected and anesthesized with a subcutaneous injection of lidocaine hydrochloride. An incision 9 cm long was made perpendicular

to and beginning 5 to 6 cm laterally from the midline to expose the fat pad. A fat sample of at least 10 g was removed by means of a scalpel. An antibiotic powder was applied to the wound and the skin was closed with a continuous locking stitch of silk suture. Sutures were removed 7 to 10 days after biopsy. Tissue samples were maintained at 37 to 39°C in physiological saline until incubated or homogenized.

A jugular blood sample was collected into a 20-ml heparinized-vacuum tube before each biopsy. The blood was centrifuged at 1000 x g for 15 minutes. Plasma was drawn off with a pipet and stored at -20°C. The steers were also weighed before each biopsy.

Incubation procedure

A 100- to 200-mg section of adipose tissue was incubated as previously described (Pothoven and Beitz, 1973). The lipid was extracted as before and washed by the method of Folch et al. (1957). After the fourth washing, the chloroform-methanol was evaporated, and the residue was dissolved in 10 ml of toluene-PPO (0.3%)-POPOP (0.017%) scintillation solution for counting.

Enzyme determinations

Adipose tissue was homogenized in three volumes of 0.15 M KCl in 50 mM Tris buffer (pH 7.4) with a Virtis 45 homogenizer at high speed for 20 seconds. The homogenate was centrifuged at 12,000 x g for 10 minutes at room temperature. An aliquot of the supernate was used for measuring acetyl-CoA carboxylase activity, and the remainder was centrifuged at 100,000 x g for 60 minutes at 4°C.

Acetyl-CoA carboxylase (EC 6.4.1.2) was assayed by the method of

Dakshinamurti and Desjardins (1969) with some of the modifications described by Anderson, Kauffman and Kastenschmidt (1972). Final concentrations of reagents were 0.1 mM EDTA, 8 mM $MgCl_2$, 3 mM reduced glutathione, 5 mM ATP, 5 mM potassium citrate, 0.6 mg/ml bovine serum albumin (Fraction V), 0.2 mM acetyl-CoA and 10 mM $NaH^{14}CO_3$ (0.4 μCi/μmole) buffered with 50 mM Tris (pH 7.6). Final volume of the reaction mixture was 1.0 ml containing 0.75 ml of supernate. After a 30-minute preincubation with citrate at 37°C, the reaction was started by adding ATP, acetyl-CoA and $NaH^{14}CO_3$. The blank contained no ATP and acetyl-CoA. Aliquots of 0.2 ml were removed at 1 and 2 minutes and delivered rapidly into scintillation vials containing 0.3 ml of 0.3 M HCl. The vials were then heated at 85°C for 30 minutes. Then 0.2 ml of 1 M Tris (pH 10.5) was added, followed by 15 ml of Bray's scintillation solution (Bray, 1960). Activity was calculated as nmoles bicarbonate fixed per minute per milligram soluble protein.

Acetate thiokinase (EC 6.2.1.1) was assayed by the method described by Murthy and Steiner (1972). The complete incubation system contained 70 mM Tris buffer (pH 8.2), 6 mM potassium acetate, 4 mM ATP, 100 μM CoA, 0.2 M NH_2OH (pH 8.0) and 2 mM each of NaF, $MgCl_2$ and reduced glutathione in a total volume of 1.0 ml containing 0.85 ml of supernate. This mixture was incubated for 60 minutes at 37°C. The generated acetyl-CoA was trapped with hydroxylamine, and the resulting acetohydroxymate was measured by the method described by Lipmann and Tuttle (1945). Acetate and coenzyme A were omitted from the mixture for the blanks. The acetohydroxymate concentration was measured spectrophoto-

metrically at a wavelength of 540 nm. Activity was expressed as nmoles acetyl-CoA produced per hour per milligram soluble protein.

Glucose-6-phosphate (G-6-P) dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate (6-PG) dehydrogenase (EC 1.1.1.44) were assayed by the method of Glock and McLean (1953). G-6-P dehydrogenase and 6-PG dehydrogenase activities were measured together and then 6-PG dehydrogenase activity alone. G-6-P dehydrogenase activity was determined by difference. NADP-isocitrate dehydrogenase (EC 1.1.1.42) activity was measured by the procedure of Ochoa (1955). Activities of these three enzymes were expressed as nmoles NADPH produced per minute per milligram soluble protein.

Soluble protein concentration of the supernate was measured by the Lowry procedure (Lowry et al., 1951).

Free fatty acid concentration of plasma was determined by the colorimetric micromethod described by Falholt, Lund and Falholt (1973). To determine plasma volatile fatty acid concentrations, the plasma was deproteinized by adding 5 ml of plasma to a 50-ml centrifuge tube containing 5 ml 6% $Na_2WO_4,2H_2O$ (Folin and Wu, 1919), 4 ml 0.5 N H_2SO_4 and 1 ml 10 mM iso-valeric acid (used as an internal standard). The tubes were stoppered and shaken vigorously. After 15 minutes, the material was centrifuged at 8000 x g for 15 minutes. The supernate was filtered, made alkaline with a few drops of 20% KOH and freeze-dried. Samples were stored at $4^{\circ}C$ until analyzed. The residue was taken up with a minimum of 5% metaphosphoric acid and 2 drops of 9 M H_2SO_4. Volatile fatty acid concentrations were measured with a

gas-liquid chromatograph with a hydrogen flame ionization detector containing a 2.0-meter column packed with 15% FFAP (Wilkins Instrument and Research Inc., Walnut Creek, Calif.) coated on 60/80 mesh acid-washed Chromosorb W (Applied Science Labs., State College, Pa.) using a fluidizer. Column temperature was 145°C and injector temperature 225°C. Nitrogen was the carrier gas. A standard solution containing acetic, propionic, butyric and iso-valeric acids of known concentrations was used to establish the relative detector response between the internal standard and the others. Knowing the concentration of iso-valeric acid added initially, the concentration of the other volatile fatty acids could be calculated.

Statistical analysis

The data were analyzed by least-square analysis of variance and linear regression on days (Snedecor and Cochran, 1967).

Results

The six steers used in this study lost an average of 45 kg in weight during the 8-day fast. Most of this weight loss was due to a decrease of rumen fill. The steers regained their prefasting weight (550 kg) by seven or eight days of refeeding. Their average weight was 586 kg at slaughter (8 weeks of refeeding). Appendix Table 3 gives the individual animal weights for each biopsy period.

Acetate incorporation into long-chain fatty acids in adipose tissue during fasting and refeeding is shown in Figure 1. The individual values for all measurements are given in Appendix Tables 4 to 12. The rates of acetate incorporation into fatty acids after

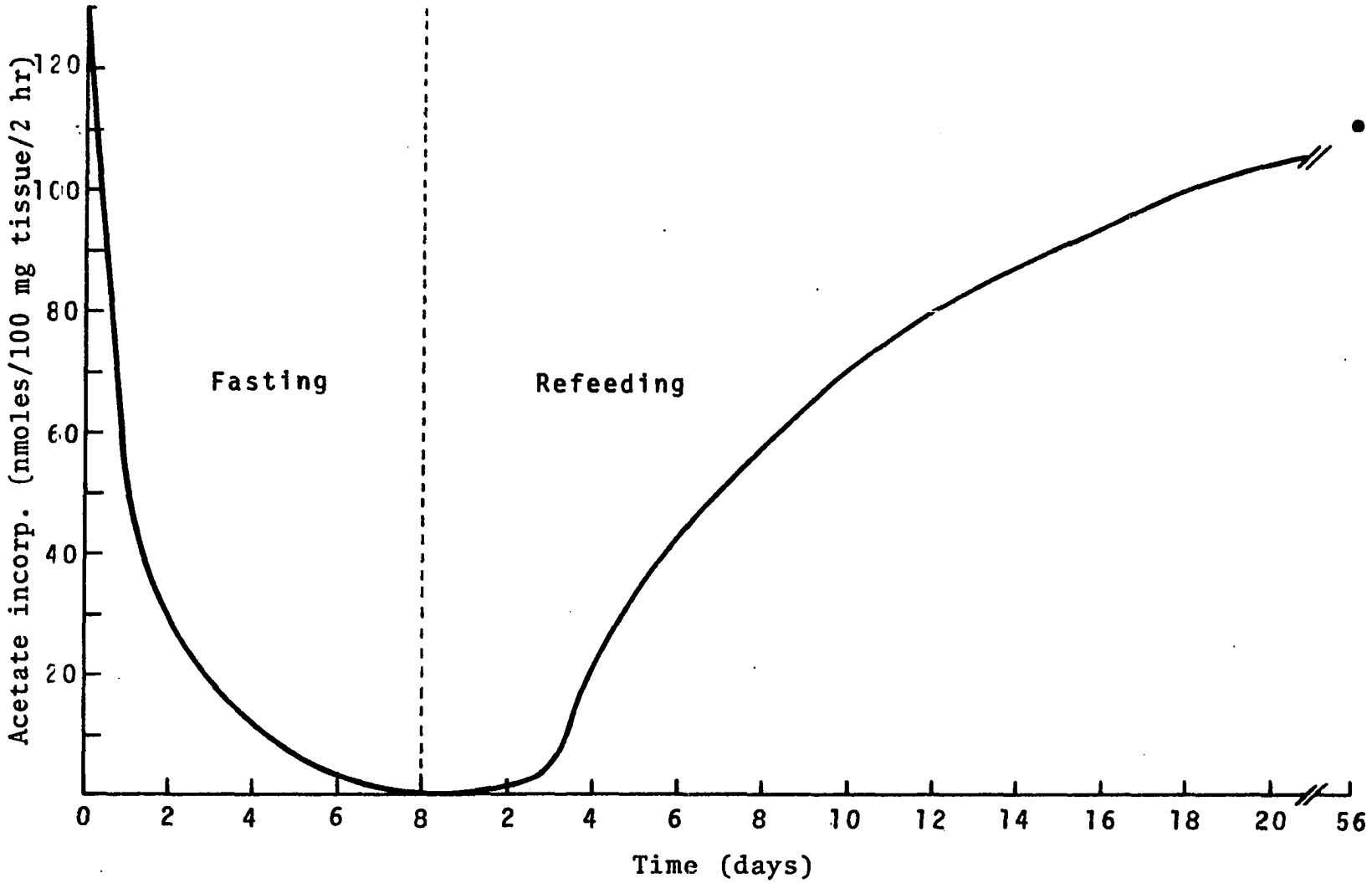

Figure 1. Incorporation of acetate into long-chain fatty acids in adipose tissue of fasted and fasted-refed steers. Pooled standard error = ± 8.6.

1, 4 and 8 days of fasting were 40%, 10% and 1% of the initial rate, respectively. The rate of fatty acid synthesis did no begin to increase until after 3 days of refeeding, which coincided with when feed intake of the steers was above maintenance for energy. Capacity for fatty acid synthesis increased rapidly after 3 days of refeeding up to about 18 days, and then gradually leveled. After 6 and 12 days of refeeding, the rates of acetate incorporation into fatty acids were 38% and 71% of the value at 56 days of refeeding, respectively. After 21 days of refeeding, the rate of fatty acid synthesis was essentially the same as that at 56 days, which was similar to that before fasting. The decrease in the rate of fatty acid synthesis during fasting was more rapid than the increase during refeeding.

Figure 2 shows changes in the activity of acetyl-CoA carboxylase in adipose during fasting and refeeding. The curve of the activity during fasting is very similar to that of acetate incorporation into fatty acids. Acetyl-CoA carboxylase activity decreased rapidly upon fasting and was 1% of the control after 6 days of fasting. Acetyl-CoA carboxylase activity did not increase until after 3 or 4 days of refeeding, reached a maximum greater than the control value at 16 days, and then decreased to a value 40% of the control at 56 days.

Acetate thiokinase activity remained unchanged during fasting and the first 7 days of refeeding (Figure 3). Then, the activity of acetate thiokinase increased steadily to a value twice that of the control after 21 days of refeeding. The activity at 56 days was similar to that at 21 days.

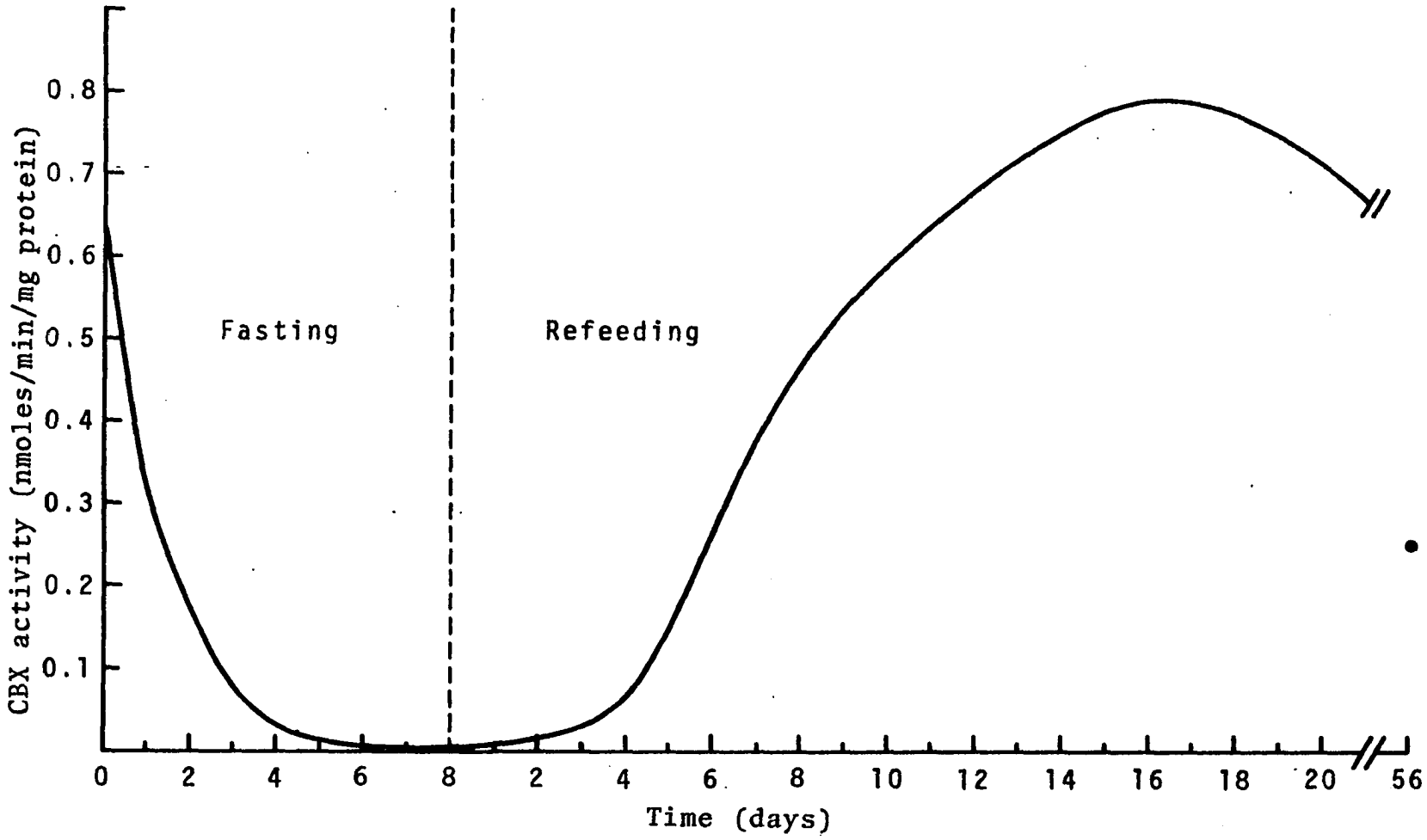

Figure 2. Activity of acetyl-CoA carboxylase (CBX) in adipose tissue of fasted and fasted-refed steers. Pooled standard error = ± 0.048.

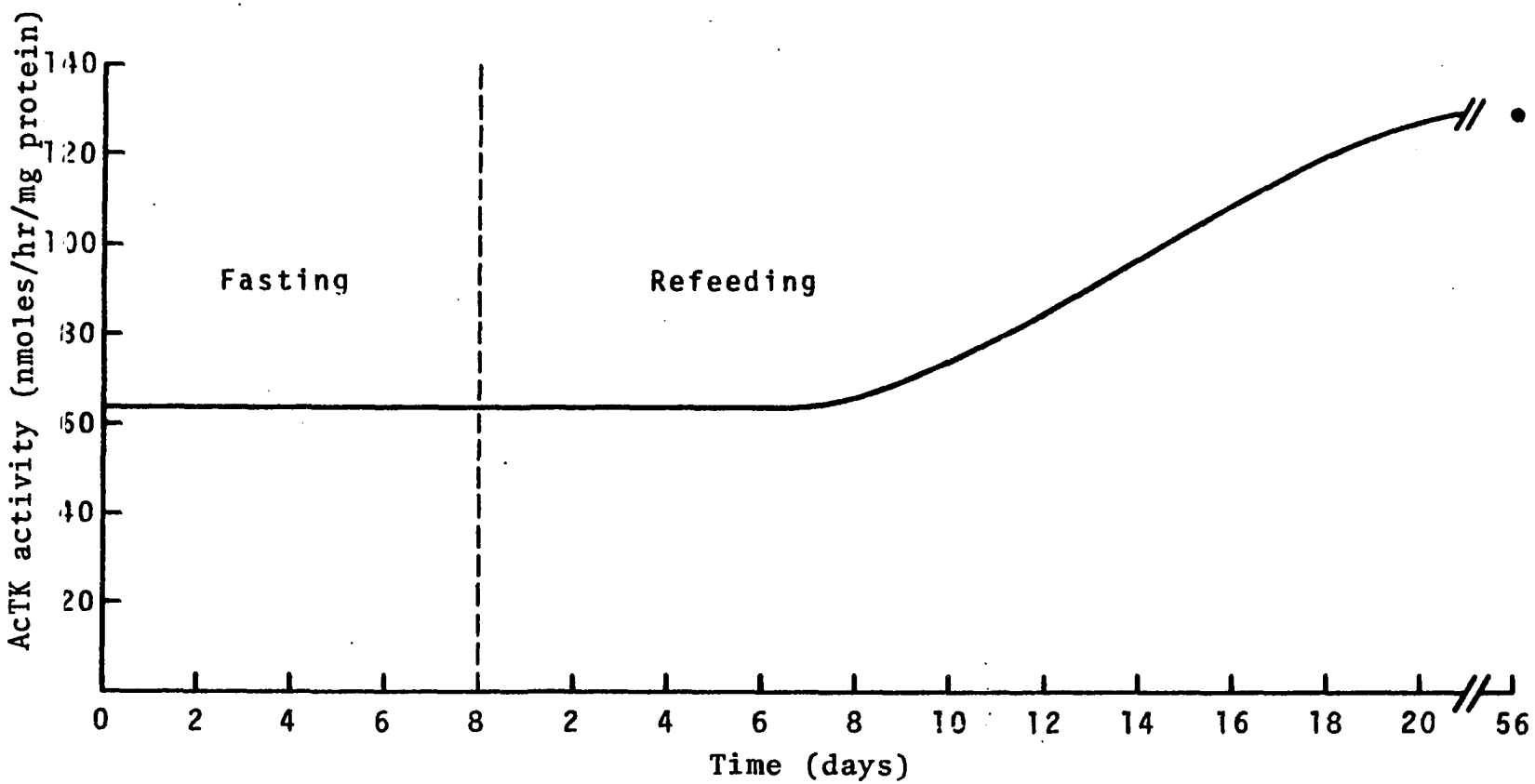

Figure 3. Activity of acetate thiokinase (AcTK) in adipose tissue of fasted and fasted-refed steers. Pooled standard error = ± 4.9.

Activities of the NADPH-generating enzymes G-6-P dehydrogenase, 6-PG dehydrogenase and NADP-isocitrate dehydrogenase are shown in Figures 4, 5 and 6, respectively. G-6-P dehydrogenase and 6-PG dehydrogenase responded similarly to fasting and refeeding. Activities of both enzymes did not decrease until after two days of fasting and reached minimum activities of about 30% of the controls. Upon refeeding, the activity of G-6-P dehydrogenase increased to a peak at 7 days, then decreased back down to the fasting level at 12 days before increasing again. At 21 days, G-6-P dehydrogenase activity was still only 70% of that at 56 days. After 56 days of refeeding, activity was similar to that of the control. 6-Phosphogluconate dehydrogenase activity changed similarly to G-6-P dehydrogenase during refeeding; however, the maximum at 7 days and minimum at 12 days were not as pronounced. Activity of isocitrate dehydrogenase decreased to about 55% of the control during fasting and gradually returned to normal during refeeding.

Concentration of free fatty acids in plasma rapidly increased to a maximum level of 450% of the control after 4 days of fasting (Figure 7). After one day of refeeding the concentration nearly returned to the control value. The concentration leveled at about 180 μmoles/liter and remained at this level through the remainder of the biopsy period. At 56 days, the level of plasma free fatty acids was slightly elevated probably due to the stresses of slaughter.

Concentration of acetate in plasma decreased rapidly with fasting (Figure 8). After 4 days of fasting, plasma acetate reached its lowest concentration (33% of the control). The concentration increased

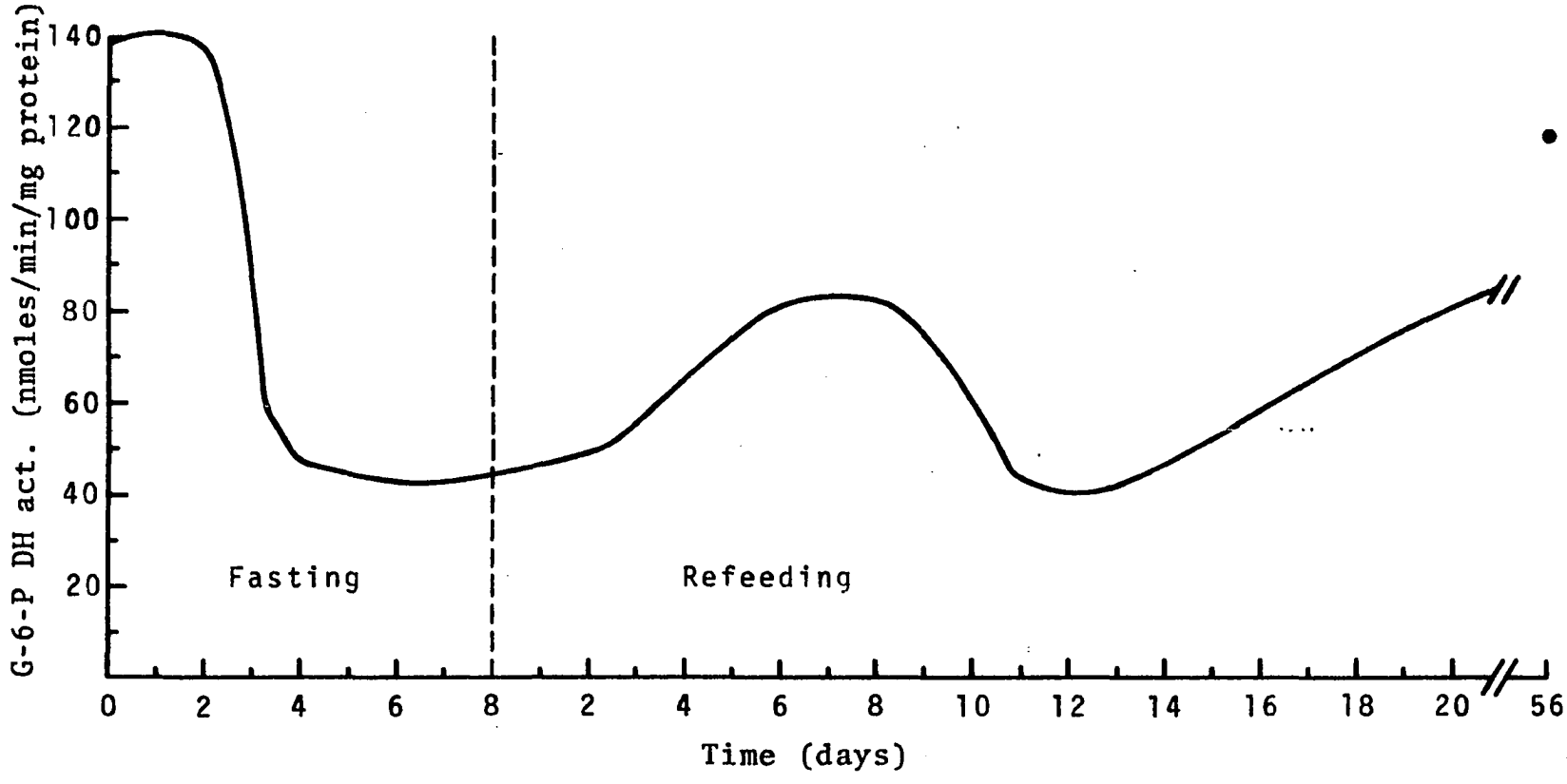

Figure 4. Activity of glucose-6-phosphate dehydrogenase (G-6-P DH) in adipose tissue of fasted and fasted-refed steers. Pooled standard error = $\pm$ 6.6.

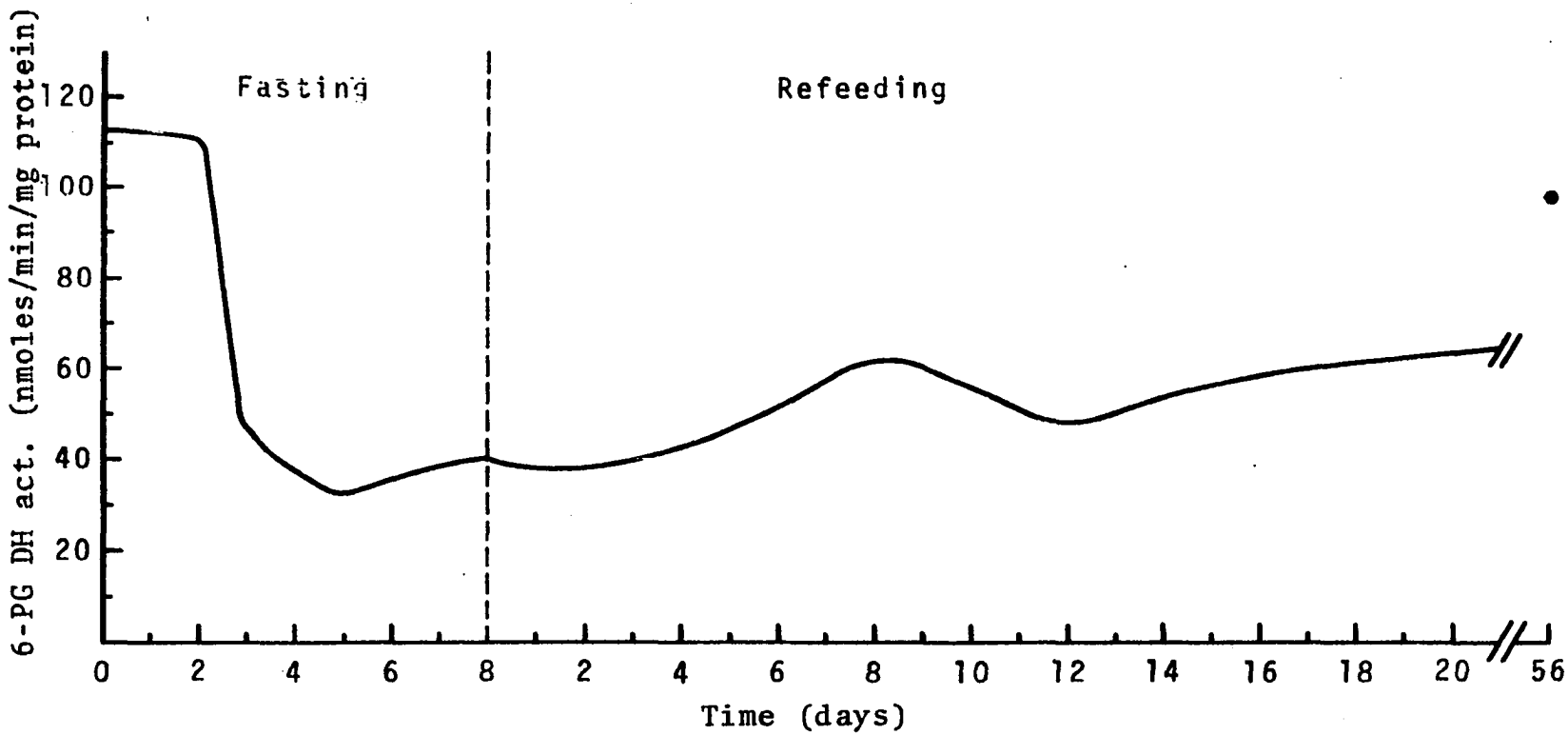

Figure 5. Activity of 6-phosphogluconate dehydrogenase (6-PG DH) in adipose tissue of fasted and fasted-refed steers. Pooled standard error = ± 7.3.

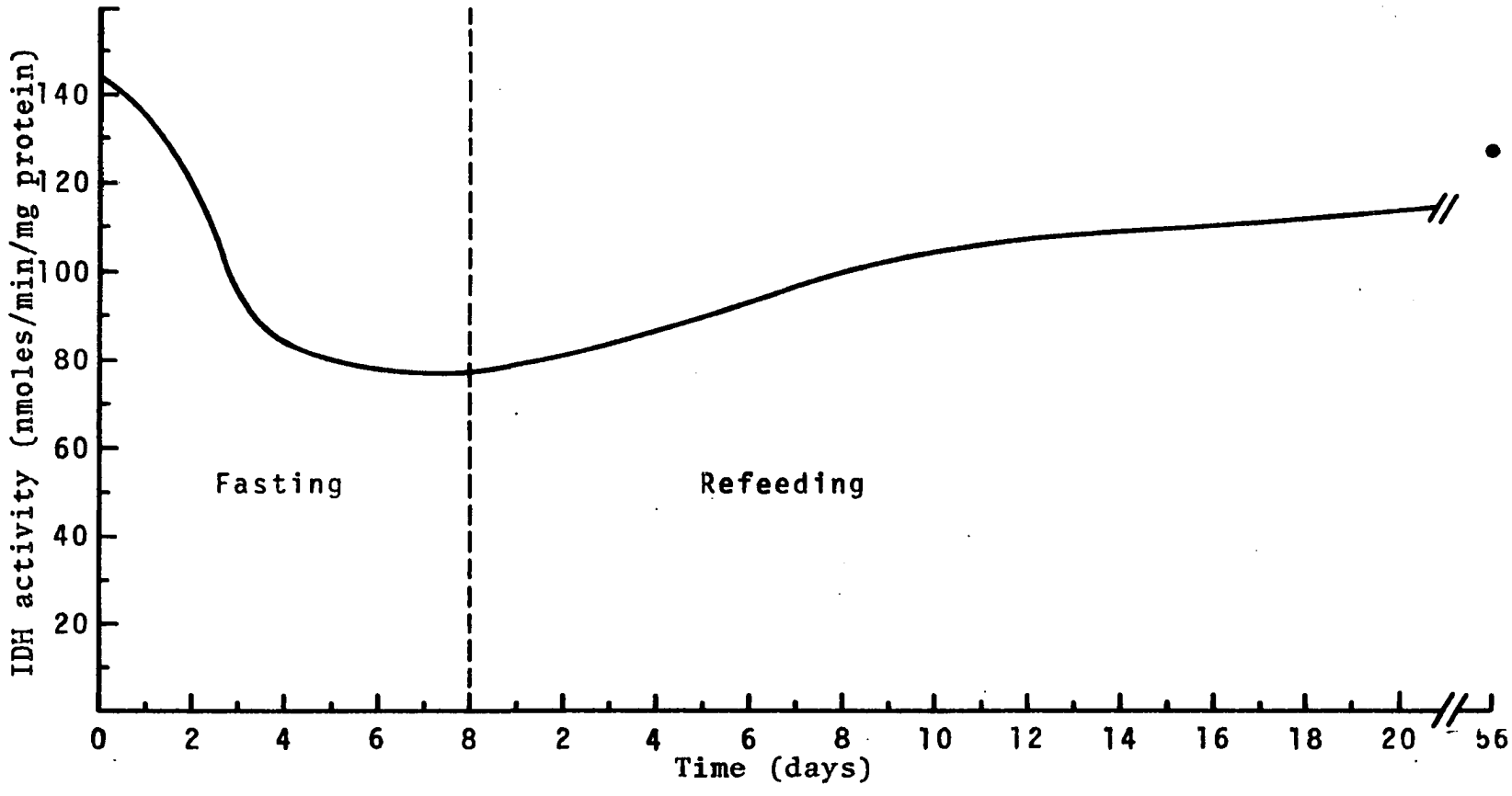

Figure 6. Activity of NADP-isocitrate dehydrogenase (IDH) in adipose tissue of fasted and fasted-refed steers. Pooled standard error = ± 5.5.

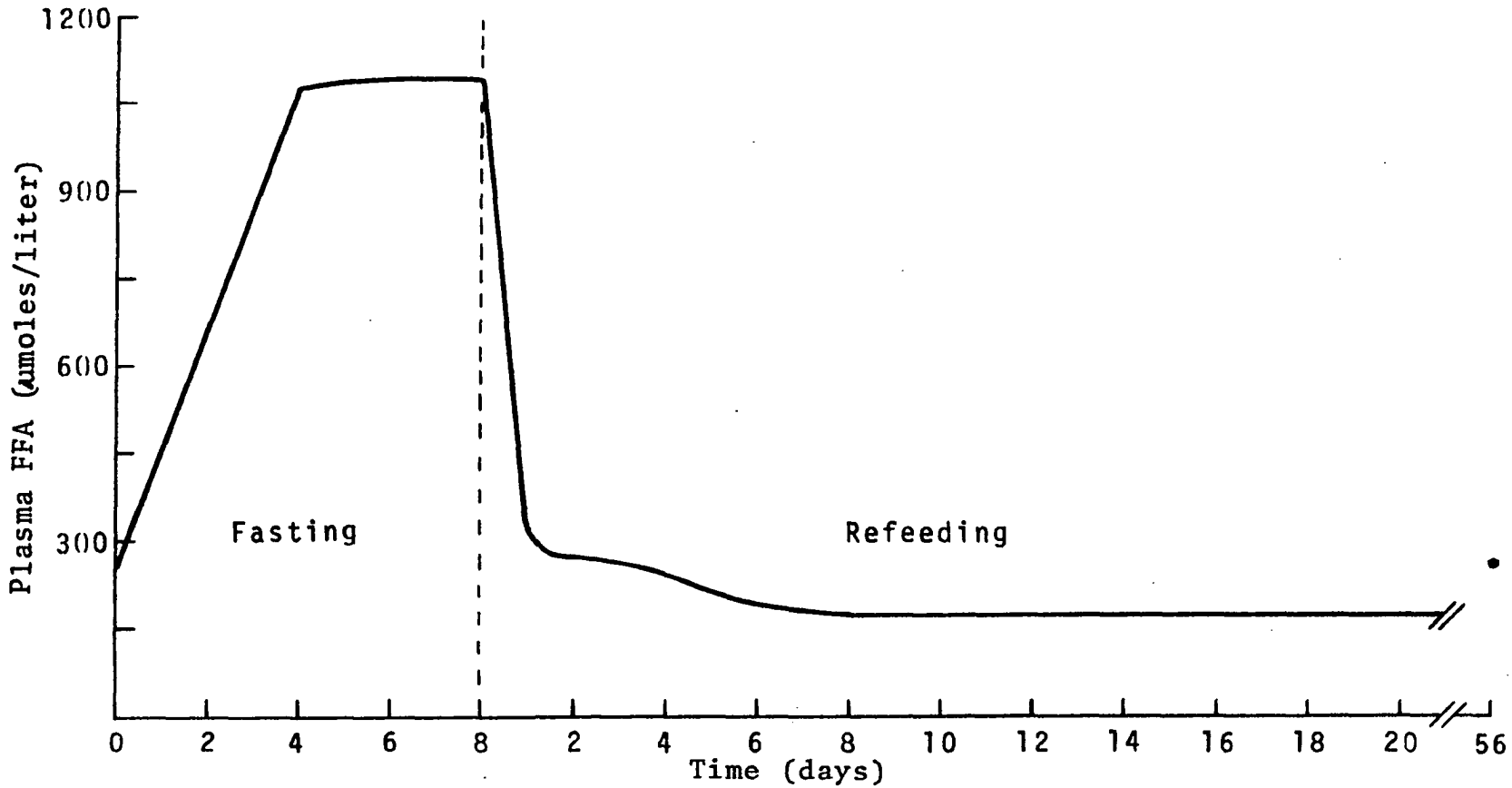

Figure 7. Concentration of plasma free fatty acids (FFA) in fasted and fasted-refed steers. Pooled standard error = ± 44.

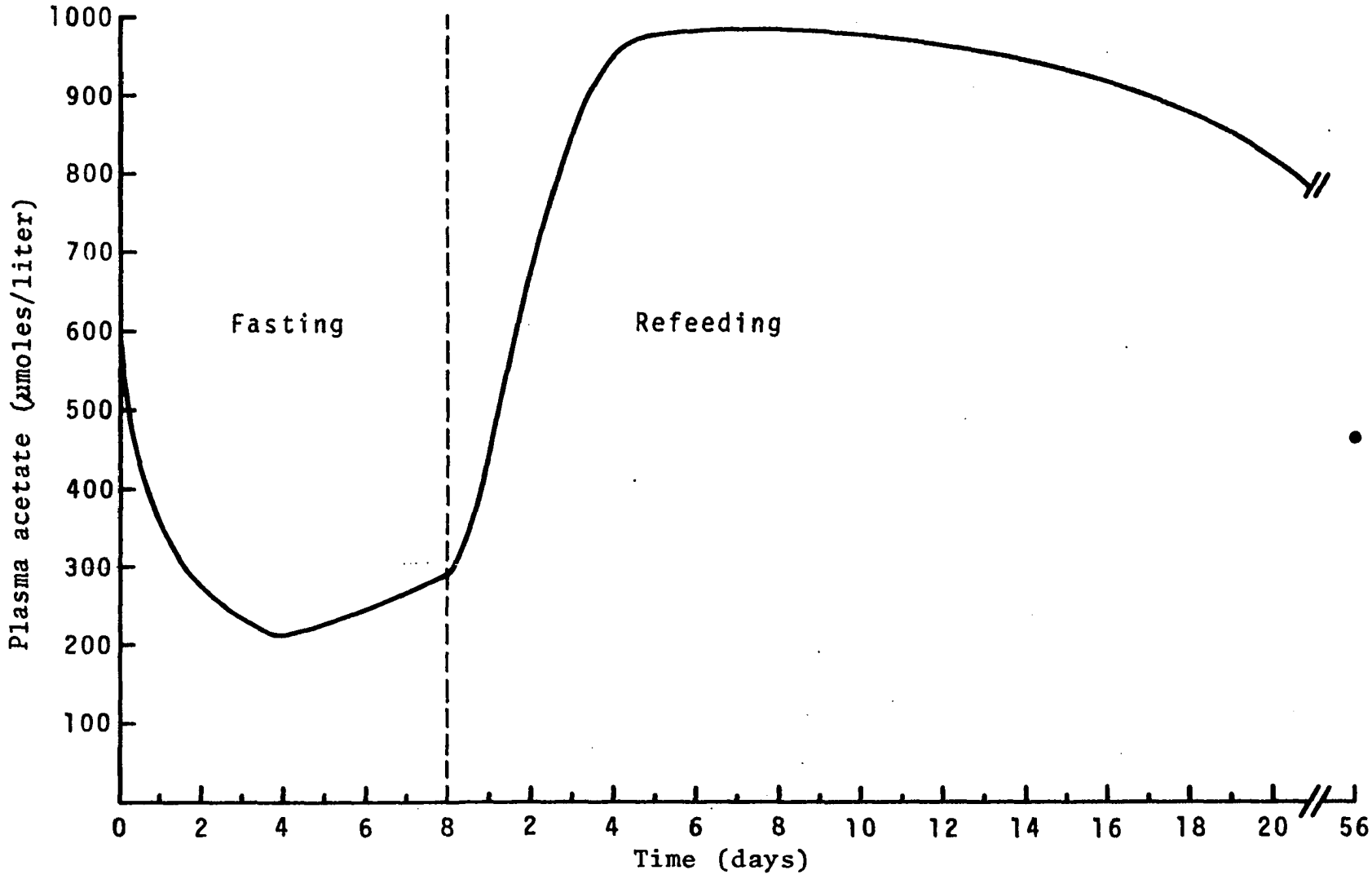

Figure 8. Concentration of plasma acetate in fasted and fasted-refed steers. Pooled standard error = ± 29.

slightly during the remainder of the fasting period. Plasma acetate increased very rapidly during the first 4 days of refeeding, reaching a value 150% of the control, and then leveled off for 5 or 6 days before returning to the control value. Plasma acetate returned to the control level sometime between 21 and 56 days of refeeding. During the first 8 days of refeeding, propionate was also present in the jugular blood plasma. The concentration of propionate remained relatively constant at 20 to 24 μmoles/liter plasma for these 8 days but then could not be measured after this time.

Discussion

Changes in the rate of fatty acid synthesis and in some of the factors involved in fatty acid synthesis occur soon after fasting in bovine subcutaneous adipose tissue. After one day of fasting, acetate incorporation into fatty acids decreased 60%, activity of acetyl-CoA carboxylase decreased 50%, concentration of plasma acetate decreased 45% and concentration of plasma free fatty acids increased 100% (Figures 1, 2, 7 and 8). Even though the rumen was feeding nutrients into the circulation for several days after fasting had begun, fatty acid synthesis started to decrease within one day of fasting. Buchanan-Smith and Horney (1971) also reported a 60% decrease in lipogenesis in ovine perirenal fat after a 24-hour fast. Ingle et al. (1973) reported a 35% decrease in fatty acid synthesis in subcutaneous adipose of fattening sheep after a 24-hour fast. They also determined that growing lambs (39 kg) achieved a fasted state by 60 hours after food removal and fattening lambs (52 kg) not until after 96 hours of

starvation.

Fatty acid synthesis increased more slowly with refeeding than did the decrease with fasting. The first two days of refeeding caused no change in acetate incorporation into fatty acids. An increase did not occur until the third day. On the third day of refeeding, the steer's feed intake was slightly above maintenance for energy which is reflected in the large increase in fatty acid synthesis on the fourth day. Like the lag in fatty acid synthesis recovery after refeeding, acetyl-CoA carboxylase activity recovery also was delayed two days. There was a slight overshoot of carboxylase activity above the control value, but the activity returned to the control value by 21 days of refeeding. Ingle et al. (1973) reported acetyl-CoA carboxylase activity in adipose of sheep to be 64, 29 and 12% of the control after 1, 4 and 8 days of fasting, respectively, compared with 50, 7 and 1%, respectively, found in this study. The activity at slaughter was markedly lower than the control. A possible reason is that these assays were conducted on samples taken at slaughter and a period of about an hour elapsed between when the animal was killed and when the sample was taken. The fat samples were still warm so this should not have been a problem. Perhaps the anoxia condition elicited responses that inactivated the enzyme which citrate could not reverse or that significant proteolytic degradation occurred before assay.

Activities of the NADPH-generating enzymes decreased less than and later than the decrease in fatty acid synthesis and in activity of acetyl-CoA carboxylase. There seemed to be a two-day lag after fasting

before the activities of the pentose phosphate pathway dehydrogenases decreased. Ingle et al. (1973) also showed a lag after fasting before the activities of these enzymes decreased. During fasting they decreased to about 30% of their prefasting levels. NADP-isocitrate dehydrogenase activity was 55% of its prefasting activity after the 8-day fast. Seemingly, the activities of these three enzymes do not decrease more than that observed in this study. In another study, activities of G-6-P dehydrogenase and NADP-isocitrate dehydrogenase in subcutaneous adipose of steers fasted three weeks were 40% and 67% of that in fed steers, respectively (Pothoven and Beitz, 1973).

Activity of acetate thiokinase did not change during fasting and the first 7 days of refeeding and then increased to a value twice that of the prefasting samples (Figure 3). Ingle et al. (1973) showed that acetate thiokinase activity decreased to 50% of the prefasting value in adipose of sheep fasted for 4 days. One possible explanation for the values observed in this study would be that the technique for assaying the enzyme was improved. Evidence against this idea is that on most days there were two samples analyzed, and on several days one sample would come from before and another after 7 days of refeeding. The corresponding samples would have values similar to others of the same time period. Another possible explanation would be that acetate thiokinase activity was increased by the abnormally high levels of acetate present in the blood during refeeding. The concentration of plasma acetate reached a level 50% higher than the prefasting level and remained high for the first 21 days of refeeding (Figure 8).

Plasma acetate concentration dropped to a low of 210 μmoles/liter after 4 days of fasting from the prefasting level of 635 μmoles/liter and then increased to 290 μmoles/liter after 8 days of fasting (Figure 8). The K_m of acetate thiokinase for acetate is 200 μmolar in bovine heart mitochondria, so if the K_m is the same in adipose then acetate level would not be limiting (Webster, 1966). The rumen will continue to supply acetate for some time after fasting, but after the supply of acetate from the rumen stops the plasma acetate must come from endogenous sources. Palmquist (1972) reported a 40% decrease in plasma acetate in sheep after a 48-hour fast.

The acetate pool is small in ruminants; the steer can have a variable acetate pool size of 300 to 600 mg per kilogram body weight (Williams, 1960; Lee and Williams, 1962). Turnover of the acetate pool is rapid with a $t_{\frac{1}{2}}$ of 4.0 to 1.6 minutes and pool turnover time of 2 to 6 minutes in steers (Lee and Williams, 1962) and $t_{\frac{1}{2}}$ of 1.3 minutes and turnover time of 2 minutes in sheep (Sabine and Johnson, 1964). So, plasma acetate would soon be exhausted if there was no endogenous acetate produced. Endogenous acetate production can account for 25% of the acetate turned over in sheep and cattle (Annison and White, 1962; Lee and Williams, 1962; Kronfeld, 1968). Entry of endogenous acetate in sheep with emptied rumens was 40 to 50% of the value for the total entry of acetate obtained in the same animals after feeding (Annison and White, 1962). Based upon these observations and the fact that the rumen was empty, endogenous production of acetate probably accounted for most of the plasma acetate present after 4 days of fasting.

The increase in plasma acetate observed between 4 and 8 days of fasting coincides with the maximum concentration of plasma free fatty acids present (Figure 7). The animal was obtaining a significant portion of its energy requirement from long-chain fatty acids during fasting and at the same time the fatty acids were probably supplying the acetate in the blood. Annison and White (1962) and Palmquist (1972) reported labelling of blood acetate during infusion of sodium (1-^{14}C) palmitate in fasted sheep. After a 24-hour fast, only 6% of the expired CO_2 in sheep was derived from acetate compared to 35% or more after 3 to 6 hours of refeeding (Annison and Lindsay, 1961). Endogenous acetate is derived from endogenous fatty acid catabolism in liver and fat depots. Raising the concentration of blood acetate or glucose reduces entry of endogenous acetate in starved sheep which suggests that oxidation of free fatty acids contributes substantially to the entry of endogeous acetate.

Normally, propionate is metabolized by the liver and little propionate will be found in jugular vein blood (Cook and Miller, 1965). Seemingly, after an 8-day fast the enzymes in the liver needed to adapt to metabolizing propionate upon refeeding. The concentration of propionate remained relatively constant the first 8 days of refeeding and then decreased so that it could no longer be measured on the tenth day. No measurement was made on the ninth day of refeeding.

Of the enzymes studied, acetyl-CoA carboxylase was most highly correlated ($P < 0.01$) with acetate incorporation into fatty acids. The two curves representing the decrease in fasting and the recovery during

refeeding (Figures 1 and 2) could almost be superimposed on each other. Evidence has suggested that the conversion of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase is the rate-limiting step in fatty acid synthesis (Numa et al., 1961; Ganguly, 1960). The quantity of total enzyme present (active and inactive) may have control over the day-to-day regulation of fatty acid synthesis. Nakanishi and Numa (1970) found the half life of acetyl-CoA carboxylase to be 59 hours in fed rats and 31 hours in fasted rats, while Majerus and Kilburn (1969) found a half life of 18 hours in fasted rats. The decrease in acetyl-CoA carboxylase observed in the in vitro assays cannot be explained by mere inactivation but rather by a decrease in total enzyme because the enzyme is fully activated by citrate before it is assayed.

The simultaneous decrease in available acetyl-CoA and increase in free fatty acids or fatty acyl-CoA derivatives may reduce synthesis of acetyl-CoA carboxylase and increase its rate of degradation. Bortz and Lynen (1963) have proposed that the inhibition of acetyl-CoA carboxylase by fatty acyl-CoA derivatives was due to competition with acetyl-CoA for the active site on the enzyme. The decrease in available acetyl-CoA and increase in fatty acyl-CoA derivatives would amplify the competition effect. Yeh and Leveille (1970) reported that in chicks plasma free fatty acid level reached a maximum before the rate of fatty acid synthesis was minimized and started to decline after maximal depression of fatty acid synthesis. Ballard (1972) showed that the concentration of glycerol-1-phosphate decreased when rats were fasted and increased greatly upon refeeding which suggested that fatty acid

esterification was controlled by the rate of glycerol-1-phosphate generation.

Activities of the NADPH-generating enzymes measured also decreased but after fatty acid synthesis had already decreased. These enzyme activities were lower because of a lesser need for NADPH during reduced fatty acid synthesis. Another factor that could have caused decreases in these enzymes along with acetyl-CoA carboxylase is the greatly increased level of fatty acyl-CoA derivatives present. The validity of inhibitions caused by fatty acyl-CoA derivatives has been questioned (Srere, 1965; Taketa and Pogell, 1966). Fatty acyl-CoA derivatives are very nonspecific in regard to the enzymes they inhibit. Their inhibitory properties are explained by their detergent-like action which produces conformational changes of various enzymes, and therefore, may not be physiologically significant. But in a fasting state when the levels of fatty acyl-CoA derivatives and free fatty acids are increased many fold, their regulatory role may become important.

As in other animal species, fatty acid synthesis in adipose tissue of cattle seems also regulated by acetyl-CoA carboxylase. The question still remains, however, as to what factors are able to quickly turn off acetyl-CoA carboxylase synthesis and probably hasten its degradation. An increase in the inhibitor, fatty acyl-CoA derivatives, probably plays a role. Also, the decrease in available fatty acid precursor may be important in causing a decrease in acetyl-CoA carboxylase. All the factors such as inhibitors, cofactors, activators and substrate level that control the activity of acetyl-CoA carboxylase

have not been studied thoroughly enough to fully understand its role in regulation of fatty acid synthesis.

SUMMARY

The main goals of this work were to study changes in capacity for fatty acid synthesis in adipose tissue of restricted-fed steers; and changes in rates of fatty acid synthesis and in activities of some of the enzymes involved in fatty acid synthesis in adipose tissue of fasted and refed steers. The long-term objective of this project is to determine the regulating factors of fatty acid synthesis and fat deposition. The results of the two experiments conducted can be summarized as follow:

1. Adipose tissue from restricted-fed steers had lower rates of fatty acid synthesis compared to adipose from ad libitum-fed steers.
2. Fasting decreased the rate of fatty acid synthesis and activity of acetyl-CoA carboxylase within 24 hours and reduced them to less than 1% of the normal rate after 8 days.
3. Fatty acid synthesis and acetyl-CoA carboxylase did not begin to increase significantly until the animal was at above energy maintenance in food intake.
4. Activities of G-6-P dehydrogenase, 6-PG dehydrogenase and NADP-isocitrate dehydrogenase were reduced 70, 64 and 45% after 8 days of fasting and did not return to fed levels as rapidly as fatty acid synthesis or acetyl-CoA carboxylase after refeeding.

5. Plasma free fatty acids responded quickly to fasting, increased 350% in 4 days, and returned to normal just as rapidly with refeeding.
6. Plasma acetate decreased 67% after 4 days of fasting and then started to increase slightly the remainder of the fasting period. Plasma acetate reached a level 150% above the normal with 4 days of refeeding and did not return to normal until after 21 days of refeeding.
7. These data suggest that free fatty acids and substrate level regulate acetyl-CoA carboxylase which limits fatty acid synthesis.

More work is necessary to fully elucidate all the mechanisms by which fatty acid synthesis and fat deposition are controlled. Some aspects that could be studied are (1) a detailed study of the regulation of acetyl-CoA carboxylase activity, (2) infusion of acetate and/or glucose during fasting to determine the role of substrate level, and (3) determination of the exact role of plasma free fatty acids on regulation of fatty acid synthesis. The ultimate goal is the ability to regulate fat deposition in meat producing animals possibly by manipulating the feeding regimen or by chemical means.

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APPENDIX

Appendix Table 1. Incorporation of acetate into long-chain fatty acids in adipose tissue of beef steers

Breed[1]	Animal wt (kg)	Adipose Tissue Sites			
		Backfat	Perirenal	Omental	Intermuscular
		----- nmoles acetate/100 mg tissue/2 hr -----			
A	106	248[2]	196	210	57
A	116	90	174	140	72
C	116	664	218	350	105
C	114	8	6	2	5
Mean	113	252	149	176	60
A	235	412	96	80	85
A	242	376	108	93	148
C	215	169	38	46	59
C	231	915	290	186	392
Mean	231	468	133	101	171
A	367	622	147	410	566
A	357	586	34	169	70
C	373	362	31	120	127
C	361	729	189	183	86
Mean	364	575	110	151	106
A-R	361	263		145	
A-R	361	111		237	
C-R	356	210		48	
C-R	367	103		21	
Mean	362	172		112	
A	493	101		38	
A	515	225		74	
C	507	229		37	
C	500	334		29	
Mean	504	222		45	
A-R	517	64		14	
A-R	504	63		24	
C-R	504	56		3	
C-R	510	292		116	
Mean	509	119		39	

[1]A refers to steers from an Angus sire, C to steers from a Charolais sire, R to those restricted-fed.

[2]Values are means of triplicate samples.

Appendix Table 2. Days individual animals were sampled

Sample Number	Animal Number					
	10	36	42	46	49	51
	Days fasted					
1	0	0	0	0	0	0
2	4	1	3	2	1	1
3	6	2	5	3	3	4
4	7	8	7	7	8	8
	Days refed					
5	3	1	4	2	3	4
6	5	5	11	8	7	7
7	11	7	17	16	10	12
8	20	19	24	20	22	21
9	38	62	59	50	58	55

Appendix Table 3. Weights of steers during the experiment

Sample Number	Animal Number					
	10	36	42	46	49	51
	kg					
1	593	578	569	518	525	517
2	553	553	553	498	510	498
3	540	555	541	485	511	485
4	536	517	531	472	499	477
5	564	529	553	497	515	497
6	574	568	580	520	530	520
7	598	568	586	525	534	513
8	611	590	583	535	554	517
9	635	640	619	535	551	538

Appendix Table 4. Incorporation of acetate into long-chain fatty acids in adipose tissue of fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	-------- nmoles acetate/100 mg tissue/2 hr ----------					
1	382[1]	124	51	100	58	50
2	10	75	12	1	37	12
3	6	36	3	34	14	15
4	1	1	2	0	4	7
5	0	3	4	0	8	68
6	46	13	55	46	13	117
7	93	20	86	192	78	143
8	87	72	9	171	31	106
9	266	99	137	115	100	109

[1]Values are means of triplicate samples.

Appendix Table 5. Activity of acetyl-CoA carboxylase in adipose tissue of fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	----- nmoles HCO_3^- carboxylated/min/mg protein -----					
1	0.35[1]	1.27	0.68	0.83	0.04	0.68
2	0.03	0.45	0.02	0.16	0.02	0.44
3	0.02	0.22	0.00	0.15	0.03	0.03
4	0.01	0.01	0.03	0.02	0.04	0.04
5	0.02	0.01	0.05	0.05	0.06	0.04
6	0.04	0.17	0.42	0.10	0.26	0.22
7	0.35	0.68	0.36	1.37	0.64	0.54
8	1.62	1.02	0.16	0.18	0.26	1.01
9	0.44	0.25	0.18	0.22	0.39	0.23

[1]Values are means of triplicate samples.

Appendix Table 6. Activity of acetate thiokinase in adipose tissue of fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	----- nmoles acetyl-CoA produced/hr/mg protein --------					
1	43	26	106	74	54	31
2	0	61	61	114	94	10
3	26	43	70	71	51	49
4	5	45	56	146	88	67
5	18	60	31	65	15	70
6	64	58	39	76	65	191
7	96	25	76	158	81	98
8	106	177	133	125	166	178
9	99	142	141	128	114	126

[1]Values are means of triplicate samples.

Appendix Table 7. Activity of glucose-6-phosphate dehydrogenase in adipose tissue of fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	nmoles NADPH produced/min/mg protein					
1	111[1]	142	103	155	100	226
2	16	179	75	159	260	100
3	42	148	42	85	66	75
4	24	45	49	71	54	35
5	40	90	22	16	50	34
6	62	94	36	63	133	57
7	31	103	42	84	58	16
8	55	69	88	101	78	94
9	158	331	199	90	59	125

[1]Values are means of duplicate samples.

Appendix Table 8. Activity of 6-phosphogluconate dehydrogenase in adipose tissue of fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	nmoles NADPH produced/min/mg protein					
1				122[1]		104
2				114		98
3			32	64	31	62
4		33	44	70	42	47
5	24	35	42	40	49	47
6	34	70	46	65	115	65
7	53	52	44	77	54	36
8	54	69	66	65	49	65
9	50	303	205	38	80	55

[1]Values are means of duplicate samples.

Appendix Table 9. Activity of NADP-isocitrate dehydrogenase in adipose tissue of fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	nmoles NADPH produced/min/mg protein					
1	140[1]	147	103	188	69	150
2	72	109	95	192	137	165
3	40	108	76	85	86	92
4	21	107	76	103	61	95
5	48	112	70	65	73	82
6	81	119	103	108	261	109
7	77	87	74	128	103	76
8	99	120	88	104	123	114
9	100	214	165	73	141	103

[1]Values are means of duplicate samples.

Appendix Table 10. Concentration of plasma free fatty acids in fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	umoles/liter					
1	250[1]	150	285	230	550	320
2	1200	425	905	625	865	550
3	1285	695	903	615	1050	1025
4	1160	815	1135	870	1422	1045
5	360	280	295	255	460	300
6	160	210	160	180	260	185
7	60	182	210	220	175	175
8	130	180	145	170	180	140
9	180	178	230	360	365	510

[1]Values are means of triplicate samples.

Appendix Table 11. Concentration of plasma acetate in fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	umoles/liter					
1	508[1]	621	794	828	546	520
2	195	346	350	241	394	278
3	245	284	497	401	208	226
4	181	222	593	359	328	323
5	576	461	1202	788	1093	734
6	548	693	998	966	898	750
7	909	600	977	927	1027	840
8	568	590	780	636	1032	1058
9	551	384	549	344	447	501

[1]Values are means of duplicate samples.

Appendix Table 12. Concentration of plasma propionate in fasted and fasted-refed steers

Sample Number	Animal Number					
	10	36	42	46	49	51
	umoles/liter					
5	20[1]	22	22	22	24	22
6	53	34		22		20
7		6				

[1]Values are means of duplicate samples.