

INFLUENCE OF *IN VITRO* ELAIDIC ACID OR *TRANS*-VACCENIC ACID UPTAKE
AND LACTOGENIC HORMONE STIMULATION ON FATTY ACID CONTENT OF
MOUSE MAMMARY CELLS

by

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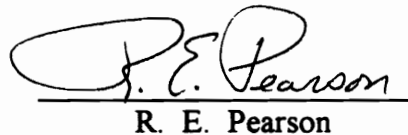
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(ABSTRACT)

The objective of the study was to examine the effects of *trans*-9-octadecenoic acid (elaidic) and *trans*-11-octadecenoic acid (*trans*-vaccenic) on uptake and alteration of exogenous fatty acids by mouse mammary epithelial cells. Cells from a subclone of the COMMA-D cell line were plated on uncoated plastic petri dishes and grown to confluence. Supplemental fatty acids bound to bovine serum albumin were added to the medium applied to the confluent cell cultures. Treatments included 200 μ M octadecanoic acid (C18:0) as a control and 100 μ M C18:0 with one of the following μ M ratios of *cis*-octadecenoic acid (*cis*-C18:1) to elaidic or *trans*-vaccenic: 100:0, 75:25, 50:50, 0:100. In addition, all treatments were conducted with or without lactogenic hormone supplementation. The cellular protein to DNA ratio and total amount of fatty acids per mg protein were decreased ($P < .05$) by addition of lactogenic hormones. In treatments without hormone supplementation, however, the total amount of cellular fatty acids per mg protein was decreased ($P < .05$) by addition of either *trans*-C18:1 isomer. Results indicated a significant ($P < .05$) relationship between the concentration of *trans*-C18:1 in the media and

uptake of *trans*-C18:1 isomers, and retroconversion of *trans*-C18:1 to *trans*-C16:1. The slopes of the lines for cellular C16:0, *cis*-C16:1, and *cis*-C18:1 were less ($P < .05$) than zero as concentration of *trans*-C18:1 in the media increased. However, *trans*-C18:1 isomers did not influence the proportion of polar and nonpolar lipids synthesized by the cells. It appears that *trans* fatty acids may depress milk fat output by decreasing *de novo* fatty acid synthesis and *cis*-C18:1 content.

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CHAPTER 1

REVIEW OF LITERATURE

Introduction

Increasing the understanding of nutrient metabolism in the dairy cow is important for more efficient milk production and manipulation of the nutrient content of milk. Health conscious consumers are especially concerned about the fat composition of milk. Consumers are increasing their demand for products that are low in total and saturated fat. In order for the dairy industry to meet this demand, a better understanding of the relationship between dietary fat and milk fat composition in dairy cows is needed. Increasing the polyunsaturated fatty acid (PUFA) content of milk fat by dietary means is very difficult. This is due to ruminal biohydrogenation, which results in flow of primarily saturated and *trans* fatty acids to the duodenum, regardless of the PUFA content of the diet consumed by the cow. The *trans* fatty acids produced from incomplete biohydrogenation of PUFA have been shown to depress milk fat synthesis (Selner and Schultz, 1980; Wonsil et al., 1994). In addition, there are potential negative effects of *trans* fatty acids on human health. This review summarizes the current understanding of the metabolic effects of dietary fat and *trans* fatty acids, as well as their influence on milk fat composition in dairy cows. The influence of milk fatty acids and *trans* fatty acids on

human health also are discussed.

Fatty acid nomenclature

Stearic acid is a saturated 18 carbon fatty acid and will be designated in shorthand as C18:0 throughout the review. Other saturated fatty acids will be referred to in the same manner. Unsaturated fatty acids contain one or more double bonds (designated C18:1, C18:2, etc.), and each bond can have a *cis* or *trans* configuration. The position of double bonds in fatty acids is determined by counting from the carboxyl end of the molecule. Elaidic acid, for example, contains a *trans* double bond between the ninth and tenth carbon and is referred to as *trans*-9-C18:1; whereas, *trans*-vaccenic acid contains a double bond at the 11th carbon and is designated *trans*-11-C18:1. Oleic acid contains a *cis* double bond at the ninth carbon and is referred to as *cis*-9-C18:1. Linoleic, linolenic, and arachidonic acid will be referred to in the review as C18:2, C18:3, and C20:4, respectively. Designations for location and type of double bonds within these PUFA will be added to their shorthand notation only when relevant to the discussion in this review.

General aspects of milk fat synthesis

Fatty acid composition of a typical dairy cow diet is different from the composition of milk fat. Palmquist (1991) determined that when dietary fat intake does not exceed the amount secreted in milk, approximately 55 to 60% of the dietary fatty acids are transferred to milk. Milk fatty acid composition is the combination of: 1) fatty acids synthesized by the mammary gland, 2) fatty acids synthesized by rumen microorganisms,

3) endogenous fatty acids from adipose, 4) biohydrogenation of unsaturated fatty acids in the rumen, and 5) mammary gland desaturase activity (Grummer, 1991). Ruminal hydrogenation and mammary gland stearoyl CoA desaturase activity are major factors that influence milk fatty acid composition. Estimates for ruminal hydrogenation of PUFA range from 60 to 90% (Bickerstaffe et al., 1972; Mattos and Palmquist, 1977; Murphy et al., 1987). The primary product of hydrogenation is C18:0. Given the extensive hydrogenation of PUFA in the rumen and the relatively minor amount of C18:1 formed by intestinal desaturase, extensive conversion of C18:0 to C18:1 presumably occurs in mammary tissue to ensure sufficient fluidity of milk fat for efficient secretion from the mammary cell. It has been estimated that 30 to 40% of oleic acid in milk is synthesized from absorbed stearic acid in mammary tissue by stearoyl CoA desaturase (Kinsella, 1970). The ratio of C18:1 to C18:0 in milk typically is 2:1 to 3:1, whereas the ratio in triglyceride-rich lipoproteins circulating in plasma is 1:2 (Grummer, 1991).

Kinsella (1970) examined stearoyl CoA desaturase activity in microsomes isolated from lactating bovine mammary tissue. Experimental conditions causing a reduced desaturase activity, such as increases in the incubation mixture pH and removal of NADH, depressed triglyceride synthesis and increased fatty acid incorporation into phospholipids in the microsomes. Furthermore, stimulation of desaturation by decreasing the pH of the incubation mixture and including NADH and L- α -glycerolphosphate, increased incorporation of fatty acids into triglyceride. It was suggested that a specific quantity of endogenous *cis*-9-C18:1 from desaturation of C18:0 may be the critical factor in

controlling triglyceride production. Therefore, reduced availability of acetate and glucose in mammary tissue due to high concentrate diets or metabolic disorders, such as ketosis, may result in a lower production of NADH and NADPH, which would impair desaturase activity and limit milk triglyceride production.

Ruminal biohydrogenation of fatty acids

Biohydrogenation of unsaturated fatty acids in the rumen has major consequences on the fatty acid composition of milk fat. Biohydrogenation is the process in which ruminal microorganisms hydrogenate *cis*-C18:1, C18:2, and C18:3 primarily to C18:0 and the intermediate metabolite *trans*-11-C18:1. Bickerstaffe et al. (1972) found the conversion of *cis*-C18:1, C18:2, and C18:3 to C18:0 in the rumen of lactating goats to be 88, 85, and 76%, respectively. In addition, the conversion of *cis*-C18:1, C18:2, and C18:3 to *trans*-11-C18:1 was found to be 3, 6, and 18%, respectively. Mattos and Palmquist (1977) reported that 60-73% of C18:2 was hydrogenated in lactating dairy cows. Biohydrogenation results in a greater amount of C18:0 and *trans*-11-C18:1 flowing to the duodenum than entering the rumen from the diet (Wu et al., 1991).

A free carboxyl group is required for biohydrogenation to occur (Hawke and Silcock, 1969). Calcium salts of fatty acids are partially protected from biohydrogenation due to the limited dissociation of calcium salts at normal rumen pH (Wu et al., 1991). Wu et al. (1991) observed that biohydrogenation of calcium salts of fatty acids (57%) was

reduced compared to fatty acids in an animal-vegetable blend (87%). This resulted in decreases in C18:0 and *trans*-11-C18:1 flowing to the duodenum, and an increase in C18:2 flow. The biohydrogenation of *cis*-C18:1, C18:2, and C18:3 was found to decrease linearly as calcium salts in the diet increased, and to increase linearly as amount of animal-vegetable blend increased in the diet. Feeding unprotected vegetable and fish oils has been shown to elevate *trans*-C18:1 fatty acids in milk fat (Banks et al., 1980; Wonsil et al., 1994; Jenkins et al., 1995). Jenkins et al. (1995) observed that feeding butylsoyamide, a fatty acyl amide resistant to ruminal biohydrogenation, inhibited the increase in *trans*-C18:1 concentrations in plasma and milk, as well as the decrease in milk fat percent associated with feeding soybean oil. Furthermore, feeding butylsoyamide increased the plasma and milk concentrations of C18:2.

The biohydrogenation of C18:2 and C18:3 is a two-step process involving bacteria with differing biohydrogenation capabilities. Hazlewood et al. (1976) grouped bacteria according to their biohydrogenation capabilities. Group A bacteria primarily hydrogenate C18:2 and C18:3 to *trans*-C18:1. Group B bacteria hydrogenate *cis*- and *trans*-C18:1 to C18:0. Thus, members of both groups of bacteria are required to completely hydrogenate C18:2 and C18:3 to C18:0.

Effects of dietary fat on milk fat synthesis

Approximately 50% of milk fatty acids are synthesized *de novo* in the mammary

gland from acetate and β -hydroxybutyrate and the remainder are extracted from the blood. Also, approximately half of the initial four carbons for *de novo* synthesis are derived directly from β -hydroxybutyrate and the remainder are derived via synthesis from acetate (Palmquist et al., 1969). Banks et al. (1983) stated that the short-chain and most medium-chain fatty acids are believed to be synthesized *de novo* in the epithelial cells of the mammary gland while a portion of C16:0 and most fatty acids with 18 carbons or more are derived from the blood (DePeters, 1993). Approximately 88% of the fatty acids derived from blood are of dietary origin and the remainder are from adipose tissue (Palmquist and Mattos, 1978).

Supplemental fat is included in diets of dairy cows to increase the energy density of the diet, thereby improving the efficiency of milk production per unit of dry matter intake. Dietary fat can either increase or decrease the concentration of fat in milk. This is dependent upon the physical form, fatty acid composition, and amount of dietary fat. Mohamed et al. (1988) observed a decrease in milk fat percentage when soybean or cottonseed oil (4% of dietary DM) was included in the diet, but not when the whole seed was fed. Theories to explain oil induced milk fat depression include decreased ruminal acetate:propionate ratio as a result of reduced fiber digestibility (Shaw and Ensor, 1959), increased production of *trans*-11-C18:1 in the rumen from hydrogenation of unsaturated long-chain fatty acids (Selner and Schultz, 1980), and long-chain fatty acid inhibition of mammary acetyl CoA carboxylase activity (Palmquist and Jenkins, 1980). Mohamed et al.

(1988) suggested that feeding oil as part of an intact seed may result in a slower release of oil in the rumen, thereby reducing the production of *trans* C18:1 fatty acids in the rumen and the severity of milk fat depression.

Feeding yellow grease at 3.5% of dietary DM did not alter milk fat percentage, but feeding at 7% depressed milk fat percentage (DePeters et al., 1987). The proportion of fatty acids with 4 to 14 carbons decreased as the amount of fat in the diet increased, and the fatty acid decrease was suggested to be more severe when dietary fat contained a high proportion of unsaturated fatty acids (Grummer, 1991). Thus, *de novo* synthesis of fatty acids in the mammary gland was reduced. The proposed metabolic regulators of *de novo* synthesis of short and medium-chain fatty acids include alteration of acetyl CoA carboxylase activity by long-chain fatty acids and the influence of rumen fermentation on the supply of acetate and β -hydroxybutyrate (as indicated above). Absence of a reduction in short and medium-chain fatty acid yields following intravenous infusion of triglycerides implied the effect was of ruminal origin (Storry et al., 1969). Inert dietary fats, such as calcium salts of fatty acids, prilled fats, saturated fatty acids, and hydrogenated tallow, are assumed to be resistant to rumen microbial action and have no effect on fiber digestion (Grummer and Maurer, 1987). However, Banks et al. (1984) observed a decrease in *de novo* fatty acid synthesis in the mammary gland after feeding saturated fatty acids and hydrogenated tallow. In addition, Wrenn et al. (1978) observed a decrease in *de novo* fatty acid synthesis after feeding tallow protected from rumen microbial attack by

formaldehyde treatment. Furthermore, Palmquist et al. (1969) determined that β -hydroxybutyrate contributes a maximum of 8% of milk fatty acid carbon and concluded that a deficiency of β -hydroxybutyrate is not a causative factor in depressed production of milk fat in cows fed a high grain, low roughage diet. Thus, the reduction in *de novo* synthesis when rumen inert fat is fed is most likely due to changes in mammary gland metabolism rather than events in the rumen.

McGuire et al. (1995) examined the validity of the glucogenic-insulin theory, which proposes that milk fat depression is caused by elevated insulin concentrations during feeding of high grain diets. Propionate production is increased due to the increased intake of soluble carbohydrate (starch). As a result of increased propionate supply, the rate of gluconeogenesis and insulin release increase. Although insulin has no influence on the uptake of metabolites for milk fat synthesis by the mammary gland, it has dramatic effects on lipid synthesis in adipose tissue. Insulin increases *de novo* lipogenesis, uptake of fatty acids from blood, and reesterification. Insulin also inhibits rates of lipolysis in adipose tissue. Therefore, this theory is based on the concept that insulin partitions substrates for lipogenesis to adipose tissue and results in an inadequate supply of substrates for synthesis of milk fat. To examine this theory, McGuire et al. (1995) subjected cows to a hyperinsulinemic-euglycemic clamp over a 4 day period to examine the effects of insulin on milk yield and composition. Insulin was maintained at a concentration that was fivefold greater than that of the baseline period. Glucose

concentration was maintained within 10% of baseline concentrations. Milk yield did not change and milk fat secretion remained relatively constant with no significant effects on the concentration or yield of milk fat in spite of the chronic elevation in circulating insulin. McGuire et al. (1995) suggested that the increases in lipid synthesis that occur in adipose tissue during milk fat depression likely were due to the increase in energy intake associated with high concentrate diets rather than being a cause of milk fat depression.

Fatty acid extraction by the mammary gland

Cant et al. (1993) measured plasma metabolite arteriovenous (A-V) differences across the mammary glands of cows fed yellow grease at 4% of dietary DM. The supplemental fat did not significantly affect plasma acetate concentration or mammary uptake. However, plasma triglyceride concentration was elevated 61%, mammary triglyceride uptake was increased, and cows produced milk fat with a greater proportion of long-chain fatty acids. Plasma nonesterified fatty acids (NEFA) and β -hydroxybutyrate concentrations were increased 22 and 21%, respectively, due to supplemental dietary fat. Supplemental fat also reduced the A-V differences for glucose and NEFA across the mammary gland, with no effect on acetate, and increased the A-V differences for triglyceride and β -hydroxybutyrate. The authors speculated that the increase in plasma triglyceride was due to fat absorption from the gut being greater than the absorption by the mammary gland. The plasma NEFA concentration probably was increased due to

increased triglyceride hydrolysis with incomplete uptake of the liberated NEFA. For example, increased adipose lipoprotein lipase activity may have contributed to the increase in plasma NEFA concentration. It is unlikely that the increase in plasma β -hydroxybutyrate concentration was due to increased ruminal production. Instead, it was likely due to an increased rate of hepatic ketogenesis as a result of the increased plasma NEFA concentration.

The fatty acid composition of triglyceride in arterial and mammary venous blood was found to be different in the lactating cow (Gluscock and Welch, 1974). Thus, it appears that the mammary gland shows a preference for extracting some triglyceride fatty acids more than others. Thompson and Christie (1991) examined the extraction of plasma triglycerides by the mammary gland of the lactating cow. The mean arterial plasma triglyceride concentration was 88 $\mu\text{mol/L}$ and mammary venous plasma triglyceride concentration was 43 $\mu\text{mol/L}$. The extraction of saturated triglyceride fatty acids was 74% of C18:0, 48% of C16:0, and 31% of C14:0. This preference agrees with data from Gluscock and Welch (1974), which indicated a greater proportion of C18:0 and *trans*-11-C18:1 (74%) than *cis*-9-C18:1 (43%) was extracted. Thompson and Christie (1991) observed no differences in arterial or mammary venous concentrations for 18 carbon PUFA.

Thompson and Christie (1991) speculated that preferences for triglyceride fatty acid extraction by the mammary gland may be due to the uptake mechanism. Lipoprotein

lipase hydrolyses the *sn*-1 position of the triglyceride most rapidly. Subsequently, the *sn*-3 position is hydrolyzed to yield *sn*-2 monoglycerides and NEFA. This phenomenon brings forth the theory that the uptake may be dictated by the structure of the triglyceride supplied to the mammary gland. Triglycerides in sheep plasma were found to have C18:0 and *trans*-C18:1 esterified more prominently in the *sn*-1 and *sn*-3 positions, whereas C16:0 and C14:0 were more prevalent in the *sn*-2 position (Christie et al., 1984).

Effects of insulin and PUFA on lipogenic enzyme mRNA concentrations

This section of the review summarizes studies that examined the effects of fatty acids and insulin on lipogenic mRNA concentrations in hepatocytes, adipocytes, and lymphocytes. The results of these studies may be similar to what occurs in the mammary gland under the same conditions. There is evidence that nutrients and hormones influence lipogenic gene expression in liver and adipose tissue (Girard et al., 1994). Weaning rats to a high-carbohydrate, low-fat diet results in a large increase in the activity of fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), and ATP-citrate lyase (ATP-CL) in liver (Taylor et al., 1967) and adipose tissue (Coupe' et al., 1990). However, weaning rats to a high-fat, low-carbohydrate diet prevents the increase in activities of FAS, ACC, and ATP-CL in liver and adipose tissue. Iritani et al. (1993) observed increases in the levels of lipogenic enzyme mRNA preceding the increases in their activities. In addition, Fukuda et al. (1992) found that the concentrations of FAS, ACC, malic enzyme, and glucose-6-

phosphate dehydrogenase mRNA in cultured rat hepatocytes were increased 8 to 10-fold when insulin and glucose were added to the medium. This evidence suggests that the change in lipogenic enzyme activity is due to an increase in the rate of transcription of the genes encoding for lipogenic enzymes. There is evidence that the increase in plasma insulin that results from feeding a high-carbohydrate, low-fat diet causes the increase in lipogenic enzyme mRNA concentrations (Storey, 1978; Hiremagalur, 1992).

Foufelle et al. (1992) found that weaning rats to diets containing PUFA prevented an increase in FAS mRNA in the liver but not in adipose tissue. However, weaning rats to saturated long-chain fatty acids or medium-chain fatty acids did not prevent the increase in FAS and ACC mRNAs in the liver. Fukuda et al. (1992) observed that concentrations of FAS, ACC, malic enzyme, and glucose-6-phosphate dehydrogenase mRNA were more effectively reduced when C18:2 or C20:4 was added to the medium of cultured rat hepatocytes than when C18:1 was added. In addition, C16:0 did not reduce the mRNA concentrations of the lipogenic enzymes. Apparently, PUFA inhibit the transcription of lipogenic enzymes in the liver.

Tebbey and Buttke (1992) found that the addition of C20:4 to the medium of cultured murine lymphocytes resulted in a decrease in endogenous C18:1 synthesis. Further investigation revealed that the cause of this decrease was due to a reduction in stearoyl CoA desaturase activity which coincided with a decrease in stearoyl CoA desaturase mRNA levels. It was concluded that C20:4 regulates stearoyl CoA desaturase

expression by inhibiting transcription.

Effects of *trans* fatty acids on milk fat synthesis

There is evidence that *trans*-C18:1 fatty acids depress milk fat percent in lactating dairy cows (Selner and Schultz, 1980; Gaynor et al., 1994; Wonsil et al., 1994; Kalscheur et al., 1995) and mice (Teter et al., 1990; Teter et al., 1995). Selner and Schultz (1980) found that feeding a fat containing 49% *trans*-C18:1 to dairy cows resulted in an increase in *trans*-C18:1 content in milk fat (2.6 to 11.2%) and a decrease in milk fat percent (3.9 to 3.1%). There also were reductions of C8:0, C12:0, and C14:0 in the milk fat. The milk fat percentage returned to control levels 2 weeks after removal of *trans*-C18:1 from the diet.

Wonsil et al. (1994) showed that *trans* fatty acids, whether derived from the diet or incomplete biohydrogenation of unsaturated fatty acids, decreased milk fat percent linearly with: 1) amount of *trans*-C18:1 flowing to the duodenum and 2) concentration of *trans*-C18:1 in milk fat. The acetate to propionate ratio was not affected by treatment. Therefore, the decrease in milk fat production could not be attributed to a decrease in acetate production. Teter et al. (1989) also showed that an increase in the *trans*-C18:1 milk fat content due to feeding a high concentrate diet resulted in a decreased milk fat percent. Kalscheur et al. (1995) showed that feeding a buffer with a high concentrate diet increased rumen pH and milk fat percent with a concomitant decrease in milk and

duodenal *trans* fatty acid levels. Gaynor et al. (1994) infused 750 g/d of a mixture of *cis* or *trans* fatty acids into the abomasum of lactating dairy cows. The *trans* fatty acid infusion resulted in a lower milk fat percentage and yield, and a higher milk citrate concentration when compared with the *cis* fatty acid infusion. In addition, there was an increase in the concentration of *trans*-C18:1 in the milk fat following the *trans*-C18:1 infusion. Romo et al. (1993) also showed that infusing *trans* fatty acids abomasally resulted in a lower milk fat percentage. In addition, it was suggested that the *trans* fatty acid infusion caused a shift in the energy deposition from milk fat to tissue, whereas the *cis* infusion caused an increase in milk energy.

Teter et al. (1990) examined the effects of feeding *trans* or *cis* fatty acids on milk fat in lactating mice. The milk fat percentage of *trans*-fed mice was found to decline within 4 d of the *trans* fatty acids appearing in milk fat. Furthermore, the removal of *trans* fatty acids from the diet resulted in a decrease in the level of *trans* fatty acids in milk fat and the milk fat percentage increasing to control levels. Teter et al. (1995) fed mice either the *cis*-9, *cis*-11, *trans*-9, or *trans*-11 isomer of C18:1 for 4 d. The amount of *cis*-11 present in milk fat increased from 2% to 10% when fed, and *trans*-9 and *trans*-11 increased from .1% to 6 and 3%, respectively, when fed. The *trans* isomers were not absorbed as well as the *cis* isomers, because fecal content of *trans* isomers was greater (65% of fecal fatty acids) when compared with that of the *cis* isomers (30%).

The mechanism by which *trans* fatty acids depress milk fat production has not been

determined. The data from Selner and Schultz (1980) suggest that *trans* fatty acids cause milk fat depression by reducing *de novo* synthesis of short-chain fatty acids. The increase in milk citrate concentration observed by Gaynor et al. (1994) following the *trans* infusion was probably due to a reduction in the NADPH requirement for fatty acid synthesis; thus, supporting the theory that *trans* fatty acids decrease *de novo* synthesis of fatty acids in the mammary gland. Hagemeister (1990) suggested that *trans* fatty acids not only affect *de novo* synthesis of fatty acids, but also desaturation of long-chain fatty acids in the mammary gland. Timmen and Patton (1988) suggested that a decrease in the availability of C4:0 to C10:0 may result in increases in the C18:1 content of milk to meet the liquidity requirements of cytoplasmic fat droplets. However, Gaynor et al. (1994) found no difference in milk *cis*-C18:1 concentrations between *cis* and *trans* treatments. Gaynor et al. (1994) conclude that *trans*-C18:1 fatty acids inhibit milk fat synthesis by reducing *de novo* fatty acid synthesis, and by decreasing the activity of acyl transferase, which results in a reduced formation of triglycerides in mammary tissue. Askew et al. (1971) showed that ¹⁴C-labeled C16:0 esterification by mammary homogenates was inhibited to a greater extent by *trans*-C18:1 than *cis*-C18:1.

Milk fatty acids and human health

O'Donnell (1989) reported a summary of the Wisconsin Milk Marketing Board's 1988 Milk Fat Roundtable in which 15 researchers from industry and academia discussed

milk fat technology. This group concluded that the "ideal" nutritional milk fat, based on current dietary recommendations, would contain less than 10% PUFA, up to 8% saturated fatty acids (SFA), and the remainder (>82%) monounsaturated fatty acids (MUFA). Typical milk fat from a dairy cow is approximately 5% PUFA, 70% SFA, and 25% MUFA (Grummer, 1991). Obviously, there is a large discrepancy between the "ideal" and actual milk fat content. It is unlikely that the "ideal" milk fat composition will be achieved through dietary manipulation. However, feeding supplemental fat to dairy cows can result in changes in milk fat composition. From a human nutrition standpoint, these changes may be neutral, desirable, or undesirable with respect to plasma cholesterol concentration.

Feeding supplemental fat to dairy cows often results in an increase in 18-carbon fatty acids and a decrease in fatty acids synthesized *de novo* in the mammary gland (C6:0 to C16:0). The decrease in C12:0 to C16:0 is desirable due to their hypercholesterolemic effects. However, the reduction in C6:0 to C10:0 fatty acids may be an undesirable effect due to their potential hypocholesterolemic effects (Grummer, 1991). The extent to which C16:0 content of milk fat varies is dependent on the amount of C16:0 in the supplemented fat, because this fatty acid can be synthesized *de novo* or extracted from blood lipids derived from the diet. A recent study suggested that C16:0 may be neutral or even hypocholesterolemic (Borlak and Welch, 1994). Increasing the amount of 18-carbon fatty acids in milk fat is also a desirable effect due to their neutral or hypocholesterolemic effects. It is difficult to incorporate dietary medium-chain fatty acids into milk fat since

they are predominantly absorbed into the hepatic portal vein and catabolized by the liver. Conversely, long-chain fatty acids are mainly incorporated into lipoproteins by the intestine and secreted into lymph. Leveille et al. (1967) observed a positive correlation between fatty acid chain length and ratio of lymphatic to hepatic portal vein uptake.

[There may be human health benefits from increasing the PUFA content of milk fat. Wood et al. (1993) suggested that the hypercholesterolemic effects of SFAs may be minimized when the minimal level of dietary PUFAs is between 3 and 6%. In addition, conjugated linoleic acid (*cis, trans*-9,11-C18:2) has been found to have anticarcinogenic activity and has been found in milk fat (Ha et al., 1989). It also may be beneficial to increase the omega-3 (location of double bond based on distance from the methyl end of fatty acid) fatty acids in milk fat due to the possibility that they may reduce the risk of atherosclerosis (Grummer, 1991). This is difficult to accomplish due to ruminal hydrogenation and inefficient transfer of PUFAs from blood to milk (Storry et al., 1969).

In relation to human health, there is controversy over which definition of food components to use on food labels. Currently, the chemical definition, which classifies fatty acids according to their chemical structure without regard for their physiological effect, is used. For example, (C18:0) is chemically defined as a SFA. SFA have been implicated as a causative factor in elevated blood cholesterol levels. However, C18:0 was reported to lower serum cholesterol (Bonanome, 1988). Milk fat contains approximately 12.1g of C18:0 per 100g fatty acids. O'Donnell (1993) suggested that using the physiological

definition of saturated fats instead of the chemical definition would allow the removal of C18:0 from the saturated portion of milk fat, and reduce the saturated fat content of milk fat from 51.3g (C12:0 to C18:0) to 39.2g/100g (C12:0 to C16:0) of total fatty acids. The saturated fat fraction, therefore, would contain only the hypercholesterolemic fatty acids.

Conversely, *trans* fatty acids are defined chemically as unsaturated fatty acids. However, physiologically, *trans* fatty acids have been implicated as a contributor of cardiovascular disease and should be classified as a saturated fatty acid. Mensink and Katan (1990) observed higher low-density lipoprotein (LDL) cholesterol and lower high-density lipoprotein (HDL) cholesterol in men and women consuming diets that contained 10% of daily energy intake from *trans*-C18:1 compared with *cis*-C18:1. This finding is in agreement with Wood et al. (1993) who observed an increase in LDL cholesterol in subjects consuming a hard margarine containing 29% *trans* fatty acids versus a soft margarine containing no *trans* fatty acids. Except for the *trans* fatty acids, the fatty acid content of the two margarines was similar. Thus, it is likely that the increase in LDL cholesterol was caused by the *trans* fatty acids. These data suggest that *trans* fatty acids are not metabolically equivalent to the natural *cis* isomer, and question the wisdom of using partially hydrogenated fats to replace saturated fats. The *trans* fatty acid content of milk fat is about 2g/100g of total fatty acids (primarily *trans*-11-C18:1). Reclassification using a physiological definition of saturated fat would reduce the saturated fat content of milk fat approximately 10g/100g (12g from C18:0 minus 2g from *trans*-11-C18:1), and

would reduce the MUFA content 2g/100g, whereas it would increase the saturated fat content of chemically hydrogenated vegetable oils approximately 14g/100g (20g from *trans*-9-C18:1 minus 6g from C18:0) (Enig et al., 1983). Therefore, to convey more accurate health information to consumers, an argument can be made to use the physiological definitions of food components.

Dietary *trans* fatty acids and human health

The results from studies examining the relationship between *trans* fatty acids and the risk of coronary heart disease (CHD) have been contradictory. Replacement of naturally occurring fatty acids in the diet with *trans* isomers increased concentrations of LDL cholesterol and lipoprotein (a), and lowered concentrations of HDL cholesterol (Mensink and Katan, 1990; Nestel et al., 1992). Rath et al. (1989) found human aortic atherosclerotic plaque contained significant quantities of lipoprotein (a). Mensink and Katan (1992) summarized 16 studies and found that there was a significant negative correlation between total cholesterol and *cis*-C18:1 and C18:2 intakes. Nicolosi and Dietschy (1995) suggested that it is impossible to differentiate whether the addition of dietary *trans* fatty acids at the expense of *cis*-C18:1 and C18:2 caused the increases in total and LDL cholesterol, or whether the *trans* isomers are neutral and the increase was the result of removal of *cis*-C18:1 and C18:2 from the diet.

Thomas et al. (1983) compared adipose tissue samples from patients in the UK

who died from CHD with controls who died from other causes. CHD patients had a higher content of *trans*-16:1 and a higher *trans*-16:1 to C18:2 ratio than controls. In addition, the *trans*-C18:1 content was numerically, but not significantly, higher. Willett et al. (1993) followed a group of 85,095 women who completed a dietary questionnaire in 1980. The intakes of *trans* fatty acids by the women were calculated from the questionnaires. During 8 years of follow-up, there were 431 new cases of CHD. When adjusted for age and total energy intake, intake of *trans* fatty acids was directly associated with a higher risk of CHD. The authors found that intake of *trans* isomers was strongly correlated with intake of monounsaturated fatty acids and C18:2. In addition, when these fatty acids were included in the model simultaneously with saturated fats, the association between *trans* isomer intake and CHD risk was a little stronger than that without these fatty acids. It was determined that 60% of the *trans* isomer intake was from processed vegetable oils and 40% was from animal sources. The major *trans* isomer formed during partial hydrogenation of vegetable oils is *trans*-9-C18:1 (elaidic acid) and the major *trans* isomer found in fat from ruminants is *trans*-11-C18:1 (*trans*-vaccenic acid) (Parodi, 1978). These two sources were examined separately in relation to risk of CHD. It was found that the positive overall association of *trans* isomer intake with CHD was entirely accounted for by partially hydrogenated vegetable oils. Conversely, an inverse association was observed for *trans* isomers from animal fats. Similarly, Ascherio et al. (1994) found a significant association between *trans* fatty acid intake and risk of myocardial infarction

(MI) that could not be explained by other risk factors for CHD. This association was almost entirely accounted for by *trans* isomers from hydrogenated vegetable fats, which contributed 74% of the total intake of *trans* fatty acids in the population, and intake of margarine was directly associated with risk of MI. No significant association was observed between intake of *trans* isomers from animal fats and MI. These data support the hypothesis that consumption of *trans* fatty acids from partially hydrogenated vegetable oils increase the risk of CHD.

Mann (1994) likens the atherosclerotic effects of *trans* fatty acids to a condition called familial hypercholesterolemia (FH). This condition is caused by a defect in the cell-surface lipoprotein receptors (Brown and Goldstein, 1986). This results in the cells being unable to internalize LDL cholesterol, which leads to increased cholesterol synthesis by the cells and hypercholesterolemia. In normal subjects, the early response to a vascular tissue injury is a concentration of lipid-laden inflammatory cells at the injury site, which are later replaced with connective tissue. The end results of a vascular tissue injury are fibrotic lesions with little residual lipid material. Tissue injuries in subjects with FH result in an abnormal accumulation of lipids at the site of repair (Hill et al., 1991). Mann (1994) proposed that *trans* fatty acids are environmental agents that influence injury repair by impeding the removal of the lipids. This causes genetically normal people to resemble those with FH.

Conversely, *trans* fatty acids have been shown to be associated negatively with

CHD. Roberts et al. (1995) obtained adipose tissue samples at necropsy from cases of sudden cardiac death to measure the proportions of *trans* isomers of C18:1 and C18:2. These measurements were compared to samples taken from healthy controls. The mean percentage of total *trans* isomers of C18:1 and C18:2 in adipose from CHD cases was significantly lower than in healthy controls. In fact, the adipose tissue content of *trans*-C18:1 was negatively associated with sudden cardiac death. No association with *trans* isomers of C18:2 was observed. Aro et al. (1995) examined adipose tissue from subjects in 8 European countries and Israel to determine the effect of *trans*-C18:1 fatty acids on risk of acute myocardial infarction (AMI). Adipose tissue samples were obtained from men with and without AMI. The results did not suggest a major overall effect of *trans*-C18:1 fatty acids on risk of AMI. Aro et al. (1995) suggested that the importance of dietary *trans* fatty acids may differ between countries due to differences in the origin and composition of fatty acid isomers. For example, in the study by Thomas et al. (1983), hydrogenated marine oils might have influenced the results more than hydrogenated vegetable oils.

It has been shown that *trans* fatty acids may impair the desaturation and elongation of C18:2 to C20:4 (Lawson et al., 1983). Data from Koletzko (1992) suggests that *trans* fatty acids may impair long-chain PUFA synthesis and cause a reduced birth weight in human premature infants. In addition, *trans* fatty acids have been shown to impair postnatal weight gain in rats and mice (Hill et al., 1982) and to lower birth weights

in newborn piglets when fed to sows (Opstevdt et al., 1988). Desci and Koletzko (1995) examined the effects of *trans* fatty acids on C18:2 metabolism in healthy children. There was no relation between the amount of plasma *trans*-C18:1 or total *trans* fatty acids and C18:2. However, significant inverse correlations of *trans*-C18:1 and total *trans* fatty acids to C20:4 were found. In addition, there was an inverse correlation of total *trans* fatty acids to the ratio of C20:4 to C18:2. This data suggests that *trans* fatty acids may inhibit the biosynthesis of C20:4, which is an important metabolite for tissue growth and development in children. Depending on the diet consumed by the mother (Aitchison et al., 1977), *trans* fatty acids in human milk ranged from of 2 to 18% (Jensen et al., 1978). The authors question the nutritional safety of high dietary *trans* isomer intakes in pregnant and lactating women, and in newborn infants.

In summary, the relationship between *trans* fatty acids and CHD is not completely understood. Further investigation of this relationship is necessary before strong recommendations can be made regarding *trans* fatty acid intake. However, there is strong evidence that *trans* fatty acids impair synthesis of essential long-chain PUFA, which may affect growth and development in prenatal and newborn infants.

Isolation and characterization of the COMMA-D cell line

Danielson et al. (1984) isolated and characterized a mouse mammary epithelial cell line which was designated COMMA-D. The cells were derived from normal mammary

gland tissue of BALB/c mice in the middle of pregnancy. The objective was to establish a mammary epithelial cell line that retained mammary gland-specific morphological and functional differentiation *in vitro*. Problems with previously developed tumorigenic mouse mammary cell lines were: lack of cellular outgrowth when transplanted into mammary fat pads, maintaining the growth potential of cultured cells, and overgrowth of fibroblastic cells.

COMMA-D cells cultured on plastic exhibited cuboidal epithelial-like morphology. Monolayers were formed with cells in close contact to each other. Domes, which were thought to be formed from fluid secretion by the cells, were found in confluent cultures. COMMA-D cells embedded in collagen gels produced three-dimensional duct-like outgrowths, which were similar to those produced by normal mouse mammary cells. It was determined that few, if any, myoepithelial-like cells were part of the COMMA-D population.

Casein was inducible in cells grown in collagen and the caseins produced were indistinguishable from those of normal mouse mammary cells. However, the COMMA-D cells and normal mouse mammary cells do not produce detectable amounts of casein when cultured in collagen with no lactogenic hormones or on plastic with lactogenic hormones.

Transplantation of COMMA-D cells into cleared mammary fat pads of mice resulted in the production of intact ductal structures. The transplants also were found to respond to the stimulus of pregnancy with the development of lobuloalveolar

differentiation. The COMMA-D cell line also provides a way to investigate mammary gland biology and differentiation *in vitro*. The COMMA-D cell line has been used to study growth, morphology, and lipid synthesis induced by growth factors, hormones, and extracellular matrix (Gibson and Baumrucker, personal communication). In addition, insulin-like growth factor (IGF) binding, as well as the mitogenic response to IGFs has been examined in the COMMA-D cell line (Hadsell, et al. 1994). COMMA-D cells have been used also to study growth, morphology, and gene expression following gene transfection (Danielson et al., 1989; Wolff, et al., 1992). Studies examining the p53 tumor suppressor gene have been performed utilizing the COMMA-D cell line (Jerry et al., 1994), as well as studies examining gene expression following hormonal induction (Horseman et al., 1992).

Summary and objectives

It is evident that further research needs to be conducted to increase our understanding of the effects that dietary fat has on milk fat synthesis. This understanding is important because it will allow dairy producers to increase efficiency of milk production while producing a food which benefits the health of the consumers. There is strong evidence that dietary or ruminally derived *trans*-C18:1 fatty acids depress milk fat synthesis. The cause of this depression has not been determined. In addition, there is a need for additional information concerning metabolism of *trans*-C18:1 isomers (elaidic and

trans-vaccenic) at the cellular level. Therefore, the present study used mouse mammary epithelial cells (COMMA-D), in the presence or absence of lactogenic hormones, to examine the effects of *trans*-9-C18:1 or *trans*-11-C18:1 fatty acids on: 1) uptake and alteration of exogenous fatty acids, and 2) incorporation into neutral and polar lipids.

CHAPTER 2

INFLUENCE OF *IN VITRO* ELAIDIC ACID OR *TRANS*-VACCENIC ACID UPTAKE AND LACTOGENIC HORMONE STIMULATION ON FATTY ACID CONTENT OF MOUSE MAMMARY CELLS

INTRODUCTION

Dietary *trans* isomers of C18:1 fatty acids depress bovine (Selner and Schultz, 1980; Gaynor et al., 1994; Wonsil et al., 1994) and murine (Teter et al., 1990) milk fat yield, but the metabolic alterations that cause the depression have not been determined. Selner and Schultz (1980) found that feeding a fat containing 49% *trans*-C18:1 to dairy cows resulted in an increase in *trans*-C18:1 content of milk fat and a decrease in milk fat percent. Wonsil et al. (1994) found that milk fat percent decreased linearly with amount of *trans*-C18:1 flowing to the duodenum and concentration of *trans*-C18:1 in milk fat. The negative linear relationship suggests that the level of *trans* fatty acids presented to the bovine mammary gland and incorporated into milk fat dictates the extent of milk fat depression. Milk fat percent was depressed by *trans*-C18:1, whether derived from the diet or incomplete biohydrogenation of unsaturated fatty acids. In addition, Gaynor et al. (1994) infused either 750 g of *cis*- or *trans*-C18:1 into the abomasum of lactating dairy cows daily and observed a depressed milk fat percentage and yield when cows received the *trans* treatment. Teter et al. (1990) reported a decline in murine milk fat percentage within 4 d of feeding *trans* fatty acids. Furthermore, when *trans* fatty acids were removed from the diet, *trans* fatty acid levels in milk fat decreased

and milk fat percentage increased to control levels.

Dietary or ruminally derived *trans* fatty acids appear to inhibit milk fat synthesis by suppressing the desaturase pathway in the mammary gland (Hagemeister 1990). Stearoyl-coenzyme A desaturase catalyzes the conversion of C18:0 to *cis*-C18:1 in mammary tissue. Substitution of *trans*-C18:1 for C18:0 may depress the activity of this enzyme, thereby altering the fatty acid composition and physical properties of triacylglycerides in milk (Kinsella 1972, Timmen and Patton 1988). The data from Selner and Schultz (1980) suggest that *trans* fatty acids cause milk fat depression by reducing synthesis of short-chain fatty acids. Gaynor et al. (1994) observed an increased concentration of citrate in milk in response to abomasal infusion of *trans*-C18:1 when compared with *cis*-C18:1 infusion. This may be due to *trans*-C18:1 more effectively inhibiting fatty acid synthesis in mammary tissue, which results in a reduction in the NADPH requirement and elevated milk citrate concentrations. In addition, Askew et al. (1971) demonstrated that *trans*-C18:1 inhibited ¹⁴C-labeled C16:0 esterification by mammary homogenates to a greater extent than *cis*-C18:1. Thus, it appears that *trans*-C18:1 fatty acids may affect milk fat secretion by regulating C18:0 desaturation, *de novo* fatty acid synthesis, and triglyceride synthesis. The present study used mouse mammary epithelial cells, in the presence or absence of lactogenic hormones, to examine the effects of elaidic or *trans*-vaccenic acid on: 1) uptake and alteration of exogenous fatty acids, and 2) incorporation into neutral and polar lipids. Elaidic and *trans*-vaccenic acids were selected for comparison because they are the *trans*-C18:1 isomers produced during partial hydrogenation of vegetable oils and incomplete ruminal biohydrogenation, respectively.

MATERIALS AND METHODS

Cell culture

Mouse mammary epithelial cells (MME), a subclone of the COMMA-D cell line, were plated on 100 mm uncoated plastic petri dishes (Corning, Corning, NY) and grown to confluence. Culture medium was Dulbecco's Modified Eagle's Medium (pH 7.4) containing Ham's F12 Nutrient Mixture with L-glutamine and 15mM Hepes (Sigma, St. Louis, MO). The medium was supplemented with sodium bicarbonate (14.3 mM), sodium acetate (3.1 mM), fetal bovine serum (FBS) (10 mL/L), apo-transferrin (10 mg/L), penicillin (5,000 U/L)/streptomycin (5 mg/L), and gentamicin (10 mg/L) (Sigma). The complete medium mixture was filter sterilized through .2 μ surfactant free cellulose acetate (SFCA) filters (Nalge, Rochester, NY). Medium supplemented with lactogenic hormones contained bovine insulin (50 ng/mL), hydrocortisone (500 ng/mL) (Sigma), and bovine prolactin (1 μ g/mL) (NIDDK, Baltimore, MD).

Supplemental fatty acids were bound to bovine serum albumin according to Spector (1986) and added to the medium. Medium containing fatty acid-albumin complexes was filter sterilized through .45 μ SFCA filters, applied to the confluent cell cultures, and changed daily for 3 d. There were 32 plates for each treatment. Medium containing 200 μ M octadecanoic acid (C18:0) was applied as a treatment to determine the desaturating capacity of the cells. All other treatment mixtures contained 100 μ M C18:0 and one of the following μ M ratios of *cis*-octadecenoic acid (*cis*-C18:1) to elaidic or *trans*-vaccenic acid: 100:0, 75:25, 50:50, 0:100 (Table 1). All treatments were conducted with media supplemented

with (16 plates) or without (16 plates) lactogenic hormones. Within each group of 16 plates, four sets of three plates each were used for fatty acid analysis by gas chromatography.

Cellular protein and DNA content were determined on each of four plates.

Cell collection and fractionation

Cell detachment from the plastic petri dishes was initiated by removing media and rinsing the monolayer with phosphate buffered saline (PBS). The PBS contained NaCl (137 mM), KCl (2.7 mM), KH_2PO_4 (1.5 mM), and Na_2HPO_4 (10 mM). The solution was adjusted to a pH of 7.4 and autoclaved. The cells then were covered with a sucrose buffer for 15 min at room temperature. The sucrose buffer contained Na_2HPO_4 (100 mM), EDTA (10 mM), sucrose (28.2 mM), and bovine serum albumin (1g/L) (Sigma). After removal of the sucrose buffer, the cell monolayer was rinsed again with PBS. The cell monolayer was covered with Hank's Balanced Salt Solution (without calcium chloride, magnesium sulfate, and sodium bicarbonate) containing trypsin /EDTA (.5%/.2%) (Sigma) and incubated at 37°C for 20 min. Medium containing FBS was added to the mixture to inhibit trypsin activity. The mixture was transferred to a conical tube and centrifuged at 1200 x g for 5 min in a Precision Universal Centrifuge (GCA Corporation, Chicago, IL). The supernatant was decanted, the cell pellet was resuspended in PBS, and centrifuged at 1200 x g for 5 min. The cells were washed a second time by the same procedure. Cells in PBS from the three plates for fatty acid analysis were combined, transferred to a glass screw top tube, and centrifuged at 1200 x g for 5 min. The supernatant was decanted and cells were frozen at -20°C until analyzed for

fatty acid content. Cells from plates designated for protein and DNA determination were suspended in 1 mL PBS, then homogenized using a hand-held Potter-Ehvehjem-type Teflon® pestle glass homogenizer. The homogenate was frozen at -70°C until analyzed for DNA and protein content.

DNA and protein determinations

Cell DNA and protein content were determined to express the amount of cellular fatty acids on a per DNA (µg) and per protein (mg) basis. Cell DNA was determined using the fluorometric method described by Labarca and Paigen (1980). Cellular protein content was determined using the microtiter plate protocol of the Pierce Bicinchoninic Acid Protein Assay (Pierce, Rockford, IL). Samples were incubated for 1 hr at 37°C. Absorbance was read at 560 nm on a Titertek Multiscan® MCC/340 microtiter plate reader (Eplab, Finland).

Fatty acid analysis

Cellular fatty acids were methylated by direct transesterification (Outen et al., 1976) and undecenoic acid was used as the internal standard. Samples were injected by auto sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard Co., Sunnyvale, CA). Samples were split 100:1 onto a 30 m SP2380 fused silica capillary column with .25 mm i. d. and .20 µm film thickness (Supelco, Inc., Bellefonte, PA). Injector and detector temperatures were set at 225°C. A temperature program initiated runs at 60°C, warmed to 205°C at 5°C/min, held for 12 min, then warmed

to 215°C at 5°C/min and held for 5 min, and then warmed to 220°C at 5°C/min and held for 2 min. Identification of peaks was based on relative retention times of a commercial standard with the addition of palmitelaidic, elaidic, and *trans*-vaccenic acids (Nu-Chek Prep, Inc., Elysian, MN). The fatty acid standards were used to construct a calibration table via a Hewlett Packard 3396A integrator (Hewlett Packard Co., Avondale, PA). Response and recovery factors were used to determine weight percentages of individual fatty acids in the analyzed samples.

Triglyceride analysis

Lipids were labelled by adding 10 μCi (1 $\mu\text{Ci}/\text{mL}$ medium) of $[1,2,3]\text{-}^3\text{H}$ -glycerol (40 $\mu\text{Ci}/\text{mmol}$, Dupont/NEN, Boston, MA) to the medium on d 2 of treatment. Cells were incubated at 37°C for 48 hr, then harvested. Lipids were extracted according to Bligh and Dyer (1959). Radioactivity in washed lipid extracts was determined in BetaMax scintillation fluid (ICN, Irvine, CA). Lipids were separated on HPTLC plates (Whatman). Plates were developed in hexane-diethyl ether-acetic acid (85:15:1.5, v/v/v) for separation of neutral lipids, and in methyl acetate-n-propanol-chloroform-methanol-0.25% aqueous KCl (25:25:28:10:7, by volume) for separation of polar lipids (Heape et al., 1985). After development, separated constituents were made visible by brief charring with cupric acetate-phosphoric acid (Fewster et al., 1969). Radioactive constituents were detected by fluorography. Plates were sprayed with Enhance (DuPont/NEN) and exposed to X-ray film for 3 to 6 days at -80°C. Cell protein for the triglyceride analysis was determined by the

modified Lowry procedure (Markwell et al., 1978).

Statistical analysis

Data were analyzed using the general linear models (GLM) procedure of SAS (1985). The model included effect of media fatty acids, hormone supplementation, replicate, and the media fatty acid by hormone interaction. Nonorthogonal contrasts were used to test the relationship between individual and total cellular fatty acids and cellular protein and DNA in response to increasing concentration of *trans*-C18:1 isomer in the medium. Contrasts also were used to determine differences in cellular fatty acid content and cellular protein and DNA content between the two *trans*-C18:1 isomer treatments. In addition, differences in cellular fatty acid content and cellular protein and DNA were determined between media supplemented with or without lactogenic hormones. Data from the control treatment were not included in the analysis of cellular *trans*-16:1 content. Data from the control and the *trans*-vaccenic acid treatments were not included in the analysis of cellular elaidic acid content. Data from the control and the elaidic acid treatments were not included in the analysis of cellular *trans*-vaccenic acid content. These treatments were excluded because they were equal to zero (no synthesis or uptake of the fatty acid by the cells), which biased the error mean square downward (they contributed no error sum of squares but did contribute degrees of freedom). Removing these observations minimized heterogenous variance problems. Bonferroni F-values were used to determine significance of individual contrasts at $P < .05$.

RESULTS

Protein and DNA content per plate

Total protein and DNA contents, expressed as $\mu\text{g}/\text{plate}$, were greater ($P < .05$) for the *trans*-C18:1 fatty acid treatments than for the control (Tables 2 and 3). The relationship between the elaidic acid concentration in the media and DNA content was significant ($P < .05$) for the treatments containing no hormones, but the corresponding relationship for the treatments containing hormones was not significant ($P \geq .05$) (Table 4 and Figure 1). In addition, the relationship between elaidic or *trans*-vaccenic acid concentration in the media and cellular protein content was significant ($P < .05$) for the treatments containing no hormones, but the corresponding relationship for the treatments containing hormones was not significant ($P \geq .05$) (Figures 2 and 3). Protein and DNA were greater ($P < .05$) for the *trans*-vaccenic acid treatments than for the elaidic acid treatments. Lactogenic hormone supplementation decreased ($P < .05$) protein content per plate in the treatments containing 50 and 100 μM *trans*-C18:1, but not in the 0 or 25 μM *trans*-C18:1 treatments. However, lactogenic hormone supplementation increased ($P < .05$) the DNA content in all treatments except for the 50 μM *trans*-vaccenic treatment. As a result, the overall protein to DNA ratio was decreased ($P < .05$) in treatments containing lactogenic hormones.

Uptake of *trans*-C18:1 isomers

The slope of the line for the cellular *trans*-C18:1 content per mg protein was

greater ($P < .05$) than zero as concentration of either *trans*-C18:1 isomer in the media increased (Figure 4). The rate of uptake of elaidic acid was greater than uptake of *trans*-vaccenic acid. Uptakes of the *trans*-C18:1 fatty acids were not different ($P \geq .05$) between media supplemented with or without lactogenic hormones.

Total cellular fatty acid content

Total cellular fatty acid content per mg protein was lower ($P < .05$) for the *trans*-C18:1 fatty acid treatments than for the control (Tables 5, 6, and 7). However, total cellular fatty acid content per mg protein was greater ($P < .05$) for the elaidic acid treatments than for the *trans*-vaccenic acid treatments. Overall, lactogenic hormone supplementation decreased ($P < .05$) total cellular fatty acid content per mg protein for all treatments. As indicated in Figures 5 and 6, the presence of lactogenic hormones in the media apparently depressed total cellular fatty acid content to a minimum level. Therefore, the relationship between total cellular fatty acids and *trans*-C18:1 concentration of the media was significant only when the media did not contain lactogenic hormones.

Cellular C18:0 content

Cellular C18:0 content per mg protein was greater ($P < .05$) for the control than for the *trans*-C18:1 treatments (Table 5). There were no overall differences ($P \geq .05$) in cellular C18:0 content between the elaidic and *trans*-vaccenic acid treatments. However,

the slope of the line for cellular C18:0 content was less ($P < .05$) than zero as concentration of elaidic acid in the media without lactogenic hormone supplementation (Figure 7). A similar decrease in response to *trans*-vaccenic acid was noted (Figure 8), but in this case there also was a significant ($P < .05$) relationship when the media contained lactogenic hormones.

Cellular *cis*-C18:1 content

Cellular *cis*-C18:1 content per mg protein was greater ($P < .05$) for the control than for the *trans*-C18:1 treatments (Table 5), and for the treatments without lactogenic hormone supplementation compared to treatments with hormone supplementation. There were no overall differences in cellular *cis*-C18:1 content between the elaidic and *trans*-vaccenic acid treatments. However, the slope of the line for cellular *cis*-C18:1 content was less ($P < .05$) than zero as concentration of elaidic acid in media without lactogenic hormone supplementation increased (Figure 9), and as concentration *trans*-vaccenic acid in media with or without hormone supplementation increased (Figure 10).

Cellular *cis*-C16:1 and content

Cellular *cis*-C16:1 content per mg protein was greater for the control than for the *trans*-C18:1 treatments (Table 5). There were no differences ($P \geq .05$) in cellular *cis*-C16:1 content between the elaidic and *trans*-vaccenic acid treatments, or between treatments supplemented with and without lactogenic hormones. However, the slope of

the line for cellular *cis*-C16:1 content was less ($P < .05$) than zero as concentration of elaidic (Figure 11) or *trans*-vaccenic (Figure 12) acid in media with or without lactogenic hormone supplementation increased.

Cellular C16:0 content

Cellular C16:0 content per mg protein was greater for the control than for the *trans*-C18:1 treatments (Table 5). There were no differences ($P \geq .05$) in cellular C16:0 content between the elaidic and *trans*-vaccenic acid treatments, or between treatments supplemented with and without lactogenic hormones. The slope of the line for cellular C16:0 content was less ($P < .05$) than zero as concentration of elaidic (Figure 13) or *trans*-vaccenic (Figure 14) acid in media with or without lactogenic hormone supplementation increased.

Cellular *trans*-C16:1 content

Cells from the control treatment contained no *trans*-C16:1. Cellular *trans*-C16:1 content per mg protein was greater ($P < .05$) for elaidic acid treatments than for *trans*-vaccenic acid treatments (Table 5). Lactogenic hormone supplementation resulted in decreased ($P < .05$) cellular *trans*-C16:1 content. There was no cellular *trans*-C16:1 measured for the 25 μ M *trans*-vaccenic acid treatment with or without hormone supplementation. The slope of the line for cellular *trans*-C16:1 content was greater ($P < .05$) than zero as concentration of elaidic or *trans*-vaccenic acid in the media increased

(Figure 15). The slope was greater ($P < .05$) for the elaidic acid treatments without hormone supplementation than with hormone supplementation. The slopes in response to *trans*-vaccenic acid treatments with versus without hormone supplementation were not different ($P \geq .05$).

Polar and nonpolar lipid fractions

Overall, there appeared to be no difference in the cellular polar and nonpolar lipid fractions when the control treatment was compared with treatments containing unsaturated fatty acids (Table 9). In addition, increasing the concentration of elaidic or *trans*-vaccenic acid in the media did not influence the proportion of polar and nonpolar lipids synthesized by the cells.

DISCUSSION

Lactogenic hormone supplementation apparently maximized cell number per plate for all treatments. This is evident from the slope for DNA concentration being near zero as concentration of *trans*-C18:1 in the media increased (Figure 1). This is expected considering the known mitogenic effects of lactogenic hormones. As a result, lactogenic hormone supplementation decreased the overall protein to DNA ratio. This is indicative of an increased cell number and a decreased cell size. Increasing the *trans*-vaccenic acid concentration in the media did not affect the DNA content per plate. However, the slopes of the lines for cellular DNA and protein content were greater ($P < .05$) than zero as the concentration of elaidic acid in the media increased. This relationship was caused by a large increase in the recovery of cellular DNA and protein for the 100 μ M elaidic acid treatment. Greater recovery of cells for this treatment may have been due to greater cell density.

The significant ($P < .05$) positive relationships between cellular elaidic or *trans*-vaccenic acid and their concentration in the media indicates that the *trans*-C18:1 fatty acids were indeed extracted by the mouse mammary cells. This finding is supported by the data from Thompson and Christie (1991), which showed uptake of *trans*-vaccenic acid by the mammary gland of lactating dairy cows. In addition, Panigrahi and Sampugna (1993) found Swiss mouse fibroblast 3T3-L1 cells to readily incorporate exogenous *trans*-C18:1 fatty acids. Elaidic acid apparently was taken up to a greater extent than *trans*-vaccenic acid. This finding may have human health significance. Willett et al. (1993) observed a

direct association between intake of *trans* fatty acids and risk of CHD. It was determined that the positive association between *trans* isomer intake and CHD was entirely accounted for by partially hydrogenated vegetable oils. Furthermore, an inverse association was observed between *trans* isomers from animal fats and CHD. Similarly, Ascherio et al. (1994) observed a significant association between *trans* fatty acid intake and risk of myocardial infarction, which was entirely attributed to *trans* isomers from hydrogenated vegetable fats. Perhaps the greater uptake of elaidic acid that occurred in the mouse mammary cells also occurs in cells of the human, which may be part of the explanation for the detrimental effects of partially hydrogenated vegetable oils on coronary health. In addition, Desci and Koletzko (1995) suggested that *trans* fatty acids may inhibit the biosynthesis of C20:4, which is important for tissue growth and development. The authors questioned the nutritional safety of high dietary *trans* isomer intakes in pregnant and lactating women. If uptake of elaidic acid by human mammary tissue responds similar to mouse tissue in the present study, it may warrant a recommendation for lactating women to limit intake of partially hydrogenated vegetable oils.

Increasing the media concentration of elaidic or *trans*-vaccenic acid without lactogenic hormone supplementation resulted in the slopes of the lines for total cellular fatty acid content being less ($P < .05$) than zero. This finding is in agreement with *in vivo* studies using lactating mice and lactating dairy cows. Teter et al. (1990) fed *trans* or *cis* fatty acids to lactating mice and found the milk fat percentage to decline within 4 d of the *trans* fatty acids appearing in milk fat. Removing the *trans* fatty acids from the diet

resulted in the milk fat percentage increasing to control levels. Panigrahi and Sampugna (1993) reported that replacing *cis*-C18:1 with *trans*-C18:1 in growth media reduced fat accumulation in mouse fibroblast 3T3-L1 cells. In addition, Atal et al. (1994) found body weights, epididymal fat pad weights, and perirenal fat yield to be lower for male mice fed *trans* fatty acids compared to male mice fed no *trans* fatty acids. Wonsil et al. (1994) used lactating dairy cows to show that *trans* fatty acids, whether derived from the diet or incomplete biohydrogenation of unsaturated fatty acids, decreased milk fat percent linearly with: 1) amount of *trans*-C18:1 flowing to the duodenum and 2) concentration of *trans*-C18:1 in milk fat. Studies have been performed to determine the mechanisms by which *trans* fatty acids reduce fat accumulation. Cromer et al. (1995) studied the effects of *trans* fatty acids on lipolysis and glucose utilization by rat adipocytes. Compared with *cis*-C18:1, elaidic and *trans*-vaccenic acids were found to reduce glucose oxidation to carbon dioxide and the amount of glucose converted to cell lipid. In addition, lipolytic rates were increased by replacing *cis*-C18:1 with either of the *trans* isomers. Koga et al. (1994) found *trans* monoene fatty acids to be selectively concentrated into only a few membrane lipid fractions instead of being incorporated uniformly into all tissue phospholipids. This selective incorporation of some fatty acid isomers into membrane lipids may cause disruption of normal membrane function and result in alterations of cell metabolism. Gaynor et al. (1994) infused either *cis* or *trans* fatty acids into the abomasum of lactating dairy cows and observed a decreased milk fat percentage for the *trans* treatment. It was speculated that reduced synthesis of fatty acids and reduced activity of acyl transferase in

mammary tissue contributed to the depressed milk fat percentage for the *trans* treatment. The findings of these studies may explain the reduced fat accumulation when *cis*-C18:1 in animal diets or growth media is replaced with a *trans* isomer.

The slope of the line for total cellular fatty acid content was less ($P < .05$) than zero as media concentration of *trans*-vaccenic acid increased with lactogenic hormone supplementation. The slopes of the lines were near zero for the elaidic acid treatments with lactogenic hormone supplementation. This may have been due to the hormones causing a maximum level of lipid synthesis despite the presence of elaidic acid in the media.

The slope of the line for cellular C18:0 content was less ($P < .05$) than zero as concentration of elaidic or *trans*-vaccenic acid in the media increased without lactogenic hormone supplementation. Panigrahi and Sampugna (1993) found that mouse fibroblast cells grown in the presence of *trans* fatty acids contained less C18:0 than cells grown without *trans* fatty acids despite the *trans* fatty acid media containing more C18:0. The decreased amount of C18:0 appeared to be compensated for by the presence of *trans* fatty acids. Atal et al. (1994) observed a reduction in the proportion of C18:0 in only the polar lipid fraction of epididymal fat pads of mice fed *trans* fatty acids compared to mice fed no *trans* fatty acids. The investigators also found the sum of *trans*-C18:1 plus saturated fatty acids in the polar lipids of mice fed *trans* fatty acids to be similar to the amount of total saturated fatty acids in mice fed no *trans* fatty acids. However, this finding was not observed in the nonpolar lipid fraction. The inverse relationship between the cellular content of C18:0 and *trans* fatty acids may be related to the similarity of their properties.

Both *trans*-C18:1 and C18:0 are linear molecules with high melting points compared to *cis*-C18:1. In addition, Emken (1984) found that both saturated and *trans* fatty acids are preferentially incorporated into the 1-acyl position of phospholipids. Thus, C18:0 and *trans*-C18:1 may compete for incorporation at the same location on the phospholipid molecule, which would also explain the decrease in cellular C18:0 as *trans*-C18:1 in the media increased.

There was a significant ($P < .05$) negative relationship between cellular *cis*-C18:1 content and amount of elaidic or *trans*-vaccenic acid in the media. This may be partly due to the amount of *cis*-C18:1 in the media being replaced by the *trans* fatty acids. Atal et al. (1994) also observed lower proportions of *cis*-C18:1 in epididymal fat pads of mice when 50% of the *cis*-C18:1 in the control diet was replaced with *trans* fatty acids. Following separation of the polar and nonpolar lipid fractions, it was found that the incorporation of *trans*-C18:1 was accompanied by a decrease in the proportion of *cis*-C18:1 in the nonpolar lipids but not in the polar lipids. Atal et al. (1994) suggested that perhaps the ability of acylating enzymes to recognize *trans* fatty acids differs greatly for different lipid classes. Relative to percentages present in the diet, proportionately more *cis*-C18:1 was present in the epididymal fat pads isolated from mice fed the *trans* fatty acids than from mice fed no *trans* fatty acids. This suggests that preferential extraction of *cis*-C18:1 may have occurred in the fat pads of mice fed the *trans* fatty acids. This suggestion is in contrast to findings concerning the mammary gland. Thompson and Christie (1991) found that a greater proportion of triglycerides containing *trans*-vaccenic acid were extracted by the mammary

gland than triglycerides containing *cis*-C18:1. In addition, Hagemeister (1990) suggests that *trans* fatty acids decrease fat synthesis in the mammary gland by decreasing the desaturation of long chain fatty acids by the desaturase enzyme. Tebbey and Buttke (1992) found that the addition of C20:4 to the media of cultured murine lymphocytes resulted in a decrease in endogenous *cis*-C18:1 synthesis. The cause of this decrease was due to a reduction in stearoyl CoA desaturase activity. It was concluded that C20:4 regulates stearoyl CoA desaturase expression by inhibiting transcription. Perhaps *trans*-C18:1 fatty acids regulate stearoyl-CoA desaturase activity in a similar manner.

There was a significant ($P < .05$) negative relationship between cellular *cis*-C16:1 content and amount of elaidic or *trans*-vaccenic acid in the media with or without lactogenic hormone supplementation. Since there was no *cis*-C16:1 supplemented in the media, the only possible sources of *cis*-C16:1 were desaturation of C16:0, which was synthesized *de novo*, and retroconversion of *cis*-C18:1, which was present in the media. The decrease in C16:0 and *cis*-C18:1, which occurred as the media concentration of elaidic or *trans*-vaccenic acid increased, would reduce the substrates available for *cis*-C16:1 production. In addition, the decrease in desaturase activity has already been discussed as a possible mechanism for decreased lipid accumulation when *trans* fatty acids are present in the diet or media.

There was a significant ($P < .05$) negative relationship between cellular C16:0 content and amount of elaidic or *trans*-vaccenic acid in the media. Since there was no C16:0 supplemented in the media, this decrease had to be the result of decreased *de novo*

synthesis. Hagemeister (1990) found that *trans* fatty acids appear to depress milk fat synthesis by suppressing the *de novo* synthesis of fatty acids from acetate and butyrate. Wonsil et al. (1994) also observed a decrease in the milk fat content of C16:0 when the incorporation of *trans*-C18:1 fatty acids into milk fat increased. Gaynor et al. (1994) also observed a decrease in the milk fat content of C16:0 when *trans* fatty acids were infused into the abomasum of dairy cows when compared to the *cis* fatty acid infusions. This decrease in the C16:0 content was accompanied by an increased concentration of citrate in the milk. This finding suggests that the decrease in C16:0 was due to a reduction of *de novo* synthesis in the mammary gland. The increased citrate concentration is due to a reduction in the NADPH requirement for fatty acid synthesis, reduced isocitrate dehydrogenase activity, and reduced tricarboxylic acid activity in mammary tissue. As a result, citrate may accumulate in the cell and be released into the milk. A similar increase in milk citrate concentration was observed by Banks et al. (1990) when soya oil or soya oil fatty acids were fed to dairy cows resulting in an increased milk fat content of *trans*-C18:1 fatty acids. These studies support the concept of *trans* fatty acids being potent inhibitors of *de novo* fatty acid synthesis in the mammary gland.

There was a significant ($P < .05$) positive relationship between cellular *trans*-C16:1 content and amount of elaidic or *trans*-vaccenic acid in the media. There was no *trans*-C16:1 supplemented in the media. The product of C16:0 desaturation is *cis*-C16:1. Therefore, the only possible source of cellular *trans*-C16:1 is peroxisomal β -oxidation, also referred to as retroconversion, of the *trans*-C18:1 isomers present in the media. Hovik and

Osmundsen (1987) define retroconversion as the chain shortening of fatty acids that are poorly oxidized by mitochondrial β -oxidation. In the present study, the greater amount of cellular *trans*-C16:1 present for the elaidic acid treatments corresponds to the greater uptake of elaidic acid by the cells. This supports the suggestion that the *trans*-C16:1 isomers are arising from the retroconversion of the *trans*-C18:1 isomers. To our knowledge, retroconversion has not been shown to occur in mouse mammary cells. However, Panigrahi and Sampugna (1993) have observed retroconversion of media derived *trans*-C18:1 isomers to *trans*-C16:1 in Swiss mouse fibroblast 3T3-L1 cells. Panigrahi and Sampugna (1993) suggested that the decreased accumulation of cellular lipid observed may have resulted from induction of peroxisomal β -oxidation by the *trans* fatty acids in the media.

Increasing the concentration of elaidic or *trans*-vaccenic acid in the media did not influence the proportion of polar and nonpolar lipids synthesized by the cells. However, Panigrahi and Sampugna (1993) observed a decreased fat accumulation in Swiss mouse fibroblast 3T3-L1 cells cultured in the presence of *trans* fatty acids, which was primarily due to a decreased nonpolar lipid content of the cells. A similar finding was observed by Atal et al. (1994) in the epididymal fat pads of male mice fed *trans* fatty acids. In the present study, the triglycerides synthesized by the cells were only about 10% of the total lipid produced. Perhaps there was not enough triglyceride synthesis by the cells to show a more significant decrease than phospholipid synthesis. Therefore, it can only be concluded that the *trans* fatty acids caused an equal decrease in phospholipid and triglyceride

synthesis in the mouse mammary cells.

SUMMARY AND CONCLUSIONS

Addition of *trans*-C18:1 isomers to the media did not influence the proportion of polar and nonpolar lipids synthesized by mouse mammary epithelial cells. As media concentration of *trans*-C18:1 isomers increased: amounts of cellular *trans*-C16:1 and *trans*-C18:1 increased, amounts of cellular C16:0, *cis*-C16:1, C18:0, and *cis*-C18:1 decreased, amounts of total cellular fatty acids decreased, and the ratio of *cis* isomers to saturated plus *trans* isomers decreased.

After uptake, *trans*-C18:1 isomers were apparently retroconverted to *trans*-C16:1 isomers. There was no *trans*-C16:1 present in the media and the product of C16:0 desaturation is *cis*-C16:1. Therefore, the source of the cellular *trans*-C16:1 has to be from the retroconversion of *trans*-C18:1 isomers present in the media. In addition, *trans*-C18:1 isomers caused a significant reduction in *de novo* fatty acid synthesis. The decrease in the ratio of *cis* isomers to saturated fatty acids and their *trans* isomers would indicate an increase in the melting point of cellular lipids.

Further investigations need to be conducted to determine the mechanisms by which *trans* fatty acids reduce lipid accumulation in cells. Possible mechanisms include: a reduction in acetyl CoA carboxylase activity, a reduction in stearoyl CoA desaturase activity, a reduction in acyl transferase activity, or alteration of cell metabolism following incorporation of *trans* fatty acids into membrane phospholipids.

Table 1. Concentration of supplemental fatty acids in treatment media applied to confluent mouse mammary epithelial cell cultures.

Treatment	Supplemental Fatty Acid (μM)			
	18:0	<i>cis</i> 18:1	Elaidic	<i>trans</i> -Vaccenic
Control	200			
0 <i>trans</i>	100	100		
25 E	100	75	25	
50 E	100	50	50	
100 E	100		100	
25 V	100	75		25
50 V	100	50		50
100 V	100			100

Table 2. Amounts of cellular protein, DNA, and the protein to DNA ratio in mouse mammary epithelial cells in response to fatty acid supplementation with or without lactogenic hormones

Treatment	Cell Fraction (mg)		
	Protein	DNA	Protein:DNA
Control	1.87	.34	5.68
Control (H) ^a	1.45	.56	2.57
0 <i>trans</i>	2.09	.45	4.60
0 <i>trans</i> (H)	2.58	.75	3.42
25 E	2.17	.39	5.58
25 E (H)	2.11	.60	3.51
50 E	2.29	.39	5.82
50 E (H)	2.11	.58	3.68
100 E	3.38	.76	4.48
100 E (H)	2.23	.73	3.09
25 V	2.76	.39	7.07
25 V (H)	2.85	.84	3.41
50 V	3.95	.89	4.43
50 V (H)	2.62	.81	3.28
100 V	2.86	.45	6.42
100 V (H)	2.88	.78	3.74
SE ^b	.15	.03	.29

^aTreatment supplemented with insulin (50ng/mL), prolactin (1µg/mL), and hydrocortisone (500ng/mL).

^bPooled standard errors for means of all treatments.

Table 3. Non-orthogonal contrasts for cellular protein and DNA content, and the protein to DNA ratio, between fatty acid treatments and within fatty acid treatments with or without lactogenic hormones

Contrast	Cell Fraction ^a		
	Protein	DNA	Protein:DNA
Control ^b vs. E + V ^c	*	*	-
E vs. V	*	*	-
25 E vs. 25 V	*	*	-
25 -H ^d vs. 25 +H ^e	-	*	*
50 E vs. 50 V	*	*	*
50 -H vs. 50 +H	*	-	*
100 E vs. 100 V	-	*	*
100 -H vs. 100 +H	*	*	*

^a Significance determined using Bonferroni F-values. * ($P < .05$), - ($P \geq .05$).

^b 200 μ M C18:0 with and without lactogenic hormones.

^c All unsaturated fatty acid treatments (E = elaidic acid, V = *trans*-vaccenic acid) with and without lactogenic hormones.

^d Media mixtures not supplemented with lactogenic hormones.

^e Media mixtures supplemented with lactogenic hormones - insulin (50 ng/mL), prolactin (1 μ g/mL), and hydrocortisone (500ng/mL).

Table 4. Tests of linear slopes of cellular protein, DNA, and their ratio in response to changing concentration of elaidic acid (E) and *trans*-vaccenic acid (V) with or without lactogenic hormone supplementation, with and without hormone supplementation together, and comparing with versus without hormone supplementation.

Contrast	Cell Fraction ^a		
	Protein	DNA	Protein:DNA
E (-H only) ^b	*	*	NC
(+H only) ^b	-	-	NC
(-H and +H) ^c	*	*	-
(-H vs. +H) ^d	*	*	-
V (-H only) ^b	NC	NC	NC
(+H only) ^b	NC	NC	NC
(-H and +H) ^c	*	-	-
(-H vs. +H) ^d	-	-	-

^a Significance determined using Bonferroni F-values. * ($P < .05$), - ($P \geq .05$), NC = Not contrasted.

^b Test of linear slopes for treatments with (+H) or without (-H) lactogenic hormone supplementation was performed only if a significant difference was detected for treatments with hormone versus those without hormones.

^c Test of linear slopes of cell fraction versus treatment concentration with (+H) and without (-H) lactogenic hormone supplementation.

^d Difference in linear slopes for treatments with (+H) versus without (-H) lactogenic hormone supplementation.

Table 5. Amounts of cellular fatty acids in mouse mammary epithelial cells in response to fatty acid supplementation of media with or without lactogenic hormones

Treatment	Cellular Fatty Acid (μg / mg protein)							TOTAL
	16:0	<i>t</i> 16:1	<i>c</i> 16:1	18:0	<i>t</i> 18:1 (E)	<i>t</i> 18:1 (V)	<i>c</i> 18:1	
Control	20.4	0	7.9	19.1	0	0	74.1	121.5
Control (H) ^a	19.8	0	7.9	11.8	0	0	50.2	89.7
0 <i>trans</i>	26.1	0	12.5	17.8	0	0	89.0	145.4
0 <i>trans</i> (H)	18.9	0	9.0	8.3	0	0	41.1	77.2
25 E	14.9	5.7	7.2	12.9	3.6	0	68.4	112.8
25 E (H)	18.0	3.8	6.4	9.5	2.5	0	43.2	83.3
50 E	18.9	8.2	6.8	11.6	4.0	0	63.4	112.8
50 E (H)	16.1	4.9	4.5	8.5	4.0	0	34.0	72.1
100 E	14.7	15.7	3.7	10.0	8.8	0	40.5	93.5
100 E (H)	15.6	10.9	3.6	9.9	9.9	0	31.0	80.9
25 V	16.7	0	6.6	10.7	0	1.5	57.6	93.2
25 V (H)	16.8	0	7.4	9.9	0	2.2	44.8	81.2
50 V	18.2	3.1	5.7	9.9	0	2.8	44.7	84.4
50 V (H)	12.1	2.1	5.1	7.2	0	2.8	32.0	61.3
100 V	13.8	5.9	4.3	10.0	0	6.1	46.9	87.1
100 V (H)	13.1	6.5	3.4	7.2	0	5.2	26.5	61.9
SE ^b	.6	.4 .5 ^c	.6	.9	.2 .3 ^d	.2 .3 ^e	3.7	6.8

^aTreatment supplemented with insulin (50 ng/mL), prolactin (1 μg /mL), and hydrocortisone (500ng/mL).

^bPooled standard errors for means of all treatments.

^cPooled standard error for means of all treatments excluding control.

^dPooled standard error for 0 *trans* and elaidic acid (E) treatments only.

^ePooled standard error for 0 *trans* and *trans*-vaccenic acid (V) treatments only.

Table 6. Analysis of variance for supplemental fatty acid (FA) treatments, lactogenic hormone treatments, and fatty acid*hormone interaction

Item	Cellular Fatty Acid ^a							Total
	16:0	<i>t</i> 16:1	<i>c</i> 16:1	18:0	<i>t</i> 18:1 (E)	<i>t</i> 18:1 (V)	<i>c</i> 18:1	
FA	**	**	**	**	**	**	**	**
Hormone ^b	*	**	**	**	-	-	**	**
FA*Hormone	*	**	*	**	*	*	**	**
df ^c	7	6	7	7	3	3	7	7

^a Significance determined using Bonferroni F-values, * (P < .05), ** (P < .01), - (P ≥ .05).

^b See Table 2 for listing of all fatty acid and hormone treatments.

^c Degrees of freedom for FA and FA*hormone.

Table 7. Non-orthogonal contrasts between fatty acid treatments and within fatty acid treatments with or without lactogenic hormones

Contrast	Cellular Fatty Acid ^a							TOTAL
	16:0	<i>t</i> 16:1	<i>c</i> 16:1	18:0	<i>t</i> 18:1 (E)	<i>t</i> 18:1 (V)	<i>c</i> 18:1	
Control ^b vs. E + V ^c	*	NC	*	*	NC	NC	*	*
E vs. V	-	*	-	-	NC	NC	-	*
25 E vs. 25 V	-	*	-	-	NC	NC	-	-
25 -H ^d vs. 25 +H ^e	-	-	-	-	-	-	*	*
50 E vs. 50 V	-	*	-	-	NC	NC	-	-
50 -H vs. 50 +H	-	*	-	*	-	-	*	*
100 E vs. 100 V	-	*	-	-	NC	NC	-	-
100 -H vs. 100 +H	-	*	-	-	-	-	*	*

^a Significance determined using Bonferroni F-values. * (P < .05), - (P ≥ .05), NC = Not contrasted.

^b 200 μM C18:0 with and without lactogenic hormones.

^c All unsaturated fatty acid treatments (E = elaidic acid, V = *trans*-vaccenic acid) with and without lactogenic hormones.

^d Media mixtures not supplemented with lactogenic hormones.

^e Media mixtures supplemented with lactogenic hormones - insulin (50 ng/mL), prolactin (1 μg/mL), and hydrocortisone (500ng/mL).

Table 8. Tests of linear slopes of cellular fatty acid content in response to changing concentration of elaidic acid (E) and *trans*-vaccenic acid (V) with or without lactogenic hormone supplementation, with and without hormone supplementation together, and comparing with versus without hormone supplementation.

Contrast	Cellular Fatty Acid ^a							Total
	16:0	<i>t</i> 16:1	<i>c</i> 16:1	18:0	<i>t</i> 18:1 (E)	<i>t</i> 18:1 (V)	<i>c</i> 18:1	
E (-H only) ^b	NC	*	NC	*	NC	NC	*	*
(+H only) ^b	NC	*	NC	-	NC	NC	-	-
(-H and +H) ^c	*	*	*	*	*	NC	*	*
(-H vs. +H) ^d	-	*	-	*	*	NC	*	*
V (-H only) ^b	NC	NC	NC	NC	NC	NC	NC	NC
(+H only) ^b	NC	NC	NC	NC	NC	NC	NC	NC
(-H and +H) ^c	*	*	*	*	NC	*	*	*
(-H vs. +H) ^d	-	-	-	-	NC	-	-	-

^a Significance determined using Bonferroni F-values. * (P < .05), - (P ≥ .05), NC = Not contrasted.

^b Test of linear slopes for treatments with (+H) or without (-H) lactogenic hormone supplementation was performed only if a significant difference was detected for treatments with hormone versus those without hormones.

^c Test of linear slopes of cellular fatty acid versus treatment concentration with (+H) and without (-H) lactogenic hormone supplementation.

^d Difference in linear slopes for treatments with (+H) versus without (-H) lactogenic hormone supplementation.

Table 9. Percentages of total ^3H -glycerol incorporation into recovered phospholipid and triglyceride fractions by mouse mammary cells in response to replacing oleic acid (0 *trans*) in the medium with elaidic acid (E) or *trans*-vaccenic acid (V).

Treatment	Percent ^a	
	Phospholipid	Triglyceride
Control	22	2.4
0 <i>trans</i>	22	2.5
50 E	21	2.0
100 E	20	2.0
50 V	21	2.3
100 V	23	2.5

^a Represents percent of recovered radioactivity in the respective fraction.

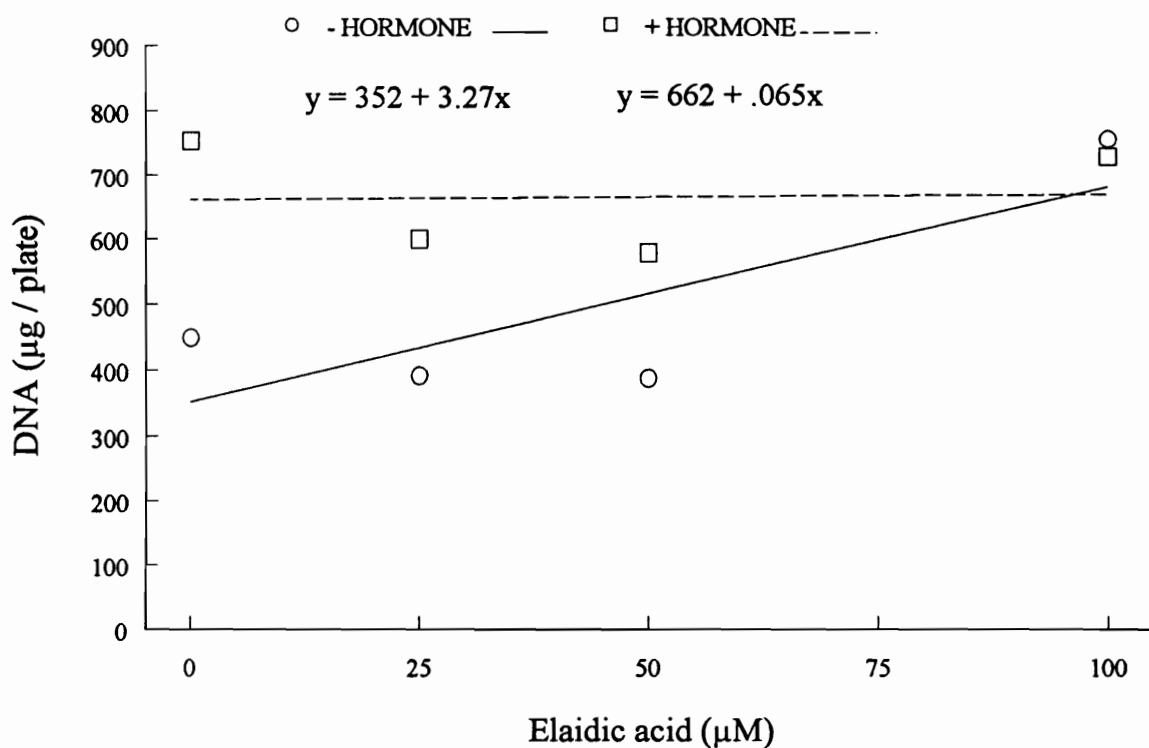


Fig. 1. Influence of elaidic acid in medium with or without lactogenic hormone supplementation on cellular DNA content. The relationship for the - Hormone treatment is significant ($P < .05$). The relationship for the + Hormone treatment is not significant ($P \geq .05$).

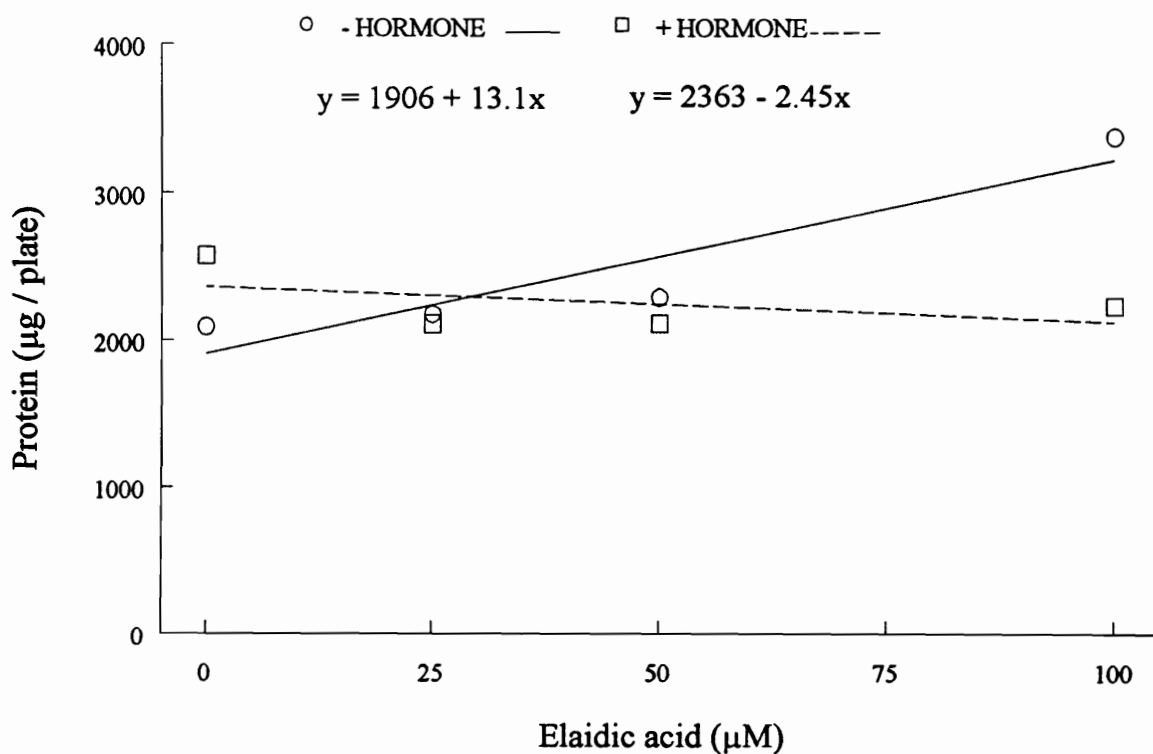


Fig. 2. Influence of elaidic acid in medium with or without lactogenic hormone supplementation on cellular protein content. The relationship for the - Hormone treatment is significant ($P < .05$). The relationship for the + Hormone treatment is not significant ($P \geq .05$).

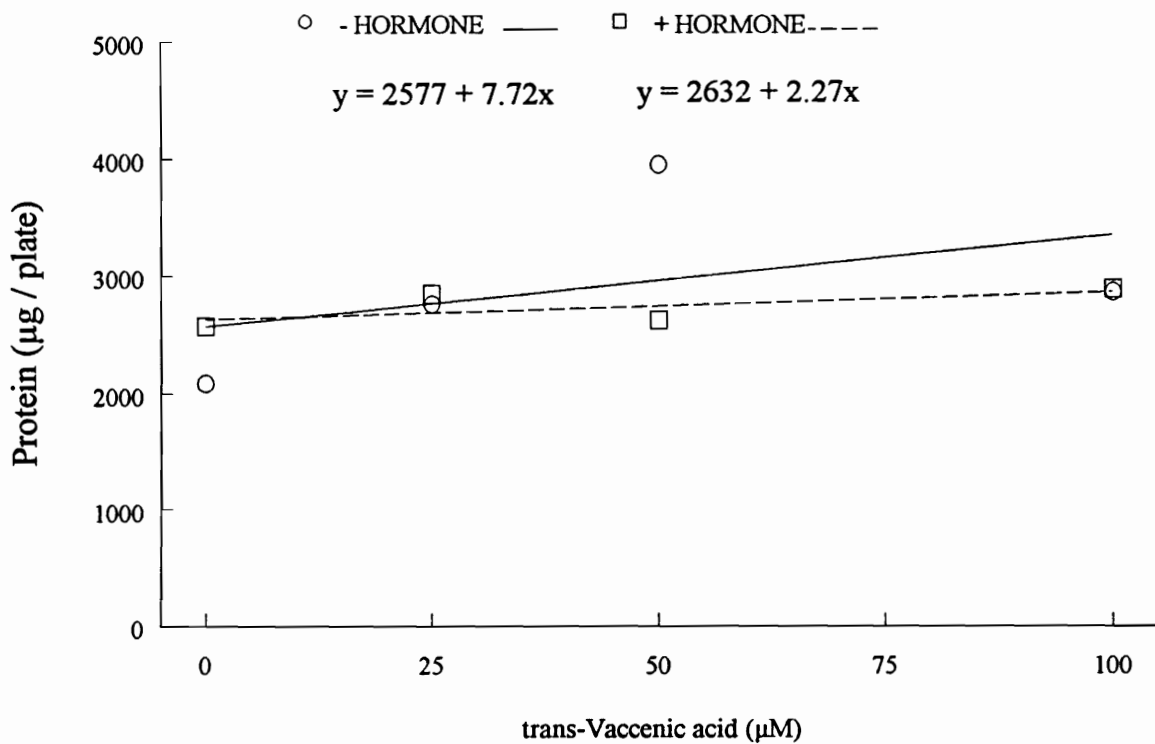


Fig. 3. Influence of *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on cellular protein content. The relationship for the - Hormone treatment is significant ($P < .05$). The relationship for the + Hormone treatment is not significant ($P \geq .05$).

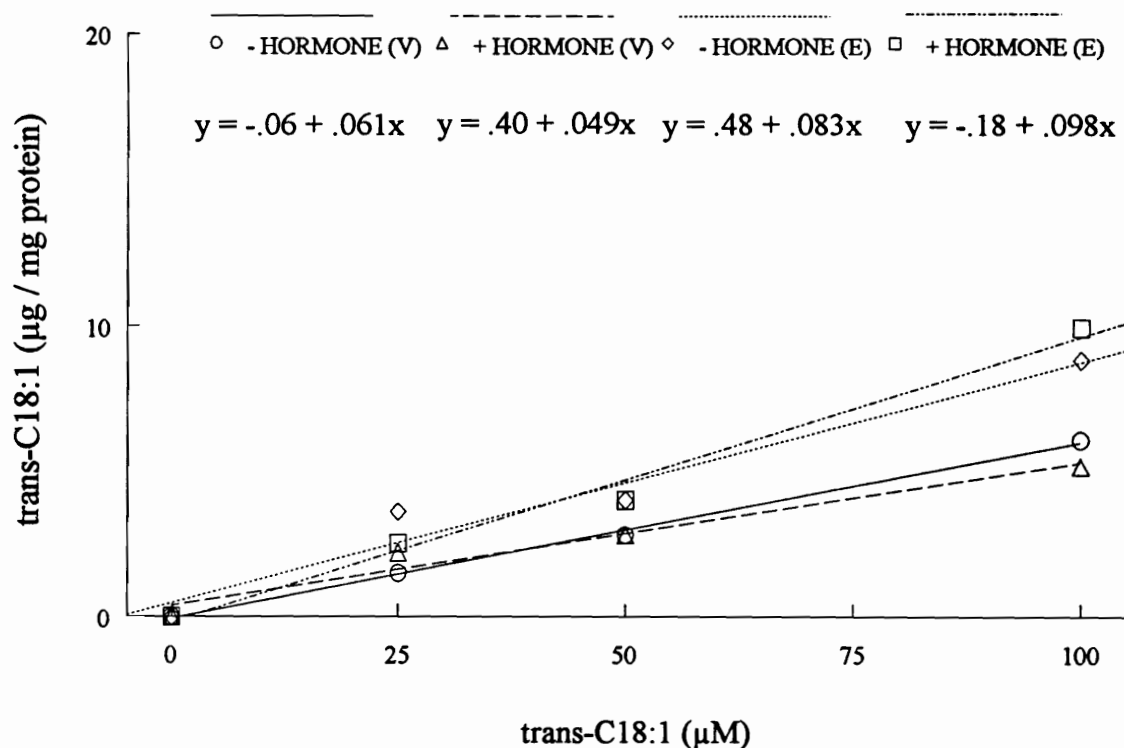


Fig. 4. Influence of elaidic or *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on cellular *trans*-C18:1 content. The relationships for the - Hormone and + Hormone treatments are significant ($P < .05$). The lines for the - Hormone and + Hormone treatments do not differ from each other for elaidic or *trans*-vaccenic acid supplementation ($P \geq .05$).

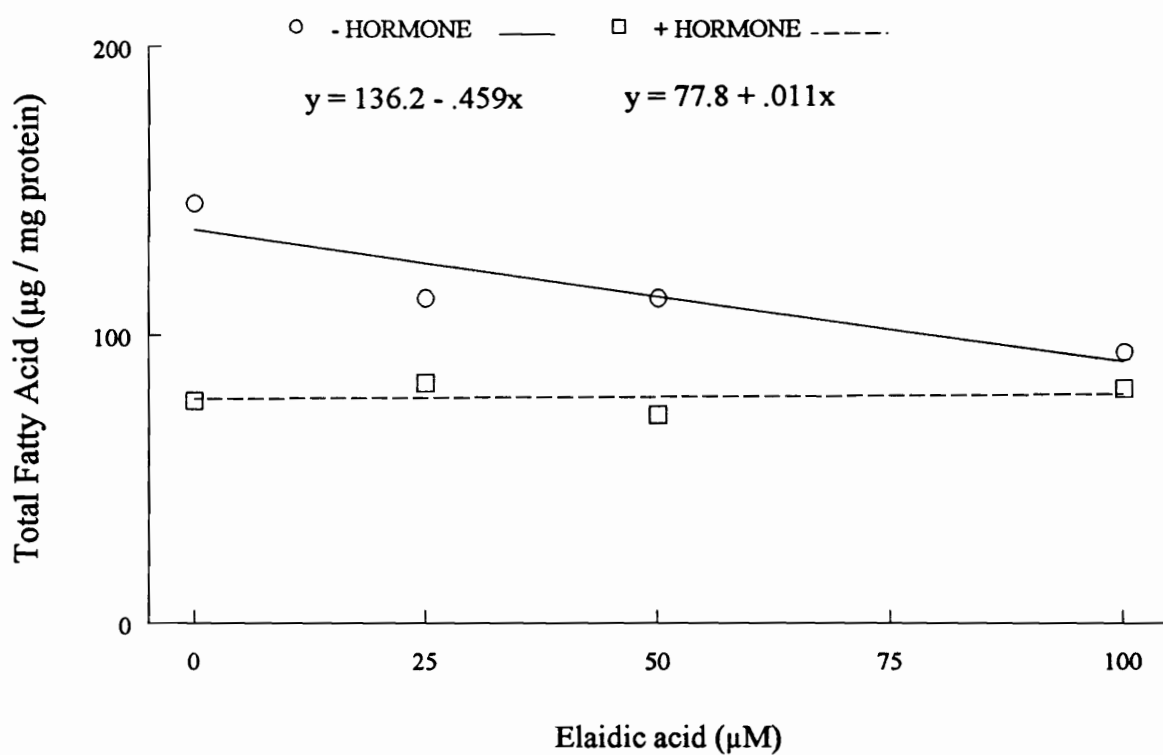


Fig. 5. Influence of elaidic acid in medium with or without lactogenic hormone supplementation on total cellular fatty acid content. The relationship for the - Hormone treatment is significant ($P < .05$). The relationship for the + Hormone is not significant ($P \geq .05$).

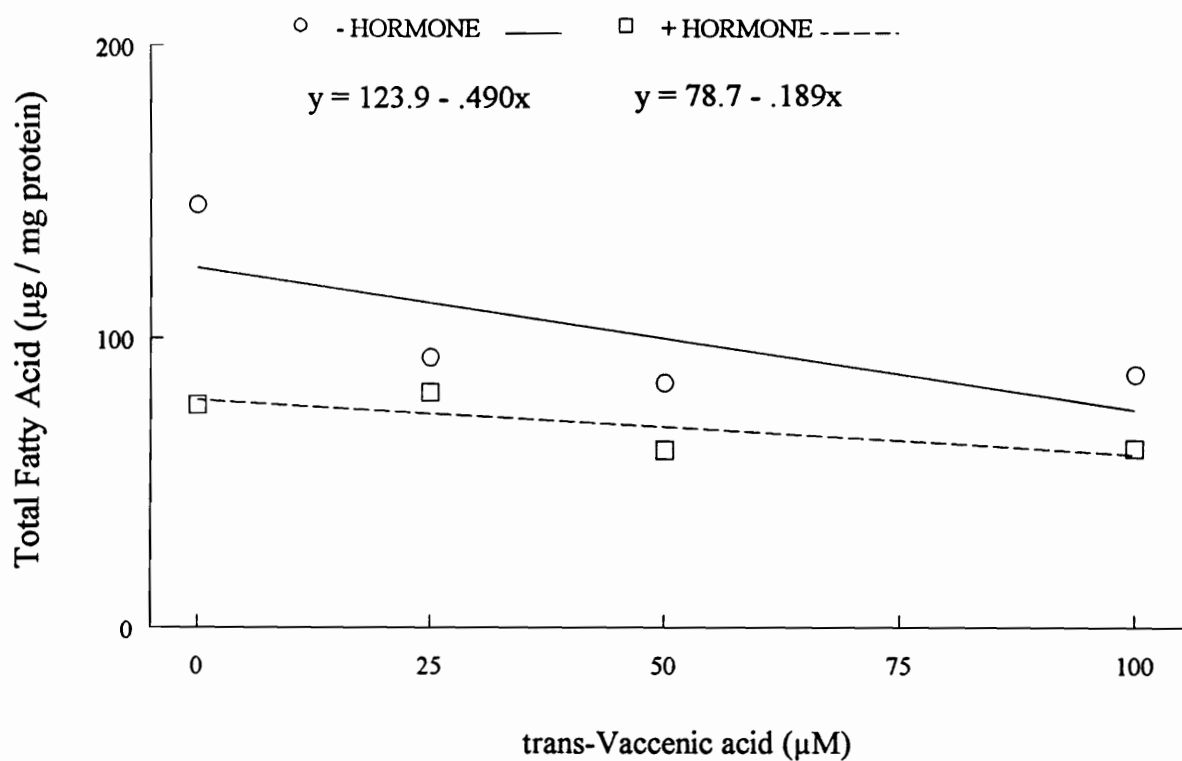


Fig. 6. Influence of *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on total cellular fatty acid content. The relationships for the - Hormone and + Hormone treatments are significant ($P < .05$). The lines do not differ from each other ($P \geq .05$).

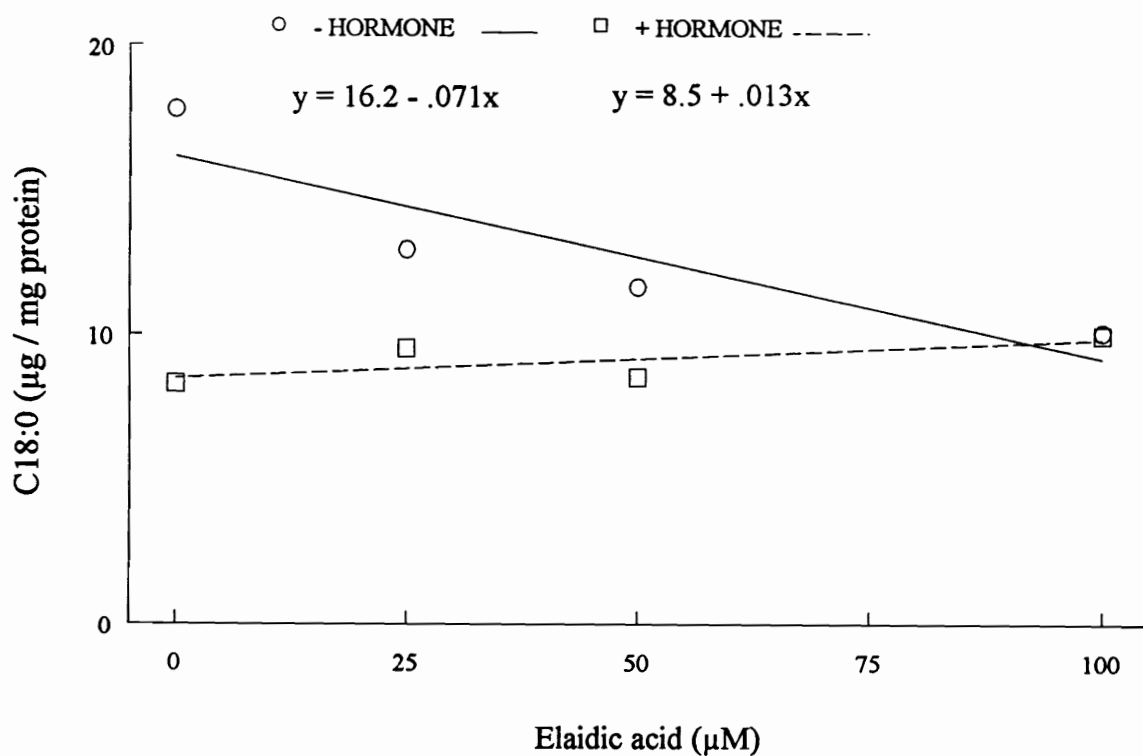


Fig. 7. Influence of elaidic acid in medium with or without lactogenic hormone supplementation on cellular C18:0 content. The relationship for the - Hormone treatment is significant ($P < .05$). The relationship for the + Hormone is not significant ($P \geq .05$).

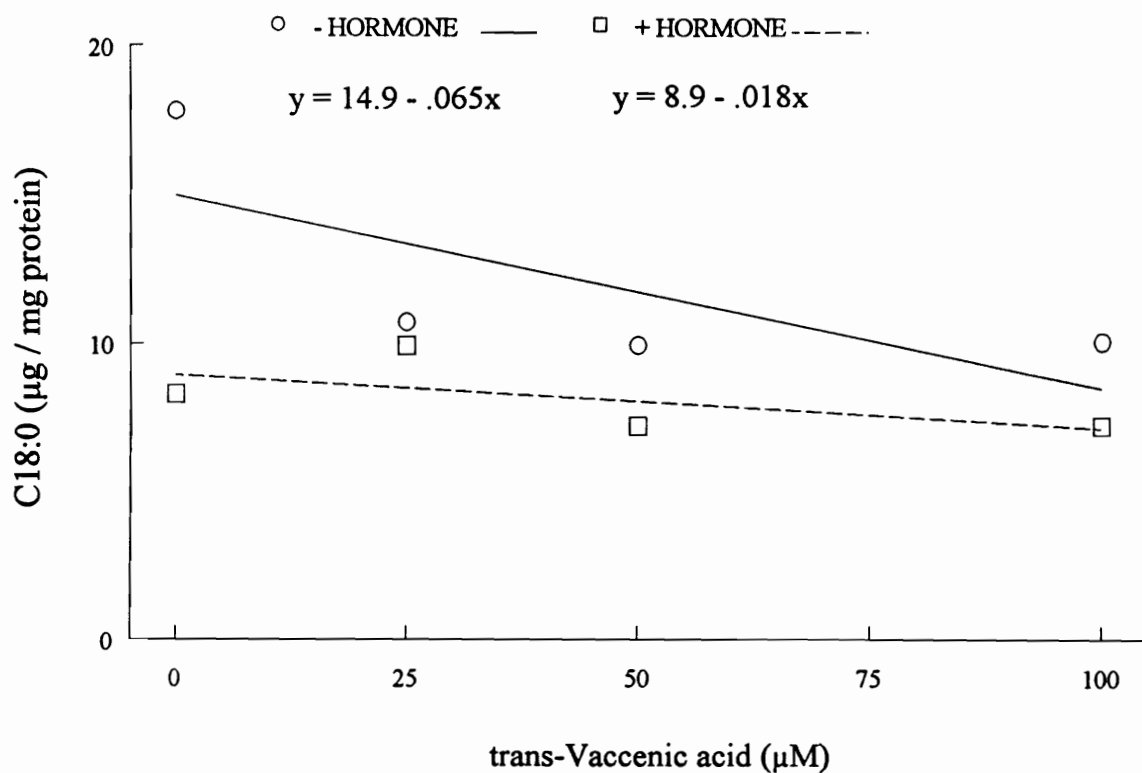


Fig. 8. Influence of *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on cellular C18:0 content. The relationships for the - Hormone and + Hormone treatments are significant ($P < .05$). The lines do not differ from each other ($P \geq .05$).

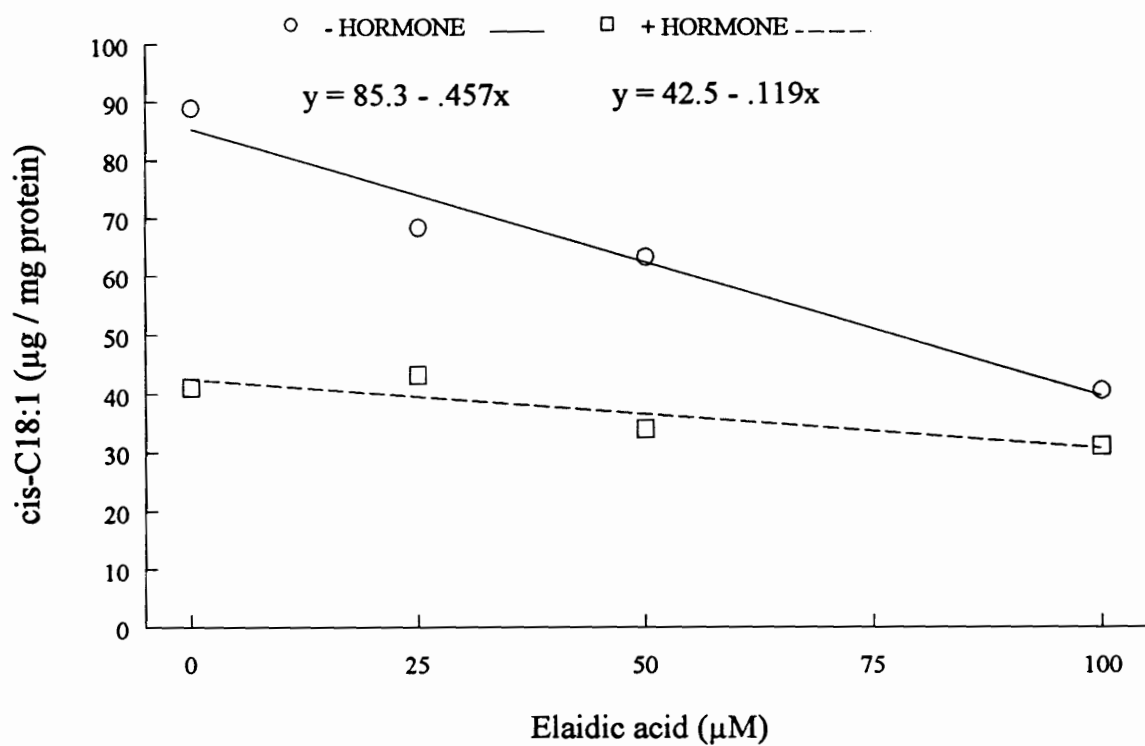


Fig. 9. Influence of elaidic acid in medium with or without lactogenic hormone supplementation on cellular *cis*-C18:1 content. The relationship for the - Hormone treatment is significant ($P < .05$). The relationship for the + Hormone is not significant ($P \geq .05$).

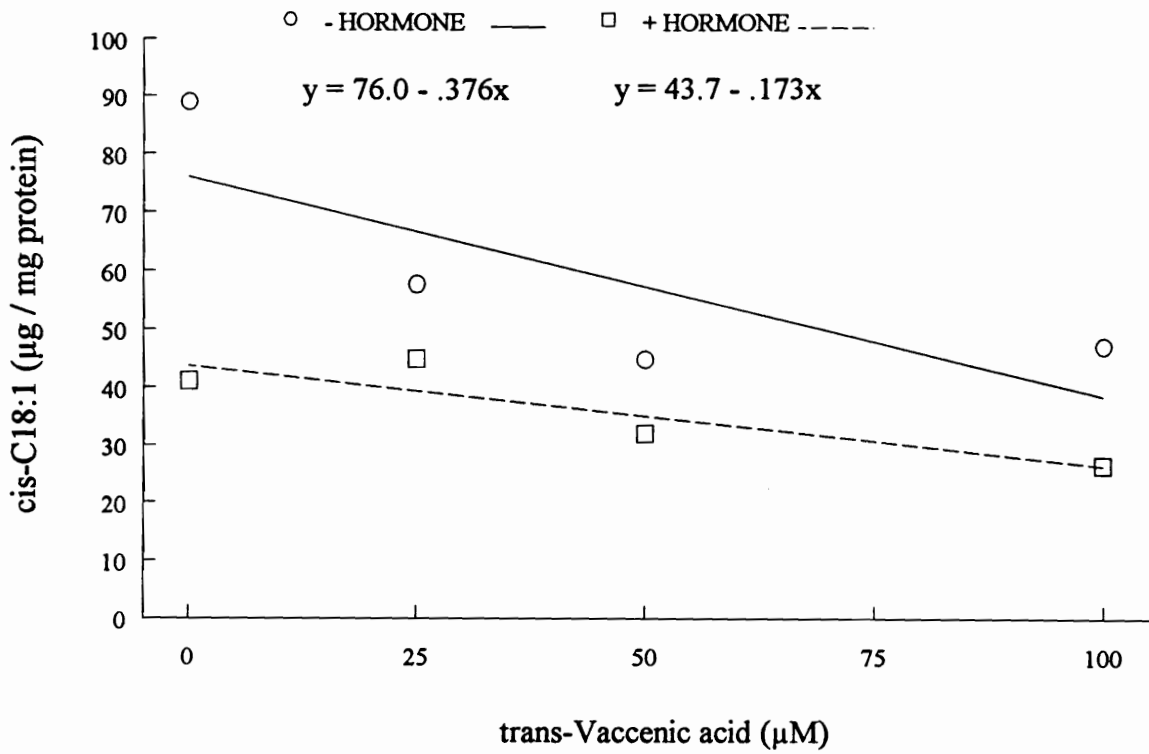


Fig. 10. Influence of *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on cellular *cis*-C18:1 content. The relationships for the - Hormone and + Hormone treatments are significant ($P < .05$). The lines do not differ from each other ($P \geq .05$).

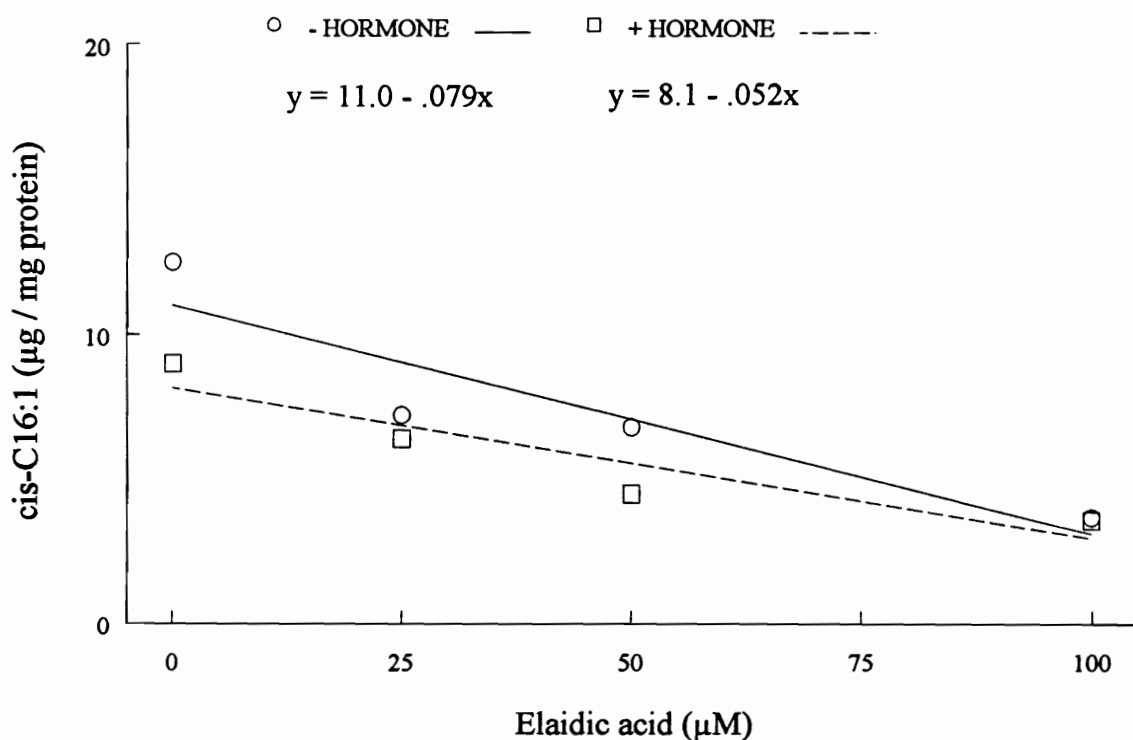


Fig. 11. Influence of elaidic acid in medium with or without lactogenic hormone supplementation on cellular *cis*-C16:1 content. The relationship for the - Hormone treatment is significant ($P < .05$). The relationship for the + Hormone is not significant ($P \geq .05$).

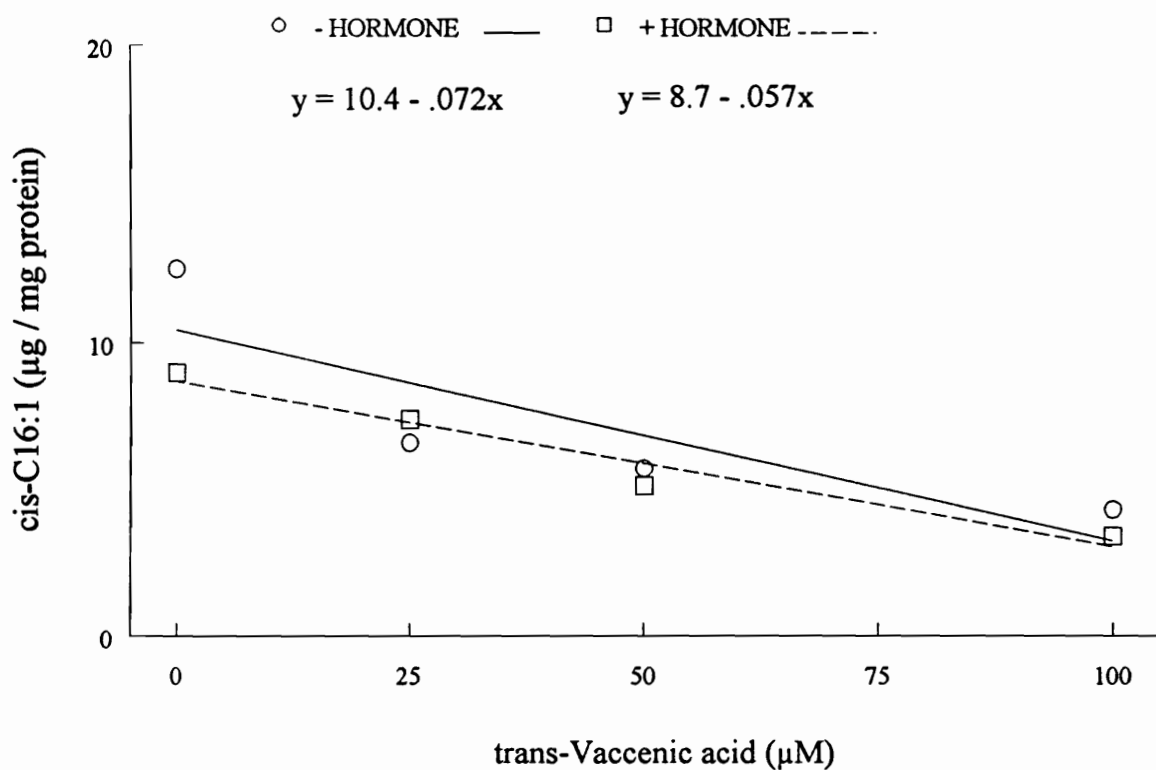


Fig. 12. Influence of *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on cellular *cis*-C16:1 content. The relationships for the - Hormone and + Hormone treatments are significant ($P < .05$). The lines do not differ from each other ($P \geq .05$).

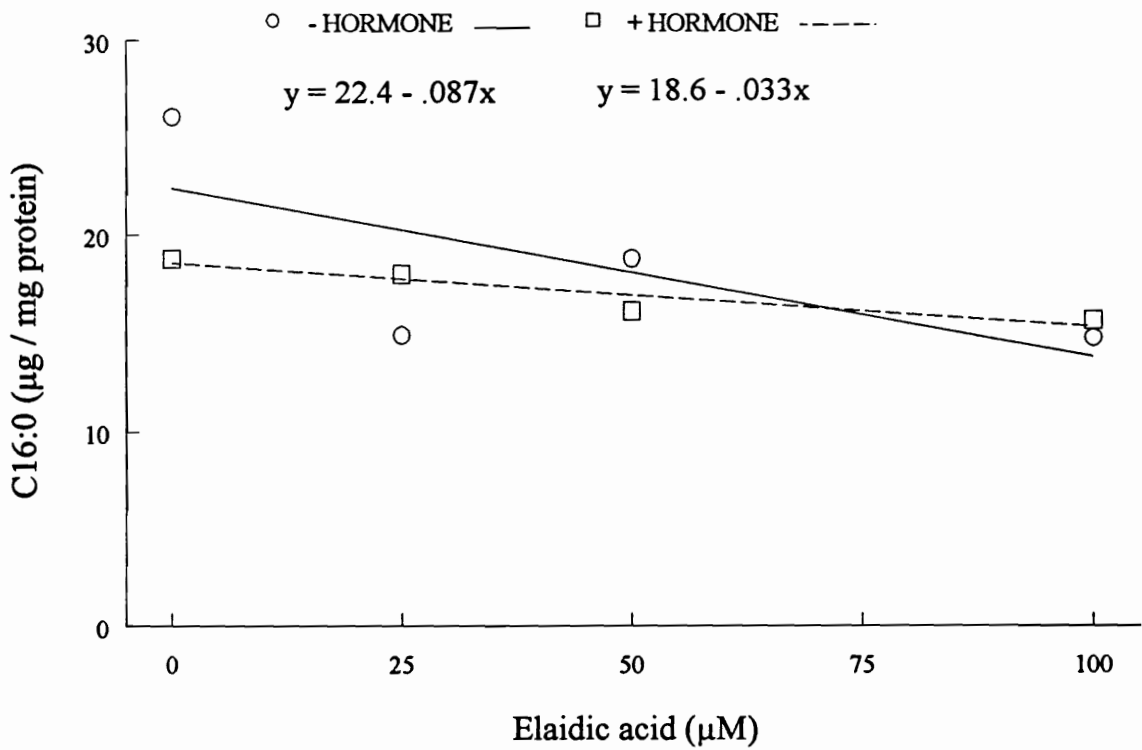


Fig. 13. Influence of elaidic acid in medium with or without lactogenic hormone supplementation on cellular C16:0 content. The relationships for the - Hormone and + Hormone treatments are significant ($P < .05$). The lines do not differ from each other ($P \geq .05$).

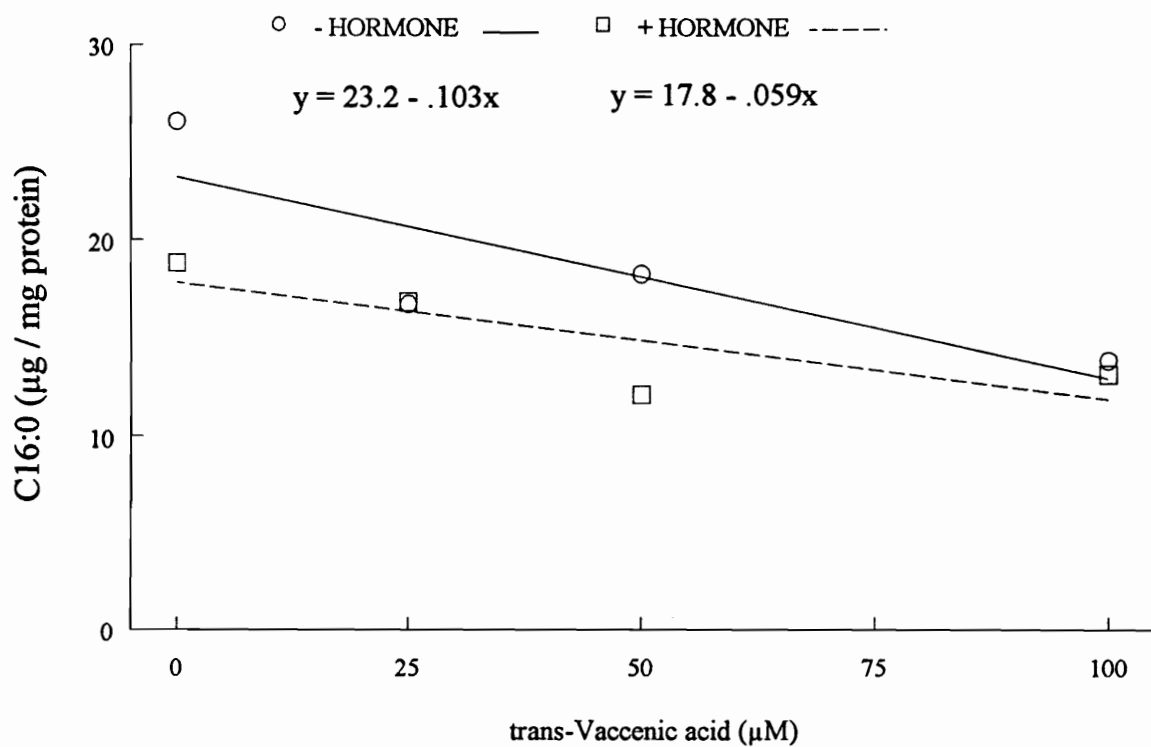


Fig. 14. Influence of *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on cellular C16:0 content. The relationships for the - Hormone and + Hormone treatments are significant ($P < .05$). The lines do not differ from each other ($P \geq .05$).

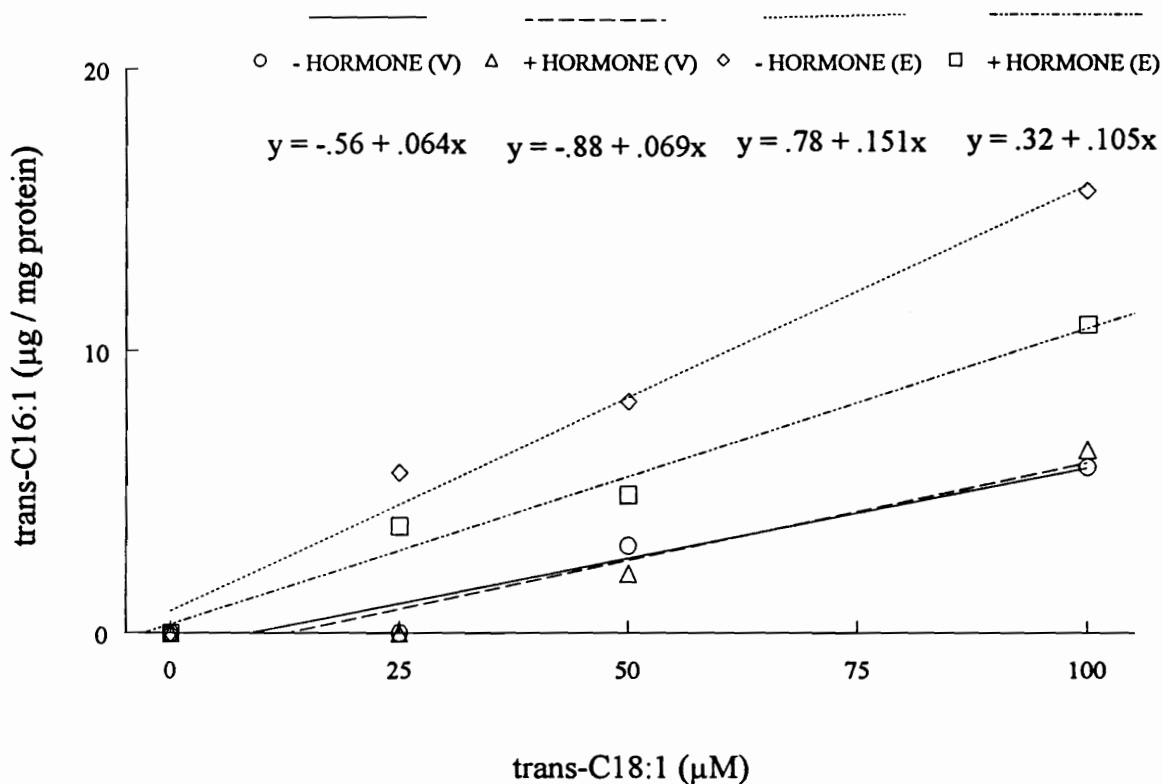


Fig. 15. Influence of elaidic or *trans*-vaccenic acid in medium with or without lactogenic hormone supplementation on cellular *trans*-16:1 content. The relationships for all treatments are significant ($P < .05$). The lines for the - Hormone and + Hormone treatments for elaidic acid supplementation are different from each other ($P < .05$). The lines for the - Hormone and + Hormone treatments for *trans*-vaccenic acid supplementation are not different from each other ($P \geq .05$).

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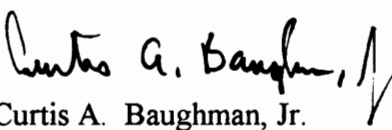
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