

AN ABSTRACT OF THE THESIS OF

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Title: The Cholesterol Content of Muscle and
Adipose Tissue From Country Natural Beef

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The cholesterol content (mg/100 g wet tissue) of the longissimus dorsi muscle and the subcutaneous adipose tissue of "Country Natural Beef" and regularly produced beef was determined by a spectrophotometric method. Proximate analysis (moisture, fat and protein contents) of both types of beef was also determined.

Country Natural Beef (natural beef) is produced without the use of hormones or antibiotic feed additives and with a feedlot-finishing period of 50-85 days versus 120-150 days for the regularly produced beef.

Samples of natural beef were taken from the 12th rib of the right side of each carcass (N = 20) at 48 hr post mortem. They were vacuum packaged, frozen and stored at -20°C until analyzed. An equal number of regular beef

(control) samples were obtained from a local food market.

The proximate analysis results show that the mean moisture and protein contents of the natural beef muscle (74.15% and 22.31%, respectively) were significantly ($P < 0.001$ and $P < 0.01$, respectively) higher than those of the control (71.56% and 21.02%, respectively). Conversely, the mean muscle fat content of the natural beef (2.92%) was significantly ($P < 0.01$) lower than that of the control (6.19%). For the adipose tissue, both moisture and fat contents of the natural beef (11.21% and 83.40%, respectively) were lower, but not significantly ($P > 0.05$), than those of the control beef (12.57% and 84.76%, respectively).

Data of this study show that the difference between mean muscle cholesterol content of the natural beef (56.91 mg/100 g) and the control beef (56.49 mg/100 g) was not statistically significant ($P > 0.05$). However, the cholesterol content of the adipose tissue of the natural beef (106.75 mg/100 g) was significantly ($P < 0.01$) lower than that of the control beef (113.08 mg/100 g). The adipose tissue was found to contain nearly twice as much cholesterol as the muscle tissue (overall mean values of 109.9 and 56.7 mg/100 g, respectively).

Even though the natural beef had an average intramuscular fat content of 3% versus 6% for the control beef, the mean cholesterol content of the natural beef

muscle was almost identical to that for the control.

Although a reduction of feedlot-finishing days reduced the intramuscular fat deposition in the natural beef, it did not influence muscle cholesterol content.

The Cholesterol Content of Muscle and Adipose
Tissue From Country Natural Beef

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THE CHOLESTEROL CONTENT OF MUSCLE AND ADIPOSE TISSUE
FROM COUNTRY NATURAL BEEF

INTRODUCTION

The cholesterol content of foods has become a major concern within the food industry because of an apparent relationship between elevated levels of serum cholesterol and heart disease (Brown, 1968). It is likely that a lower dietary intake of cholesterol may reduce the risk of heart disease, especially for those individuals who are highly susceptible to chronic atherosclerosis (LRCP, 1984).

Cholesterol is present primarily in foods of animal origin. The main sources of cholesterol in the American diet are meat, egg, poultry, fish and dairy products (Sweeney and Weihrauch, 1976). Beef is a major constituent in the American diet; therefore, increasing attention has been directed toward the investigation of the cholesterol content in beef.

Despite the interest in cholesterol, only a limited amount of information is available on the cholesterol content of beef. Although Stromer et al. (1966), Tu et al. (1967) and Terrell et al. (1969) investigated the

cholesterol content in lean beef, the results varied considerably. Their data ranged from 36 to 78 mg/100 g of wet tissue. More recent studies were conducted to determine the cholesterol content in both beef muscle and adipose tissue. A highly significant difference between the cholesterol content in muscle (58.2 mg/100 g) and adipose tissue (101.7 mg/100 g) was found by Eichhorn et al. (1986a). Slightly higher, but similar, results were published earlier by Rhee et al. (1982b) who reported a cholesterol content for muscle and adipose tissue of 62.4 and 114.3 mg/100 g wet tissue, respectively.

Reduction of saturated fat and cholesterol in the diet has been advocated as a possible means of reducing serum cholesterol (Reiser, 1973; Creasey, 1985). Based on this idea and an opportunity to improve their economic conditions by fulfilling an increasing consumer demand for leaner meat, a group of about 30 Oregon ranchers joined together in March of 1986 to form an association to promote the marketing of their beef raised naturally on Oregon grasslands, without hormones or antibiotic feed additives. This beef is now sold under the label of "Country Natural Beef".

Since a positive relationship exists between the length of time cattle are maintained in the feedlot and the percentage of total body fat (Hyer et al., 1986), it is obvious that a reduction in feedlot time will decrease fat

deposition. Thus Country Natural Beef should have less total fat since it is produced in approximately one-half the fattening feedlot time (50-85 days) than that of typical USDA choice or regular beef (120-150 days). Whether less time in the feedlot will also reduce the cholesterol content of muscle and adipose tissue of beef is not known.

The purpose of this study was to determine the cholesterol content of muscle and adipose tissue of Country Natural Beef in relation to that contained in the regular beef, i.e., beef from animals fed 90-150 days in the feedlot period to slaughter. Proximate analysis of both types of beef was also determined.

LITERATURE REVIEW

BIOLOGICAL FUNCTIONS OF CHOLESTEROL

Cholesterol (cholest-5-en-3 beta-ol) is the major sterol present in mammalian cells. Cholesterol is a universal and essential constituent of animal cell membranes and it can be produced by practically all mammalian cells (Smith et al., 1983). The planar nature of the cholesterol molecule (Figure 1) presumably allows it to fit somewhat snugly within any of the animal membranes which contain phospholipids and proteins. A 1:1 ratio of cholesterol to phospholipids is usually favored in natural membranes although relative low ratios of cholesterol to phospholipids are found in plasma membranes and subcellular membranes of nuclei (Ashworth et al., 1966; Lee et al., 1973).

The presence of cholesterol in the membrane alters membrane structure and function. Cholesterol serves as an essential stabilizing constituent and regulates membrane permeability, probably by affecting the internal viscosity and molecular motion of lipids in the membrane. For instance, cholesterol in biological membranes decreases the permeability of phospholipid vesicles to chloride ions, glucose and monovalent cations (Demel, 1968; DeGier, 1968, 1969) and increases the electrical capacitance and

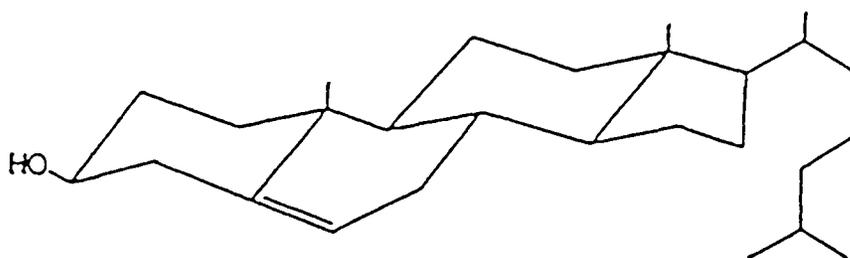


Figure 1. The planar nature of the cholesterol molecule. (From Sabine, 1977)

resistance (Ohki, 1969). It also has been shown with black lipid films that the presence of cholesterol decreases the water permeability (Finkelstein, 1967). In addition, cholesterol can inhibit the activity of reconstituted preparations of (Na^+ - and K^+ -) ATPase (Papahadjopoulos et al., 1973).

The effects of cholesterol on membrane fluidity seem to have profound effects on the aggregation, lateral diffusion and coupling of components such as the signal transduction systems confined to plasma membranes (Gibbons et al., 1982). Also, cholesterol may keep the hydrocarbon chains of phospholipid bilayers in a state of intermediate fluidity over a wide temperature range for some animals. This permits cell membranes to function optimally in the face of sudden changes in environmental temperature. A proposal from studies on artificial membranes suggests that cholesterol may diminish the effect of changes in the pH and cation concentrations of the aqueous medium on the physical state of phospholipid bilayers. This effect may help to maintain intracellular membranes in an optimal state of fluidity (Myant, 1981).

It is well known that cholesterol is important as the precursor of bile acids, steroid hormones and vitamin D. Most of the cholesterol is transformed by the liver into various bile acids which function as a "detergent" to enhance the digestion of lipid-soluble compounds and to

regulate the cholesterol synthesis in the liver. Steroid hormones and vitamin D synthesized from cholesterol are very important for the regulation of a variety of fundamental metabolic processes. Furthermore, esterified cholesterol serves as a source of cholesterol for steroid hormone synthesis and it may also store surplus cholesterol in a form which can not interact with membranes (Myant, 1981).

CHOLESTEROL ABSORPTION, SYNTHESIS AND METABOLISM

Cholesterol Absorption

In humans, the maximum absorption of cholesterol occurs in the upper intestine. It enters the intestinal tract from two major sources --- the diet and bile. Intake of cholesterol in the American diet, which is derived entirely from animal foods (meat, eggs, milk and cheese) averages from 500 to 750 mg per day while biliary outputs of cholesterol range from 750 to 1250 mg per day (Bennion et al., 1975; Grundy, 1972).

For the absorption of cholesterol to occur from the diet, it first must be mixed with bile salt and the hydrolytic products of phospholipids and triacylglycerols (triglycerides) in the duodenum to form micelles. Free cholesterol is dissolved in the hydrophobic center of the

micelle, and is thus transported to the intestine where it is released to the intestinal cells. At this point, cholesterol is absorbed and esterified in the mucosal cells. The cholesterol esters are transported by the lymph system. The mechanisms for cholesterol absorption are shown in Figure 2. An excellent summary on the absorption sequence of cholesterol is given by Sabine (1977).

Bile acids exert a detergent effect in the micelle. They play a crucial role in the absorption of the cholesterol. However, due to the strong hydrophobic property of cholesterol, its absorption is incomplete even when bile acids are present. Usually, only 30 to 60 percent of the intestinal cholesterol enters body pools (Grundy et al., 1971, 1977).

It is notable that plant sterols can affect cholesterol absorption by three possible mechanisms: (1) displacing cholesterol from mixed micelles, (2) competing for its uptake by the mucosal cell membrane, and (3) inhibiting cholesterol esterification in the mucosa. The amounts of endogenous and dietary cholesterol absorbed from the intestine are increased by the feeding of fat (Treadwell et al., 1968).

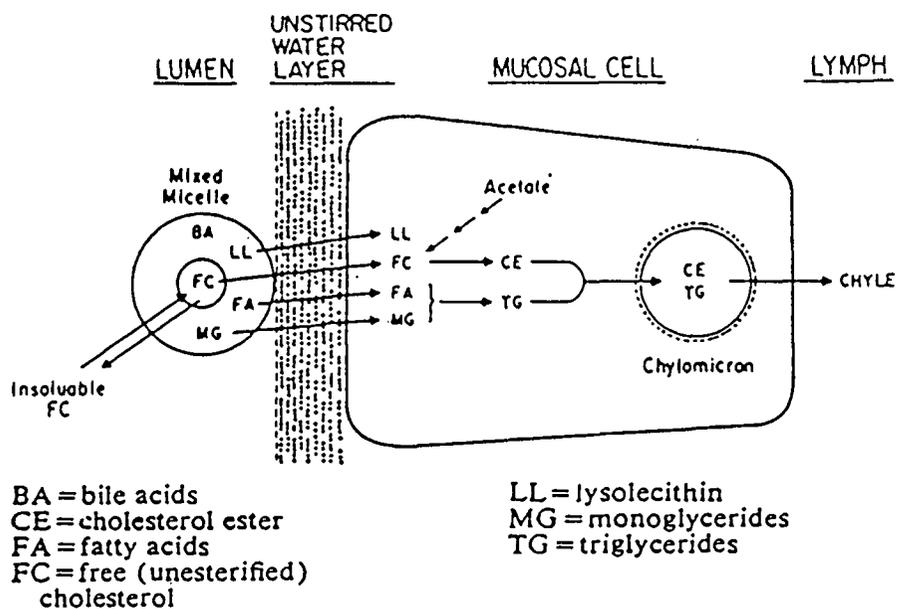


Figure 2. Mechanisms of cholesterol absorption. (From Grundy, 1978)

Cholesterol Synthesis

Most mammalian tissues can synthesize cholesterol endogenously. In the tissues, cholesterol is synthesized from acetyl-CoA in cytosol. Acetyl-CoA is derived from the intramitochondrial oxidation of pyruvate and fatty acids. The initial steps in cholesterol synthesis usually involve the sequential formation of acetyl-CoA, 3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) and mevalonic acid. It is generally accepted that the conversion of HMG-CoA to mevalonic acid, catalyzed by HMG-CoA reductase, is the step that determines the rate of cholesterol synthesis (Smith et al., 1983).

The liver and the gastrointestinal tract appear to be the two major sources of the endogenously synthesized cholesterol. In the squirrel monkey, for example, cholesterol synthesis in these two organs accounts for about 97 percent of the total cholesterol synthesized by all organs and tissues (Gibbons et al., 1982). It has been suggested that at least one-half of the whole body synthesis occurs in the human liver (Dielschly et al., 1971). The synthesis of cholesterol in the liver is regulated by its own cholesterol concentration, known as a feedback inhibition of HMG-CoA reductase. The activity of this enzyme apparently depends largely on amounts of cholesterol absorbed by the intestine and transported to the liver. Furthermore, hepatic cholesterologenesis may be

also regulated by bile acids and food intake (Grundy, 1978).

Cholesterol Metabolism

The main products of cholesterol metabolism are bile acids, steroid hormones and vitamin D. Approximately 80 percent of cholesterol is transformed by liver tissue into various bile acids. Quantitatively, cholic acid and chenodeoxycholic acid are the main products derived from cholesterol (Smith et al., 1983). The biological functions of bile acids are: (1) contribute to the homeostatic regulation of the amount of cholesterol in the whole body, (2) play a role in cholesterol and lipid-soluble compounds digestion by formation of micelles, and (3) regulate cholesterol synthesis in the intestinal mucosa and possibly in the liver (Gibbons et al., 1982).

Steroid hormones represent only a minor pathway in the catabolism and elimination of cholesterol from the body; however, they are important for the regulation of a variety of fundamental metabolic processes. For instance, they are important in cell differentiation, in the development of reproductive tissues, and in enabling the animal to adapt to changing environmental conditions. Vitamin D is essential for the control of calcium and phosphate metabolism (Sabine, 1977).

In tissue metabolism, it is currently recognized that low density lipoprotein (LDL) is the carrier for transporting cholesterol into the tissue where the lipoprotein is degraded and free cholesterol is released. Smith et al. (1983) described these activities in detail.

CHOLESTEROL AND HEALTH

Although cholesterol has an important role in the human body because of its biological functions, several adverse effects of cholesterol have also been reported (Sabine, 1977; Naber et al., 1980; Ansari et al., 1982). Many studies appear to show that the cholesterol level in the body may serve as an indicator of the health status of humans. Since there is a statistical positive relationship between high serum cholesterol levels and heart disease (Brown, 1968; Sabine, 1977), many researchers have proposed that a high intake of dietary cholesterol is one of the risk factors of heart disease because diet can affect serum cholesterol (Zanni et al., 1987) and heart attack risk increases with elevated serum cholesterol levels (Olendzki et al., 1981; Gasner and McCleary, 1982; Creasey, 1985). According to Creasey (1985), the American Heart Association and American Health Foundation have recommended diet modification to reduce consumption of dietary fat and cholesterol although the cause and effect relationship has

not been clearly established.

Most recently, a report by Findlay (1987) confirmed that people with lower cholesterol levels live longer and with a reduced risk of heart disease. However, there is a strong indication that cholesterol levels decline in people who have cancer and other illnesses, except heart disease.

Cholesterol oxides have received much attention in recent years in view of biological actions associated with the ethiology of certain human diseases (Smith, 1981). Cholesterol is easily oxidized when exposed to oxygen and heat and/or catalyzed by cholesterol oxidases (Sabine, 1977). Those oxidized products are considered potentially harmful (Imai et al., 1980). They have been directly correlated with atherogenesis (Baranawski et al., 1982) and indirectly with cytotoxic, mutagenic and carcinogenesis (Chen et al., 1974; Chan et al., 1980; Ansari et al., 1982; Chan et al., 1974). Vajdi (1979) reported that the main cholesterol oxide found in beef when heated is cholesta-3,5-dien-7-one.

CHOLESTEROL CONTENT IN BEEF

Distribution

Cholesterol is always the major sterol present in animal tissues. More than 95% of the total sterol of

adipose tissue is cholesterol (Myant, 1980). Most of the cholesterol in the whole body is present in free form. Cholesterol may be also present in esterified forms which are conjugated with fatty acids, glycosides, sulphate esters and partly bound to proteins mainly in the adrenal cortex, brain, plasma, kidney and skin. Nervous tissues and the brain, together, contain the largest proportion of the total body cholesterol of any single tissue. However, significant levels are found in skeletal muscle, skin and liver by virtue of their relatively large total mass (Gibbons et al., 1982).

In addition to cholesterol, the sterol fraction of the lipid of mammalian tissues contains varying amount of sterols and methyl sterols related metabolically to cholesterol, e.g. 7-dehydrocholesterol, cholestanol and lathosterol, etc. (Demel et al., 1976). Those noncholesterol sterols are either intermediates in the terminal stages of the biosynthesis of cholesterol or the products of its enzymic or non-enzymic modification (Gibbons et al., 1982).

Cholesterol content varies with different tissues and parts of the body as discussed in the following two sections.

In Muscle Tissue

Values reported in the earlier literature for the cholesterol content (wet wt basis) of beef longissimus dorsi (LD) muscle were quite varied: 36-46 mg/100 g (Stromer et al., 1966), 46-57 mg/100 g (Hood and Allen, 1971), 53-60 mg/100 g (Tu et al., 1967), and 78 mg/100 g of wet tissue (Terrell et al., 1969). Rhee et al. (1982a) investigated the relationships of marbling level (intramuscular fat) and the cholesterol content of raw beef LD muscles. Eight marbling levels ranging from "Practically Devoid" to "Moderately Abundant" which were equivalent to intramuscular lipid contents of 2.7 to 12%, respectively, were studied. They reported cholesterol values of 51.8 mg/100 g of wet tissue for samples rated "Practically Devoid" of marbling and 65.9 mg/100 g for the "Moderately Abundant" marbled samples. Only samples with "Practically Devoid" of marbling (2.7% lipid) contained significantly less cholesterol than did samples with any of the other seven marbling levels (lipid contents ranging from 3.6 to 12%). More recently, Eichhorn et al. (1986a) completed a comparative study of the cholesterol contents of the LD muscle of 34 crossbred bull and 35 steer carcasses. A mean cholesterol value of 58.3 mg/100 g of wet tissue was reported for all samples. Sex condition differences were not significant. Kunsman et al. (1981) found that

mechanically deboned meat (MDM) and lean beef had mean cholesterol contents of 153.3 and 50.9 mg/ 100 g of tissue, respectively. The higher cholesterol content in MDM was attributed to the inclusion of some spinal cord material. It has been reported that the muscles with higher lipid contents also are higher in cholesterol (Hood and Allen, 1971). This aspect is covered in the following section.

In Adipose Tissue

Adipose tissue is the major mammalian cholesterol storage organ which contains significantly higher levels of cholesterol than muscle (Myant, 1981). Most of the cholesterol in adipose tissue is present in the free or unesterified form. The proportion of the total cholesterol in adipose tissue that is present in the adipocytes increases with age and body weight (Farkas et al., 1973).

The cholesterol content of beef adipose tissue varies with anatomical location. Subcutaneous adipose tissue and perinephric adipose tissue were reported to have cholesterol levels of 101.7 and 89.7 mg/ 100 g of tissue (wet basis) respectively (Eichhorn et al., 1986a). Rhee et al. (1982b) found that the intramuscular (marbling) fat contained, on the average, 70.9% lipid and 104.6 mg of cholesterol/100 g of wet tissue. Similar results were obtained for intermuscular (seam) fat (71.3% lipid and

105.2 mg of cholesterol/100 g of wet tissue) while higher values (82.1% lipid and 112.3 mg of cholesterol/100 g of wet tissue) were found for the subcutaneous fat. They concluded that the differences in total lipid and cholesterol content between intramuscular fat and intermuscular fat were non-significant while differences between the subcutaneous and intramuscular fat fractions were statistically significant ($P < 0.05$).

The Effects of Feeding, Age and Sex

There are many unanswered questions regarding the cholesterol content of beef as influenced by dietary factors, age and sex of the animal. It is generally agreed that different feeding conditions can affect the cholesterol content in some of the animal tissues. Kunsman et al. (1981) found a significant difference in the cholesterol content of beef marrow between grain-fed (150.6 mg/100 g of wet tissue) and grass-fed (119.6 mg/100 g of wet tissue) animals. Eichhorn et al. (1986b) found that feeding groups effects were more pronounced in adipose tissue for cholesterol content than in muscle tissue. Restricted feeding produced higher ($P < 0.01$) values for cholesterol content of both perinephric and subcutaneous adipose tissue, compared with ad libitum feeding. They reported that the cholesterol content of muscle tissue was

not affected significantly by breed type or by level of nutrition. Moreover, they concluded that altering the cholesterol content of bovine muscle tissue appears to be a more difficult task than producing changes in adipose tissue. This statement is supported by the results of an earlier study (Weyant et al., 1976) which showed that the level of polyunsaturated fatty acids in beef muscles could be increased through dietary means without altering the cholesterol content of muscle tissues.

Most of the available evidence suggests a positive relationship between cholesterol and the lipid content of muscle. Both Hood et al. (1971) and Eichhorn et al. (1985a) reported that muscles higher in fat were also higher in cholesterol. Since there is a strong relationship between the number of feedlot days and body fat(%) of beef (Hyer et al., 1986), the total cholesterol content tends to increase as the body fat increases. However, Tu et al. (1967) found that the cholesterol content of beef muscle increased only slightly with increasing fat levels.

There is a lack of agreement concerning the relationship between the age of the animal and the muscle cholesterol content (Sweeney and Weihrauch, 1976). Del Vecchio et al. (1955) reported that younger animals were higher in muscle cholesterol content than older animals. However, Stromer et al. (1966) and Hood and Allen (1971) found the cholesterol content of beef longissimus dorsi

muscle did not change with maturity. The latter findings are supported by the data of Eichhorn et al. (1986a, b) who reported that the cholesterol content of two different muscles of 7 - 10 year old mature cows were similar to corresponding values for young bulls and steers less than two years of age.

The relationship between the sex of the animal and the muscle cholesterol content is confusing. Terrell et al. (1969) reported that the muscles of steers were higher in cholesterol than were the same muscles of heifers. Conversely, Hood and Allen (1971) found that the longissimus dorsi muscle of heifers contained higher ($P < 0.01$) levels of cholesterol compared with both bulls and steers. In the Hood and Allen study, differences in the cholesterol values of the longissimus dorsi muscle of bulls and steers were not statistically significant. Eichhorn et al. (1986a, b) also reported nonsignificant differences in the longissimus dorsi muscle cholesterol contents between bulls and steers. However, they found the cholesterol content of longissimus dorsi muscle of mature cows (54.9 mg/100 g) to be slightly lower than that for bull and steers (58.3 mg/100 g).

DETERMINATION METHODS

Cholesterol is usually classified as a neutral lipid

due to its chemical and physical properties. It is strongly hydrophobic, almost insoluble in water (0.2 mg/100 ml water) but soluble in non-polar organic solvents (e.g., hexane, ether, chloroform, etc.) and fat or oil (Stecher et al., 1968). It also can associate with fatty acids, glycosides, sulphate esters and proteins. These bondings tend to change cholesterol solubility and affect its extraction from tissues (Gibbons et al., 1982).

Cholesterol is easily oxidized in air under a variety of conditions (Finocchiaro and Richardson, 1983). Thus, care must be taken to prevent autoxidation and enzymatic changes in tissue lipids, primarily before extraction solvents are added to the tissue. The storage of cholesterol extracts at low temperature is also recommended.

From a food science point of view, more interest is placed on the total cholesterol content of foods rather than on either the free or ester forms. The latter would be hydrolyzed into free forms in the human stomach and any cholesterol esters that might enter the intestine would be hydrolyzed rapidly by pancreatic cholesterol esterase (Treadwell et al., 1968). Many of the procedures for determining cholesterol contents in foods were initially developed for measuring cholesterol levels in blood serum. Through considerable experimentation and adaptation, appropriate procedures for determining the cholesterol

contents of various foods have been developed (Sweeney and Weihrauch, 1976).

Extraction of Cholesterol

It is generally believed that a mixture of polar-nonpolar solvents extract cholesterol more efficiently than when either type of solvent is used alone. Although nonpolar solvents can readily extract lipids or cholesterol, not all of the cholesterol esters and triglycerides can be extracted from tissue with nonpolar solvent. This may be due to the fact that hydrophobic regions, in some cases, are surrounded by polar regions and, hence, impervious to nonpolar solvents. Therefore, to increase quantitative extraction of tissue lipids or cholesterol, a polar solvent, usually in combination with a nonpolar solvent, is necessary. The polar solvent disrupts the hydrogen bonding, allowing free access of the nonpolar solvent to cholesterol esters (Nelson, 1975). Many organic solvents have been used for the extraction of lipids or cholesterol from biological materials. Solvents used include acetone (Szent Gyorgyi, 1923), ethanol-ethyl ether (1:1) (Kelsey, 1939), acetone-ethanol-ether (4:4:1) (Kabara et al., 1961), benzene (Schwartz et al., 1967), chloroform-methanol (Bondjers et al., 1971), etc. However, chloroform-methanol is widely used for the quantitative

extraction of lipids. In meat, a portion of the cholesterol is bound to protein in lipoprotein complexes and lipid in ester forms (Punwar, 1975; Myant, 1981). Thus the cholesterol extraction procedure must remove not only cholesterol in the lipid fraction of the animal tissues but also any cholesterol that may be bound with protein as lipoprotein.

Thus far, two extraction methods have been used mostly in the cholesterol analysis of animal tissues. In the first method, Folch et al. (1957) introduced a chloroform-methanol (2:1, v/v) system for extracting lipids from animal tissue. Folch's method includes a step in which the lipid extract is washed with physiological saline solution equilibrated with the solvent. This is useful for removing water-soluble compounds which may interfere with the later determination of cholesterol. This method is relatively simple for both extraction and purification of lipids from most tissues. It is very critical to precisely follow the steps listed for Folch's method. Although the 1:20 volume ratio of sample to solvent is recommended by Folch, a sample to solvent ratio at 1:25 or 50 facilitates the complex extraction of all the lipids in the tissue in a single step. However, the smaller this ratio, the higher the extraction efficiency (Nelson, 1975). Also in the washing procedure, Folch emphasized that the proportions 8:4:3 by volume of chloroform:methanol: water are critical

and must be kept constant. The losses of lipid in white tissue, gray tissue and muscle in the washing procedure are 0.3%, 0.6% and 2%, respectively. Even though the total loss of lipid is less than 5%, this loss can be eliminated by using the proper salt solution in washing procedure. The main criticisms of Folch's method are the use of a large, inconvenient volume of solvent (Bligh et al., 1959) and the low recovery of polar lipids, especially phospholipids. However, excellent recovery was obtained with cholesterol esters (Jaeger et al., 1981). Sabine (1977) stated that little or no cholesterol should be lost if Folch's procedure is followed precisely.

The second extraction method which is also widely used was developed by Bligh and Dyer (1959). It is a variation of the Folch procedure and uses the same solvent system. However, the Bligh and Dyer technique uses much less solvent. They recommended that the volume ratio of sample to solvent in extraction be 1:5.5 and after dilution, the proportions of chloroform:methanol:water should be kept in 2:2:1.8. However, this method uses a re-extraction step to ensure complete recovery of the lipids from tissue residue because of the low solvent to tissue ratio used in this procedure.

More recently, Schmid et al. (1973) reported that benzene-methanol or toluene-ethanol mixtures were good for extracting free fatty acids and neutral lipids rather than

polar lipids (phospholipids), but whether these solvent systems are efficient as chloroform-methanol has not been proven.

A direct saponification of the meat sample for determining cholesterol without an extraction procedure was reported by Adams et al. (1986). The results showed that it produces comparable or slightly higher cholesterol results than the current AOAC method (1984) of the meat samples examined.

Separation of Cholesterol

Saponification of the crude lipid extract of meat for determining total cholesterol content is necessary to convert the cholesterol esters to the free forms by hydrolysis. After this step, the separation of the cholesterol from saponifiable substances can be done by a non-polar organic solvent. In some cases, Zak (1977) found that the extracting fluid (biphasic liquid-liquid extraction) will not completely separate both free and esterified cholesterol without saponification. Cholesterol esters may cause errors in the final colorimetric determination when using the Liebermann-Burchard (L-B) or Zatkis-Zak method (1953) since unsaturated acids and other substances (e.g., hemoglobin) may also produce color with those reagents (Rhodes, 1959; Searcy and Bergquist, 1960).

It is necessary to saponify the lipid extract by mixing with 15% to 50% NaOH solution in ethanol at 85-90 C. Reports of the saponification time range widely from 10 minutes (Rhee et al., 1982b) to 60 minutes (Kunsmann et al., 1981). In spite of those differences, complete saponification of lipid in the extract is necessary to eliminate turbidity in the colored solution so it can be read in the colorimeter. Tu et al. (1967) reported that slight turbidity of the solution can cause large errors in the observed cholesterol content. After saponification, the cholesterol--in the unsaponifiable fraction -- is extracted by a nonpolar organic solvent (e.g., hexane or benzene) to separate the cholesterol from the saponifiable fraction.

Although digitonin and tomatine have been used extensively in clinics for determining free and esterified cholesterol in blood serum (Edwards et al., 1964), most of the studies on cholesterol content in meat have employed saponification procedures to separate and purify cholesterol (Kovacs et al., 1979; Rhee et al., 1982a, b; Eichhorn et al., 1986a, b). This may be due to the fact that procedures using digitonin or tomatine are slow, cumbersome and lacking in sensitivity since any phospholipid present in the extract would hinder the washing out of excess digitonin. Digitonin can react with the color reagent to produce a color (Okey, 1930). Thus, if digitonin is used, complete washing is necessary. One

exception was a study done by Weyant (1976) who used digitonin successfully to precipitate cholesterol for determining cholesterol content of polyunsaturated meat.

Cholesterol also can be isolated by various chromatographic methods, for instance, column, thin layer and gas-liquid chromatography (GLC) (Capella et al., 1960; Goad and Goodwin, 1966; Avigan et al., 1963a; McNair, 1975). These chromatographic methods provide a good separation of free cholesterol and its esters from other compounds, based on differences in affinity or polarity (Myant, 1981). However, these methods may be more time-consuming and costly. Therefore, these methods have not been widely used for cholesterol determination in foods except for the GLC method.

Measurement of Cholesterol

Colorimetric and gas-liquid chromatography methods are most widely used for the determination of cholesterol in foods at present. Most of the colorimetric methods are based either on the Liebermann-Burchard (L-B) reaction (Myant, 1981) which is carried out in an acetic acid-sulfuric acid-acetic anhydride medium or on the Zlatkis-Zak (Z-Z) reaction (Zlatkis et al., 1953) which is carried out in acetic acid-sulfuric acid-ferric salt. Burk et al. (1974) investigated the mechanisms of the L-B and Z-Z color

reactions for cholesterol and reported that both reactions have a similar oxidative mechanism, each yielding, as oxidation products, a homologous series of conjugated cholestapolyenes. They suggested that the colored species observed in these two systems were enylic carbonium ions formed by protonation of the parent polyenes. Thus, the red product (maximum wavelength 563 nm) typically measured in the Z-Z reaction is evidently a cholestatetraenylic cation and the blue-green product (maximum wavelength 620 nm) in the L-B reaction is the pentaenylic cation.

Some workers (Krishnamoorthy et al., 1979; and Eichhorn et al., 1986a, b) have used the L-B method to determine the cholesterol content in meat. However, this method has been criticized because the final color of the reaction is unstable. It is temperature-dependent, time-dependent and light labile. The intensity of the color is also influenced by the presence of moisture in the cholesterol solvent and color reagent and by the proportion of esterified to free cholesterol in the sample since cholesterol esters yield a stronger L-B reaction than free cholesterol (Kabara, 1962; Martensson, 1962). An excellent critical review of those methods was published by Tonks (1967).

The Z-Z method has been widely used to determine cholesterol in meat by many workers (Tu et al., 1967; Weyant et al., 1976; Cho, 1983). This color reaction is more sensitive and the color is far more stable than L-B

reaction (Zak, 1977). Since the free cholesterol and its esters give an equal color in the reaction (Zlatkis et al., 1953), the method may be used for the assay of total cholesterol without a saponification step. However, Searcy and Bergquist (1960) indicated that the reaction may lack specificity. Protein, unsaturated fatty acids, hemoglobin and steroids may also react with the Z-Z color reagent to produce a color which may affect the accuracy of the results.

In addition to the L-B and Z-Z methods, many other color reactions for cholesterol assay have been proposed. Wulfert (1947) used a p-dimethylamino benzaldehyde, salicylaldehyde and sulfuric acid mixture for the determination of cholesterol. Gordon (1951) proposed a bromine dye-sulfuric acid-triphenyl methane color reagent and Hanel et al. (1955) used a zinc chloride-acetic acid-acetyl chloride reagent which was assumed to be specific for cholesterol. Anthrone-digitonide was used as a color reagent by Vahouny et al. (1960). Searcy and Bergquist (1960) also used a ferrous sulfide-acetic acid-sulfuric acid color reagent which was found to be less susceptible to interfering substances. However, those methods have not been widely used in cholesterol analysis in meats.

The cholesterol in meat has been quantitatively determined by gas-liquid chromatography (GLC) with flame

ionization detector as the free cholesterol (Kovacs, 1979; Kunsman et al., 1981; Karkalas et al., 1982), as an acetate derivative and as a trimethylsilyl (TMS) derivative (Miettinen et al., 1965; Punwar et al., 1978). The quantitative determination is usually made by comparison of either peak height or peak area with that of a standard of known concentration. Because of their greater specificity, methods for determining cholesterol based on GLC are usually more accurate than colorimetric procedures (Sweeney and Weihrauch, 1976).

An enzymatic method was proposed for the clinical assay of cholesterol (Roschlau et al., 1974; De Hoff et al., 1978; Cho, 1983) since hydrolysis of cholesteryl esters by saponification is generally considered to be unsatisfactory, owing to the formation of reducing substances (Myant, 1981). The basic principle involves the selective and quantitative oxidation of cholesterol by cholesterol oxidase from certain bacteria. This oxidase catalyzes the oxidation of cholesterol to cholest-4-en-3-one derivative and an equimolar amount of hydrogen peroxide in the presence of oxygen. Thus, the determination of cholesterol can be done based either on quantitative measurement of the cholest-4-en-3-one by a colorimetric method or GLC or on the measurement of the amount of hydrogen peroxide produced. This method eliminates the necessity for time-consuming isolation and

saponification steps and eliminates the use of hazardous chemicals (Hoff, 1978). Cho (1983) reported that the enzymatic method is reproducible and his results of cholesterol analysis correlated well with those obtained by the Z-Z method. However, no reports were found in the literature on the determination of cholesterol content in meat by the enzymatic method.

Other methods for cholesterol assay were also reported, for instance, gravimetry (Sperry et al., 1963), titration (Okey, 1930), fluorometry (Roberts, 1968) etc. However, they have been used little for analysis of cholesterol in meat.

METHODS AND MATERIALS

SAMPLES

Twenty "Country Natural Beef" animals were slaughtered and dressed by standard commercial procedures at the Welch Meat Co., Portland, Oregon. One rib steak (2.5 cm thick) was taken at the 12th rib of the right side of each carcass at 48 hr post mortem. Steaks were vacuum packaged, mechanically frozen and stored at -20°C until needed.

Due to extenuating circumstances (financial constraints), we were not able to obtain control samples at the slaughter plant simultaneously with the natural beef samples. Thus meat samples serving as the control were obtained from a local food market. Although the control (regular beef) samples were obtained under conditions less than ideal in respect to experimental design, they do represent beef that is normally sold at the retail level. Hence, they do serve as a meaningful point of reference in this study.

SAMPLE PREPARATION

Frozen steaks were thawed at 4°C for 12 hr after which the longissimus dorsi (LD) muscle was excised from each steak. After removal of adhering fat and connective tissue,

the LD muscle was cut into thin strips and ground through a grinding plate having orifices of 9 mm diameter, mixed thoroughly and reground twice through a 3.5 mm plate. Subcutaneous adipose tissue was also sampled at the 12th rib cut. Samples consisted of approximately 10 grams of tissue which were finely sliced and minced for subsequent analysis.

The controls were sampled in the same manner as that for the "Country Natural Beef".

PROXIMATE ANALYSIS

Moisture (in vacuo) and protein (macro-Kjeldahl) contents of muscle samples were determined in duplicate by standard AOAC (1984) methods. For determining the moisture content of adipose tissue, however, samples were first dried at 70°C for 12 hr and then for an additional 12 hr at 105 C.

Total lipids were extracted from each sample of finely ground muscle tissue (2.8 g) and minced adipose tissue (1.4 g) by the Folch procedure (Folch et al., 1957) using chloroform-methanol (2:1, v/v) as the extraction solvent. Extracts from both muscle and adipose tissue samples were washed with distilled water rather than a salt solution. After this step, extracts were diluted to a total volume of 50 ml with the chloroform-methanol solution. Two

ml-aliquots of each lipid extract were placed in small glass test tubes in a water bath maintained at 45°C and evaporated to dryness under a gentle stream of nitrogen. The lipid content of each muscle and adipose tissue sample was determined gravimetrically in duplicate. All components for proximate analysis were expressed on a wet weight basis.

CHOLESTEROL ISOLATION

A 5 ml-aliquot of the lipid extract prepared by the Folch procedure was freed of solvent by vacuum aspiration while the glass test tube containing the extract was warmed in a 45°C water bath. Five ml of freshly prepared 15% (w/v) KOH in 90% ethanol was added to each tube containing the lipid residue. The tube was flushed with nitrogen and sealed with a glass stopper. The lipid residue was saponified by heating in a water bath at 90°C for 30 min (Eichhorn et al., 1986a). Upon cooling, 5 ml of distilled water was added to each tube. The unsaponifiable material was extracted three times successively with 5, 4, 3 ml of hexane. Hexane was removed from the extract by vacuum aspiration while the tube was warmed in a water bath at 45°C.

CHOLESTEROL ASSAY

Preparation of Reagents

All chemicals used in this study were reagent grade.

Color reagent: A stock ferric chloride solution was prepared by dissolving 2.5 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in concentrated (85%) orthophosphoric acid and diluted to 100 ml with the same acid. Four ml of the stock ferric chloride solution was diluted to 50 ml volume by the addition of concentrated sulfuric acid. This was the final composition of the color reagent (Zak et al., 1957).

Standard cholesterol solution: Pure cholesterol was purchased from Nu-Chek, Inc., Elysian, MN.. The purity of this material was confirmed by thin layer chromatography (Lisboa, 1969) prior to its use. One hundred mg of pure, dry cholesterol was dissolved in glacial acetic acid and made to 100 ml volume with the same acid. This solution was then diluted 1:10 with glacial acetic acid to yield a final concentration of 0.1 mg per ml.

Cholesterol Standard Curve

Precise amounts of the standard cholesterol solution, 1.0, 2.0, 3.0, and 4.0 ml, were pipetted into test tubes and diluted with 5.0, 4.0, 3.0, and 2.0 ml of glacial

acetic acid respectively. Four ml of the color reagent was added to each tube and agitated on a Vortex mixer for 15 sec and then cooled for 10 min in an ice-bath. The absorbance of each tube was read against a reagent blank at 550 nm in a Bausch & Lomb (Spectronic 20) spectrophotometer. The standard curve was prepared by plotting absorbance values against cholesterol concentration.

Cholesterol Determination

Six ml of glacial acetic acid was added to each tube containing unsaponifiable residue (obtained from cholesterol isolation procedure) and mixed well on a Vortex mixer. Four ml of color reagent was added per tube and agitated for 15 sec on the Vortex mixer and then cooled for 10 min in an ice-bath for color development. Absorbance was determined spectrophotometrically (Spectronic 20) at 550 nm against reagent blank (Zak et al., 1957). Each sample was determined in duplicate.

Total cholesterol was calculated as follows:

$$\text{mg cholesterol/100 g wet tissue} = (A \times V_o \times 100) / (K \times V \times W_o)$$

where A = absorbance; V_o = total lipid extract volume (ml); K = slope of standard cholesterol curve; V = volume of lipid extract used (ml); and W_o = sample weight (g).

Recovery Test of Cholesterol Determination

A standard cholesterol solution was prepared by dissolving pure, dry cholesterol in chloroform-methanol (2:1, v/v) to yield a final solution contained 1 mg cholesterol/ml.

Cholesterol recovery from both adipose and muscle tissue was evaluated by adding appropriate amounts of the standard cholesterol solution to known weights of sample prior to lipid extraction. These samples were treated by the same procedures as described previously.

STATISTICAL ANALYSIS

Data were analyzed by the t-test and the means were separated by the method of Devore and Peck (1986). The correlation coefficients between each two variables were calculated by linear regression. Also, the Statistical Interactive Programming System (SIPS) (Rowe and Brenne, 1982) was used to analyze the overall correlation coefficients among cholesterol, fat, moisture and protein contents.

RESULTS AND DISCUSSION

PROXIMATE ANALYSIS

The results of the proximate analysis (moisture, protein and lipid contents) of the longissimus dorsi muscle and subcutaneous adipose tissue samples are expressed on a wet weight basis. Each value listed in Tables 1, 2 and 3 is the average of duplicate determinations. The "Country Natural Beef" is referred to hereafter as natural beef.

Data for the moisture content of muscle and adipose tissue are presented in Table 1. The moisture content of the muscle ranged from 72.81 to 75.25% for the natural beef and from 67.39 to 73.48% for the control samples. Mean moisture values were 74.15% and 71.56% for the natural and control groups respectively. These results are similar to those reported for the same muscle by Tu et al. (1967) and Rhee et al. (1982a), 69-74% (mean = 72.0%) and 68-76% (mean = 71.0%) respectively. In the current study, the mean moisture content of the muscle of the natural group was significantly ($P < 0.01$) higher than that of the control. One possible explanation for this difference is that the control samples were obtained from a local food market and may have lost some moisture during the aging period of 5 to 7 days. Another factor that will be discussed later is that fat levels are inversely related to moisture contents. Since the muscle lipid content of the control group was

TABLE 1. Moisture content of muscle and adipose tissue of natural and control beef^a

Sample Number	<u>Muscle tissue</u>		<u>Adipose tissue</u>	
	<u>Natural %</u>	<u>Control %</u>	<u>Natural %</u>	<u>Control %</u>
1	74.25	72.75	10.86	12.18
2	74.25	73.25	11.64	12.08
3	74.75	70.48	13.80	12.06
4	74.15	70.83	11.02	15.82
5	74.80	70.80	8.90	12.68
6	75.05	73.01	14.20	12.58
7	75.15	71.62	13.00	11.41
8	75.25	72.69	12.65	10.38
9	72.81	73.48	9.29	11.85
10	74.35	72.78	8.18	14.38
11	74.68	72.62	9.57	16.40
12	73.14	67.39	13.81	15.74
13	72.94	69.86	13.02	14.75
14	74.30	72.66	12.76	9.68
15	73.12	72.31	10.34	9.59
16	72.93	72.60	9.85	11.82
17	73.78	72.91	11.13	10.70
18	74.60	72.41	9.07	10.90
19	74.44	68.66	8.10	14.98
20	74.24	68.14	12.97	11.38
MEAN	74.15	71.56	11.21	12.57
SD ^b	0.78	1.81	1.98	2.08
SL ^c	S (P<0.001)		NS (P>0.05)	

^a Mean of duplicate determinations.

^b Standard deviation.

^c Significance level; NS = not significant, S = significant.

much higher than that of the natural beef, the former would be expected to show a lower moisture content.

The subcutaneous adipose tissue contained a much smaller percentage of moisture than muscle (Table 1). The mean moisture contents of the adipose tissue of the natural and control groups were 11.21 and 12.57% respectively. The difference between the two groups was not statistically significant ($P > 0.05$). The results obtained in this study are lower than the mean moisture value of 15% reported by Rhee et al. (1982b) for 25 subcutaneous adipose tissue samples.

Muscle protein values are presented in Table 2. The protein content of the natural beef ranged from 20.93 to 23.48% with a mean value of 22.31% which was significantly higher ($P < 0.01$) than the mean value of 21.02% for the control beef. For the latter group, the protein content ranged from 18.27 to 23.90%. The muscle protein values of this study were comparable to the data (21.1%) compiled by Richardson et al. (1980) for lean muscle samples taken from the 11-12th beef rib section.

The protein content of the adipose tissue was not determined because most external adipose tissue is trimmed away prior to sale and usually is not consumed per se.

Fat content data for both muscle and adipose tissue are given in Table 3. The fat content of the subcutaneous adipose tissue ranged from 77.68 to 87.37% for the natural

TABLE 2. Protein content of muscle tissue
of natural and control beef^a

<u>Sample No.</u>	<u>Natural %</u>	<u>Control %</u>
1	23.48	23.70
2	22.58	23.90
3	22.70	20.70
4	23.20	19.52
5	22.90	21.78
6	21.98	20.32
7	21.66	21.54
8	21.82	21.00
9	22.10	21.90
10	23.22	20.87
11	22.52	21.93
12	22.10	19.37
13	22.40	19.47
14	21.90	21.63
15	21.98	21.93
16	21.92	21.07
17	22.22	21.05
18	22.53	20.87
19	22.05	18.27
20	20.93	19.67
MEAN	22.31	21.02
SD ^b	0.60	1.39
SL ^c	S (P<0.01)	

^a Mean of duplicate determinations.

^b Standard deviation.

^c Significance level; S = significant.

group and from 80.29 to 88.19% for the control beef. The mean fat content (83.41%) of the adipose tissue of the natural beef was not significantly ($P>0.05$) different from that of the control (84.76%). The means of this study were slightly higher than that (81.4%) reported by Rhee et al. (1982a) but lower than the mean of 90.7% recorded by Tu et al. (1968).

Table 3 shows that fat content of the natural beef muscle ranged from 2.04 to 4.51% with a mean value of 2.92%. For the control beef, muscle fat content ranged from 2.64 to 10.82% with a mean value of 6.19%. The mean fat content of the muscle of the natural group was significantly ($P<0.01$) lower than that of the control beef. In fact, the average muscle fat content of the control beef was twice that of the natural beef. Generally, decreasing activity and increasing the plane of nutrition, both of which occur during the feedlot-finishing period, result in increasing deposition of lipids in the muscle tissue (Lawrie, 1966). Thus these data support the assumption that a reduction in the feedlot-finishing time from 120-150 days to 50-85 days decreases the fat content in the muscle tissue.

In respect to the proximate composition of beef muscle, the sum of moisture, protein and lipid is relatively constant. Any increase in the lipid content is accompanied by a corresponding decrease in the content of protein

TABLE 3. Fat content of muscle and adipose tissue of natural and control beef^a

Sample Number	Muscle tissue		Adipose tissue	
	Natural %	Control %	Natural%	Control%
1	2.42	3.37	85.22	85.46
2	2.82	2.64	82.87	85.38
3	2.52	8.08	80.44	86.12
4	2.28	8.59	85.23	81.51
5	2.15	7.47	82.16	84.75
6	2.24	5.07	77.68	84.62
7	2.27	5.62	81.78	87.34
8	2.71	7.26	81.64	85.73
9	4.40	4.86	85.82	86.04
10	2.60	3.83	85.84	83.26
11	2.40	5.40	85.56	81.23
12	3.24	10.54	82.43	80.29
13	3.79	9.37	80.38	85.47
14	2.89	3.74	83.10	88.19
15	4.51	3.26	85.57	85.74
16	4.07	4.73	87.37	84.66
17	4.00	4.16	83.92	86.68
18	2.26	4.18	82.88	86.86
19	2.54	10.82	85.92	80.78
20	2.04	10.76	82.14	85.02
MEAN	2.92	6.19	83.40	84.76
SD ^b	0.81	2.70	2.42	2.23
SL ^c	S (P<0.01)		NS (P>0.05)	

^a Mean of duplicate determinations.

^b Standard deviation.

^c Significant level; NS = not significant, S = significant.

and/or moisture (Bodwell and Anderson, 1986). These statements are reflected in the data of the present study because the natural beef muscle had significantly ($P < 0.01$) higher levels of both protein and moisture and a significantly ($P < 0.01$) lower fat content than the control beef.

CHOLESTEROL PROCEDURE

The first phase of the cholesterol assay was to examine some of the parameters affecting the accuracy of the procedure. The literature (Searcy and Bergquist, 1960; Radin and Gramza, 1963; Sweeney and Weihrauch, 1976) indicates that both temperature and time of color development are extremely critical. In preliminary studies on color development, it was found that the absorbance of the color reaction changed with temperature and time. Figure 3 shows the absorbance spectra (wavelength versus absorbance) of a standard cholesterol solution mixed with the sulfuric acid-ferric chloride color reagent and measured at 30 nm intervals from 340 to 700 nm at two different time intervals. The spectral curve shown by the solid line having a maximum absorbance peak at 490 nm was obtained immediately at the end of the 10 min incubation or color development period in an ice bath. The spectral curve plotted with the broken line was obtained after the sample

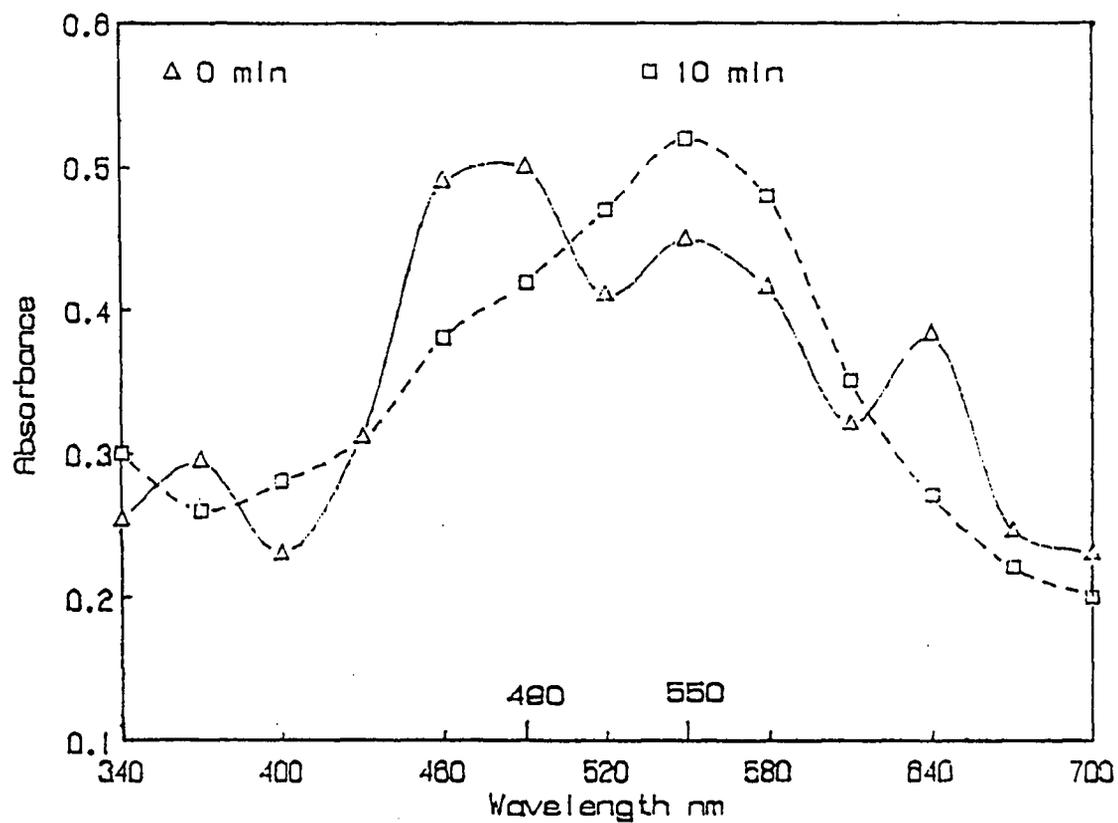


Figure 3. Standard cholesterol spectra.

Δ and \square refer to 0 min and 10 min exposure to ambient temperature after color development, respectively.

was removed from the 10 min incubation period in the ice bath and allowed to stand an additional 10 min at ambient temperature. It had a maximum absorbance peak at 550 nm. The absorbance value at 550 nm of the latter curve was about 0.05 of an absorbance unit higher than that obtained for colored solution measured at the end of the color development period with no exposure to ambient temperature. The spectral curve (broken line) obtained with the 10 min exposure time to ambient temperature and having a maximum absorbance peak at 550 nm was very similar to that recorded in an earlier study by Radin and Gramza (1963) who found the maximum absorbance to occur between 540 and 560 nm. Thus a wavelength of 550 nm and 10 min exposure time to ambient temperature were the conditions selected to measure the absorbance of the cholesterol color reaction in this study.

The absorbance peak at 490 nm, shown on the spectral curve (solid line) for the reaction solution measured immediately after removal from the ice bath, was not evident in the curve (broken line) obtained for the reaction solution allowed to stand an additional 10 min at ambient temperature. It appears that an intermediate compound was formed in the color reaction which was responsible for this 490 nm peak. With increased exposure to ambient temperature, the compound was converted to another form which showed less absorbance at 490 nm. Burke

et al. (1974) proposed a sequential reaction of the Zak color reagent with cholesterol. They indicated that there was an intermediate trienylic cation reaction with the ferrous ion which had a maximum absorbance at 478 nm. This intermediate compound may have been responsible for the absorption peak at 490 nm in our study. Since the rates of the color reactions are strongly temperature dependent and increase rapidly with increasing temperature, the temperature and color developing time must be standardized or there will be distinct variations in the amount of color produced. The same observations were emphasized by Tonks (1967).

Proper mixing after adding the color reagent to the cholesterol-acetic acid sample solution was very important to obtain satisfactory results. Care must be taken not to produce bubbles which if present, can distort the results. Results of mixing time trials indicated that mixing time of 15 sec was optimum for this step. Thus a 15 sec mixing time was used in this study to achieve proper mixing of the color reagent with the cholesterol sample solution.

Upon completion of the cholesterol isolation procedure, care must be taken to make certain that the isolated cholesterol is free of water and hexane since they interfere with the subsequent color development. Complete removal of water and hexane is required to prevent large increases in absorbance values which were observed when

water or hexane or both were present in the final mixture of color reagent and cholesterol.

Care must also be taken to prevent the oxidation of cholesterol during the determination procedure since cholesterol can be oxidized. The resulting oxidation products can result in a lower melting point and changes in solubility characteristics to affect the color reaction. It was observed that when the cholesterol extract was unduly exposed to air at 45 C or higher, an opaque solution (milk-like) developed during the final color reaction, which distorted absorbance values to give erroneous results.

The cholesterol standard curve is shown in Figure 4. This curve demonstrates that the proportionality of the color developed in this reaction followed Beer's law ($r = 0.99$) for cholesterol concentrations ranging from 0.0 to 0.4 mg/ml. This curve was used to quantify the cholesterol contents of both muscle and adipose tissue of this research.

The rate of cholesterol recovery was determined for the analytical procedure. A known amount of pure cholesterol was dissolved in chloroform-methanol (2:1) solution and added to the tissue samples quantitatively. The mixtures were run through the entire analytical procedure as described earlier. The average rate of cholesterol recovery was about 90% for muscle tissue and 88% for adipose

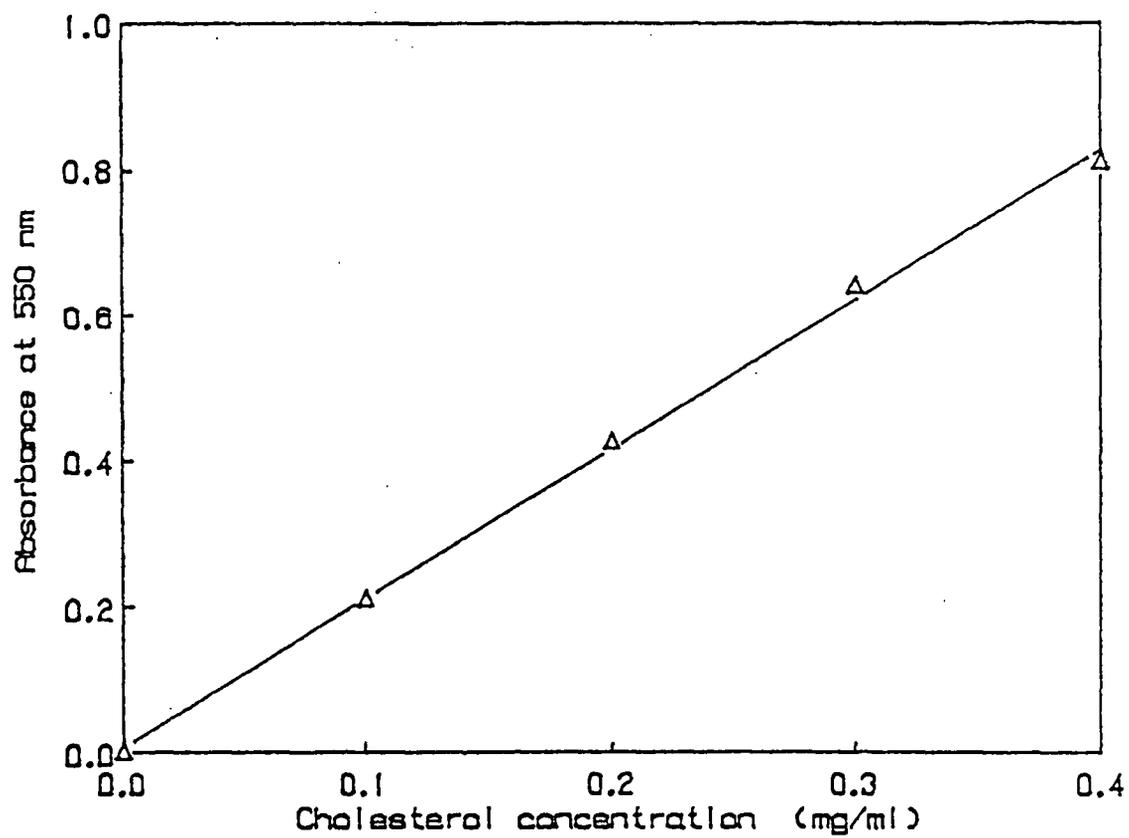


Figure 4. Cholesterol standard curve.

tissue. In a previous study, Jaeger et al. (1981) determined the rate of cholesterol recovery with radiolabeled compounds. They found that best results were obtained with cholesterol esters. The recovery rate was 86% after filtration and 84% after subsequent washing. Eichhorn et al (1986a) also reported that when randomly chosen samples were re-homogenized and re-extracted three times with chloroform, less than 2% additional cholesterol was recovered each time. No detectable cholesterol was recovered from randomly chosen samples that were re-extracted three times with hexane following saponification.

CHOLESTEROL CONTENT

Table 4 shows that the cholesterol values (wet weight) of the natural beef muscle ranged from 52.34 to 60.68 mg/100 g as compared to a range of 50.34-63.90 mg/100 g for the control. The difference between the mean muscle cholesterol content of the natural (56.91 mg/100 g) and the control beef (56.49 mg/100 g) was not statistically significant ($P>0.05$). The data obtained in this study are similar to those published in earlier studies. Mean cholesterol values reported for the same muscle from comparable animals were 56.0 mg/100 g by Tu et al. (1967), 61.3 mg/100 g by Rhee et al. (1982a) and 58.3 mg/100 g by

TABLE 4. Cholesterol content of muscle and adipose tissue of natural and control beef (mg cholesterol/100 g wet sample)^a

Sample Number	Muscle tissue		Adipose tissue	
	Natural	Control	Natural	Control
1	56.28	58.22	113.24	124.46
2	58.53	58.61	104.38	123.71
3	59.79	61.06	103.72	119.81
4	58.23	63.90	118.00	119.76
5	58.71	57.26	102.56	112.22
6	57.99	56.57	104.79	113.01
7	59.62	56.10	103.50	121.14
8	57.89	55.99	101.84	101.95
9	53.59	57.31	109.46	111.38
10	53.41	55.86	112.66	101.62
11	53.05	52.32	108.43	100.72
12	55.66	58.92	114.30	111.56
13	53.36	54.84	110.02	112.28
14	60.56	51.61	106.79	106.96
15	60.20	50.34	100.15	107.70
16	60.68	52.90	111.63	111.60
17	59.84	51.67	99.43	111.82
18	54.72	53.56	98.46	120.57
19	52.34	61.54	109.04	110.80
20	53.56	61.21	102.64	118.44
MEAN	56.91	56.49	106.75	113.08
SD ^b	2.93	3.74	5.45	7.15
SL ^c	NS (P>0.05)		S (P<0.01)	

^a Mean of duplicate determinations.

^b Standard deviation.

^c Significance Level; NS = not significant, S = significant.

Eichhorn et al. (1986a). Thus the cholesterol content of the longissimus dorsi muscle was not affected by a 40-50% reduction in the number feedlot-finishing days since the mean muscle cholesterol contents were almost identical for the natural and control groups.

Cholesterol values (Table 4) of the adipose tissue of the natural beef group ranged from 98.46 to 118.00 mg/100 g wet tissue while the range for the control group was 100.72 to 124.46 mg/100 g. The mean adipose cholesterol value for the natural beef (106.75 mg/100 g) was significantly lower than that (113.08 mg/100 g) for the control group. The mean cholesterol value of the adipose tissue of the control group agrees closely with that (114.3 mg/100 g) of Rhee et al. (1982b) but is higher than the mean value (101.7 mg/100 g) reported by Eichhorn et al. (1986a). In a later study, Eichhorn et al. (1986b) found that the overall mean of the cholesterol content of the adipose tissue of mature cows (7 to 10 years) was 124.5 mg/100 g wet tissue. The animals in that particular study were three to five times older than the animals (2 to 2.5 years) studied in their previous investigation (Eichhorn et al., 1986a).

The finding that the cholesterol content of the adipose tissue of the control group was significantly ($P < 0.01$) higher than that of the natural group in the current study strongly suggests that increasing the length of the feedlot-finishing time also results in an increased

deposition of cholesterol in the adipose tissue.

Results of the current study also show a marked difference in the cholesterol content between muscle and adipose tissue. The mean cholesterol value of all muscle samples was 56.7 mg/100 g wet tissue versus that of 109.9 mg/100 g for adipose tissue. Since adipose tissue contains nearly twice as much cholesterol as muscle tissue, this indicates that adipose tissue is the major repository for cholesterol storage.

The SIPS computer software program was used to determine the overall correlation coefficients between cholesterol content and fat, moisture and protein contents. Table 5A shows that the correlation coefficients between the variables for the muscle tissue within the natural beef groups were low, 0.33 or less. However, correlation coefficients were more pronounced for the muscle tissue of the control group. For the latter, cholesterol content was positively correlated ($r=0.62$) with fat content and negatively correlated with both moisture ($r=-0.55$) and protein ($r=-0.35$) contents. Also, fat content was negatively correlated with moisture ($r=-0.91$) and protein ($r=-0.80$) levels, and moisture content was positively correlated with protein ($r=0.71$) for the muscle tissue of the control group.

In regard to adipose tissue (Table 5B), correlation coefficients between the natural and control groups were in

Table 5. Correlation coefficients among experimental variables^a

A. Muscle tissue

	<u>Moisture%</u>	<u>Protein%</u>	<u>Cholesterol</u> ^b
Natural beef:			
Fat%	-0.33	-0.18	0.17
Moisture%		0.07	0.09
Protein%			-0.06
Control beef:			
Fat%	-0.91	-0.80	0.62
Moisture%		0.71	-0.55
Protein%			-0.35

B. Adipose tissue

	<u>Moisture%</u>	<u>Cholesterol</u> ^b
Natural beef:		
Fat%	-0.76	0.39
Moisture%		-0.10
Control beef:		
Fat%	-0.87	0.25
Moisture%		-0.13

^a Linear regression method used.

^b mg/100 g wet tissue.

closer agreement than they were for the muscle tissue. Moisture content was negatively correlated with fat content of both the natural and the control groups. In contrast to muscle tissue, a higher correlation coefficient existed between cholesterol content and adipose fat content of the natural samples ($r=0.39$) than for the control group ($r=0.25$), although both values were rather low.

The regression equation for mg cholesterol per 100 g wet tissue (Y) on percentage fat (X) for the muscle tissue of natural beef in this study was $Y = 54.95 + 0.66X$, and for the muscle tissue of the control beef was $Y = 51.18 + 0.86X$. Rhee et al. (1982a) found the regression equation of $Y = 54.73 + 0.87X$, and Tu et al. (1967) reported $Y = 48.9 + 1.7X$ for muscle tissue. The regression equation of adipose tissue for natural beef and control beef were also determined in this study; $Y = 34.16 + 0.87X$ for the natural beef and $Y = 46.18 + 0.79X$ for the control beef. No regression equations for beef adipose tissue were found in the literature.

PRACTICAL IMPLICATIONS

In the present study, the cholesterol content of the muscle tissue was not significantly different between natural and control beef. Conversely, the cholesterol content of the subcutaneous adipose tissue of the control

group was found to be significantly ($P < 0.01$) higher than that of the natural beef. An apparent cause for this higher level of cholesterol may be the result of the longer period of time (120-150 days) these animals were kept in the feedlot-finishing phase than the natural beef (50-85 days). The longer feedlot-finishing time for the control group allowed for greater fat accretion accompanied by a parallel increased deposition of cholesterol in the adipose tissue. Moreover, the mean lipid content of the muscle tissue of the control group (6.19%) was significantly higher ($P < 0.01$) than that of the natural beef (2.92%). This also suggests that the longer feedlot-finishing time accommodated an increased deposition of intramuscular (marbling) fat.

In a study in which cholesterol content of the longissimus dorsi muscle in eight marbling-score categories was determined, Rhee et al. (1982a) observed only one significant difference, i.e., muscles "Practically Devoid" of marbling contained significantly ($P < 0.05$) less cholesterol (wet weight basis) than muscles with higher marbling scores. A summary of Rhee's data is listed in Table 6. No significant differences were found between steaks from any of the other seven marbling groups.

Although marbling scores were determined by three highly trained evaluators, these are visual ratings that must be considered as subjective values. Hence, they are somewhat at variance with the data for the analytical

determination of total lipid content.

In a later study concerning the cholesterol content of intermuscular (seam) and subcutaneous fat of beef, Rhee et al. (1982b) obtained data (Table 7) that were very similar to their earlier results (Rhee et al., 1982a) in respect to cholesterol contents of 62.4 and 114.3 mg cholesterol/100 g wet tissue for muscle and adipose tissue, respectively. Furthermore, the carcasses from which samples were taken in both of Rhee's studies were handled in a manner (4-6 days post mortem aging) similar to that for the control samples (5-7 days post mortem aging) of the current study.

Rhee et al. (1982a) stated that consumers need not be concerned about the marbling content of beef (trimmed of subcutaneous fat) relative to its cholesterol content. They concluded that although raw steaks with "Practically Devoid" marbling had significantly ($P < 0.05$) less cholesterol than did steaks with any of the other seven marbling scores, it was of little practical importance since the availability of beef with that little marbling is low. However, since publication of their data in 1982, there has been an organized, systematic move by the beef industry to produce leaner beef such as that characterized by the natural type in this study. Thus beef with a lower level of marbling is steadily becoming more available at retail markets.

The data of the current study and that of Rhee et al.

TABLE 6. Moisture, total lipid and cholesterol values^a (wet weight basis) for raw beef longissimus steaks having different amounts of marbling (From Rhee et al., 1982a)

Marbling score	Moisture%	Lipid%	Cholesterol ^b
Moderately abundant	67.56 f	12.08 a	64.74 a
Slightly abundant	68.92 e,f	9.57 b	62.48 a
Moderate	68.02 f	11.69 a	61.43 a
Modest	70.82 c,d	6.76 c	65.88 a
Small	69.99 d,e	7.95 b,c	64.00 a
Slight	72.07 c	6.06 c	59.95 a
Traces	73.71 b	3.63 d	60.06 a
Practically devoid	75.55 a	2.73 d	51.77 b

^a Mean value of data from 10 steaks per marbling group.
Means in a column which are not followed by the same letter are significantly different ($P < 0.05$).

^b mg cholesterol/100 g wet sample.

TABLE 7. Moisture, total lipid and cholesterol values^a (wet weight basis) of raw beef longissimus steak fractions (From Rhee et al., 1982b)

Sample type	Moisture %	Lipid %	Cholesterol ^b
Muscle tissue	69.6	10.8	62.4
Adipose tissue	15.0	81.4	114.3

^a Mean value of data from 25 samples.

^b mg cholesterol/100 g wet sample.

(1982a, b) suggest that a large proportion of the cholesterol in beef longissimus dorsi muscle is present in structural lipids such as cell membranes and intracellular structures rather than concentrated in the marbling or intramuscular fat. It is also apparent that altering the cholesterol content of beef muscle is a difficult task (Eichhorn et al., 1986b) because data of the current study show that both the natural and control beef muscles contain essentially identical cholesterol levels although the control group has twice as much intramuscular fat as the natural beef.

Total cholesterol intake from beef may be reduced by trimming away subcutaneous adipose tissue since it contains twice as much cholesterol as muscle tissue. However, the benefit or significance of removing the subcutaneous fat should be considered from the standpoint of triacylglycerols intake rather than that of cholesterol intake. According to Rhee et al. (1982b), the removal of the subcutaneous fat eliminates a significant intake of triacylglycerols which provide not only calories but also the starting material for cholesterol synthesis in the body.

CONCLUSIONS

The cholesterol content and proximate composition of the longissimus dorsi muscle and the subcutaneous adipose tissue of both natural and regular beef were determined. From the data obtained in this study, the following conclusions were drawn:

1. The 40-50% reduction in the feedlot-finishing time significantly ($P < 0.001$) reduced the intramuscular fat content of the natural beef muscle, which was accompanied by increases in both the moisture and protein contents. However, there was essentially no difference in the moisture and fat contents of the adipose tissue between the natural and control beef.

2. The reduction in the feedlot-finishing time did not affect the muscle cholesterol content of the natural beef since its mean cholesterol level was almost identical to that of the control. However, the decrease in the feedlot-finishing time resulted in a lower cholesterol level of the adipose tissue since the cholesterol content of the natural beef adipose tissue was significantly ($P < 0.01$) less than that of the control.

3. On an overall basis, the adipose tissue was found to contain nearly twice as much cholesterol as the muscle tissue.

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