Skin Lipids of Puppies as Affected by Kind and Amount of Dietary Fat

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ABSTRACT Clinical evidence of a dietary requirement for linoleic acid in maintenance of a healthy skin prompted a study of fatty acid distributions in skin of 84 young dogs fed diets with and without linoleic acid. When weaning puppies were fed diets deficient in linoleic acid for 2 months, monoene fatty acids in whole skin greatly exceeded levels for saturated fatty acids. Linoleic and arachidonic acid levels were lower than those in new born puppies. Levels of these fatty acids decreased further in skin and serum after 4 months when definite deficiency signs were evident, but levels remained approximately the same during longer feeding periods. Small amounts of linoleic acid were always present in skin and serum. Step-by-step increases in dietary linoleate were reflected in increased levels of this fatty acid in triglycerides, cholesterol esters, and phospholipids in skin and serum. During linoleic acid-deficient states, 5,8,11-eicosatetraenonic acid was always present in serum, but it was observed infrequently in skin and only in the phospholipid fraction. In deficient states arachidonic acid was present in serum and skin, but it was observed less frequently in small amounts only in skin phospholipids. When ethyl arachidonate was fed for 2 months after weaning, it was observed in skin and serum, but during recovery from the linoleic deficient state, it was not noted in skin after a 2-month feeding period. Phospholipids make up a small fraction of skin lipids, but changes in their fatty acid patterns appear to reflect alterations which occur in epidermal cells during a dietary deficiency of linoleic acid.

Dietary linoleate has been shown to be required for the maintenance of a healthy skin in young infants (1, 2) and many young animals (3-11). When the diet is deficient in linoleic acid, the most universal and striking clinical sign is dry, thickened and scaly skin. Among the histologic alterations which develop are increased density of the keratin layer with parakeratosis and thickening of the epidermal layer. Mitosis is evident in early stages of the deficiency, but in advanced deficiency there is increased thickening of the epidermis without mitotic activity (12). These changes appear to indicate alterations in the normal maturation of the epidermal cells. Many feeding studies have demonstrated the reversibility of the gross and histologic changes in skin by incorporation of linoleic acid in the diet. Numerous investigators have also reported the chemical components of skin, particularly surface lipids, ductal or sebaceous gland lipids, and components of epidermis of various species. This literature has been reviewed by Nicolaides and co-workers (13-16), who have contributed additional comprehensive data. However, there has been little attempt to correlate the fatty acid composition of whole skin with dietary regimen. In view of evidence for the essentiality of linoleic acid in the maintenance of healthy skin, a 4-year study was undertaken to determine major fatty acid components in skin of young puppies (a) at birth, (b) when the diet fed from the time of weaning is deficient in linoleic acid, (c) when the diet fed from the time of weaning contains 1% or more of the calories as linoleic acid, and (d) when the diet fed during recovery from the deficient state contains 2% or more of the calories as linoleic or arachidonic acid. No attempt was made to characterize the many components present in epidermis or

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epidermal flakes other than the saturated and unsaturated fatty acids identifiable by gas-liquid chromatography of known standards.

The young puppy was chosen as the experimental animal because it is particularly susceptible to a dietary deficiency of linoleic acid, and serial skin biopsies can be taken from the same animal for histology and lipid analysis during the development and during the curative stages of linoleic acid deficiency. Also, periodic changes in distribution of fatty acids in blood serum which reflect changes in linoleic acid intake can be followed.

**EXPERIMENTAL CONDITIONS AND METHODS**

Experimental conditions. Beagle puppies from the time of weaning were fed isocaloric diets which were considered fully adequate in calories, protein, vitamins, and minerals, but differed in the kind and amount of fat (17). Five diets were deficient in linoleic acid, one having 30% of the calories as hydrogenated coconut oil, one low in fat (1% of the calories), and 3 diets low in fat to which sufficient linolenic, oleic, or arachidonic acid was added to supply 2% of the calories. Diets which contained linoleic acid were prepared by isocaloric substitution of sucrose in the low fat diet with butter, lard, soy bean lecithin, corn oil, or safflower oil to provide one to 16% of the calories as linoleic acid. Likewise, during the period of recovery from the deficient state, trilinolein, lard, soy bean lecithin, corn oil or safflower oil was substituted isocalorically for sucrose to provide 2% or more of the calories as linoleic acid. During recovery, ethyl arachidonate was fed in place of linoleic acid to 2 dogs. The fats and oils chosen as dietary sources of linoleic acid represent types consumed in American households.

Diets were fed at the normal caloric level for growing puppies (17). Immunization and care of the animals also were the same as previously described (17).

Skin biopsies. To determine the most feasible site for detecting early signs of deficiency, skin biopsies were taken from the abdomen, interscapular and thigh areas. Under local anesthesia, crescent shaped biopsies were dissected along the fascial layer and any subcutaneous fat was carefully removed. One-half of each specimen was placed in 10% formalin with 1% calcium acetate for histologic examination and the other half was frozen immediately in a tightly stoppered vial and stored at −10°C until analyzed. Histologic sections of skin were stained with hematoxylin and eosin for general characterization. Staining with Sudan black for possible appearance of phospholipids, aldehyde fuchsin for characterization of elastin tissue, toluidine blue for pigment content and Hale's dialyzed iron preparation for mucopolysaccharides did not provide additional information.

Young dogs show the earliest gross signs of linoleic acid deficiency on the abdomen, then on the thigh and last in the interscapular area. Histologic examination of the skin from these 3 areas for 5 animals confirmed the differences in degree of deficiency signs that were observed grossly. Extraction of lipids from whole skin showed the interscapular area to be highest and the abdominal area to be lowest in total fatty acid content. Abdominal skin tended to be highest in saturated and lowest in monoene and linoleic acids. Interscapular skin was consistently highest in linoleic acid. The observations correlated well with the degree of gross and histologic changes for the 3 areas. Since sequential biopsies could be taken alternately from the dorsal surface of each thigh, and gross appearance, histology and fatty acid composition of the skin were typical for the nutritional state of the animal, all of the following data represent results for skin biopsies taken from the dorsal surface of the thigh.

Skin fractions. Although the most marked histopathologic changes in skin occur in the epidermal layer during the development and during the curative stages of linoleic acid deficiency, attempts to separate epidermis and dermis in dog skin by a combination of 2 methods (18, 19) were very unsatisfactory. By histologic examination of the remaining dermis, it was estimated that 70 to 95% of the epidermis was removed in only 10 of 24 skins. Compari-

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son of the results from analysis of the saponifiable components in epidermis and dermis of these 10 specimens with those from silicic acid fractions of whole skin showed fatty acid patterns in epidermis to be similar to those of cholesterol esters in whole skin and patterns in dermis to be similar to those of triglycerides in whole skin. Also, epidermis and cholesterol esters of whole skin contained high percentages of unidentified components which were not noted in dermis or triglycerides of whole skin. Carbon numbers of these components calculated from retention times were in the same range as those observed in epidermal flakes removed from linoleic acid-deficient dogs and conform to those reported by other workers (15) in human surface lipids as C13,5 to C21,5 straight-chain or branched-chain fatty acids. Characterization of fatty acids in dog skin in relation to dietary fat, therefore, has been confined to the total fatty acids and fatty acids obtained by silicic acid separations of lipid extracts from whole skin.

Chemical analyses. In preparation of whole skin for analysis, hair was carefully removed with fine scissors, and the skin was cut into small bits, weighed, and then ground by hand in a porcelain mortar with washed, ignited sea sand. Analyses included total cholesterol (20), total fatty acids (21), and, when possible, silicic acid separation of the triglyceride, cholesterol ester, and phospholipid fractions (22). Fatty acids from each of the above fractions were methylated at 70° with 2% sulfuric acid in methanol. The methyl esters were chromatographed in a Beckman GC-2A instrument using 183-cm diethylene glycol succinate columns at 220° and helium as the carrier gas. Peaks were identified by comparison with retention times of known methyl esters. The 5,8,11-eicosatrienoic acid was identified by its conformity to the theoretical retention time of an eicosatrienoic acid which appeared in serum from linoleic acid-deficient animals and subsequently disappeared from serum of the animals after feeding linoleic acid. This retention time also conformed to that of 5,8,11-eicosatrienoic acid reported by Ackman (23). Peak areas were quantitated both by triangulation and by measurement of peak heights and relative retention times.

Correction factors were applied to compensate for difference in detector response to individual fatty acid components. Detector response was linear within a given instrument sensitivity setting and injected sample sizes were chosen to allow use of a single sensitivity setting.

Fasted blood specimens were drawn at intervals of 2 months when skin biopsies were taken and the serum fatty acids prepared and chromatographed by the same procedures used for skin.

RESULTS

The data represent observations and analysis of skin and blood serum for 85 dogs over a period of 6 to 8 months from the time of weaning. Most dogs reach maturity at approximately 8 months of age; hence the data were obtained during the entire growth period when the requirement for linoleic acid appears to be the greatest.

Total cholesterol and fatty acids in whole skin and blood serum. Since fatty acids in blood serum readily reflect either the absence of fat in the diet or the composition of the dietary fat and since nutrients for the skin are derived from serum, comparisons were made between fatty acid components in serum and those of the skin. Figure 1 demonstrates the relative differences in the amount of cholesterol and fatty acids derived from the various lipid fractions in skin and serum under 3 dietary conditions: when the diet was low in fat and when 30% of the calories was provided as hydrogenated coconut oil or corn oil. Total cholesterol in skin was very low (1 to 2% of dry weight) for all puppies. Total fatty acid content of skin varied considerably, but mean values for newborns and puppies fed the low fat diet were lower than when hydrogenated coconut oil or corn oil diets were fed. Silicic acid separation of the triglycerides, phospholipids, and cholesterol esters in skin showed that triglycerides constituted the major fraction of lipids in whole skin for all puppies. No blood serum was available for the newborns; however, in blood serum of the other 3 groups, over 40% of the total fatty acids was derived from phospholipids. In skin of newborns, phospholipid fatty acids made

up 8% of the total fatty acids but after a
4-month feeding period, this decreased to 1
to 2%. Cholesterol ester fatty acids were
consistently lower in skin than in serum.
The amount of the fraction containing free
fatty acids, mono- and diglycerides was ap-
proximately the same in skin and serum.

**Saturated and unsaturated fatty acids in
skin and serum of puppies fed diets defi-
cient in linoleic acid.** Fatty acids in skin
of newborn puppies were compared with
those in skin of puppies fed linoleic acid-
deficient diets for 2 months at which time
minimal gross and histologic changes usu-
ally become evident. Figure 2 illustrates
mean values for distribution of saturated
and unsaturated fatty acids in skin of 5
puppies at birth, five fed the diet low in fat,
three fed the diet containing 30% of the
calories as hydrogenated coconut oil, and
each of 3 puppies fed the low fat diet with
2% of the calories as methyl linolenate, tri-
olein or ethyl arachidonate. Expressed as
the percentage of the total fatty acids in
skin, monoene fatty acids, principally oleic,
greatly exceeded the level for saturated
acids and in some instances were much
higher than in blood serum. Skin of new-
borns contained more linoleic and arachi-
donic acid than did the skin of puppies fed
the linoleic acid-deficient diets for 2 months.
The extremely low level of linoleic acid in
skin and serum of puppies fed 2% of the
calories as methyl linolenate, triolein, and
ethyl arachidonate was of special interest
because the mother of these puppies was
fed the low fat diet during her pregnancy
and period of lactation. Mean serum levels
of linoleic and arachidonic acids for these
puppies at weaning were very low — 3.4
and 3.0% of the total fatty acids, respec-
tively — whereas, 5,8,11-eicosatrienoic acid
made up 10.4% of the total fatty acids.
Also of special interest was the identifica-
tion, by retention time, of linoleic acid in
skin when methyl linolenate or triolein was
fed. The puppy fed triolein had previously
received methyl linolenate in his diet. Al-
though the latter fatty acid had disappeared
from blood serum, it was still present in the
skin. The 5,8,11-eicosatrienoic acid which
is characteristic of a dietary deficiency of
linoleic acid was not identified in the total
fatty acids of skin at this age, although
serum levels indicated synthesis of this
metabolite by all of the puppies. Arachi-
donic acid was present in the total fatty acids in skin of newborns, of 3 puppies fed the low fat diet (3/5) and of the puppy fed ethyl arachidonate. In the latter instance the arachidonate level in serum was 21% of the total fatty acids. Gross and histologic appearance of the skin was normal for this animal. Gross and histologic deficiency changes were minimal for the other young puppies.

Saturated and unsaturated fatty acids in skin and serum of puppies fed diets with and without linoleic acid for 4 months. When weanling puppies are fed diets deficient in linoleic acid for 4 months, definite and often marked gross and histologic alterations in skin are evident (24). Fatty acid composition of these grossly abnormal skins was compared with healthy skin at the time of birth and skin of puppies fed diets which provided low, moderate and generous amounts of linoleic acid. Figure 3 demonstrates mean values for saturated and unsaturated fatty acids in skin for 5 newborns and in skin and serum after feeding the following diets for 4 months: 11 puppies, low fat; 12 puppies, hydrogenated coconut oil; 4 puppies, butter fat; 4 puppies, fresh lard; 5 puppies, corn oil. Fat in the latter 4 diets provided 30% of the calories with linoleate contributing 0.1, 1.0, 4, and 16% of the calories, respectively. Saturated fatty acids in skin of puppies fed the low fat diet remained relatively unchanged from the 2- to 4-month period. Saturated fatty acids increased in skin of animals fed hydrogenated coconut oil as a result of increased deposition of lauric and myristic acids during the 2- to 4-month feeding period. Except for puppies fed the hydrogenated coconut oil diet, the level of monone fatty acids in skin decreased step-by-step with increasing levels of dietary linoleate just as it did in blood serum. When the diet was low in fat or contained hydrogenated coconut oil, linoleic acid in skin and serum were lower after 4 months than after the 2-month period. It increased both in skin and serum with step-by-step increases in dietary linoleate. Small amounts of octadecatrienoic acid identified by retention time as linolenic acid were
noted in the skin of puppies fed butter fat or corn oil. The 5,8,11-eicosatrienoic acid which appeared in serum of animals fed the low fat, hydrogenated coconut oil or butter fat diets was observed in the skin of only one puppy fed the low fat diet (1/11). Arachidonic acid, which is present in relatively large amounts in skin at the time of birth, was not identified in the total fatty acids of skin from puppies fed deficient diets for 4 months. Only trace amounts of this fatty acid were noted occasionally in skin of the puppies fed lard or corn oil. Arachidonic acid was still present in the serum of all animals.

Figure 4 illustrates the distribution of linoleic, linolenic, 5,8,11-eicosatrienoic and arachidonic acids in the total, triglyceride, phospholipid and cholesterol ester fatty acids of skin from the same 6 groups of dogs after a 4-month feeding period. Linoleic acid was found in all silicic acid fractions. However, in contrast with the usual observation in serum, cholesterol esters in skin did not consistently show the highest percentage of linoleic acid. Also as previously discussed, cholesterol esters differed from other silicic acid fractions in skin in having many unidentified components. Octadecatrienoic acid, which appeared in the total fatty acids of skin from puppies fed butter fat or corn oil, was found in small amounts quite consistently in the triglyceride fraction of skin. The 5,8,11-eicosatrienoic acid was found only in the phospholipids from skin of 2 animals (2/11) fed the low fat diet. Arachidonic acid which was prominent only in the total fatty acids in skin of newborns was present in the phospholipids of skin from each group of animals but not always for each animal. In addition, the silicic acid fraction which was composed of free fatty acids, mono- and diglycerides also showed linoleic acid for all animals but arachidonic acid was found infrequently in this fraction.

**Fatty acid composition of skin after feeding periods of 6 to 8 months.** When the feeding period was continued from 4 to 6 or 8 months with the same diet, there
were no major changes in distribution of saturated or unsaturated fatty acids in skin. With diets low in fat, linoleic acid decreased slightly but at no time did it disappear completely from the total fatty acids in skin. Arachidonic acid, however, was found infrequently only in phospholipids of skin after prolonged feeding of a diet lacking linoleic acid. Small amounts of both linoleic and arachidonic acid were present in the total fatty acids of blood serum under all dietary conditions and at all times.

Although there were no marked changes in fatty acid composition of skin during the 4- to 8-month feeding periods, the general condition of the dogs fed diets deficient in linoleic acid became progressively poor after 4 months. After 6 to 8 months there was loss of hair, extensive desquamation, an unkempt appearance and tremulousness. Histologically, the skin showed variable degrees of acanthosis, abnormal keratin, slight-to-moderate epidermal thickening, moderate to numerous sebaceous glands, and round cell infiltration in the upper dermis. Most of the puppies fed butter fat remained in good condition throughout the period of study, but showed some degree of dryness of the skin with very fine desquamation. Histologic changes were minimal with slightly increased density of the keratin and very slight thickening of the epidermis. General health and appearance of the skin and hair were excellent for most of the animals fed diets containing lard, soybean lecithin, corn oil or safflower oil which provided 4% or more of the calories as linoleic acid.

Saturated and unsaturated fatty acids in skin and serum after feeding linoleic or arachidonic acids to dogs showing severe signs of linoleic deficiency. To detect early changes in distribution of fatty acids in skin and serum during the period of recovery from essential fatty acid deficiency, linoleic or arachidonic acid was incorporated into the diet of 14 dogs at a level of 2% of the calories for a period of 2 months. Groups of 2 dogs were fed ethyl arachidonate, corn oil, safflower oil, or fresh lard; and groups of 3 dogs were fed trilinolein or soybean lecithin. Figure 5 illustrates the mean levels for saturated, monoene, linoleic, 5,8,11-eicosatrienoic and arachidonic...
acids in the total fatty acids of skin and serum. Levels for monoene fatty acids in skin exceeded those in serum. The relatively low levels for linoleic acid in skin were of the same order of magnitude as those for skin after feeding deficient diets for 4 to 6 months. Except for the 2 dogs fed ethyl arachidonate, serum levels for linoleic acid were much higher than those observed during the deficient state. No 5,8,11-eicosatrienoic or arachidonic acids were identified in the total fatty acids of skin. The presence of small amounts of 5,8,11-eicosatrienoic acid in serum of all animals indicated incomplete recovery from the deficient state. Silicic acid separation of triglycerides, cholesterol esters, and phospholipids in skin showed linoleic acid in all fractions, but 5,8,11-eicosatrienoic acid was not identified in any fraction. Arachidonic acid was noted only occasionally in the phospholipids for animals fed corn oil, soybean lecithin or lard. Interpretation of the presence of arachidonic acid in the phospholipids of skin from these animals as an indication of superior curative effects of corn oil, soybean lecithin and lard over those of safflower oil, trilinolein, and ethyl arachidonate does not appear justified at this time. Gross appearance of all animals showed considerable improvement. Skin in the abdominal area was smooth, soft, and pliable; but extensive desquamation and some loss of hair were still evident in the interscapular area. Histologically, skin from the thigh showed variable degrees of recovery with improved keratin, thinning of the epidermis, and mild acanthosis. Keratin plugs were still evident in many hair follicles. There was little change in the number and size of the sebaceous glands.

Recovery from the deficient state was notably more rapid for animals fed corn oil, safflower oil, or soybean lecithin when linoleate intakes were 5, 8, or 12% of the calories than when the intake was 2% of the calories. Under these conditions, lin-
oleic acid levels in skin were 20 to 30% of the total fatty acids. Arachidonic acid was found occasionally in the phospholipids of skin. It is not clear from the data whether differences in fatty acid composition of the dietary fats and oils other than their linoleic acid content influenced the deposition of linoleic or arachidonic acid in skin.

**DISCUSSION**

This study was designed to compare fatty acid constituents in healthy skin of growing puppies fed diets containing linoleic or arachidonic acid with those in abnormal skin of puppies fed diets deficient in these essential fatty acids. No attempt was made to follow metabolites of cholesterol synthesis or of the keratinization process. The data represent fatty acids derived from the dermis, the epidermis, and the sebum which also may be affected by the diet.

Linoleic acid levels in skin decreased markedly when the dogs were fed diets deficient in this fatty acid; however, it did not disappear completely from skin or serum even after prolonged deficiency (6 to 8 months). Arachidonic acid, which was present in the total fatty acids of skin from all puppies at the time of birth, was still present in the serum but was not detected in the total fatty acids of skin after the puppies had been fed a diet deficient in linoleate for a period of 4 months. The small amount of arachidonic acid observed occasionally in phospholipids of these skin biopsies was not detectable in the total fatty acids. The relatively high level of arachidonic acid in skin of newborn puppies was of special interest in view of reports for significantly higher levels of this fatty acid in serum of normal infants at birth than in the serum of their mothers during the third trimester of pregnancy (25, 26). Although no correlations were demonstrated by Hansen and co-workers (25) between dietary intakes of 30 mothers during the third trimester and serum lipids in their neonates, arachidonic acid in serum of the mothers was significantly lower in the third trimester than 2 to 9 weeks postpartum. Effects of dietary fat on arachidonic acid metabolism during pregnancy and the significance of high arachidonate levels in tissues of newborns require further investigation.

Triglycerides make up the major lipid fraction in whole skin and reflect the composition of dietary fat to the greatest extent. Constituents of cholesterol esters are similar to those of epidermis and show a high content of unidentified components which are not found in dermis or in the triglyceride fraction of whole skin. Some of these components are observed in normal and in abnormal skin and may represent metabolites of the keratinization process. Phospholipids which make up the smallest fraction in skin reflect fatty acid metabolites that are considered characteristic of the intake of linoleic acid. Both arachidonic acid which normally is synthesized in the body from linoleic acid (27) and 5,8,11-eicosatetraenoic acid which is synthesized when the diet is lacking linoleic acid (28) are observed principally in phospholipids of skin. Changes in fatty acid composition in this fraction of skin correlate well with histologic alterations which occur in epidermal cells during a dietary deficiency of linoleic acid. Other silicic acid fractions may reveal intermediate metabolites of linoleic acid. For example, the octadecatrienoic acid found in the triglyceride and cholesterol ester fractions of skin from animals fed butter fat, corn oil, and soybean lecithin may prove to be, in part, an intermediate metabolite in the conversion of linoleic to arachidonic acid (29). Hence, future studies on the role of linoleic acid in the maintenance of healthy skin should include distribution of fatty acid metabolites in the phospholipid, triglyceride, and cholesterol ester fractions of whole skin.

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**LITERATURE CITED**


