

Feeding enriched omega-3 fatty acid beef to rats increases omega-3 fatty acid content of heart and liver membranes and decreases serum vascular cell adhesion molecule-1 and cholesterol levels

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Abstract

Dietary intake of the long-chain omega-3 fatty acids prevents the development of heart disease. In this study, we evaluated whether feeding beef from cattle fed an omega-3 fatty acid–enriched diet to rats had health benefits. Cattle raised on a 10% flaxseed diet have high amounts of α -linolenic acid in their muscle tissue when compared to cattle fed a control diet of corn. Twenty, weanling, Sprague-Dawley rats were fed one of two diets ($n = 10$ in each group), one diet containing fat from beef of cattle fed 10% flaxseed and the other beef from cattle fed a conventional corn ration. The diets contained 10% fat from the cooked beef using a modification of the American Institute of Nutrition diet formulated in 1976 diet for a 5-week period. There was a statistically significant increase in the amount of membrane docosahexaenoic acid in the livers of the rats on the diet of beef from cattle fed flaxseed and a statistically significant increase in the amount of membrane arachidonic acid in the hearts of the controls. There were also strong, positive trends for the increases in the amounts of membrane docosahexaenoic acid in the hearts and membrane linoleic acid in the livers of the rats on the diet of beef from cattle fed flaxseed when compared with controls. Serum cholesterol and vascular cell adhesion molecule-1 levels were decreased in rats fed the beef from cattle fed flaxseed. These findings suggest that agriculture practice of feeding a high omega-3 fatty acid diet to cattle can produce positive health benefits to the consumer.

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1. Introduction

Dietary intake of long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) protects against heart disease [1]. Heart health benefits are obtained by eating a diet high in long-chain omega-3 fatty acids results through the physiological properties of the biological compounds formed during either

the cyclooxygenase or lipoxygenase pathways [2]. The positioning of the double bonds from the methyl end of the fatty acid chain causes the omega-3 and omega-6 fatty acids to be handled differently by the body. The end products of the cyclooxygenase pathway, thromboxanes, when made from omega-3 fatty acids, are less biologically active than thromboxanes formed from the omega-6 fatty acids [3,4].

The shorter-chain omega-3 fatty acid, α -linolenic acid (ALA), can be converted to EPA, and the EPA is converted to DHA [3–5]. However, controversy exists on whether an appreciable conversion occurs. If a large conversion of ALA

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to the longer-chain omega-3 fatty acids does occur, then individuals can obtain the same heart health benefits from eating foods high in ALA. Although it is believed that only 5% of ALA is converted to DHA [4], there have been reports that ALA in itself may be anti-inflammatory [6]. A study whereby hypercholesterolemic human subjects were fed extra ALA resulted in reduced inflammatory responses as evidenced by decreased intracellular adhesion molecule-1, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and C-reactive protein [7]. Furthermore, several large studies such as the National Heart, Lung, and Blood Institute; the Nurses' Health Study; and the Health Professional Follow-Up Study have shown a reduced risk of heart disease when eating more ALA in the diet [8-10]. However, many people avoid eating fish, the best source of long-chain omega-3 fatty acids [1]. Therefore, if eating foods high in the shorter-chain omega-3 fatty acids, such as flaxseed, gives positive heart health outcomes, it would benefit the public [11,12]. Making foods rich in ALA can help increase the level of this fatty acid in the diet.

Researchers had success in creating a cattle carcass with higher levels of shorter-chain omega-3 fatty acids than conventionally fed cattle by feeding the cattle a 10% flaxseed diet [13]. Furthermore, differences existed in the ratio of omega-3 to omega-6 fatty acids between the cattle fed flaxseed and the cattle fed a control diet of corn. However, it remained to be determined if fatty acid changes in the membrane phospholipids of those that ate the beef with the higher levels of ALA occurred. Therefore, our objective in this study was to show that rats fed beef with high levels of ALA have increased amounts of EPA and DHA in their hearts and livers when compared with controls and whether factors such as blood cholesterol and one measure of inflammation, VCAM-1, was affected. Here, we report that feeding a diet composed of beef from flax-fed cattle to rats results in positive health outcomes.

2. Methods and materials

2.1. Design

To meet our objective, we used 2 treatment groups, measured body weight weekly and at the time of sacrifice, and collected and weighed the hearts and livers. All methods were approved by the Institutional Animal Care and Use Committee at Kansas State University. Our study used 2 dietary treatment groups, a modified beef diet made of 10% fat from flaxseed-fed cattle and a control diet made from the control-fed cattle.

2.2. Flax-fed cattle and beef sampled

In a study with 80 steers with an initial weight of 743 lb, cattle were fed diets consisting of 0%, 5%, 10%, and 15% ground flax on a dry weight basis with or without supplementation of 200 IU of vitamin E per day (2×4

design). Most of the diets were corn-based. Cattle were fed these diets in individual pens for 120 days. After slaughter, the entire rib section was removed for meat quality analysis. After this initial study, it was determined to use rib-eye steaks from cattle fed 10% flax and without flax that were supplemented with vitamin E. Supplementing with vitamin E improved the red color of the meat, and feeding flax above 10% (15%) resulted in a significant decrease in feed to gain ratio.

2.3. Treatment groups

For this experiment, we used 20 male, weanling, Sprague-Dawley rats housed in an American Association for the Accreditation of Laboratory Animal Care-approved facility. The animals were divided randomly into 2 dietary treatment groups: (1) one group fed a diet where beef from cattle fed a control corn-based diet and (2) an experimental group where beef from cattle fed a 10% flax-based diet were studied. The American Institute of Nutrition diet formulated in 1976 diet [14] was modified to make these diets using beef from the Department of Animal Sciences and Industry at Kansas State University. After analysis, there was 807 μg ALA/g tissue from the 10% flaxseed-fed cattle and 161 μg ALA/g tissue from the control cattle. The final diets consisted of 10% fat from cooked beef, 20% protein from cooked beef and casein, 50% sucrose, 15% cornstarch, and the remaining amount as vitamins, minerals, choline, and methionine. All of the fat was supplied by the beef to meet the 10% fat level. Approximately 16% of the protein came from beef, and the remainder came from casein for a total of 20% protein. No corn oil was added to the diet because the aim of the study was to evaluate the beef fat per se. The rats received food and water ad libitum. The rats were kept in wire-bottom cages and were monitored daily. A 12-hour light and dark cycle was used.

2.4. Fatty acid extraction and preparation of fatty acid methyl esters

After 5 weeks from the start of the study, the rats were anesthetized by using pentobarbital as the anesthetic agent, and death was caused by exsanguinations. The hearts and livers were collected and weighed. The tissue was put into glass tubes and homogenized in phosphate-buffered saline. The samples were stored at -20°C . The 1959 protocol by Blish and Dyer [15] was used to extract lipid followed by *trans*-esterification of only the tissue phospholipids fraction. The phospholipids were separated from the neutral lipids by applying the samples to packed Unisil (Clarkson Chemical Company, Williamsport, Pa) columns and eluting with methanol. Pentadecanoic acid (50 nmol; 15:0) was added to each lipid sample that contained 200 to 600 nmol fatty acid. Methanolic hydrochloric acid (1 mL 3 mol/L) was added to each tube and then bubbled with nitrogen gas. The samples were heated at 78°C for 30 minutes. Then, 2 mL water and 3 mL pentane were added, and the mixture was

shaken vigorously. The solvents were allowed to separate, and the upper pentane layer was removed and placed in tubes with sodium sulfate. The aqueous sample had pentane added again to remove any fatty acid methyl esters (FAME). The pentane samples were transferred to clean tubes and dried down until gas chromatography analysis. Fats from the control and modified beef diets were also extracted and prepared for FAME analysis using the same method except the fractions were not separated.

2.5. Fatty acid analysis

Gas chromatography was performed on the FAME samples dissolved in 50 μ L carbon disulfide. The FAME of the hearts, livers, and diets were separated on a gas chromatograph (model 5890, Hewlett-Packard, Avondale, Pa) equipped with a SP2380 capillary column (Supelco, Bellefonte, Pa) and ChemStation software (Agilent, 2003, A.10.02 Revision, New Castle, Del). Fatty acids were identified using a standard mixture with 20 FAME, 68D standard solution (Nu-Check-Prep, Elysian, Minn), and individual FAME, methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, gamma linolenate, methyl arachidonate, methyl eicosapentaenoate, and methyl docosahexaenoate. The temperature program was 160°C for 10 minutes, raised at 10°C/min to 180°C, and then after 1 minute raised to 220°C at 5°C/min, with a final time of 2 minutes. A split ratio of 20:1 was used for all injections. Identification of the peak areas for the individual FAME (using ChemStation software) occurred, and results were expressed as relative weight percents when compared to 50 nmol/sample of an internal standard, 15:0.

2.6. Serum cholesterol and VCAM analysis

Blood was collected from anesthetized rats using a cardiac puncture. The blood was allowed to clot and then centrifuged at 400 \times g to obtain serum. Total cholesterol was determined from the serum using a commercial kit from Wako Chemicals USA, Inc (Richmond, Va). The assay uses the cholesterol oxidase method.

For VCAM determination, serum had albumin removed before using a Western blot. The serum was dialyzed using a Slide-A-Lyzer (Pierce, Rockford, Ill) and then applied to an Affi-gel Blue Gel column of 100 to 200 mesh (BioRad, Hercules, Calif). After removal of the albumin, 20 μ g of protein from each sample was loaded on a 7.5% SDS-PAGE mini-gel (Bio-Rad, Inc), then the protein was transferred to nitrocellulose using a semidry method. The membrane was probed using as a primary antibody goat antihuman VCAM (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) and used donkey antigoat (Santa Cruz Biotechnology) as the secondary antibody. Detection was via chemoluminescence followed by capturing the images with an Alpha Inotech analyzer and density determination of the VCAM signal.

2.7. Statistical analysis

The body weights of the 2 treatment groups were analyzed using a *t* test for independent samples using Statistical Analysis Software (SAS/STAT User's Guide, 2004, Version 9.1.2, SAS Institute, Cary, NC). The same analysis was used for the fatty acid amounts in the hearts and livers between the 2 treatment groups as well as for the fatty acid amounts in the diets. The following fatty acids were analyzed in the heart and liver samples and presented as means \pm standard error of the mean: palmitic acid, stearic acid, oleic acid, linoleic acid, ALA, arachidonic acid, EPA, and DHA. In addition to the previous 8 fatty acids, other fatty acids in the diet were analyzed. *P* value of .05 or less was considered statistically significant.

3. Results

None of the animals died before termination of the study. Body weight of the rats in the modified beef diet group (294 g) was not significantly different (*P* > .05) from that of the control group (288 g).

3.1. Diet fatty acids

Table 1 shows the fatty acid amounts in the diets. There was a statistically significant (*P* < .05) increase in the

Table 1
Mean fatty acids in control and modified beef diets

Fatty acid	Control (mg/100 g)	Modified beef (mg/100 g)
Lauric acid (12:0)	2.4	2.9
Myristic acid (14:0)	178	228
Myristolic acid (14:1)	27	35
Palmitic acid (16:0)	1373	1302
Palmitoleic acid (16:1)	176	180
Stearic acid (18:0)	773	746
Elaidic acid (18:1n9t)	28	42
18:1n11t	16	40
Oleic acid (18:1n9c)	1753	1608
Linoleic acid (18:2)	228	282
γ -Linolenic acid (18:3)	2.4	–
α -Linolenic acid (18:3)*	15	103
Arachidic acid (20:0)	6.9	5.5
Eicosenoic acid (20:1)	10.5	11.1
Eicosadienoic acid (20:2)	9.0	7.5
Dihomo- γ -linolenic acid (20:3n6)*	12.1	6.5
20:3n3*	–	3.6
Arachidonic acid (20:4)	32	23
EPA (20:5)*	3.0	6.3
Docosapentaenoic acid (22:5n3)*	5.6	11.1
DHA (22:6)*	1.9	2.8
CLA 10t, 12c	2.0	3.6
CLA 9t, 11t	11.2	14.0
CLA 9c, 11t	6.3	7.9
n-3 fatty acids*	23	127
n-6 fatty acids	305	349
n-6:n-3 fatty acids*	13:1	2.8:1

* *P* \leq .05 as determined by *t* test.

amount of ALA, 20:3n3, docosapentaenoic acid, DHA, and EPA in the modified beef diet when compared to the control diet, but lower levels of dihomo- γ -linolenic acid.

3.2. Serum cholesterol levels

Rats fed the modified beef diet had significantly lower serum cholesterol levels (77 ± 1.8 mg/100 mL) compared with rats fed the control diet (107 ± 7.4 mg/100 mL).

3.3. Serum VCAM levels

Rats fed the modified beef diet had significantly ($P < .05$) lower VCAM levels compared with rats fed the control diet (Fig. 1).

3.4. α -Linolenic acid amount

The amount of ALA was not significantly different ($P > .05$) between the 2 treatment groups in either the hearts or livers of the rats. The amount of ALA was the same in the hearts of the modified beef diet group and the control group (16 nmol/100 g tissue), and the amount varied little in the livers: modified beef diet group (51 nmol/100 g tissue) and control group (44 nmol/100 g tissue).

3.5. Liver fatty acids

As shown in Table 2, we found that the amount of linoleic acid and DHA were higher in the modified beef diet group when compared with the controls. The higher amount of DHA was statistically significant with a P value of .001 or less. The P value of .0633 for the increased amount of linoleic acid shows a strong, positive trend.

3.6. Heart fatty acids

As shown in Table 2, we found that the higher amount of arachidonic acid was statistically significant in the controls with a P value of .0006 or less. The amount of DHA

Table 2

Phospholipid fatty acids in liver and hearts of rats fed control or modified beef diets (mean \pm SE)

Fatty acid	Control (nmol/100 g), n = 10	Modified beef (nmol/100 g), n = 10
Liver		
Palmitic acid (16:0)	261 \pm 20.4	259 \pm 19.2
Stearic acid (18:0)	350 \pm 31.5	378 \pm 43.3
Oleic acid (18:1n9c)	143 \pm 28.4	149 \pm 22.6
Linoleic acid (18:2)**	30 \pm 7.3	63 \pm 13.0
ALA (18:3)	44 \pm 4.1	51 \pm 13.6
Arachidonic acid (20:4)	212 \pm 33.5	213 \pm 25.3
DHA (22:6)*	67 \pm 10.0	168 \pm 21.9
Heart		
Palmitic acid (16:0)	148 \pm 17.3	132 \pm 13.2
Stearic acid (18:0)	239 \pm 18.0	189 \pm 26.8
Oleic acid (18:1n9c)	101 \pm 12.7	78 \pm 7.9
Linoleic acid (18:2)	74 \pm 6.1	68 \pm 12.7
ALA (18:3)	16 \pm 2.3	16 \pm 1.8
Arachidonic acid (20:4)*	164 \pm 17.7	81 \pm 9.1
EPA (20:5)	10 \pm 2.1	8 \pm 1.3
DHA (22:6)**	38 \pm 3.2	51 \pm 6.4

* $P \leq .05$ as determined by t test.

** $P \leq .07$ as determined by t test.

showed a nonsignificant increase in the modified beef diet group ($P = .0677$).

4. Discussion

These results suggest that eating beef from cattle fed flaxseed increases the amount of the long-chain omega-3 fatty acid DHA in the membrane phospholipids of the heart and liver. The ratio of dietary n-6:n-3 is similar to what Zhao et al [7] reported in their study using human subjects to have a positive impact upon health indicators. Our findings also suggest a statistically significant increase in the arachidonic acid content of the control hearts and a strong, positive trend for the increase in linoleic acid in the livers when eating the modified beef diet with high amounts of ALA. Although the amount of ALA in the hearts and livers of the rats did not differ between the 2 treatment groups, after analysis we were able to show that the modified beef diet had a statistically significant higher amount of ALA when compared to the control diet and other longer chain omega-3 fatty acids. Therefore, the modified beef diet rats ate more ALA than the controls, but also consumed more EPA, DHA, and docosapentaenoic acid. Although it remains uncertain if the higher levels of the longer chain omega-3 fatty acids in the organs were due to diet, the ALA levels were 7-fold higher in the modified beef diet, and DHA was less than 2-fold greater. This suggests that for liver DHA, where there was a greater than 3-fold enrichment, conversion of ALA to the longer chain fatty acids were likely.

The lower cholesterol levels in the modified beef diets could be likely due to increased polyunsaturated fatty acids that that diet had compared to the control diet, but this is speculation. The results of the lower VCAM-1 in the rats fed

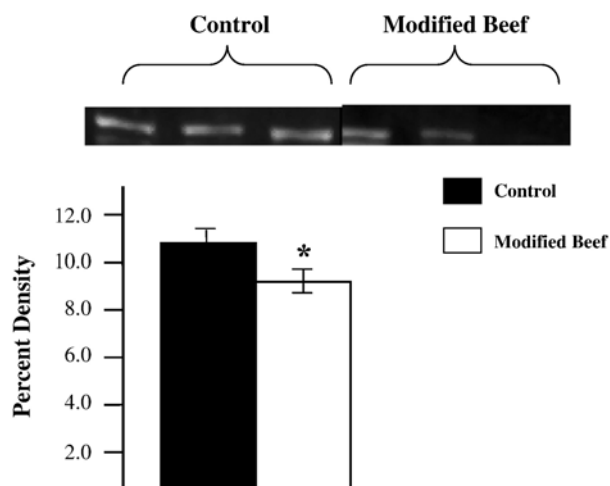


Fig. 1. Serum VCAM levels for control and modified beef diet rats (* $P \leq .05$).

the flax modified diet is in agreement with the findings of Zhao et al [7], where humans fed ALA had lower levels of inflammatory compounds such as C-reactive protein, E-selectin, intracellular adhesion molecule-1, and VCAM-1. Collectively, these results imply a protective role of omega-3 fatty acids from ALA sources, including that from beef of cattle fed 10% ground flaxseed.

Previous studies show little conversion of ALA to the longer-chained omega-3 fatty acids EPA and DHA [4,5]. It has been advocated that the best way to achieve the benefits of omega-3 fatty acids is by eating foods high in the long-chain omega-3 fatty acids EPA and DHA. As shown in Table 2, we were able to show there was an increased amount of the DHA in the hearts and livers of the rats on the modified beef diet. The increase was statistically significant in the livers, and a strong, positive trend was seen in the hearts. One reason for this may be the greater levels of EPA and DHA in the modified beef diet. Another reason could be due to a conversion of ALA to DHA.

We also found an increase in arachidonic acid in the hearts of the control rats. In this case, the results may imply an efficient conversion of linoleic acid to arachidonic acid because there was no difference in the 2 diets for either of these 2 fatty acids. Furthermore, the livers of the rats on the modified beef diet showed an increase in linoleic acid. α -Linolenic acid and linoleic acid use the same pathway for conversion to long-chain fatty acids. A possible mechanism for the increased amount of linoleic acid in the livers of the modified beef diet group may result from an increased use and conversion of ALA to DHA. This would impede conversion of linoleic acid to arachidonic acid, thus causing linoleic acid levels to remain high in the liver.

The level of omega-3 fatty acids in the cooked beef per 100 g was 44 mg for the non-flax-fed cattle beef and 83 mg for the 10% flax-fed beef. For the flax-fed beef, half of this was in the form of ALA and the remainder in EPA and DHA. Using these values, two 3-oz servings of beef per day would supply 140 mg of omega-3 fatty acids daily. Although the content doubled, individuals with heart disease are urged to consume 1 g of EPA plus DHA per day [16]. Kris-Etherton et al [17] reported that 1.6 g/d of omega-3 fatty acids was consumed by Americans, and 1.4 g was due to ALA, with only 0.2 mg coming from EPA and DHA. The American Heart Association recommends for those individuals without heart disease that they eat a variety of fish at least twice a week. Include oils and foods rich in ALA (flaxseed, canola, and soybean oils; flaxseed and walnuts) [16]. It would appear to be important to try and improve the omega-3 fatty acid content of the entire food supply when possible to help reach this goal.

In conclusion, our study was the first to look at the effects of eating a high-ALA diet of beef from cattle fed flaxseed and the impact on long-chain omega-3 fatty acid composition of EPA and DHA in the membrane phospholipids of the heart and liver using a rat model. We were able

to show that an increase in DHA occurs in the hearts and livers of rats fed a high-ALA diet from cattle fed flaxseed. Improving the nutritional quality of beef, in this case omega-3 fatty acids, by simply changing a part of the cattle's diet was demonstrated.

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