

# **Critical Reviews in Food Science and Nutrition**



ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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**To cite this article:** Christopher P. Haskins, George Henderson & Colin E. Champ (2018): Meat, eggs, full-fat dairy, and nutritional boogeymen: Does the way in which animals are raised affect health differently in humans?, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2018.1465888

To link to this article: <a href="https://doi.org/10.1080/10408398.2018.1465888">https://doi.org/10.1080/10408398.2018.1465888</a>

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Publisher: Taylor & Francis

**Journal**: Critical Reviews in Food Science and Nutrition

**DOI**: https://doi.org/10.1080/10408398.2018.1465888

Meat, eggs, full-fat dairy, and nutritional boogeymen: Does the way in which animals are raised affect health differently in humans?

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Financial Disclosures: None declared

Conflicts of Interest: CEC receives income from health books that he wrote.

Key words: diet quality, dietary recommendations, cancer, animal food

Running Title: Does the way in which animals are raised affect health differently in humans?

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#### Abstract:

**Background:** Food recommendations to improve cancer prevention are generally based on epidemiologic data and remain inconsistent. These epidemiologic studies, while controversial, have generally produced results that caution against the consumption of high-fat foods, including eggs, red meat, and full-fat dairy, such as butter and cheese. Yet, limited data exist assessing the

quality of individual sources of these foods and the effect each has after its consumption. This study set out to assess the impact sources of food within the same groups from animals raised differently on variables associated with health in human studies.

**Methods and Materials:** A search was conducted through MEDLINE, Embase, and PubMed. In total, twenty-nine studies met inclusion criteria, measuring physiologic changes in humans after consuming animal products following animal diet manipulation. A meta-analysis was attempted to assess the differences between the cohorts in these studies, but was aborted due to poor study quality, vast differences in study design, and a limited number of studies.

**Results:** Studies varied by animal, animal diet manipulation, food product, and overall design. Significant differences were present between groups eating the same food (cheese, beef, eggs, and butter) from animals raised differently, including levels of: conjugated linoleic acid, omega-3 fatty acids (alpha linoleic acid [ALA], docosahexaenoic acid [DHA], and eicosapentaenoic acid [EPA]), and inflammatory factors (triacyl glycerol [TAG], interleukin-6 [IL-6], interleukin-8 [IL-8], tumor necrosis factor [TNF], and C-reactive protein [CRP]). Lipid levels were minimally affected.

Conclusions: This work highlights differences in human health markers after consumption of the same foods from animals raised differently. Overall, lipid levels remained relatively neutral, but significant changes in inflammatory and other serum markers and phospholipids were present. Future studies and dietary recommendations should consider how animals are raised, as this can produce different effects on health markers.

## **Introduction:**

Dietary recommendations for cancer prevention, including food types and macronutrient composition, vary significantly between sources. These recommendations are based on epidemiologic studies with results that differ drastically based on data evaluation methods, dietary assessment techniques, and inherent biases. Initial massive epidemiologic studies performed by Doll and Peto found an association between several foods and cancer, including coffee, fish, and dietary fat. While they cautioned against the extrapolation of the association of dietary fat and breast cancer with population-wide recommendations due to a strong connection with Westernization, enthusiasm within the medical field was escalating following the massive public health overhaul occurring largely due to Sir Richard Doll's monumental epidemiologic study linking smoking and lung cancer. A natural progression led to the attempts to link other lifestyle behaviors with cancer risk using the powerful tool of epidemiology.

However, as Doll and Peto initially cautioned, multiple studies assessing hundreds of thousands of individuals did not confirm their initial findings. In fact, while dietary fat was linked to colon cancer via several epidemiologic studies, the link with breast cancer was disproven multiple times. Several studies reveal an adverse relationship when dietary fat is replaced with carbohydrate, illustrating the interaction between numerous variables. Furthermore, even dietary recommendations to reduce dietary fat and increase fruit and vegetable consumption to reduce the risk of breast and other cancer have been inconsistent, at high risk of confounding, and often unsupported by the data. Vegetable studies have, however,

begun to suggest that specific cruciferous and allium vegetables may reduce the risk of cancer, but overall studies have been inconsistent.

While newer vegetable-based data imply that specific types of foods from the same grouping may have a different impact on cancer risk, little is known about how different techniques of raising the same types of animals could lead to diverse health effects after the consumption of their meat, eggs, or dairy products. Multiple studies have shown that food products from animals raised on pasture instead of grains contain significantly higher amounts of nutrients that may protect against cancer, like omega-3 fatty acids and conjugated linoleic acid. Due to the general recommendation against dietary fat consumption over the past several decades, there has been a lack of scientific dialogue regarding the differences pasture-raised animal byproducts may have on health and cancer prevention. In this work, we set out to perform the first thorough assessment of the differing effects on measurable values in humans after the consumption of eggs, butter, cheese, and meat from animals raised differently.

# **Method and Materials:**

A systematic review, comparison, and analysis was performed of all studies where subjects were fed animal-based foods including butter, eggs, meat, and cheese, but randomized to different groups based only on the mechanisms by which the animals were raised. We performed multiple searches through MEDLINE, Embase, and PubMed to identify eligible trials published from database inception until March 6, 2013. Multiple searches were employed as many articles that fit the inclusion criteria did not share common index terms. Inclusion criteria consisted of two components: an experimental manipulation of animal diet consisting of tangible food changes, and a physiologic measurement in humans after consuming the animal products. Studies that did not directly change animal diet or analyze a measurable parameter in humans were excluded. Studies that added synthetic vitamins as the only dietary change were excluded. Similarly, any study that only assessed subjects' subjective taste preferences of the different food products were also excluded Initially, search results revealed 31 studies on which nine met inclusion criteria. These nine studies were then analyzed and any additional studies that were referenced within that met inclusion criteria were also included. Due to the complexity of the indices, reviewing the paper's sources provided the majority of studies in this review. Twentynine studies therefore met initial criteria. The studies were reviewed independently by multiple authors (CH, CC) to assess inclusion criteria.

A meta-analysis was attempted to assess the differences between the cohorts in these studies, but was aborted due to design differences, poor study quality, and a limited number of studies. The studies included different animal sources such as red meat, poultry, and dairy products. They also differed drastically in subject inclusion criteria, diet composition, and physiologic parameters measured. As such, any statistical analysis was also aborted.

All studies were assessed focusing on the quantification of the multiple factors that have been shown to modify cancer risk, including conjugated linoleic acid (CLA), omega-3 fatty acids (alpha linoleic acid [ALA], docosahexaenoic acid [DHA], and eicosapentaenoic acid [EPA]), and inflammatory factors (triacyl glycerol [TAG], interleukin-6 [IL-6], interleukin-8 [IL-8], tumor necrosis factor [TNF], and C-reactive protein [CRP]). The effect, if any, on serum cholesterol levels was included for discussion purposes only as they have been poorly correlated with cancer risk. The results for each study, if applicable, will be discussed under the categories 1) CLA

with effects on lipids and cytokines, and 2) Omega-3 fatty acids, as these were the predominant factors assessed in the studies examined.



#### **Results:**

# Conjugated Linoleic Acid:

CLAs are 18-carbon fatty acids converted from linoleic acids in foliage and conjugated by bacteria in the gut of ruminant animals. Preclinical data reveal CLA as a potential effector in multiple physiologic processes including fat metabolism, atherosclerosis, cholesterol, blood pressure, carcinogenesis, and immunomodulation. 13,14,15,16,17

The most robust data on CLA is on fat metabolism. In vitro studies suggest CLA increases the efficiency of fatty acid uptake from tissues for lipolysis and inhibits the progression of adipogenesis. In a multitude of animal studies that range from exercise to the obese, these regulatory roles are accomplished through similar mechanisms, including the activation of PPAR-gamma and decreased expression of fatty acid binding and transporter proteins. <sup>19,20,21</sup> Furthermore, studies reveal improvements in body fat composition, insulin sensitivity, and blood lipid levels after CLA supplementation. <sup>20,22</sup> Up-regulation and activation of PPAR-gamma also increases expression of uncoupling proteins (UCPs) of the respiratory chain, <sup>23</sup> serving as an additional potential mechanism by which CLAs may increase energy expenditure and lipolysis. In humans, however, the effect of CLA supplementation on body mass reduction is mixed. <sup>24,25,26</sup>

Multiple in vitro studies suggest anticarcinogenic properties of CLAs, through their effect on cell-cycle control, apoptosis, hormone levels, cellular proliferation, and angiogenesis.<sup>27</sup> Animal studies reveal potent anti-tumor effects of CLA, particularly in breast cancer.<sup>28–31,32,33–35</sup> CLA isomers have differing effects on tumorigenesis, suggesting the ratio of CLA isomers is critical in supplements.<sup>36</sup> Other studies point to both CLA isomers as having anticarcinogenic properties.<sup>34</sup> Supplementing foods augmented with CLA, like enriched butter, decreases tumorigenesis, tumor volume, and metastasis from breast cancer in rat studies.<sup>19,25</sup> Data suggest CLA mixtures originating from ruminant products have higher antiproliferative potency towards tumor cells than pure synthetic preparations.<sup>38</sup>

Data in humans is limited. Serum levels of CLA are inversely related to breast cancer risk, and dietary consumption of CLA is associated with lower rates of breast cancer.<sup>39</sup> A population study of over 60,000 Swedish women associated higher intakes of high-fat dairy food – naturally containing high levels of CLA – with a reduced risk for colorectal cancer.<sup>40</sup> For every two additional servings of high-fat dairy, the risk of colorectal cancer fell by 13%. However, the same database provided no evidence of reduced risk of breast cancer.<sup>41</sup> A study in Finnish women revealed that serum CLA was associated with a significantly decreased risk of breast cancer.<sup>39</sup>

#### Dairy:

Overall, ten studies examined the effect of CLA from butter and cheese on serum levels of CLA (Table 1). Of the studies examined, five out of 10 directly measured serum CLA in subjects and three measured CLA in food products only. CLA was significantly increased in all cases. The other two studies used dietary changes documented to increase CLA in the literature, as described below for each study.

Sofi et al. used a crossover design randomizing 10 subjects to the consumption of weekly 200g of pecorino cheese versus a commercially available cow cheese for 10 weeks each. A traditional Italian cheese, pecorino is naturally rich in CLA and produced from the milk of sheep fed on grass. Study participants that consumed the CLA-rich cheese for ten weeks experienced

significant decreases in the inflammatory factors IL-6, IL-8, TNF- $\alpha$ , and vascular endothelial factor (VEGF), a potent angiogenic factor linking inflammation and cancer, and whose overexpression is key in cancer initiation and progression.

Desroches et al. enrolled sixteen subjects in an 8 week crossover study using CLA-enriched butter to assess changes in lipid profile and body composition. <sup>45</sup> Sunflower seeds were added to standard dairy cow feed to induce a ten-fold increase in CLA levels in their milkfat (4.22g CLA/100g butter fat versus 0.38g CLA/100g butter fat). The subjects ate a standard American diet (15% protein, 45% carbohydrate, and 40% fat) with the two butters incorporated. Both groups experienced a nonsignificant decrease in total cholesterol and no appreciable change in abdominal fat mass on CT scan.

Burdge et al. used a double-blind, crossover study with 31 men to evaluate changes in plasma CLA levels following cattle feed manipulation. <sup>46</sup> They added sunflower seeds and fish oil to standard cattle feed for 7 days to increase CLA in the dairy fat. Subjects then consumed the milk-fat in various forms of dairy products. After 6 weeks, CLA was significantly increased in both the plasma and cellular lipids of subjects from the modified milk-fat dairy.

Tholstrup et al. enrolled 42 men in a 5 week, randomized, parallel intervention study. The experimental groups consisted of butter enriched with a specific CLA known as vaccenic acid (3.6g vaccenic acid/day), from the addition of sunflower seeds to cow feed. The butter was baked into breads and pastries, and subjects consumed 115g of fat/day. The vaccenic acid-rich diet decreased both total and HDL cholesterol compared to controls (6% and 9%, respectively), with no change in the HDL:total cholesterol ratio. In addition, serum levels of CLA incorporated into TAGs, cholesterol esters, and phospholipids were significantly increased from the vaccenic acid-enriched butter.

Tricon et al., in a very similar study, enrolled 32 healthy middle-aged men in a double-blind, crossover study to eat dairy products enriched in CLA from sunflower and fish oils added to cow feed. There were no significant effects found on the lipid profile after consuming high-fat CLA enriched dairy.<sup>48</sup>

Tholstrup et al. modified Danish cow feed in order increase oleic and stearic acid, and lower palmitic and myristic acid within the cows' milk. 49 Although data are mixed, oleic and steric acid are felt to have a limited effect on serum cholesterol, while palmitic and myristic acid are felt to raise it. 50 Eighteen men were randomized to a daily diet with 25g per meal of butter containing the modified milkfat or the same diet with 25g of conventional milkfat butter. After 4 weeks, there was no change in low-density lipoproteins (LDL), high-density lipoproteins (HDL), or APO-B. However, significantly elevated levels of CLA were incorporated into serum triacylglycerol in the modified milk fat group.

Ritzenthaler et al. enrolled 44 healthy, lactating women in an 8-week, parallel intervention study design that randomized subjects to three groups. Each group ate 113g of experimental cheddar cheese, either with control, low, or high levels of CLA, achieved by replacing grains in cow feed with grass, which significantly increases CLA in cows' milk. After 8 weeks, rumenic acid (RA, a type of CLA) was significantly increased in the plasma of the high- versus low-CLA cheese diet group. RA was also increased in the milk of the lactating women on the high-CLA cheese diet. Of note, no differences were seen between the groups regarding cardiovascular disease risk factors, total cholesterol, TAG, or lipoprotein concentrations.

Meat:

Four studies in total assessed the impact of consuming meat from differently raised cows, including one study using kangaroo meat to represent a wild source. Only one study assessed changes in CLA composition, most studies focused on lipid changes, and the study utilizing kangaroo meat assessed changes in inflammatory factors in study subjects.

An Australian group, Arya et al., measured post-prandial serum markers in humans after consumption of Kangaroo meat or Wagyu beef.<sup>51</sup> Kangaroo meat was used as an example of a lean game meat from animals consuming their native wild diet. Their meat is felt to improve lipid levels,<sup>52</sup> whereas Wagyu beef is a common hybridized breed conventionally raised with standard cow feed in an effort to marbleize their fat content to increase flavor.<sup>53</sup> The Kangaroo meat was also found to have high levels of CLA. Using a crossover design, ten subjects were randomized to either 100g of kangaroo or Wagyu beef, along with a baked potato and peas. Triacylglycerol (TAG), IL-6, TNF-α, and CRP levels were measured at baseline, and 1 and 2 hours following consumption. Six to 10 days later, the subjects ate the same meal with the other meat, in a crossover design, and repeated blood samples. The results revealed significantly increased TAG, TNF-α, and IL-6 in the serum of subjects consuming Wagyu beef compared to Kangaroo meat at both 1- and 2-hour intervals.

Brown et al. tested 18 women with two diets differing in pasture-fed vs. grain fed beef and dairy, measuring daily CLA and blood lipids at the end of the 56-day trial. <sup>54</sup> Inclusion criteria included women aged 18-39 with BMIs ranging from 20-30, with normal or mildly increased blood cholesterol. Subjects were randomized to two groups eating a diet controlled for energy and macronutrient composition, differing only in pasture vs. grain-fed cattle. CLA in the diet was increased in the pasture-fed diet to 1.17g/day compared to 0.35g/day. There were no significant differences in subjects' blood lipids, body composition, or insulin sensitivity between the two groups.

Gilmore et al. tested grain-fed beef patties with higher MUFA content versus pasture-fed beef patties with higher SFA content. Thirty healthy males between the ages of 23 and 60 were enrolled. Subjects were tested in a two period, five-week intervention, randomized crossover design with a four-week washout. Five beef patties were supplied each week to each participant. Total energy and macronutrient composition were maintained throughout the study. Body weight, BMI and total body fat were unchanged throughout the study. Grain-fed, high MUFA beef significantly increased plasma HDL-C compared to pasture-fed, from 1.17 to 1.24. There was no change in triglyceride, total cholesterol, or LDL-C. HDL particle size was decreased with both interventions, with no change in LDL particle size. Particle counts were not reported.

Weill et al. compared diets of multiple animal products with the addition of linseed oil to conventional feed and the effect of circulating n-3, CLA, and plasma lipids in participants. A double-blind, randomized, crossover study of 75 healthy volunteers was conducted. The study consisted of two, 35-day study periods with an 18-day washout. In subgroups, 50 individuals ate entirely food allocated from the study design, and 25 individuals ate a "half-dose" (2/3 meals from the study design) to adjust for any dose-effects. Five percent linseed oil was added to standard, conventional, grain-feed of dairy cows, laying hens, pigs, chickens, and lambs. In subjects who ate the linseed-oil fed animal products, serum CLA and plasma and erythrocytes n-3 fatty acids were significantly increased. The same trend was observed in the "half-dose" group. There were no changes in total cholesterol or TAG.

# Omega 3 Fatty Acids

Omega-3 (n-3) fatty acids account for a relatively small proportion of fat within the diet. After their consumption, they are incorporated into the cellular membrane, leading to a measurable increase in n-3 content. They are generally considered beneficial to multiple organ systems including the brain and cardiovascular system,<sup>56</sup> and give rise to numerous beneficial processes, including a reduction in inflammation via downregulation of inflammatory cytokines a reduction in triglycerides, along with antithrombotic and antiarrhythmic effects.<sup>57,58</sup> Along these lines, an elevated ratio of n-6:n-3 fatty acids has been associated with an increased risk of several cancers;<sup>59</sup> as n-3 compete with n-6 to reduce the expression of eicosanoids, like prostaglandin, and COX-2, changes that can act to reduce the risk of cancer.

Epidemiologic data across multiple countries suggest that cultures who consume significant amounts of fish, and thus higher n-3 and less n-6 fatty acids, have lower rates of cardiovascular and other diseases. The pharmaceutical supplementation of omega-3 fatty acids, usually in the form of fish oil, has yielded mixed results in the prevention and treatment of cardiovascular disease; however, epidemiologic studies show reductions in multiple diseases, including cancer, with higher n-3 intake. 57,61

Preclinical animal studies reveal that n-3 fatty acids efficit a chemosuppressive effect on microscopic metastatic cancer cells, 62 thus illustrating the potential anticancer benefits of increasing n-3 fatty acid levels. Nineteen studies met criteria assessing changes in n-3 fatty acid levels after the consumption of varying food sources (Table 2).

Eggs:

In total, 13 studies met criteria for testing the effects of chickens raised differently to optimize n-3 content of their eggs. Most studies revealed varying changes in blood lipids. Six studies assessed change in n-3 content after consumption, all revealing significant changes.

Oh et al. tested 11 subjects consuming four eggs per day for four weeks in a crossover design. Fish oil was fed to chickens to increase n-3 fatty acids, versus standard chicken feed-fed controls. After four and eight weeks, they measured cholesterol, triglycerides, phospholipids, and blood pressure. Total cholesterol increased following control egg consumption but did not change in response to n-3 enriched eggs. VLDL cholesterol and LDL-cholesterol significantly increased following control egg but not experimental egg consumption. HDL and triglycerides were not significantly affected. Furthermore, n-3 enriched eggs decreased both systolic and diastolic blood pressure following 4 weeks, while control egg consumption had no effect.

Ferrier et al. conducted two experiments, both adding flaxseed to hens' meal to increase the omega-3 content. <sup>63,64</sup> In the first study, five subjects were fed experimental or control eggs for two weeks in a crossover design. <sup>63</sup> After 7 and 14 days, triglyceride levels fell significantly in the experimental egg group from day 0, but not the control group. Total and HDL cholesterol were significantly raised in the control group after 14 days. In the second experiment, 28 male subjects consumed four eggs per day from hens fed 0%, 10%, or 20% ground flaxseed. <sup>64</sup> The subjects were split into 3 groups and followed a cyclic Latin-square design, eating each type of experimental or control egg for two weeks at a time, followed by 2 weeks of washout. The fatty acid composition of the eggs varied between groups, with significantly more DHA and omega-3 in the 20% flaxseed eggs. The diet did not change plasma levels of cholesterol, triglyceride, or HDL; however, platelet phospholipids did change in accordance with experimental egg consumption, with significant increases in DPA, DHA, n-3, and decreases in both n-6 and n-6:n-3 ratio.

Jiang et al. enrolled 23 subjects, randomized to two groups. Each subject was fed two eggs every morning for 18 days. <sup>65</sup> Experimental eggs came from hens fed an addition of flaxseed and sunflower seed to conventional hen feed. N-3 were increased in the experimental eggs as well as plasma in the human subjects. Experimental eggs increased HDL-C and decreased triglycerides, whereas traditional eggs significantly decreased both.

Farrell et. al. conducted a study of four groups of 14 subjects, each with a different type of experimental egg. <sup>66</sup> Four groups of hens were fed a diet differing only in added oils consisting of 50g fish oil, 30g fish oil plus 10g linseed oil, 20g fish oil plus 10g each of linseed and canola oils, or the control diet with 40g sunflower oil. Eggs from chickens fed a greater percentage of fish oil contained more n-3 fatty acids. Subjects ate 7 experimental eggs per week for 24 weeks, without other dietary specifications or restrictions. At 16 and 24 weeks, BMI, HDL, LDL, triacylglycerol, blood pressure, and plasma n-3 levels were measured. Statistically significant changes were found between plasma n-3 of all experimental egg groups versus the control group.

Van Elswyk et al. recruited 44 male and female graduate students and professors from the University of Texas A&M. <sup>67</sup> Subjects were screened for cardiovascular disease, and then divided into three groups. Each group ate four eggs weekly, split into control eggs, hnoleic acid (LNA) enriched eggs or DHA enriched eggs. Hens were fed diets either devoid of n-3 for control eggs, 5% flaxseed for LNA-enriched eggs, or 1.5% menhaden oil for DHA-enriched eggs. The groups ate 4 weekly eggs for 6 weeks, followed by no eggs for a two week "washout," and then switched groups in a Latin-square design. The total experiment was 26 weeks, and subjects were blinded to the egg source. No significant differences were found between overall blood lipids or platelet responsiveness.

Surai et al. recruited forty healthy volunteers randomized to a designer egg or a control egg diet per day. 68 Designer eggs were produced from hens eating regular hen meal plus supplemented selenium, lutein, vitamin E, and DHA from tuna oil. The subjects then ate one egg per day for 8 weeks without other dietary specifications. DHA, alpha-tocopherol, lutein, and selenium were all significantly increased in the designer eggs versus the control eggs, translating to significantly increased plasma values in DHA, alpha-tocopherol, and lutein. Plasma lipid concentrations and blood pressure were not statistically different.

Lewis et al. conducted a study of 25 subjects with hypercholesterolemia but without any other diagnoses. These subjects were divided into three groups, with each group eating a low-fat diet (30% fat and 10% saturated fat) with supplementation of no eggs, 12 control eggs per week, or 12 n-3 fatty acid enriched eggs. In a Latin-square design, the groups ate each dietary modification for 6 weeks for a total of 38 weeks for the study. N-3 enriched eggs were produced by the addition of flaxseed to hen meal. Blood lipid levels were then measured. Twenty three out of 25 subjects saw no difference in total, LDL or HDL cholesterol, and no difference in triglycerides. Two subjects, termed "responders," did see a significant increase in LDL between the addition of eggs vs. no eggs. Furthermore, responders also saw a significant decrease in triglycerides after eating n-3 eggs versus no eggs.

Maki et al. tested 150 subjects with mild hypercholesterolemia. Subjects were divided into two groups that ate 10 eggs per week in addition to their normal diet. DHA levels were manipulated by the addition of marine algae to hens producing DHA-enriched eggs, increasing levels from 20 to 147 mg. The subjects' diets were not otherwise manipulated. Blood lipids were measured at 6 weeks, and both groups saw a decrease in triglycerides and increase in HDL cholesterol. The DHA-enriched eggs group had increased total cholesterol and LDL-cholesterol compared to baseline, and increased LDL-cholesterol compared to the control eggs. In an

analysis of the effects of treatment in subjects with a BMI at or less than 30, the DHA-enriched eggs produced a significantly greater decrease in triglycerides and increase in HDL-cholesterol. Additionally, although LDL-cholesterol increased in DHA-enriched eggs, small-LDL particle count did not increase as measured by NMR.

Sindelar et al. recruited 12 physically active adults in another n-3-enriched egg study. Participants were randomized into two groups, with each group eating six control or n-3 enriched eggs per week for four weeks in a crossover design. Flaxseed was added to the hens' diet to increase n-3 from 60mg in control eggs to 350mg in experimental eggs. A significant elevation in triglycerides resulted from the n-3 enriched eggs. There was no difference in total, LDL, of HDL cholesterol levels. Furthermore, ALA was significantly elevated in serum following n-3-enriched egg consumption. DHA was also elevated, though not significantly.

Gillingham et al. tested 14 hypercholesterolemic patients on statins <sup>72</sup> The subjects were randomly divided into two groups given two control eggs or two n-3-enriched eggs daily for three weeks. Both menhaden oil and ground flaxseed were added to the hen meal. Two n-3-enriched eggs contained 629mg total n-3 versus 100mg n-3 in the control eggs. Patients continued their regular diets except for avoiding alternate sources of n-3, remained on the statin, and subsequently had blood lipids measured at the beginning and end of the three-week period. The experimental egg group experienced a significant increase in HDL cholesterol from baseline. No other blood lipids were significantly changed, and no significant differences occurred between diets. Serum phospholipid fatty acid composition was also measured, with significant increases from the experimental eggs in total n-3, DHA, EPA+DHA, and a significant decrease in n-6:n-3 ratio.

Bovet et al. recruited 25 healthy individuals for a double-blind crossover study. Subjects were randomly divided into two groups; one ate five weekly control eggs or n-3-enriched eggs for three weeks, followed by three weeks of crossover. Experimental eggs were enriched with 5% tuna oil to increase the n-3 percentage from 0.9% to 7.8%. Before testing and after each three-week experimental period, blood lipids were measured. The experimentally enriched eggs produced a significant 18.3% reduction in serum triglycerides between groups. No other significant differences were found.

Burns-Whitmore et al. conducted two studies analyzing n-3-enriched eggs on human physiology. In the first study, they recruited 20 healthy lacto-ovo vegetarians to assess lutein bioavailability from n-3 enriched eggs or organic eggs versus no eggs. The study design consisted of three treatment periods of eight weeks where subjects ate six eggs per week, with four weeks of washout between treatments. The subjects' diets were tracked, but no major changes were made aside from asking them to avoid foods otherwise high in lutein. The n-3 enriched eggs were produced with flaxseed meal, and the organic eggs with soy-based organic meal. At the start and conclusion of each study period, serum lutein, beta-carotene, and zeaxanthin levels were measured. Serum lutein was significantly elevated in both egg treatment groups. Beta-carotene and zeaxanthin were not significantly changed, nor were any inflammatory factors, including in IL-1β, IL-6, TNF-α, soluble intracellular adhesion molecule-1, E-Selectin, or CRP.

In a second similar study by this group, with walnuts added for comparison. DHA content was significantly elevated following n-3-enriched eggs. Furthermore, walnuts significantly decreased triglycerides, total cholesterol, apo B particles, and HDL compared to control eggs. This difference was not significant compared to n-3-enriched eggs.

#### Meat:

Three studies assessed beef from cows raised differently, two of which assessed changes in n-3 levels in subjects. Both revealed significant increases in the group consuming meat from grass-fed cows.

Adams et al. conducted a study on high SFA beef versus high MUFA beef.<sup>76</sup> The SFA beef was produced with grass-fed cattle and the MUFA with conventionally fed cows. In a crossover design, 10 mildly hypercholesterolemic subjects ate 114g beef per week for five weeks with a three-week washout. Serum lipids were measured, revealing a significant increase in triglycerides, HDL-C, and LDL particle diameter distribution percentage from the consumption of meat from grass-fed cows.

McAfee et al. compared grass-fed and grain-fed red meat intake on serum n-3 PUFAs and analyzed any subsequent changes in blood lipids between groups.<sup>77</sup> In a double-blind study, 38 healthy volunteers were randomized into two groups for a four-week intervention consisting of approximately 470g red meat per week, grass fed vs. conventional. The study precluded fish intake, but the diets were not otherwise controlled, with nutrient intake diaries attempted. The grass-fed meat group experienced significant increases in serum platelet DHA, EPA, and total n-3 content from baseline and the control group. There were no differences in TAG, total cholesterol, or blood pressure.

As discussed above, Weill et al. compared diets of multiple animal products with the addition of linseed oil to conventional feed. Those subjects consuming this beef experienced a significant increase in plasma and erythrocytes n-3 fatty acids were significantly increased.

## Cheese:

Only two studies assessed the effect of cheese on omega-3 fatty acid levels, both by the same group. Intorre et al. tested effects of cheese produced from cows fed a combination of grass and maize silage with the addition of 5% linseed oil. Thirty volunteers were enrolled in a double-blind, randomized crossover trial and randomized to experimental cheese or control cheese for four weeks, followed by a week of washout, and then four weeks of the other cheese. They consumed three servings of 50g of cheese per week. Omega-3 fatty acids were significantly increased in the experimental cheese arm, however, there were no significant differences in the serum fatty acids. Lipid profiles were not significantly different between groups. Oxidized LDL and serum myristic acid were significantly decreased after four weeks in the experimental cheese group, while vitamin C and E levels rose significantly.

The same group, Intorre et al. published another study, also with 30 volunteers, with the same experimental design and 5% linseed cheese. The authors performed a more extensive analysis of individual fatty acids in the subjects' serum and found no significant differences over the course of the experiment.

## Discussion

To our knowledge, this is the first review assessing the available studies revealing the effects of animal-derived foods on health markers based solely on the methods of raising these animals. Multiple studies reveal that feeding randomized participants the same foods while simply changing the feed of the animals can have significant effects on serum markers and health risk factors. However, the differing physiologic effects of participants consuming these foods has yet to be incorporated into dietary recommendations. <sup>1</sup>

Current dietary recommendations for lowering cancer risk, including the American Cancer Society guidelines, generally recommend reducing fat and animal product intake with minimal focus on the source and no focus on how animals are raised. Recommendations often promote the consumption of omega-3 fatty acids from nuts and fish for their anti-inflammatory effects, however, no mention is made of the benefits of conjugated linoleic acid found largely in pasture-raised ruminants and dairy, and rather, minimization or avoidance of these sources are recommended.

Furthermore, while the avoidance of foods containing high amounts of fat, like full fat cheeses, butter, and eggs, has generally been recommended to improve health and reduce the risk of cancer, the majority of the population continues to consume these foods. For instance, the NHANES database reveals that 96% of men aged 31-50 overconsume the recommended amount of solid fat, including dairy fat. <sup>80</sup> Perhaps recommendations and a focus on the above data and foods, including fats that may provide beneficial inflammatory changes, may allow those individuals to optimize the ratio of beneficial to harmful foods to improve compliance and their overall diet. Along these lines, the above data reveal that the same sources of foods could have drastically different health effects, especially if scaled to the general population. Newer recommendations should include differentiation of the methods with which animals are raised, if there is in fact data to show a difference, as discussed above.

Recent data reveal that the American public has reduced fat intake and increased simple carbohydrate consumption to match dietary recommendations. Studies have consistently implicated increased serum glucose and insulin with an increased risk of cancer. Recommendations to avoid fatty foods, which could potentially contain many anticancer compounds, may favor a diet high in simple carbohydrates, thus elevating both serum glucose and insulin. He NHANES database furthermore reveals that dietary fat was largely replaced by an increase in simple carbohydrate consumption, which may promote an increased risk of cancer along with a host of other chronic diseases. Indeed, population studies confirm that overall fat consumption has decreased over the past several decades, while carbohydrate and fructose consumption has increased significantly. The past several decades in the carbohydrate and fructose consumption has increased significantly.

While red meat, butter, cheese, and eggs have been generally treated as food "boogeymen" with most nutritional recommendations endorsing their avoidance, these individual foods may vary significantly in nutritional value and the corresponding effect on those who consume them. If grass-fed butter, n-3 enriched eggs, or wild-game meat have a relatively neutral effect on lipid levels, whereas their conventionally raised counterparts raise serum lipids, as noted above, studies grouping these foods under the same category may need to be reexamined. These foods also provide significant improvement of serum inflammatory markers and levels of CLA and n-3 fatty acids, further questioning the veracity of older dietary recommendations. Furthermore, many plant oils contain high amounts of linoleic acid, which, while data varies, may result in a pro-inflammatory effect. Ruminants conjugate linoleic acids into a molecule that appears to be anti-inflammatory, anti-carcinogenic, and may reduce body mass.

Indeed, the varying physiological effects of the same sources of food depending on their diets, as shown above, may account for the drastically different results from similar epidemiologic studies.<sup>2</sup> Such concerning oversimplification of food sources may be one of the myriad reasons why studies have revealed, for instance, that dietary consumption of fat is not associated with breast cancer occurrence and may actually be protective.<sup>5,7</sup> These findings may collaborate with preclinical data in mice, revealing a benefit of CLA-dense dietary fat sources,<sup>89</sup>

including those from dairy sources and even beef fat from grass-fed cows.<sup>38</sup> Additionally, recent population studies support the protective link of CLA-rich dairy from cancer.<sup>39,41</sup> While data is limited, the makeup of milk derived from animals generally mirrors that of their carcass, with a similar distribution of long chain fatty acids, thus the overall food supply benefit of improving dairy and meat quality may be greater than predicted from these foods alone. Finally, some studies incorporated less than optimal techniques of increasing consumption of the dairy products, like baking them into breads or pastries,<sup>47</sup> or consuming them with insulinogenic foods,<sup>51</sup> yet still observed positive health changes in study participants. This should be further explored in future studies as similar food interactions occur in the general population.

Several limitations exist in this study. It is a review of multiple studies that differ largely and inconsistently in their methods. Moreover, they do not account for other potentially confounding variables, like other foods consumed, the effect of common additives, antibiotics, and hormones. While this level of detail was not recorded in the methods in any of these studies, antibiotics and hormone use is still common, and their potential harm remains under debate. In addition, all of these studies only lasted a matter of weeks, and long-term studies are needed to assess these changes and the impact on their consumption over a longer period. Finally, the safety and effect of augmented foods, like enriched eggs, should be verified with long-term studies before we can make firm recommendations including their promotion.

## **Conclusion:**

While red meat, butter, cheese, and eggs have been generally treated as nutritional "boogeymen" with most nutritional recommendations endorsing their avoidance, these individual foods may vary significantly in nutritional value and the corresponding effect on consumers. Grass-fed butter, n-3 enriched eggs, and wild game meat appear to have a neutral effect on serum lipids while providing a decrease in several inflammatory factors, potentially improving health. Significant data exist illustrating a marked differing physiological affect from consuming the exact same food produced from animals raised differently, and this finding could have a large impact on population-wide dietary recommendations if controlled for in future studies. More studies are needed focusing on these aspects of food production to help guide future dietary recommendations.

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# **Figure Legend:** (Animal Feed/ OR Diet/veterinary) AND (Humans/ AND Animals/) (Comparative Study [publication type] OR Cross-Over Studies/) (Humans/ AND Animals/) (Comparative Study [publication type] OR Cross-Over Studies/) ("free range" OR "cage free" OR "farm raised") Search #3: (Humans/ AND Animals/) (Comparative Study [publication type] OR Cross-Over Studies/) ("grass fed" OR "grain fed") 22 studies that did not include both animal diet changes and human physiologic measures N=9 studies Within the citations of each of the above papers, citations were analyzed for inclusion criteria N=29 studies met inclusion criteria: -Animal diet manipulation within the study design -Objective human physiologic outcomes measured

Figure 1: Medline search criteria

Table 1. CLA = conjugated linoleic acid, HDL = high density lipoprotein, TC = total cholesterol

Year	Study	Subjects	Animal Product	Animal Diet Change	CLA	Other Changes (i.e. lipids, if measured)
1998	Tholstrup	18	Butter	Silage vs. soy	Serum CLA ↑	None
2004	Ritzenthaler	44	Cheddar cheese	Increased grass %	Serum CLA ↑	None
2005	Desroches	16	Butter	Sunflower seeds	Butter CLA	None
2005	Burdge	31	Multiple dairy products	Sunflower seeds + fish oil	Serum CLA	No other measurements
2006	Tholstrup	42	Butter	Sunflower seeds	Serum CLA ↑	TC ↓, HDL ↑
2006	Tricon	32	Multiple dairy products	Sunflower seeds + fish oil	Dairy product CLA 个	None
2010	Arya	10	Red meat	Kangaroo vs. conventional wagyu	Not directly 个	↓ Inflammatory markers with game meat
2010	Sofi	10	Cheese	Pastured cheese	Cheese CLA ↑	↓ Inflammatory markers with grass fed
2011	Brown	18	Beef	Grass-fed beef	Serum CLA ↑	None
2011	Gilmore	30	Beef	Grass-fed beef	Not directly 个	HDL ↓

Table 2. HDL = high density lipoproteins, N-3 = plasma omega-3 fatty acids, TC = total cholesterol, TG = triglycerides, LDL = low-density lipoproteins, BP = blood pressure

Year	Study	Subjects	Animal Product	Animal Diet Change	Omega-3	Changes in Experimental Group (i.e. lipids, if measured)
1991	Oh	11	Eggs	Fish oil	Serum N- 3 个	TG ↓, systolic BP ↓
1992	Ferrier	5	Eggs	0, 10%, 20% Flaxseed	Serum N- 3↑	TG ↓
1993	Jiang	23	Eggs	Flaxseed, sunflower seed	Serum N- 3 ↑	HDL ↑,TG ↓
1995	Ferrier	28	Eggs	0, 10%, 20% Flaxseed	Serum N-	None
1998	Farrell	56	Eggs	Fish, linseed, canola, sunflower seed oils	Serum N- 3个	None
1998	Van Elswyk	44	Eggs	Flaxsced (linseed)	Serum N- 3 个	None
2000	Surai	40	Eggs	Selenium, lutein, vitamin E, tuna oil	Serum N- 3 ↑	Lutein, alpha-tocopherol 个
2000	Lewis	25	Eggs	Flaxseed (linseed)	Serum N- 3 个	None
2002	Weill	75)	Eggs, cheese, beef, pork, chicken, lamb	Flaxseed (linseed)	Serum N- 3 ↑	None
2003	Maki	153	Eggs	Marine Algae	Serum N- 3↑	LDL ↑
2004	Sindelar	12	Eggs	Flaxseed	Egg N-3	TG ↑
2005	Gillingham	15	Eggs	Menhaden oil and flaxseed	Serum N- 3 ↑	None

2005	Bovet	25	Eggs	Tuna oil	Egg N-3	TG ↓
2010	Burns- Whitmore	25	Eggs	Flaxseed or organic	Serum N- 3 个	None
2010	Adams	10	Beef	Grass-fed beef	Beef N-3 ↑	TG 个, HDL 个, LDL particle diameter 个
2011	McAfee	38	Red meat	Grass-fed	Serum N- 3 个	None
2011	Intorre	30	Cheese	Flaxseed (linseed)	Serum N- 3 个	Vitamin C, E 1
2013	Intorre	30	Cheese	Flaxseed (linseed)	Serum N- 3 ↑	Changes in saturated fatty acids
2014	Burns- Whitmore	20	Eggs	Flaxseed (linseed)	Serum N-	None