

A Literature Review of the Value-Added Nutrients found in Grass-fed Beef Products

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Grass-fed beef, or beef produced from cattle finished on forage only diets, has been touted as a more nutritious beef product. There are a number of reports that show grass-fed beef products contain elevated concentrations of β -carotene and α -tocopherol, increased levels of omega-3 fatty acids, a more desirable omega-3:omega-6 ratio, and increased levels of conjugated linoleic acid (CLA), all substances reported to have favorable biological effects on human health. The purpose of this article is to summarize information currently available to support the enhanced nutrient claim for grass-fed products as well as review the effects these specific nutrients have on human health.

Nutrients of interest for Grass-fed Beef

ProVitamin A: β -Carotene:

β -carotene, a fat-soluble antioxidant, is derived from the Latin name for carrot, which belongs to a family of natural chemicals known as carotenes or carotenoids. Carotenes produce the yellow and orange color found in fruits and vegetables and is converted to vitamin A (retinol) by the body. While excessive amounts of vitamin A in supplement form can be toxic, the body will only convert as much vitamin A from beta-carotene as it needs, thus beta-carotene is a safe dietary source for vitamin A supplementation. (University of Maryland Medicine, 2002)

Vitamin A is a critical fat-soluble vitamin that is important for normal vision, bone growth, reproduction, cell division, and cell differentiation (Stephens et al., 1996). Specifically, it is responsible for maintaining the surface lining of the eyes and also the lining of the respiratory, urinary, and intestinal tracts. The overall integrity of skin and mucous membranes is maintained by vitamin A, creating a barrier to bacterial and viral infection (Semba, 1998; Harbige, 1996). In addition, vitamin A is involved in the regulation of immune function by supporting the production and function of white blood cells (Ross, 1999; Gerster, 1997).

The current recommended intake of vitamin A is 3,000-5,000 IU for men and 2,300-4,000 IU for women (National Institute of Health Clinical Center, 2002; Harvard School of Public Health) which is equivalent to 900 – 1500 μ g (micrograms) (Note: DRI (dietary reference intake) as reported by the Institute of Medicine for non-pregnant/non-lactating adult females is 700 μ g and males is 900 μ g /day or 2,300 - 3,000 IU (assuming

conversion of 3.33 IU/ug). While there is no RDA (Required Daily Allowance) for beta-carotene or other pro-vitamin A carotenoids, the Institute of Medicine report suggests that consuming 3 mg of beta-carotene daily to maintain plasma beta-carotene in the range associated with normal function and a lowered risk of chronic diseases (NIH: Office of Dietary Supplements).

Descalzo et.al., 2005, found pasture-fed steers incorporated significantly higher amounts of β -carotene into muscle tissues as compared to grain-fed animals. Concentrations ranged from 0.63 – 0.45 $\mu\text{g/g}$ and 0.06 – 0.5 $\mu\text{g/g}$ for meat from pasture and grain-fed cattle respectively, a 10 fold increase in β -carotene levels for grass-fed beef. Similar data is reported by Simonne, et.al., 1996; Yang et.al., 2002a; and Wood and Enser, 1997, presumably due to the high β -carotene content of fresh forage as compared to cereal grains (Simonne et al., 1996).

Vitamin E: Alpha-tocopherol:

Vitamin E is also a fat-soluble vitamin that exists in eight different forms with powerful antioxidant activity, the most active being α -tocopherol (Pryor, 1996). Antioxidants protect cells against the effects of free radicals. Free radicals are potentially damaging by-products of the body's metabolism that may contribute to the development of chronic diseases such as cancer and cardiovascular disease.

Preliminary research shows vitamin E supplementation may help prevent or delay coronary heart disease (Lonn and Yusuf, 1997; Jialal and Fuller, 1995; Stampfer et al., 1993; Knekt et al., 1994). Vitamin E may also blocks the formation of nitrosamines, which are carcinogens formed in the stomach from nitrites consumed in the diet. It may also protect against the development of cancers by enhancing immune function (Weitberg and Corvese, 1997). In addition to the cancer fighting affects, there are some observational studies that found lens clarity (a diagnostic tool for cataracts) was better in patients who regularly use vitamin E (Leske et al., 1998; Teikari et al., 1997).

The current recommended intake of vitamin E is 22 IU (natural source) or 33 IU (synthetic source) for men and women (National Institute of Health Clinical Center, 2002; Harvard School of Public Health; ARS, United States Department of Agriculture, 2000) is necessary for biological activity. Twenty-two international units is equivalent to 15 milligrams by weight.

The concentration of natural α -tocopherol (vitamin E) found in grain-fed beef is approximately 2.0 $\mu\text{g/g}$ of muscle whereas pasture fed beef ranges from 5.0 to 9.3 $\mu\text{g/g}$ of tissue depending on the type of forage made available to the animals (Yang et al., 2002b, Arnold et al., 1992, Faustman et al., 1998). Forage finishing increases α -tocopherol levels 3-fold over conventional beef and well within range of the muscle α -tocopherol levels needed to extend the shelf-life of retail beef (McClure et al., 2002). Vitamin E, α -tocopherol, acts post-mortem to delay oxidative deterioration of the meat, i.e., a process by which myoglobin to converted into brown metmyoglobin, producing a darkened appearance to the meat.

Omega 3: Omega 6 fatty acids:

Omega-3 fatty acids are considered essential fatty acids, which means that they are essential to human health but cannot be manufactured by most mammalian species. For this reason, omega-3 fatty acids must be obtained from food.

Essential fatty acids (EFAs) are polyunsaturated and grouped into two families, the omega-6 EFAs and the omega-3 EFAs. Although there are just minor differences in their molecular structure the two EFA families act very differently in the body. While the metabolic products of omega-6 acids promote inflammation, blood clotting, and tumor growth, the omega-3 acids act entirely opposite. However, it is important to maintain a balance of omega-3 and omega-6 in the diet as these two substances work together to promote health.

There are 3 major types of omega-3 fatty acids that are ingested in foods and used by the body: α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Once eaten, the body converts ALA to EPA and DHA, the two types of omega-3 fatty acids that are most readily used by the body.

According to the University of Maryland, an inappropriate balance of these essential fatty acids (high omega-6/omega-3 ratio) contributes to the development of disease while a proper balance helps maintain and even improves health. A healthy diet should consist of roughly one to four times more omega-6 fatty acids than omega-3 fatty acids. The typical American diet tends to contain 11 to 30 times more omega-6 fatty acids than omega-3 and many researchers believe this imbalance is a significant factor in the rising rate of inflammatory disorders in the United States (Simopoulos, 1991; Simopoulos 2002).

Scientists discovered the many benefits of EPA and DHA in the early 1970's when Danish physicians observed that Greenland Eskimos had an exceptionally low incidence of heart disease and arthritis despite the fact that they consumed a high-fat diet. More recent research has established that EPA and DHA play a crucial role in the prevention of atherosclerosis, heart attack, depression and cancer (Simopoulos, 1991; Simopoulos 2002; Connor, 2000). In addition, omega-3 consumption by individuals with rheumatoid arthritis has led to the reduction or discontinuation of their ordinary treatment (Kremer, 1989; DiGiacomo, 1989).

The human brain has a high requirement for DHA, low DHA levels have been linked to low brain serotonin levels, which are connected to an increased tendency for depression and suicide. Several studies have established a clear association between low levels of omega-3 fatty acids and depression. In fact, countries with a high level of omega-3 consumption have fewer cases of depression, decreased incidence of age-related memory loss as well as a reduction in impaired cognitive function and a lower risk of developing Alzheimer's disease (Kalmijn et al., 1997a; Kalmijn et al., 1997b; Yehuda et al., 1996; Hibbeln, 1998; Hibbeln et al., 1995; Stoll et al., 1999; Calabrese et al., 1999; Laugharne et al., 1996).

There is some consensus among leading nutritionists who consider increases in chronic disease as no accident; they believe it is directly related to the change in our dietary patterns over the last 200 years. Our ancestors lived on an omega-6:omega-3 ratio of 1:1, while our current dietary habits are closer to 10-20:1 (Simopoulos, 1991; Pepping, 1999). Researchers believe the ideal omega-6 intake should be no more than 4-5 times that of our omega-3 intake. The National Institutes of Health recently published recommended daily intakes of fatty acids, specific recommendations include 650 mg of EPA and DHA, 2.22 g/day of alpha-linolenic acid and 4.44 g/day of linoleic acid. However, the Institute of Medicine has recommended DRIs for linoleic acid (omega-6) at 12- 17 g and 1.1-1.6 g for α -linolenic acid (omega-3) for adult women/men.

As with the human diet, cattle feed or the composition of the ration has a significant effect on the fatty acid profile of the final beef product. Cattle fed primarily grass enhanced the omega-3 content of beef by 60% and also produces a more favorable omega-6 to omega-3 ratio. Conventional beef contains a 4:1 omega 6:3 ratio while grass-only diets produce a 2:1 omega 6:3 ratio (French et al., 2000; Duckett et al., 1993; Marmor et al, 1984; Wood and Enser, 1997). Table 1 which shows the effect of ration on omega 6 and omega 3 fatty acid concentrations in beef, data is reported as g/100g of total fatty acids in meat produced from the various feeding regimes. The all grass diet produces the highest omega-3 concentration within the meat product while omega-6 levels stay fairly constant regardless of grain to grass ratio.

Table 1. Essential Fatty Acides by diet (g/100g of fatty acid)	Treatment				
Fatty Acid	Grass silage + 4kg conc.	1kg hay + 8 kg conc.	6 kg grass (DM basis) + 5 kg of conc.	12 kg grass (DM basis) + 2.5 kg of conc.	22 kg of grass DM
n-6 fatty acids	2.96	3.21	3.12	3.04	3.14
n-3 fatty acids	.91 ^y	.84 ^y	1.13 ^x	1.25 ^{wx}	1.36 ^w
n6:n3 ratio	3.61 ^w	4.15 ^w	2.86 ^x	2.47 ^x	2.33 ^x

^{w,x,y,z} Means within rows with common superscripts are not significantly different (P>.05) French, et al., 2000.

Rule et al., 2002, reported similar results in a direct comparison of n-3 and n-6 EFAs for cattle on grain vs. grass, i.e., grass-fed cattle produced higher percentages of omega 3 within the lipid fraction than grain-fed contemporaries.

Table 2. EFAs by diet (as % of total fatty acids)	Grass-fed	Grain-fed
n-6 fatty acids	5.66 % ^a	3.92 % ^a
n-3 fatty acids	2.90 % ^b	0.64 % ^c
n6:n3 ratio	1.95 ^d	6.38 ^e

^{a,b,c,d,e} Means within rows with common superscripts are not significantly different ($P > .01$) Rule, et al., 2002.

The amount of lipid per serving is highly variable and depends on the feeding regime, genetics and actual cut of beef, however when lipid content is standard (as in hamburger), a serving of grain-fed beef at 10% fat would provide 84 milligrams of omega-3 in a 100 gram serving according to French et al., 2000 (.84 g n-3/100g lipid; 100g serving at 10% lipid = 10g fat/serving; roughly 84 mg n-3). The same hamburger from grass-fed beef would produce 136 mg n-3/serving.

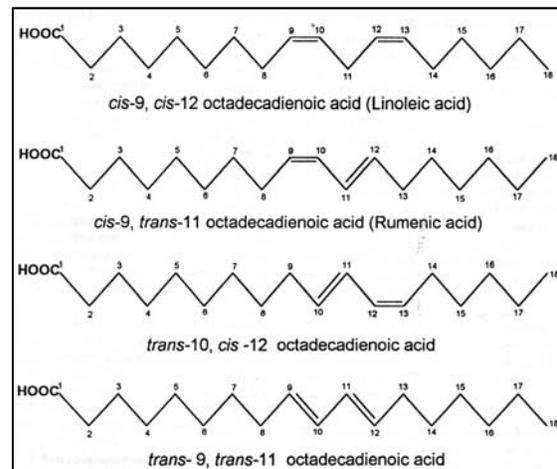
In general, grass-fed cattle are slaughtered at lighter weights than grain fed beef, producing leaner (lower fat) carcasses overall. Thus, whole cuts from grass-fed carcasses will not provide the same quantities of n-3 as described for hamburger at a constant % fat. Leaner carcasses have the advantage of an overall lower percent fat and a higher proportion of favorable unsaturated fatty acids. However, ultra lean carcasses (less than .3 inches of backfat) lead to cold shortening and reduced tenderness, in addition, lowered fat levels impact eating quality such as flavor and juiciness.

Conjugated Linoleic Acid (CLA):

The term conjugated linoleic acid and its acronym CLA is a group of polyunsaturated fatty acids found in beef, lamb, and dairy products that exist as a general mixture of positional and geometric conjugated isomers of linoleic acid (Sehat et al., 1999). These compounds are produced in the rumen of cattle and other ruminant animals during the microbial biohydrogenation of linoleic and linolenic acids by an anaerobic rumen bacterium *Butyrivibrio fibrisolvens*. (Pariza et al., 2000).

Nine different positional and geometrical isomers result from this process, of which, cis-9, trans-11 is the most abundant and is the biologically active form. Cis-9, trans-11 makes up 75% or more of the total CLA in beef (Ip, et al, 1994; Chin et al., 1992; Parodi, 1997).

Over the past two decades numerous health benefits have been attributed to CLA in experimental animal models including actions to reduce carcinogenesis, atherosclerosis, onset of diabetes, and fat body mass.



The anti-atherosclerotic evidence was first reported in CLA treated mice by Clement Ip in 1994. Ip and coworkers showed CLA levels as low as 0.05 percent of the diet can have a beneficial effect in mice. A level of 0.5 percent reduced the total number of mammary tumors by 32 percent. These results also demonstrated that CLA administered through a dietary route was effective in providing protection against cancer (Ip et al., 1994).

In a 1996 supplemental feeding study, Carol Steinhart showed a lower level of LDL (“bad”) cholesterol in both rabbits and hamsters treated with oral CLA, resulting in significantly less plaque formation in the aortic artery of treated animals (Steinhart, 1996). Presumably this reduction in plaque formation would therefore reduce the incidence of heart disease. Likewise, David Kritchevsky demonstrated that CLA levels as low as 0.1 percent of the diet can have beneficial effects by inhibiting atherogenic activity in rabbits (Kritchevsky et al., 2000). This particular study also showed a 30 percent regression of established atherosclerosis with a CLA level of 1 percent of the diet.

There is considerable data that demonstrates how CLA modulates body composition by reducing the accumulation of adipose tissue, primarily in experimental animals. In mice, rats, pigs, and now humans, dietary CLA has been shown to reduce adipose tissue depots (Dugan et al., 1999; Park et al., 1997; Sisk et al., 2001; Smedmen et al., 2001) Although there is some controversy within the human data, it is likely that dose, duration, isomeric composition, age and gender influence the outcome of CLA supplementation. For instance, lower doses (3g/day: Blankson et al., 2000) had little effect while larger doses (3.4 – 6.0 g/day) significantly reduced fat mass in humans (Zambell et al., 2000).

These ultra high doses of synthetic CLA may produce ill side-effects, with the most common being of gastrointestinal origin, although there have been reports of adverse changes to glucose/insulin metabolism and liver function in some animal studies depending on the dose and the isomer studied (Tsubooyama-Kasaoka et al., 2000; Delany et al., 1999; Clement et al., 2002; Roche et al., 2002). In humans, insulin resistance was reported with ingestion of a supplement enriched with the t10,c12 isomer, but not with a mixed preparation of predominantly c9,t11 and t10,c12 CLA isomers (Riserus et al., 2002).

CLA is found naturally in a variety of ruminant meats (French, et al, 2000) and dairy products (Dhiman, et al, 1999), due to the anaerobic activity of the rumen bacterium *Butyrivibrio fibrisolvens*. This rumen organism is responsible for the biohydrogenation of linoleic and linolenic acids into the conjugated isomers referred to as CLA. Because linoleic and linolenic acid is a precursor, diets rich in these compounds increase the concentration of the CLA within the fat depot of the animal. Lush green forages are particularly high in this precursor, therefore, **grass-fed ruminant species have been shown to produce 2 to 3 times more CLA than ruminants fed in confinement on concentrate-only diets** (French, et al, 2000; Duckett, et al, 1993; Rule, et al, 2002; Mandell et al, 1998).

Conjugated Linoleic Acid (g/100g or g/3.50oz.)			
Study	Feedlot/Concentrate	Range/Grass	Amount Increased
French, 2000	.37 ^z	1.08 ^w	2.92 X
Duckett, 1993	.82 ^c	2.2 ^d	2.69 X
*Rule, 2002	.26 ^e	.41 ^c	2.04 X

On average, grass-fed beef will provide approximately 123 mg of CLA for a standard hamburger at 10% fat. The same hamburger produced from grain-fed beef would provide 48.3 mg. (i.e., grass-fed = 1.23 g CLA/ 100g lipid; 12.3 mg/g lipid; 10% lipid/serving = 123 mg CLA).

Research to date would support the argument that grass-fed beef is higher in Vitamin A, Vitamin E, CLA and Omega 3 when lipids are compared on a gram of fatty acid/gram of lipid basis. Little work has been done to compare grass-fed cattle to grain-fed at a constant degree of fatness, most studies harvest cattle after a specific number of days on feed rather than processing cattle at a logical slaughter endpoint based on degree of fatness. Because grass-fed cattle are fed lower energy diets, they tend to fatten more slowly and are slaughtered at a lower % body fat. As percent body fat decreases so does the concentration of these important lipids like CLA and omega-3 in whole cuts of beef.

Maintaining the favorable lipid profile:

Maintaining the favorable lipid profile in grass-fed beef requires a high percentage of forages, the more green and fresh the forage, the higher the C18:2 α -linoleic and α -linolenic acid precursor will be available for n-3 and CLA synthesis. Dried, cured forages will have a lower amount of precursor, with a slightly lower level of functional lipids in the final product. However, if cattle are switched over to a diet predominantly composed of cereal grains, a significant amount of FA remodeling takes place in the intramuscular fat fraction (marbling or neutral lipid fraction viewed as fat flecks throughout the meat.) will take place within 30 days of diet transition (Duckett, et al., 1993).

To maintain high functional lipid concentrations, producers must feed forages rich in C18:2 is to maintain a high concentration of pre-cursor compounds in the ration. The precursor for the n-3 series is α -linolenic (LNA: C18:3 n-3), the higher the concentration of C18:3 n-3 in the ration, the more n-3 fatty acids will be found in the final product. Fresh forages have 10 to 12 times more C18:3 than cereal grains (French, et al., 2003). Likewise, the precursor fatty acid for CLA is linoleic acid (LA: C18:2n-3), the higher the concentration LA the diet, the higher the concentration of CLA in the meat.

Bibliography

- Arnold RN, Scheller N, Arp KK, Williams SC, Beuge DR, Schaefer DM. Effect of long or short-term feeding of α -tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristics, and beef colour stability. 1992. *J Animal Sci.* 70:3055-3065.
- Blankson H, Stakkstand J, Fagertun H, Thorn E, Wadstein J, Gudmundson O. conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr.* 2000; 130:2943-48.
- Chin SF, Liu W, Storkson JM, HaYL, Pariza MW. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J Food Comp Anal* 1992; 5:185-197.
- Clement L, Poirier H, Niot I, Bocher V, Guerre-Millo M, Krief B, Staels B, Besnard P. 2002. Dietary trans-10, cis-12 conjugated linoleic acid induces hyperinsulemia and fatty liver in the mouse. *J. Lipid Res.* 43, 1400-1409.
- Descalzo AM, Insani EM, Biolatto A, Sancho AM, Garcia PT, Pensel NA, Josifovich JA. Influence of pasture or grain-based diets supplemented with vitaminE on antioxidant/oxidative balance of Argentine beef. *Meat Science* 2005; 70:35-44.
- Dietary Guidelines Advisory Committee, Agricultural Research Service, United States Department of Agriculture (USDA). Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2000. <http://www.ars.usda.gov/dgac>
- Dugan MER, Aalhus JL, Jeremiah LE, Kramer JKG, Schaefer AL. The effects of feeding conjugated linoleic acid on subsequent port quality. *Can J Anim Sci* 1999; 79:45-51.
- Duckett SK, Wagner DG, Yates LD, Dolezal HG, May SG. 1993. Effedts of time on feed on beef nutrient composition. *J. Anim. Sci.* 71:2079-2088.
- Fautsman C, Chan WKM, Schaefer DM, Havens A. Beef color update: the role for Vitamin E. 1998. *J. Anim. Sci.* 76:1019-1026.
- French P, O'Riordan EG, O'Kiely P, Caffrey PJ, Moloney AP. 2001. Intake and growth of steers offered different allowances of autumn grass and concentrates. *Anim. Sci.* 72:129-138.
- French P, O'Riordan, EG, Monahan FJ, Caffrey PF, Moloney AP. 2003. Fatty acid composition of intra-muscular triacylglycerols of steers fed utumn grass and concentrates. *Livestock Production Science.* 81:307-317.

French P, Stanton C, Lawless F, O'Riordan EG, Monahan FJ, Caffrey PJ, Moloney AP, 2000. Fatty acid composition, including conjugated linoleic acid of intramuscular fat from steers offered grazed grass, grass silage or concentrate-based diets. *J. Anim. Sci.* 78:2849-2855.

Gerster H. Vitamin A-functions, dietary requirements and safety in humans. *Int. J. Vitam. Nutri Res.* 1997; 67:71-90.

Harbige LS. Nutrition and immunity with emphasis on infection and autoimmune disease. *Nutri Health* 1996; 10:285-312.

Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press, Washington, DC, 2001.

Institute of Medicine, Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. The National Academies Press. September 2002. Web address <http://www.iom.edu/Object.File/Master/4/154/0.pdf> or <http://www.nap.edu> for the entire document.

Ip C, Scimeca JA, Thompson HJ. Conjugated Linoleic Acid. *Cancer Supp.* 1994; 74(3):1050-1054.

Ip C, Singh M, Thompson JH, Scimeca JA. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res.* 1994; 54:1212-5.

Jialal I and Fuller CJ. Effect of vitamin E, vitamin C and beta-carotene on LDL oxidation and atherosclerosis. *Can J Cardiol* 1995; 11 Suppl G:97G-103G.

Kritchevsky D, Tepper SA, Wright S, Tso P, Czarnecki SK. Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. *J Amer Coll of Nutri* 2000; 19(4):472S-477S.

Leske MC, Chylack LT Jr., He Q, Wu SY, Schoenfeld E, Friend J, Wolfe J. Antioxidant vitamins and nuclear opacities: The longitudinal study of cataract. *Ophthalmology* 1998; 105:831-6.

Lonn EM, Yusuf S. Is there a role for antioxidant vitamins in the prevention of cardiovascular diseases? An update on epidemiological and clinical trials data. *Can J Cardiol* 1997;13:957-65.

McClure EK, Belk KE, Scanga JA, Smith GC. 2002. Determination of appropriate Vitamin E supplementation levels and administration times to ensure adequate muscle tissue alpha-tocopherol concentration in cattle destined for the Nolan Ryan tender-aged

beef program. 2002. Animal Sciences Research Report. The Department of Animal Sciences, Colorado State University.

Melhus H, Michaelsson K, Kindmark A, Bergstrom R, Holmberg L, Mallmin H, Wolk A, Ljunghall S. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk of hip fracture. *Ann Intern Med.* 1998; 129:770-778.

National Institute of Health Clinical Nutrition Center, 2002: Facts about dietary supplements: Vitamin A and Carotenoids.

Pariza MW, Park Y, Cook ME. Mechanisms of action of conjugated linoleic acid: evidence and speculation. *P.S.E.B.M.* 2000; 223:8-13.

Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza ME. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 1997; 32:853-58.

Parodi PW. Cows' milk fat components as potential anticarcinogenic agents. *J Nutr* 1997; 127:1055-1060.

Riserus U, Arner P, Brismar K, Vessby B. 2002. Treatment with dietary trans 10 cis 12 conjugated linoleic acid causes isomer specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* 25, 1516-1521.

Roche HM, Noone E, Sewter C, McBennett S, Savage D, Gibney MJ, O'Rahilly S, Vidal-Plug AJ. 2002. Isomer-dependent metabolic effects of conjugated linoleic acid: insights from molecular markers sterol regulatory element-binding protein 1c and LXR alpha. *Diabetes* 51, 2037-2044.

Ross AC. Vitamin A and Retinoids. In: Shils ME, Olson J, Shike M, Ross AC, ed. *Modern Nutrition in Health and Disease.* 9th ed. Baltimore: Williams & Wilkins, 1999.

Sehat N, Rickert RR, Mossoba MM, Dramer JKG, Yurawecz MP, Roach JAG, Adlof RO, Morehouse KM, Fritsche J, Eulitz KD, Steinhart H, Ku K. Improved separation of conjugated fatty acid methyl esters by silver ion-high-performance liquid chromatography. *Lipids* 1999; 34:407-413.

Semba RD. The role of vitamin A and related retinoids in immune function. *Nutr Rev* 1998;56:S38-48.

Simonne AH, Green NR, Bransby JI. Consumer acceptability and beta-carotene content of beef as related to cattle finishing diets. *J Food Sci* 1996;61:1254-1256.

Sisk M, Hausman D, Martin R, Azain M. Dietary conjugated linoleic acid reduces adiposity in lean but not obese Zucker rats. *J Nutr.* 2001; 131:1668-74.

Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans – metabolic effects. *J. Nutr.* 2001; 36:773-81.

Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 1993; 328:1444-9.

Stephens D., Jackson PL, Gutierrez Y. Subclinical vitamin A deficiency: A potentially unrecognized problem in the United States. *Pediatr Nurs* 1996;22:377-89, 456.

Teikari JM, Virtamo J, Rautalahti M, Palmgren J, Liestro K, Heinonen OP. Long-term supplementation with alpha-tocopherol and beta-carotene and age-related cataract. *Acta Ophthalmol Scand* 1997; 75:634-40.

Tsuyoyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, Kasai M, Ikemoto SS, Ezaki O. 2000. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* 49, 1534-1542.

University of Maryland Medicine, 2002:

<http://www.umm.edu/altmed/ConsSupplements/BetaCarotenecs.html>

Weitberg AB and Corvese D. Effect of vitamin E and beta-carotene on DNA strand breakage induced by tobacco-specific nitrosamines and stimulated human phagocytes. *J Exp Clin Cancer Res* 1997; 16:11-4.

Wood JD, Enser M. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. 1997. *British J of Nutrition* 78:S49-S60.

Yang A, Brewster MJ, Lanari MC, Tume RK. Effect of vitamin E supplementation on α -tocopherol and β -carotene concentrations in tissues from pasture and grain-fed cattle. *Meat Science* 2002a; 60(1):35-40.

Yang A, Lanari MC, Brewster M, Tume RK. Lipid stability and meat colour of beef from pasture and grain-fed cattle with or without vitamin E supplement. 2002b. *Meat Science* 60:41-50.

Zambell KL, Keim NL, Van Loan MD, Gale B, Benito P. conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure. *Lipids.* 2000; 35:777-82.