HEALTH IMPACTS OF CARDIOVASCULAR DISEASE RISK OF GRASS-FED VS. GRAIN-FED BEEF

The impact of various beef complex fatty acids on plasma and tissue lipid enrichment and lipoprotein size distribution

Bachelor Thesis I

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Abstract

Cardiovascular diseases are one of the main causes of early onset health issues that lead to death worldwide. This is in part due to the various foods humans are eating that may contribute to atherosclerotic risks. One of the foods that is a stable in the Western diet as well as other habitual diets worldwide is meat. Specifically cow beef, which in this thesis will be analyzed to a cellular extent on how it impacts lipid profiles.

Numerous research articles examine the effects that grass and grain-fed beef have on lipid profiles, investigating the concentrations of fat and fatty acid content in the beef. Thus stimulating questions as to why grain-fed beef has been shown to increase low density lipoprotein production, triacylglycerol concentrations and additional factors attributed to atherosclerosis. While studies on grass-fed beef have shown that it increases high density lipoprotein levels and poly-unsaturated fatty acid concentrations. Particularly long chain omega 3 poly-unsaturated fatty acids, which are key factors for increased lipid oxidation and decreased lipid production in humans.

In this thesis the main questions that will be answered are how does grass and grain-fed beef affect the lipidome. Furthermore, what actions do their fatty acids specifically have and how does this lead to preventing cardiovascular diseases and promoting cardiovascular health.

Key words:

Grass-fed beef, Grain-fed beef, Cardiovascular health, Cardiovascular disease, Fatty acids, Lipoprotein, Polyunsaturated fatty acids, Saturated fatty acids, Monounsaturated fatty acids, Omega-3, Omega-6.

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List of Abbreviations

ALA	Alpha linolenic acid
AA	Arachidonic acid
CVD	Cardiovascular disease
CVH	Cardiovascular health
CLA	Conjugated linoleic acid
CHD	Coronary heart disease
COX	Cyclooxygenase
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
GLA	Gamma linolenic acid
gCO2e	Greenhouse carbon dioxide gas emissions
HNF-4α	Hepatocyte nuclear factor-4 alpha
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
LA	Linoleic acid
LXR	Liver X-receptor
LCn-3PUFA	Long chain omega-3 polyunsaturated fatty acid
LDL	Low density lipoprotein
LDL-C	low density lipoprotein cholesterol
MUFA	Monounsaturated fatty acids
NF-*k*B	Nuclear factor-*kappa* B
OECD	Organisation for Economic Co-operation and Development
n-3	Omega-3
n-6	Omega-6
PPAR	Peroxisome proliferated-activator receptors
PL	Phospholipid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid

SCD	Stearoyl Coenzyme A desaturase
SREBP	Sterol-regulatory element binding protein
TAG	Triacylglycerol
VLDL	Very low density lipoprotein

1. Introduction

How do lipids effect the cardiovascular system, based on the feeding practices that influence the lipid composition of beef? Research illustrates that there are fundamental impacts to beef feeding that effect the lipid composition and fatty acid content. The various health factors that contribute to beef play an abundance of roles in diet, fatty acid composition and lipid oxidation and inflammatory pathways. The increase in cardiovascular diseases has been a major factor in recent research studying the effects that grass and grain-fed beef have on lipid profiles.

1.1. Cardiovascular disease risk

Humans have been bogged down with cardiovascular risk factors in recent decades now more than ever, with heightened cardiovascular diseases such as high cholesterol, elevated low density lipoproteins (LDL), lowered high density lipoproteins (HDL), coronary heart disease (CHD), high blood pressure, stroke and other atherosclerotic related health issues. Cardiovascular diseases (CVD) are the number one cause of death worldwide representing an estimated 17.7 million (31%) of all global deaths (1). One of the factors contributing to the health problems attributed to these health risks is the food we eat. There are three main ways that food consumption contributes to disease risk; the amount of food we eat, what we eat and how it is produced. This paper will highlight and compare the fatty acid composition of beef on the lipidomic profiles. Furthermore, shedding light on how the feeding practices of cows affects the way their body absorbs and processes essential fatty acids, thus quantifying the quality of beef. So what has changed?

1.2. Beef consumption

Between 1961 to 2014 beef consumption has risen from 0.89 to 0.98 kilograms per year per capita (2) and is steadily increasing. Not only is the consumption of beef on the rise but its effects on the environment are on average 22.01 of greenhouse carbon dioxide gas emissions per kilocalorie of beef/mutton production (gCO2e) (2). In correspondence the amount of cattle livestock has almost doubled from 1961 to 2014 from an average of 942 million to 1.47 billion worldwide (2). Out of these cattle livestock according to Dr.Dale Woerner, an assistant professor at the Center for Meat Safety & Quality at Colorado State University, about 97% of beef produced in the U.S.A are from grain-fed feedlot cows, while the remaining 3% are grass-fed (3). In comparison, a country like Australia, also one of the top ten beef producing countries, produces 39% of grain-fed feedlot cattle as reported by Meat & Livestock Australia (4). Even though Australia produces more grass-fed beef then the U.S.A, more than one in four of its' adult population is obese. Similar to Australia, New Zealand to a majority farms grass-fed cows as well and still it's adult population is more than 30% obese, according to the Organisation for Economic Co-operation and Development (OECD) (5). In exemplification, Japan that generally consumes more seafood and significantly lower intakes of meat, has an adult population that is less than 6% obese (5). Although this does not illustrate why obesity is on the rise, it does help to give an idea of what factors are into play. With this in mind, there is a wide variety of beef produce available for consumers, but is the way cows are farmed effecting human health? These statistics lead to the next question, what are the benefits of grass-fed verses grain-fed beef?

Historically, cows were fed on a grass-fed diet up until the 1940s where consumer demand increased the production of beef and as a result cows where switched to a more grain-fed diet as it was cheaper. The introduction of grain-fed diets into the farming of cows caused cows to grow faster and bigger in a shorter period of time helping farmers to produce more beef for a demanding industry. Ever since then beef consumers are used to and enjoy the taste of grain-fed beef rather than to the taste of grass-fed beef (6). Due to recent research, scientists are starting to realize the side effects of a diet consisting of grain-fed beef and thus looking into the benefits of grass-fed beef. Research worldwide acknowledges that the biological outcomes of grass-fed beef provides overall better human health outcomes, due to the beef being leaner, thus improving fatty acid (FA) composition and preventing CVD onsets (6), but there is still ongoing discussion as to why and how it is better or if it is better.

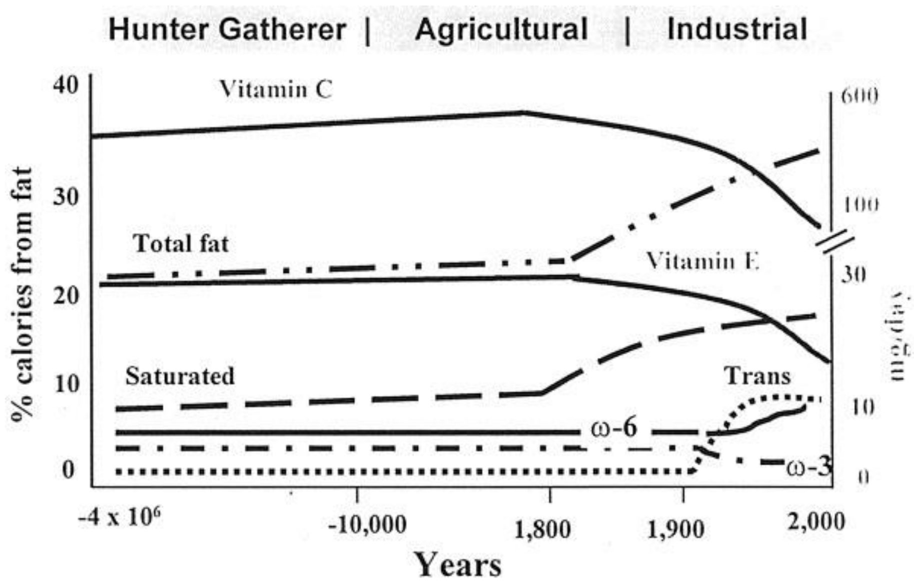
1.3. Introduction to complex fatty acids

The embarking of interest on the roles of lipids have opened doors to conceptual understandings that have changed our eating habits. Based on various research papers and their obtained evidence, this paper will illustrate and elaborate on the scope of the main thesis question. Henceforth, the main question is how does a change in beef affect lipidomic profiles? Firstly, lipids are fat like substances that are important parts of membranes found within and between each cell and myelin sheath that coats and protects the nerves. Lipids include oils, fatty acids, waxes, steroid like cholesterol and estrogen (7). Lipids used to be viewed as senseless compounds that only contributed to adipose tissue density, however since being investigated more in voluminous studies, lipids have been discovered to be very intelligent compounds that contribute to very dynamic and complex roles in the body. We now know that not all fats are created equal and their involvement in lipid metabolism contributes to various activations and inhibitions.

The different fatty acid concentrations attributed to beef types are mainly based on their feeding practices. Grass-fed beef is viewed as the healthiest and best source of fatty acid composition, because in particular its high polyunsaturated fatty acid (PUFA) levels are what make it a better option to grain-fed beef. Grain-fed beef feeds their cows soy and corn grains which contain high saturated fatty acids (SFA) and omega-6 (n-6) fatty acids (FA). These increased concentrations have been associated with increasing CVDs. In fact, grass-fed cows has a 4% increased long chain omega-3 polyunsaturated fatty acid (LCn-3PUFA) content compared to grain-fed beef.

2. Impacts of habitual diets on humans

Diet is one of the most critical and impactful modifiable lifestyle factors influencing health and disease, being related to 70% of cancer occurrences today (8). Modern diets have indeed changed since the dawning of the agricultural revolution that occurred about 10,000 years ago, which practiced similar farming techniques that are still used today. Additionally, modern human genetic makeup compared to our 40,000 year old Paleolithic ancestors has only changed about 0.005% (9). This statistic gives a good reference and hints at why worldwide onset of CVD and atherosclerotic related diseases have increased, as is illustrated in *Figure 1*. Indicating that we are indeed engaging in habitual diets that are not optimum for our health that we certainly cannot genetically and physiologically sustain. Given these circumstances what factors in modern habitual diets are causing the onset of CVDs.



(10)

Figure 1: Hypothetical scheme of fat, fatty acid (n-6, n-3, trans and total) intake (as percentage of calories from fat) and intake of vitamin E and C (mg/d).

Bondia-Pons I, Pöhö P, Bozzetto L, Vetrani C, Patti L, Aura A-M, et al. Isoenergetic diets differing in their n -3 fatty acid and polyphenol content reflect different plasma and HDL-fraction lipidomic profiles in subjects at high cardiovascular risk. Mol Nutr Food Res [Internet]. 2014 Sep

2.1. Habitual dietary implications

The lifestyle eating habits of an individual are the main reasons for negative lipid profiles. There are additional reasons that play into lipid profiles such as genetic and environmental factors, however these are variables and can be adjusted by improved lifestyle habits. The most common signs and earliest conditions associated with unhealthy habitual diets are hyperlipidemic and dyslipidaemic which stem from a variety of lifestyle factors that include daily dietary habits, activity level, environmental factors or something as rudimentary as genetic makeup. These factors lead to elevated total cholesterol, elevated triglycerides and other lipid abnormalities that could enhance CHD and related atherosclerotic risk factors (11).

A diet rich in SFA content stimulates high blood cholesterol levels contributing to increased lipoprotein profile production such as high LDL levels, increased triacylglycerol (TAG) and glucose levels. This is generally more predominant in males, resulting in a higher risk for developing CVDs, thus more likely to encounter dyslipidaemia. Moreover, men are more likely to consume greater amounts of red meat in comparison to women (12). Although it must be noted that most research has been frequently conducted in males, therefore for equal representation more research needs to be done on females in order to look into the full scope of the effects on women.

2.2. The Western diet

The most common habitual diet today is the Western diet. It has positive and negative aspects attributed to cardiovascular health and diseases.

Epidemiological studies in the Western diet indicate that beef eaters are reportedly always on the higher risk spectrum for developing CHD. This is frequently associated with high consumption of beef (140g/day), rather than the recommended serving of 500g/week (13). Statistically in the Western diet beef eaters are observed to be the unhealthiest, due to the fact that they are generally more stationary, performing less physical activity and tending to consume foods high in fat and protein, while simultaneously consuming less vegetables and fruits (14). On the other hand of the spectrum, vegetarians in the Western diet are generally more health conscious performing more physical activity and tending to consume a balanced vegetarian diet (14). Although these statements are very general they do help to give a good overview on typically how the Western diet plays into overall health. This does not mean that the consumption of beef is a direct cause of CVD, rather the type of beef plus the individuals' status of activity contributes to overall health outcomes.

The Western diet has maintained the problem of having an increased omega 6 (n-6) and a decreased omega 3 (n-3) ratio level usually around 16:1 (9), which have shown to cause an increase in CVD, such as heart disease. In comparison, hunter-gatherers generally had a 1:1 ratio of n-6 to n-3 (15), this explains why our biological needs are not being meet. The main animal product of interest discussed in this paper is beef and the various fats it contains. A key point in meeting our needs that should be taken into consideration is the type and amount of fat in the diet, as different fat types exert differing influences on lipid profiles leading to heightened cholesterol and CVD implications. Various differences in beef types, for example can produce negative effects due to the high SFA content. SFAs can also produce positive outcomes in the lipid biochemistry, where the beef cuts contain rich amounts of monounsaturated fatty acids (MUFA) like oleic acid (C18:1n-9), as well as an abundance of PUFA and essential LCn-3PUFA such as eicosapentaenoic acid (EPA) (C20:5n-3), docosahexaenoic acid (DHA) (C22:6n-3) and docosapentaenoic acid (DPA) (C22:5n-3). One type of fat of controversy that beef contains are natural trans-fats mainly trans-vaccenic acid (trans-11 18:1n-7) and oleic acid (18:1n-9), which are formed during bio hydrogenation in the rumen. Contrary to popular belief these trans-fats produce positive lipid metabolic interactions rather than the ones in baked goods which are produced by hydrogenated trans-fats that lead to atherosclerotic risk factors (16,17). Hence, there is some controversy over the negative and beneficial roles of beef derived fats on human health. Thus resulting in questions as to what types of beef are the best in order to reduce the risk of adverse effects of lipoproteins that have been identified to cause CVDs?

2.3. Effects of grass-fed and grain-fed beef on lipid composition

Grass-fed and grain-fed beef contain many of the same essential FAs, however the concentration of the levels of these essential FAs when comparing them make all the difference in health outcomes. However it must be noted, that the breed type, diet and slaughter age of any cow must always be taken into consideration when comparing any two types of cuts. It has been established that the Western diet results in the increased n-6 to n-3 PUFA ratio and therefore in consequence causes increased total cholesterol, low density lipoprotein cholesterol (LDL-C) and non-high density lipoprotein cholesterol (HDL-C) (18).

Recent research has indicating that the overconsumption of beef in the Western diet has been associated with cancers, particularly colorectal cancer in the last decade (8). Most evidence suggests that the way red meat is cooked affects its carcinogenic impact resulting in mutagenic behavior believed to be caused by heterocyclic amines and N-nitrosation production (19). In contrast, beef cooked correctly at moderate temperatures contain bioantimutagens like superoxide dismutase activity and glutathione, which is a prominent antioxidant inhibitor that reacts against the oxidative reactions of mutagen formation that can be found at higher concentrations in grass-fed beef (20,21).

Even though grass and grain-fed beef typically contain all the same FAs, their varying concentration levels are what make all the difference in one’s health. For the most part grain-fed beef generally contains higher concentrations of SFA, MUFA, n-6 and trans-vaccenic acid, meanwhile grass-fed beef contains higher concentrations of PUFA. PUFA are of interest when comparing grass and grain-fed beef because of the health benefits associated with them. Grass-fed beef contains higher levels of essential LCn-3PUFA, which means that they cannot be synthesized de novo by the human body, thus they must be consumed through diet (22). The important essential LCn-3PUFAs EPA (C20:5n-3), DHA (C22:6n-3) and DPA (C22:5n-3) found at higher concentrations in grass-fed beef, however DHA is reported to have the lowest concentrations out of the three in grass-fed beef (23). Nonetheless a great source of DHA can be found in fatty fish like salmon (14). As a side note, fish consumption is able to decrease LDL-C concentrations and increase HDL-C concentrations which result in improved HDL:LDL ratios (24). Oil from fish contains LCn-3PUFA and one of its many health benefits indicates a lowering in serum triglyceride levels in type 2 diabetics (25). All in all, the three LCn-3PUFA are essential in promoting better CVH and thus their recommended serving is 90 to 160mg/day for females and males respectively (18).

2.3.1. Nutritional health of plant and beef food sources

A vegetarian diet will probably not have the same proportional nutritional intakes as omnivore diets, yet that does not mean that one cannot receive the nutritional requirements from other sources. Hence, it has been researched and identified that the energy and protein content of vegetarian diets are similar to those of omnivore diets, indicating that meat is an optional component, rather than a necessity (26). To compare grass-fed beef contains more Se, Na, Zn, vitamin E, B12 and less Mg, P, K. Although the bioavailability of Se is good in beef, its bioavailability is way higher in plant foods (21,27). This is in part due to the variation in content of the soil composition. For example, if the soil is rich in vitamin B12 or Se, than the grass will also be rich in these essential vitamins, thus impacting the micronutrient meat quality of the cattle that graze them.

In comparison, beef contains a sufficient amount of iron in the haem form with about 15-20% absorption rate, while the non-haem iron found in plant foods only has a 1-7% absorption rate (14,28). Thus it is thought that deficiency of iron is more prevalent among vegetarians than omnivores, however this is just a general assumption and vegetarians show to make more of an effort in incorporating a balanced nutritional intake and thus reaching their daily dietary needs (13). Nonetheless beef is the easiest bioavailable source of iron absorption, although it must be noted that incorporating more vegetables with your beef can increase the iron absorption because of the non-haem form found in plants. This is thought to be possible by the iron binding capacity of cysteine in peptides in congregation with proteolysis of meat muscle production (26). Concluding, it is recommended to always include vegetables with beef in order to enhance certain nutrients and reduce the development of cancer. As studies show that vegetables and fruits provide protective actions when consumed, while reduced intake of vegetables leads to increased mutations of cancer. Therefore increasing intakes of vegetables, especially green and yellow vegetables which are rich in beta-carotene, vitamin C, calcium and dietary fiber have been shown to decrease the risks of developing breast and colorectal cancers (8,19). Zinc is another important essential mineral that is mostly found in meat, poultry and fish. It's bioavailability is enhanced when consumed with animal proteins by inhibitors like phylate and oxalate, thus being consumed less in vegan and vegetarian diets (14,29).

All in all, vegetarian diets are associated with lower cholesterol plasma levels (30), compared to omnivore diets. Vegetarian diets also have higher n-6 to n-3 ratio and lower DHA levels due to not consuming fish or beef. Therefore, good alternatives would be incorporating flaxseed or linseed oil in order to balance out the n-6 to n-3 ratio and a good source of DHA would be algae (15), that may be lacking in a vegetarian diet. In order for both vegetarian and omnivores to meet nutritional needs food sources of enriched quality must be consumed, as the whole process of differing feeding practices impacts the end result of the micronutrient quality of the foods. As the Farmingham study of 1991 concluded, “The quality and not the quantity of dietary fat may be the more potent determinant of plasma total cholesterol level” (31).

3. Fatty acid composition in food, body and role in human health

There are a variety of fats in beef that are associated with different disease risks due to their effects on CVD risk factors and the lipidome. Although based on various research articles the difference in the SFA and PUFA concentrations when comparing grass-fed beef to grain-fed beef is inconsistent. This is in part due to the variation in the quality and composition of the FA content. It has been stated that the overall consumption of grain-fed beef in general has high SFA, MUFA, n-6 and trans vaccenic acid content relative to PUFA content, thus increasing the risk of CVD. Meanwhile, multiple studies show that dietary PUFA found at higher levels in grass-fed beef repeatedly prevents and treats lipid related disorders, specifically CLA and n-3 PUFA which has clearly shown to be an effective integral in reducing plasma triglyceride levels and very low density lipoprotein (VLDL) production (32).

In regards to beef three factors must be taken into consideration when being examined which are the total fat content, P:S ratio and n-6:n-3 ratios (33). The fat content of beef has been reducing since the early 1980s due to production and social systems favoring less fat. It has been possible for industries to reduce fat content by various ways such as selective breeding, increasing the carcass lean to fat ratio, and modern butchery techniques by removing all intermuscular fat away. In the UK beef fat content has reduced by 15% and is estimated to reduce again in the next 5-10 years (33). The reduction in fat content in meat is a trend worldwide and is similar for various other foods.

3.1. Fatty acids in circulation - the role of lipoproteins in cardiovascular health

Varying FA compositions and fat content in grass and grain-fed beef have led to interests in how their storage and transport within the body, namely through lipoproteins assist in precursory pathways that propel overall health. It is a well-known fact that an increase in saturated fats causes hypercholesterolemic effects compared to unsaturated fats which are thought to induce hypocholesterolemia effects.

Notably, the main lipoproteins that have been frequently examined and studied in grass-fed beef exhibited decreased plasma total and LDL concentrations in subjects. The reason being that the high levels of cis-unsaturated fatty acids replaced saturated fatty acids, thus decreasing total LDL-C and HDL-C concentrations (15). Looking further into this replacing any of the three FAs with PUFA has shown to lower fasting serum TAG concentrations. By the same token, concentrations of MUFA in grain-fed beef were slightly higher than in grass-fed beef and interestingly like SFA, diets high in MUFA show effects of rising HDL-C concentrations (15). Returning to the rationalization that grass-fed beef promotes CVH rather than grain-fed beef, due to its lower SFA content and increased PUFAs contributing to total decrease in LDL-C and an increase HDL-C concentrations.

3.2. Saturated and Monounsaturated fatty acids

Major SFAs found in beef are stearic (C18:0), palmitic (C16:0), lauric (C12:0) and myristic (C14:0) acids, while the two major MUFA are oleic (C18:1n-9) and palmitoleic acids (C16:1n-7). However their concentrations vary based on seasonal changes, for example during the summer seasons when the grass is more abundant there is an increase in concentrations and likewise during winter seasons the concentrations become lower (16). Beef containing these major SFA are thought to be the most harmful to CVH, except stearic acid which has a neutral cholesterol effect and is correlated with reducing cholesterol when replacing carbohydrates. While lauric and myristic acid although not that prominent in beef are the most potent total and LDL-C elevating, thus decreasing total to HDL-C ratios proportionally (14,34).

Oleic acid is mainly found at higher concentrations in grain-fed beef and has been reported to promote cholesterol ester synthesis by accumulating in the adipose tissue and further elevating cholesterol levels (35). Likewise palmitic acid (C16:0) is a major hypercholestorlemic FA found at higher concentrations in grain-fed beef and like oleic acid increases elevated cholesterol levels. Stearic and oleic acid are generally responsible for the fat softness and thus influencing the palatability of beef. However they are both frequently depressed in the presence of increased PUFA levels. Since the presence of PUFA inhibits delta-9 desaturase enzyme activity, which can only be inhibited if there is a reduced abundance of stearic and palmitoleic acid that establishes a negative correlation in lipid synthesis that is thought to be desaturated by delta-9 desaturase (36), which will be elaborated upon in the next section.

3.2.1. Trans (unsaturated) fatty acids

Trans fatty acids found in beef, mainly trans vaccenic, but also palmitoleic acid increase total LDL-C and fasting TAG levels, this is thought to be possible by cholesteryl ester transfer from HDL to LDL in exchange for TAG (16). Grain-fed beef increases the proportions of trans- and cis-unsaturated fatty acids in intramuscular adipose tissue and correlates with an impaired clearance by lipoprotein lipase or an increased production of VLDL due to its adverse effects FA have on lipid synthesis metabolism (17). The types of trans fats in animal products, however are not thought to have the same detrimental effects on CVH as trans fatty acids derived from chemical hydrogenation processes.

3.3. Presence of delta-9 desaturase *or (Acyl-CoA desaturase)*

Delta-9 desaturase is an enzyme used to convert stearic acid (C18:0) into oleic acid (C18:1n-9). In a sentence, delta-9 desaturase enzyme is encoded by stearoyl coenzyme A desaturase (SCD) gene that catalyzes the synthesis of unsaturated fatty acids, which additionally may convert trans-vaccenic acid (C18:1trans-11) into its corresponding conjugated linoleic acid (CLA) isomer (C18:2cis-9,trans11), which preforms numerous anti-mutagenic activities (37). With this in mind the importance of delta-9 desaturase in gene expression and enzyme activity is essential in understanding the development of adipose tissue and catalytic activity in beef fat.

In regards to grass and grain-fed beef delta-9 desaturase experiences higher activity levels in grain-fed beef due to its higher fat, SFA and oleic acid content. In particular Waygu cows produce 8-10% of their stearic acid in adipose tissue makeup (37) with a ratio of 0.77 in MUFA to SFA development, while grass-fed beef achieved a 2:1 MUFA:SFA ratio. Based on this interpretation, the strong negative correlation between palmitoleic and stearic acid is due to delta-9 desaturase activity in adipose tissue (36). Grass-fed beef has positive effects on PUFA to SFA ratios due to trans vaccenic acid content accumulating and thus being absorbed by delta-9 desaturase, converting it into precursor CLA (6,38). This consequently decreases SFA concentrations in the grass-fed beef, due to the animals diet (39).

To summarize, delta-9 desaturase is more prominent in grain-fed beef due to more adipose tissue development, therefore increasing the conversion rate of stearic into oleic acid. Additionally, delta-9 desaturase is a primary producer of isomer CLA by biohydrogentation of PUFA in the rumen (40).

3.3.1. Importance of conjugated linoleic acid

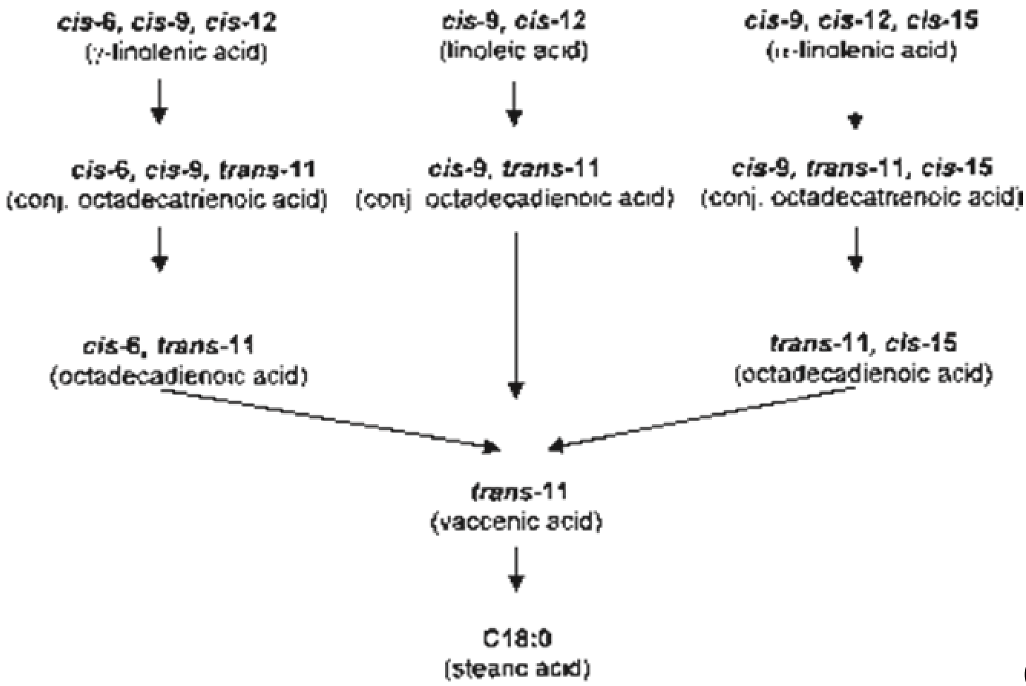
There has been significant recent research done on conjugated linoleic acid (cis-9, trans-11 18:2) (CLA) in understanding its biological mechanisms. It is of interest due to the considerable research done on its potent effects on the immune system, anti-mutagenic activity, tumor inhibition and lipid metabolism. CLA is an anti-carcinogen that is a naturally occurring major isomer (C18:2) found in ruminant cattle and by de novo synthesis in various tissues of ruminants (41,42). In its simplest explanation CLA originates from an incomplete form of biohydrogenated linoleic acid (LA) (C18:2n-6) in the rumen of cattle.

Nevertheless, delving into the detailed complexity of CLA production. The main sequential pathway begins with LA and ends with stearic acid. To clarify LA in this case acts as a competitive inhibitor for biohydrogenation inhibiting trans-11 octadecenoic acid. Proceeding with its main pathway as illustrated in *Figure 2* , the isomerization of LA to cis-9, trans-11 octadecadienoic acid is followed by the hydrogenation of cis-double bonded conjugated diene to trans-monoenoic acid (43). Likewise its other two pathways, as can be seen in the *Figure 2* below, commencing with alpha-linolenic acid (ALA) (C18:3n-3) and gamma-linolenic acid (C18:3n-6) (GLA) are hydrogenated after initial isomerization of cis-12 double bond. This isomerization is an essential step in the biohydrogenation of fatty acids containing cis-9, cis-12 double bond systems. The accumulation of trans-11 octadecenoic acid is the mutual intermediate in the biohydrogenation process of ALA and GLA in the rumen (40). While ALA is increased in the pathway cis-15 and trans-15 octadecenoic acid are accumulated, instead of trans-11 octadecenoic acid, thus being the reasoning for the high CLA concertation in cows indulging in diets high in ALA levels (44).

The simplistic *Figure 2* below illustrating the main pathways below doesn’t do justice to the multi-dynamic array of trans-octadecenoic acids performing continuously in the rumen and adipose tissues. These acids are thought to occur due to the rumen containing various specific bacterial isomerases.

However not all cows produce the same amounts of CLA. Grass-fed cows from Australia statistically produce the most CLA representing 1% of total FA, while grain-fed cows from the US have the lowest values of CLA representing 0.3-0.5% of total fat (40). The decreased levels of CLA in US cows are due to their low fiber diets that the grass-fed cows are being fed. The low fiber diets increase the amount of trans-10, cis-12 CLA isomers and decrease trans-11 octadecenoic acid formation in the rumen. Trans-10, cis-12 CLA is reported to be an inhibitor of adipose and milk fat synthesis, that any cow on a low fiber and grain-fed diet would experience (45). Although low fiber diets don’t change trans fatty acid production, they do alter the concentrations of specific important isomers in the rumen.

Returning to the pathway, the conversion of trans-11 octadecenoic acid into CLA is due to the activity of delta-9 desaturase. However before its conversion to CLA it is converted into vaccenic acid (trans-11, 18:1) which is the main trans-octadecenoic acid isomer formed by biohydrogenation of the initial C18 PUFA.



(40)

Figure 2: Predominant pathways of biohydrogenation of unsaturated C18 fatty acids.

Griinaria, J. Mikko and Bauman DE. Chapter 13: Biosynthesis of Conjugated Linoleic Acid and Its Incorporation into Meat and Milk in Ruminants. Adv Conjug Linoleic Acid Res [Internet]. 1999

In conclusion, CLA originates from delta-9 desaturase of vaccenic acid which comes from the rumen metabolism of LA in a process called biohydrogenation (46). CLA is thus an intermediate substrate from the final predominant source being LA, in addition smaller amounts of trans fats like oleic and palmitic acids are produced (16). The mechanistic pathways of CLA are still a ways away from being fully understood, however it is clear that diets containing CLA increase body protein and decrease body fat content. For example, there was a study done on rabbits that showed CLA inhibiting cholesterol induced atherosclerosis (47).

The irony that CLA is a tumor inhibitor, while LA is a tumor promotor that can be found in ruminant cattle shows how amazing nature's paradox functions, giving counteracting effects in one piece of beef, fulfilling the saying, "moderation is key".

4. Fate of dietary long chain fatty acids

Essentially, when consuming beef various FA are ingested and a wide range of intermediate biological pathways and biomarkers are activated. There are certain biomarkers in beef that play a key role in providing essential fatty acids and macronutrients in diets. It is well known that cows fed on grass diets have muscles consisting of higher n-3 PUFA concentrations, while cows fed on grain diets have muscles consisting of higher n-6 PUFA concentrations (48,49). Thus, how do these concentrations internally regulate the FA make-up?

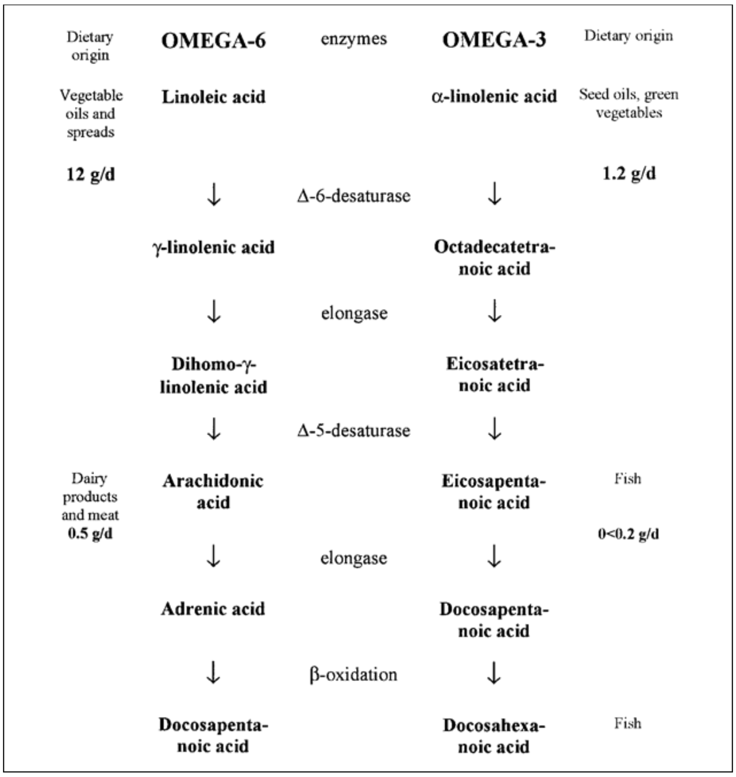
Adipose cells and fatty acids have found intelligent ways at the cellular level, not only to store and source energy but also participate in multiple complex cellular signaling and metabolic pathways. Fatty acids are able to serve as precursors for many lipid metabolic molecules and activating various gene expression patterns. This is important because pathways elaborate upon the positive and negative impacts that particular fatty acids can have on health.

4.1. Omega-3 vs. omega-6 polyunsaturated fatty acids and their inflammatory and anti-inflammatory pathways

In grass-fed beef n-3PUFA concentrations are high due to the presence of ALA (C18:3n-3) in grass, while n-6PUFA are formed from LA (C18:2n-6) found mostly in grains (50). Key biomarkers that are associated with LCn-3PUFA are EPA, DHA and DPA as well as ALA. Which are all found at higher concentrations in grass-fed beef, except DHA which had the lowest concentrations (14,23,46). Nonetheless there is significant evidence indicating their antioxidant and anti-inflammatory health outcomes that widely reduce factors of encountering CVDs by almost 20%. Grass-fed beef contains the previously stated LCn-3PUFA biomarkers but they just do not reach the level of concentration as they do with grass-fed beef. The increased LCn-6PUFA biomarkers associated with grass-fed beef are LA and arachidonic acid (AA) which are negatively correlated with fat content (48).

To elaborate further on their biological impacts n-3 and n-3 compete for the same conversion enzymes, thus having a greater quantity of n-6 in the diet directly affects the conversion of n-3 and therefore results in the increase of inflammatory related diseases. For instance, ALA which is converted into its longer chain fatty acid families EPA and DHA, and LA which is converted into its longer chain fatty acid AA (n-6) (9). However it is not as simple as *Figure 3* depicts, the pathways proceed as follows. ALA is an essential n-3 FA that plays a key role in the conversion of EPA, DHA and DPA. While EPA's main function is acting as a competitive inhibitor for pro-inflammatory AA as an eicosanoid substrate by inhibiting the production of pro-inflammatory eicosanoids, therefore inhibiting AA metabolism and altering inflammatory gene expression (9,47). While LA is a biomarker of n-6PUFA and a precursor to AA being oxidized in the cyclooxygenase (COX) or lipoxygenase pathways (32), thus the substrate for eicosanoids (30), as can be seen in *Figure 4*. Concluding, it is of incredible value to have a balance between n-6 and n-3, because they share an enzyme called delta-9 desaturase, which was previously mentioned is a key factor in adipose tissue development. Under ideal conditions this shared enzyme delta-9 desaturase preferably converts LCn-3PUFA precursor ALA into EPA and DHA, however when there are high intakes of n-6PUFA the presence of LA is abundant resulting in delta-9 desaturase focusing its actions on LCn-6PUFA (49) causing increased development of precursor AA. As well as correspondingly resulting in lower plasma levels of EPA, DPA and DHA.

With this in mind, grain-fed beef causes increased LA concentrations and excessive AA and eicosatrienoic acid (C20:3n-6) production in tissue metabolism (14,30). Eicosanoids are oxidative derivatives of PUFAs released from phospholipids of plasma membranes, that can have short term changes in proteins and have long term changes on quantity of mRNA (32,51). AA eicosanoid development are adverse because they are released as a negative response to stress, injury and infection causing unfavorable bio-physiological outcomes such as pro-aggregatory and prothrombotic, leading to a hypercholesterolemic state (9). While EPA which are found at higher concentrations in grass-fed beef counteract the AA responses by promoting heart health by preventing heart attacks and strokes(15).



(22)

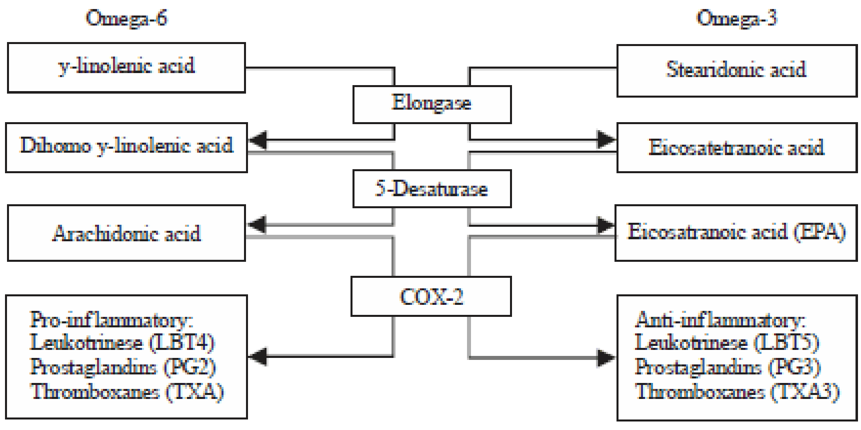
Figure 3: Pathways of omega-6 and omega-3 metabolism showing chain elongation and desaturation steps which share common enzyme systems. Showing major sources of precursor and long chain omega-6 and omega-3 PUFAs in the diet.

Williams CM, Williams CM. Dietary fatty acids and human health. Vol. 49, Ann. Zootech. 2000.

Therefore switching to grass-fed beef may increase the intake of ALA thus, reducing LA concentrations and effectively reducing its pathway competition between EPA and AA in the acylation into phospholipids (PL), thereby reducing platelet aggregation due to the over saturation of AA (14,52). Whereas a diet rich in n-3 PUFA improves some hematologic parameters associated with CVD risk. In essence a diet rich in n-3's is favored to n-6 because it decreases the pro inflammatory aspects of n-6 in various cellular pathways.

4.1.1. Specific lipid oxidation and inflammatory pathways

At the cellular level the only way that these various fatty acids are functioning is not through the dietary fatty acids themselves, rather thousands of microscopic cell signaling and gene expression pathways, depicted in *Figure 4* below. More specifically through membrane phospholipid content and/or eicosanoid production. Thanks to the various abundant essential PUFA in foods for being excellent hypolipidemic enhancers that can activate lipid oxidation and decrease gene encoding enzymes of lipid synthesis such as delta-9 desaturase and therefore repressing lipogenic genes like SCD by increasing the production of eicosanoids (51).



(15)

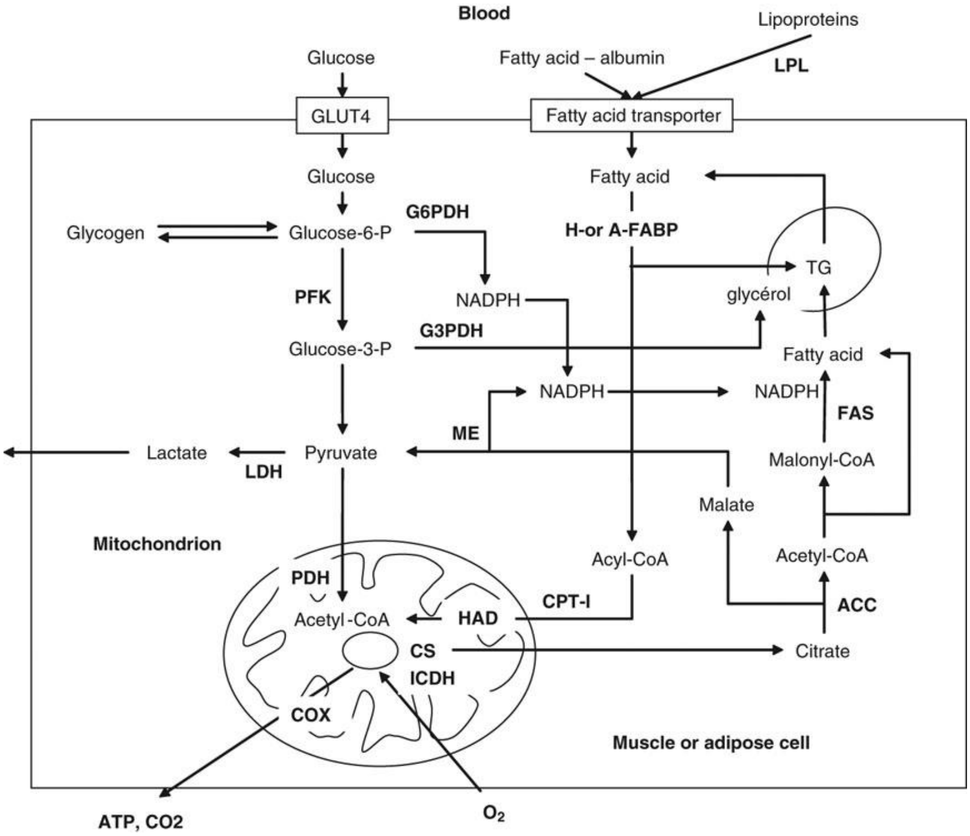
Figure 4: Mechanisms of omega-6 and omega-3 pathways. Source APS knowledge center

Bhardwaj K, Verma N, Trivedi RK, Bhardwaj S, Shukla N. Significance of ratio of Omega-3 and Omega-6 in human health with special reference to flaxseed oil. Vol. 10, International Journal of Biological Chemistry. 2016. p. 1–6.

As seen in *Figure 4* essential omega 3 and 6 fatty acids play in multiple cellular roles establishing anti- and pro- inflammatory reactions in cyclooxygenase-2 (COX-2) pathways synthesizing prostaglandins and therefore leading to a multitude of responses, some will be outlined in this section. Recent research has shown that there is a direct link for gene regulation between specific nuclear receptors as can be seen in *Figure 5,* such as peroxisome proliferated-activator receptors (PPAR), liver X-receptor (LXR) and hepatocyte nuclear factor-4 alpha (HNF-4α) that all skillfully bind to fatty acids to serve their roles in gene regulation. Even though the function and mechanisms of fatty acids is not entirely clear, it has been established that they effect the quantity of transcription factors such as sterol-regulatory element binding protein (SREBP) and nuclear factor-*kappa* B (NF-*k*B) that mediate various gene regulations (32).

To briefly describe some of the mechanisms illustrated in *Figure 5*, PPAR belongs to a steroid hormone nuclear superfamily of ligand activated transcription factors. PPARα acts in primary hepatocytes that are predominantly activated by EPA as well as LA, ALA and CLA decreasing lipid production. Secondly, LXR are a set of nuclear receptors that are mediators for gene regulation by fatty acids. It has been recently researched that n-6 PUFA inhibit prolipogenic actions of LXRs as well as decreasing transcription of SREBP-1c promoter activity. Thirdly, HNF-4α regulates glucose and lipid metabolism while PUFA in its CoA form like ALA, EPA and DHA repress its lipid and cholesterol activity. Lastly, NF-*k*B regulates PUFA positively by AA stimulating NF-*k*B translocation of target genes and regulated negatively by EPA inhibiting NF-*k*B translocation, due to EPA not being able to metabolize in the COX pathways (32).

Thus, frequently proving that PUFA increased intake is vital for activation pathways, which decrease lipid production and increase lipid oxidation. Concluding, that the ideal beef option is grass-fed beef due to its higher concentrations of incredibly beneficial PUFAs.



(53)

Figure 5: Overview of energy metabolism in muscular or adipose cells. Lipogenesis (right) is a major metabolic pathway in adipose cells. Mitochondrial oxidation is a major pathway in oxidative muscle fibres. ACC = acetyl-CoA carboxylase; COX = cytochrome-c oxydase; CPT-I = carnitine palmitoyl-transferase-I; CS = citrate synthase; ME: malic enzyme; H- or A-FABP = fatty acid-binding protein (H-FABP: heart and muscle isoform; A-FABP: adipocyte-isoform); FAS = fatty-acid synthase; G3PDH = glycerol-3 phosphate dehydrogenase; G6PDH = glucose-6-phosphate dehydrogenase; ICDH = isocitrate dehydrogenase; HAD = hydroxyacyl-CoA dehydrogenase; LDH = lactate dehydrogenase; LPL = lipoprotein lipase; PDH = pyruvate dehydrogenase; PFK = phosphofructokinase; TG = triglycerides.

Hocquette JF, Gondret F, Baéza E, Médale F, Jurie C, Pethick DW. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. animal. 2010 Feb 23.

4.2. Omega-3 and omega-6 balance

It is important to realize that not all studies agree that grass-fed beef has decreased n-6 ratios. Some articles concluded that there were increased proportions of n-3 in grass-fed cows, meanwhile n-6 levels were unchanged. In either case, the increased intake of n-3 PUFA shows signs of health benefits in immunological and neurological reports. Although this does not mean that n-6 PUFA should be completely neglected, rather the balance between n-6: n-3 should be maintained in a diet. Especially given the data presented, it is natural to assume that exponentially increasing the amount of n-3 and decreasing the amount of n-6 PUFAs in diets is key to avoiding CVD. It must be emphasized that the balance between long chain n-3:n-6 (1:1) and their essential PUFA is the goal, since they both in conjugation play a crucial role in gene expression, eicosanoid metabolism and cytokine production (9,15).

Especially since long chain n-6 and n-3 PUFA are made of AA and EPA that work in conjugation to produce eicosanoids after being metabolized. Not to mention DHA which works similarly in being anti-inflammatory (47) by lowering serum triglycerides levels and increasing HDL-C values in one study (54). Additionally improving neurological brain function by n-3PUFA regulating neurotransmitters by supplementing brain fluidity in neuronal brain function, improving retina/visual performance, enhancing nitric oxide biosynthesis and spermatozoa (54). However more research needs to be done on understanding its potential mechanistic pathways. Nonetheless for these reasons a balanced consumption of n-3's and n-6's PUFA is vital for optimum heart health and avoiding atherosclerosis and therefore resulting in a healthy lipidomic profile in individuals who consume more grass-fed beef.
Thus, concluding the importance of having these biomarkers in conjugation with one another, otherwise an imbalance will lead to adverse platelet fatty acid activity (30).

5. Conclusion

In a final analyses grass-fed beef exhibits a plethora of health benefits due to its higher n-3 PUFA content, thus being the favorable health option for humans as well as for the cows. Omega 3s enhancement of CVH works at the cellular level maintaining healthy cell proliferation and regulating a healthy lipidome.

All in all, from analyzing the path of essential LCn-3PUFA it can be seen that various FA contain more than one compound and their compounds facilitate more than one mechanistic pathway. Playing a key role in regulating anti-arrhythmic, anti-thrombotic and hypotriglyceridemia thus preventing atherosclerotic plaque buildup through anti-inflammatory effects.

For all of these pathways the impact of feeding systems on cows' beef directly influences the effects of blood lipid pathways on the lipidome and lipogenesis. Feeding systems influence the uptake of essential micro and macronutrients that are defiantly lacking in habitual diets worldwide, that need to be researched further. All things considered, beef feeding practice differences on the impact of CVD risk have not been thoroughly studied yet.

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