IMPACT OF CATTLE FEEDING-STYLE ON BEEF AND HUMAN POSTPRANDIAL INFLAMMATION

by

Meghan Leigh Spears

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Exercise and Nutrition Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2024

ACKNOWLEDGEMENTS

The author thanks all faculty, graduate, and undergraduate students who assisted in the completion of this research project. She also thanks the ladies of the Nutrition Research Laboratory for offering mental and physical support when needed.

The author offers special thanks to her parents, Scott and Maribeth, for providing support to allow my graduate work to be focused on my education as well as her grandmother for thinking this work is the coolest thing ever. Lastly the author thanks James Flynn for being a constant support in this process.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

GLOSSARY

Abbreviations

AOAC- Association of Agricultural Chemists
AUC- net area under the curve
BCAA- branched chain amino acid
BMI- body mass index
COPD- chronic obstructive pulmonary disease
CON- conventional feeding style
CRP- c-reactive protein
CVD- cardiovascular disease
FA- fatty acids
FFA- free fatty acid
GALT- gut associated lymphoid tissue
GRA- grass-fed feeding style
GM-CSF- granulocyte macrophage colony-stimulating factor
HCL- hydrochloric acid
IL- interleukin
IFN-γ- Interferon gamma
LPS- Lipopolysaccharide
MALT- mucosa associated lymphoid tissue
MUFA- monounsaturated fatty acid
NF-kB- Nuclear Factor Kappa B
PAMPs- pathogen associated molecular patterns
PEPT1- human peptide transporter 1
PKB- protein kinase B
PUFA- polyunsaturated fatty acid
ROS- reactive oxygen species
SD- standard deviation
se- standard error
SFA- saturated fatty acid
T2DM- type 2 diabetes mellitus
TAG- triacylglyceride
Th- t helper
TLR4- toll-like receptor 4
TMAO- trimethylamine-N-oxide
TNF-α- tumor necrosis factor
VLDL- very low-density lipoprotein

ABSTRACT

Purpose: Various cattle-feeding styles have arisen in recent years, impacting the sustainability and environmental practices of many producers. While these changes are known to have an impact on the environment, little is known about the direct impact cattle raised using different feeding styles has on human health. Acute response focused studies, like this, are a glimpse into the expected impact of a certain food on the diet over time. The purpose of this study is to investigate the impact of cattle-feeding style on postprandial inflammation. **Methods:** A randomized, double blind, crossover study design was used to compare grass-fed (GRA) and conventional (CON) beef. Subjects (n=10) were comprised of men and women with a healthy body mass index (BMI) and no preexisting metabolic conditions. Blood samples were collected fasted and postprandially for four hours. Blood samples were analyzed for inflammation markers (TNF-α, IL-23, IL-17, IL-10, IL-1β, IL-6, IFN-γ and GM-CSF) at hourly timepoints. To observe postprandial changes with and without consideration for cattle feeding style, the net area under the curve (AUC) was calculated. Maxchange and CMAXtime were calculated by finding the maximum value of each cytokine between hours one and hour postprandially and subtracting that from the fasting value. CMAXtime represents the time at which the maximum value of each cytokine was reached in hours. Maxchange and AUC responses were compared to zero using a one-sample t-test to determine if response was greater than fasting. **Results:** In response to beef, maxchange of all measured markers and IFNγ AUC were significantly greater than zero ($p< 0.05$). No differences were shown between GRA and CON in inflammation AUC ($p> 0.05$). **Conclusion:** This demonstrates that beef consumption does increase postprandial inflammation, but cattle-feeding style does not significantly impact this response.

CHAPTER ONE

INTRODUCTION

Development of the Problem

Changes in agricultural techniques in the interest of animal welfare and climate impact have made grass-fed beef a product of growing interest in the market. According to a 2017 report, roughly 4% of total U.S beef retail sales are from beef labeled as grass-fed[1]. It is expected that the market for grass-fed beef will continue to expand due to economic changes and personal interest in nutritional choices, with a compound annual growth rate of 4.4% between 2021 and 2025[2]. To accompany this increased interest in beef, literature has grown in the area comparing grass-fed beef to other cattle feeding styles like grain or corn-fed (conventional feeding styles). Part of this research focuses on investigating the potential differences in nutrient content such as fatty acid and lipid composition. These studies speculate that the nutritional composition of beef will have an impact on human health based on known metabolic pathways and previous research[3–5]. This information has been applied to animal models, specially focused on postprandial inflammation following beef consumption[6,7]. Inflammation plays a role in human health, regulating immune responses and possibly disrupting metabolic processes.

Inflammation that may result from consumption of beef is important to identify because chronic disease can be exacerbated by inflammation. When food is digested, reactive oxygen species are formed from the oxidation of protein and fat. These changes to the structure of the protein and/or fat produce free radicals that then cause cellular damage and inflammation[8,9]. The intake of red meat in the diet has been linked to increased systemic inflammation, accounting for

amount eaten and body composition of the individual[10]. The impact of red meat on inflammation has been studied only in rodent models to the best of our knowledge, showing that red meat consumption increases oxidative stress parameters, trimethylamine-N-oxide (TMAO), and c-reactive protein (CRP), but less work has been done on the impact in humans[7]. The gap in research currently is the need to understand the direct impact that cattle-feeding styles have on the metabolic health of the human consumer, particularly the inflammatory response to a single meal of beef from grass-fed and conventional feeding styles.

Purpose

The aim of this study is to investigate the differences between grass-fed and conventionally fed beef on acute inflammation responses in human subjects. Systemic inflammation is one of the leading causes of chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD) (particularly atherosclerosis related), and metabolic syndrome[11–13]. The influence of diet on inflammation is vast. Varying diets and specific foods have shown to effect inflammation based on the nutritional profiles comprised in a diet[14]. The fat content of a meal has been shown to influence acute inflammation. Meals both high in saturated fatty acids (SFA) and low in the n-3/n-6 polyunsaturated fatty acids ratio increase acute inflammation postpradially[15]. Amino acid composition from the diet also affects health. Proteins are susceptible to oxidative degradation, leading to inflammation. Sulfur-containing amino acids have been shown to act as antioxidants, mitigating the radical damage to cells[16]. There also may be other currently unknown factors that differentiate grass-fed and conventional beef that could impact human health. By analyzing the human postprandial inflammation response to a single

meal of beef, it can provide insight into if cattle-feeding style, grass-fed (GRA) and conventional (CON), elicit different responses.

Hypothesis

It is hypothesized that the postprandial inflammatory response will be increased from fasting in response to beef and that there will be a difference in postprandial inflammation between GRA and CON beef.

Delimitations

1. This study is age restricted to females and male sages 18-45 years old.
2. This study is restricted to individuals with a body mass index (BMI) between 18-27 kg/m^2.
3. This study observes the response to a single meal and the acute response that follows.

Limitations

1. The results cannot be generalized to those under 18 or above 45 years old.
2. The results cannot be generalized to individuals with a BMI below 18 and above 27 kg/m^2.
3. The results cannot be generalized to individuals with metabolic, cardiovascular, gastrointestinal or other chronic disease states.

Implications

1. Understanding the impact of a single meal of beef can be indicative of possible chronic implications of beef in the diet.
2. Analyzing the impact of cattle-feeding style on human postprandial inflammation informs a connection between cattle feeding and its impact on nutritional components of beef, and then that impact on the consumers postprandial response.
3. The impact of beef on postprandial inflammation relates to chronic systemic inflammation, which is linked to chronic disease states such as CVD.

CHAPTER TWO

LITERATURE REVIEW

Grass-fed Cattle Feeding Style

According to the United States Department of Agriculture, grass-fed has been defined as animals who have been fed grass and forage for their entire life span. Forage includes grain crops in the vegetative state, forbs, browse, and grass[17]. There are many versions of grass-fed methodology, including pasture grazing of the entirety of the cattle lifespan to pasture feeding finished with grain before slaughter. Cattle fed a primary grass diet are smaller in terms of weight compared to conventionally fed cattle. This is due to the absence of calorically dense finishing feed received by conventionally fed cattle. This influences the amount of cattle needed to produce similar amounts of product to other methods. Environmental impact is then affected by this, creating a larger greenhouse gas footprint compared to others[18,19]. While this feeding style has grown in popularity, the consistency between producers has been questioned in literature. The observed nutritional differences between producers using this method have been seen in fatty acid and phytochemical composition. The seasonal differences in temperature and climate, as well as grass type also influence the nutritional profiles of product[20].

Conventional Cattle Feeding Style

Conventional feeding refers to a feeding method in which animals are fed high energy grains to increase fat storage, especially in the muscle. These cattle are fed roughage and then transitioned to incorporate grain feed in the diet[5]. This results in producers having more cycles of feed-to-slaughter in a year than grass-fed producers. The primary macronutrient makeup of beef

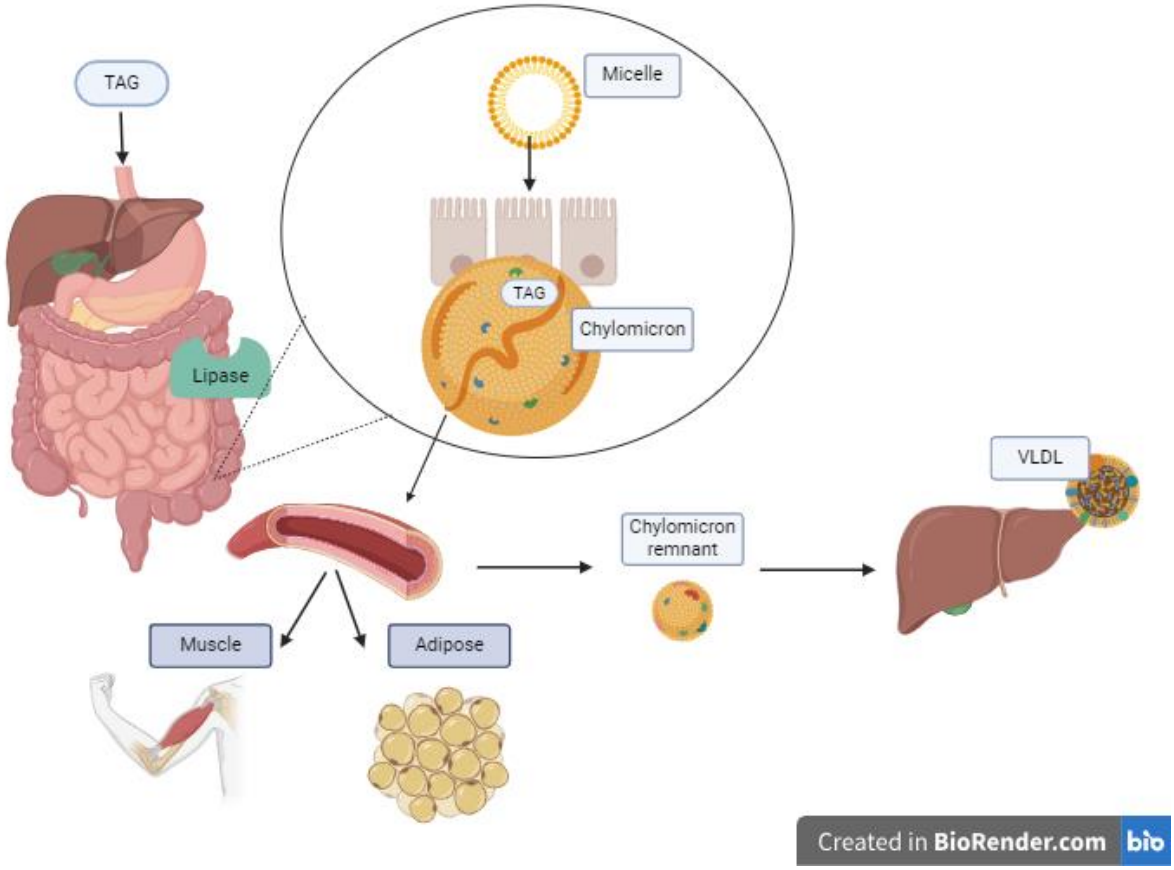
in general is protein and fat. The digestion and metabolism of these macronutrients is important to the understanding of downstream postprandial responses.

Postprandial Lipid Digestion and Metabolism

After consuming a meal containing fat, the goal of digestion is to breakdown the triglyceride unit by first removing fatty acids from the glycerol and then further digesting induvial fatty acids into small chains. Most dietary fats are consumed as triglycerides, a glycerol bonded to three fatty acids by ester bonds. The structure of the fatty acids (saturated and unsaturated) within the triglyceride can impact downstream metabolism. Saturated fatty acids have a structure that includes no double bonds in the carbon chain, while unsaturated fatty acids have one or more double bonds[21]. Digestion of triglycerides in the stomach and small intestine are catalyzed by lipase, produced by the pancreas and stomach, until free fatty acids are available for absorption. In the small intestine, free fatty acids are incorporated into micelles for transport and absorption in enterocytes. Within enterocytes large lipoproteins, known as chylomicrons, are formed as well as triglycerides reformed from fatty acids[22]. Chylomicrons are triglyceride rich particles that carry lipids from enterocytes to other tissues in the body and then the liver. Chylomicrons enter lymphatic circulation to deliver lipids to tissues like adipose and skeletal muscle for energy storage. In adipose tissue, fatty acids are stored as triglycerides and then released when fatty acids are needed as an energy production substrate. In skeletal muscle, fatty acids are stores to also be used as an energy production substrate when needed. During this transport, the chylomicron is hydrolyzed to produce smaller lipoproteins. Lipoproteins are then taken up by the liver where remaining fatty acids are reassembled as very low-density lipoprotein (VLDL) and secreted into the blood stream[23,24]. Remnants of chylomicrons are then delivered to

the liver. Figure 1 depicts this pathway. This metabolic process can take upwards of 4-5 hours, depending on the individuals fasting lipid concentrations as well as the type of meal consumed. Due to this time frame, the process is rarely complete before another meal or nutrient is consumed. Lipid metabolism is incorporated into many vital bodily functions. For energy storage and usage beta oxidation takes fatty acids and converts to acetyl-coa. Lipids also act as signaling molecules by interacting with immune cell receptors to initiate secondary responses, like T cell receptor responses. Immune cells in the digestive tract, like gut-associated lymphoid tissue (GALT) and mucosa-associated lymphoid tissue (MALT), interact with lipids through specific receptors to induce or prevent inflammatory response. For instance, SFA increase T-helper (Th) 1 production, which incites a proinflammatory response[25,26]. Another macronutrient important to understanding postprandial responses is protein.

Figure 1. Lipid Digestion and Metabolism.



Triglycerides (TAG) are consumed in the diet and digested into free fatty acids (FFA) with the help of lipase enzyme. Digested lipids are reformed and then added to chylomicrons in enterocytes for transport into lymphatic circulation. On the way to the liver, lipids are delivered to other tissues, like skeletal muscle and adipose, for energy storage. Chylomicrons then become smaller lipoproteins (chylomicron remnants). The liver further digests lipid components in the chylomicron remnant, resulting in very low-density lipoproteins (VLDL) that are transported into systemic circulation. Created with BioRender.com

Postprandial Protein Digestion and Metabolism

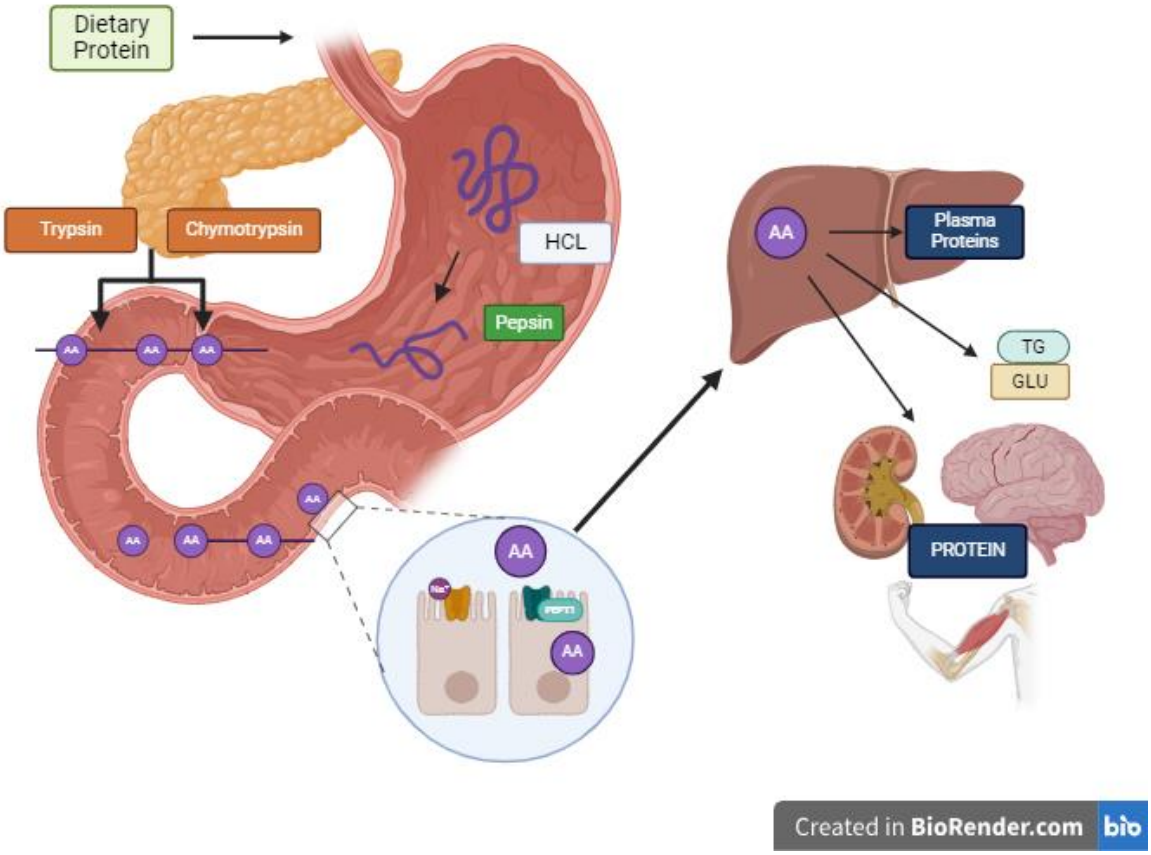
Proteins are ingested in various dietary sources, including beef products, and then digested into shorter chains of amino acids and free amino acids by protease enzymes. This begins in the stomach where ingested proteins are denatured (unfolded from the quaternary and termitary structures) by the secretions of low pH gastric juice from parietal cells in the stomach

lining. Gastric juice also activates dormant forms of protease enzymes, like pepsinogen into pepsin, and others secreted from the pancreas (trypsin and chymotrypsin) the further protein digestion[27]This is made possible by the low pH in the stomach resulting from gastric juice secretion, which denatures proteins and activates the dormant zymogen form of these enzymes. For example, pepsinogen is converted to pepsin, an active protease enzyme, by hydrochloric acid in gastric juice. The digestion of proteins stimulates pancreatic hormones secretin and cholecystokinin, which stimulates bile and pancreatic juice secretion help further breakdown polypeptides[28]. Pancreatic juice has unique protease enzymes, chymotrypsinogen and trypsinogen, that breakdown longer chains of amino acids into smaller polypeptides or free amino acids[29]. Protease enzymes (peptidases) at the brush border help to ensure sufficient digestion of compounds before absorption[30]. At enterocytes, most polypeptides and amino acids are absorbed into portal circulation, but some (like glutamine) remain to be used for intestine specific protein synthesis. A transporter, human peptide transport 1 (PEPT1), moves di- and tri-peptides across the membrane while other amino acids are transported via sodium independent and dependent transport into portal circualtion[31]. Within enterocytes, protein synthesis occurs, but remaining free amino acids are transported to the liver and muscle tissue. Figure 2 depicts this pathway. Like lipids, amino acids also interact with immune cells in the digestive tract. Depending on the amino acid, this interaction can instigate a pro or anti-inflammatory response, for example, threonine has been reported to increase the expression of proinflammatory genes while cysteine consumption has been reported to attenuate proinflammatory responses[32.]

Portal circulation delivers these amino acids and polypeptides to the liver where hepatocytes use them in a variety of ways. Protein synthesis occurs in the liver and other extra-

hepatic tissue like the kidneys, brain, and skeletal muscle. To utilize amino acids for protein synthesis, the amine group must be removed via deamination, or it can be transferred to synthesize non-essential amino acids (transamination)[33]. Protein synthesis using the amino acids and polypeptides absorbed through the digestive system and portal circulation is essential for synthesizing numerous plasma proteins, like albumin and providing amino acids for extra-hepatic protein synthesis of tissue and neurological proteins[34,35]. When in excess, amino acids can be converted into triglycerides for storage as well as into glucose for use as an energy substrate. An excess of amino acids also promotes the use of amino acids to synthesize skeletal muscle[36,37]

Figure 2. Protein Digestion and Metabolism.



Dietary protein is digested in the stomach and small intestine with the help of hydrochloric acid (HCL) and pepsin. The pancreas secretes protease enzymes and bile to further digest proteins into smaller chains and free amino acids. Sodium independent and dependent transports free amino acids across enterocytes along with specific polypeptide transport PEPT1. Some amino acids remain in enterocytes and the rest are transported to the liver via portal circulation. In the liver, the synthesis of many plasma proteins occurs. Amino acids also are transported to extra hepatic tissues like the kidneys, brain and skeletal muscles to be used in other anabolic pathways. When amino acids are in excess, they can also be used to make glucose and triglycerides. AA; amino acid. TG; triglyceride, GLU; glucose, HCL; hydrochloric acid.

Inflammatory Markers

Inflammatory markers (cytokines) measured in relation to this study include interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-17 (IL-17), interleukin-23 (IL-23), interleukin-1 beta (IL-1ß), tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ), and granulocyte-

macrophage colony stimulating factor (GM-CSF). Cytokines are signaling proteins present in immune cells, adipose cells, endothelia cells, and others[38,39]. These inflammation markers can be characterized as pro-inflammatory, and anti-inflammatory based on the impact the cytokine has on the immune system and stimuli to which they respond. Table 1 provides a description of all measured cytokines. Cytokines in these categories have a pleiotropic and synergistic effect depending on the target cell. Pro-inflammatory cytokines are produced when an immune response is required. Anti-inflammatory cytokines are tasked with reducing pro-inflammatory responses. Of the measured cytokines, IL-10 is the most potent anti-inflammatory of this group[40]. These inflammatory markers play a key role in the postprandial inflammation response to a meal.

Table 1. Description of cytokines measured including purpose, function, and pro or anti-inflammatory categorization[41–49].

Cytokine	Function and Source	Pro or Anti Inflammatory
GM-CSF	Produced by T_H1 and T_H17 lymphocytes via Jak/STAT signaling Stimulates macrophage and granulocyte mobilization.	PRO
IL-23	Produced by macrophages and antigen-presenting cells. Works with IL-17 to promote inflammation in joints, peripheral tissues, and intestines.	PRO
IL-17	Produced by $CD4^+$ and $CD8^+$ T cells in response to IL-23, regulate intestinal mucosal defenses.	PRO
IL-10	Produced by T helper cells, monocytes, dendritic cells, and macrophages. Prevents maturation of macrophages to dull inflammation.	ANTI
IL-6	Produced by macrophages, adipose tissue, and endothelial cells. Signals pro-inflammatory response to tissue damage. Anti-inflammatory capabilities by promoting IL-10 production.	PRO

Table 1 Continued.

Cytokine	Function and Source	Pro or Anti Inflammatory
IL-1ß	Produced by macrophages and monocytes, involved in cell proliferation and differentiation.	PRO
TNF-α	Produced by macrophages, involved cytokine production and cell proliferation.	PRO
IFN-γ	Produced by T cells and natural killer cells. Role in innate immune system stimulus of inflammation.	PRO

CD= cluster of differentiation.

Postprandial Inflammation

Inflammation has been measured in response to a variety of meals to better understand how different foods may impact inflammation. To establish information about timing and magnitude of inflammation responses, a challenge meal or high-fat meal (containing over 30% fat) is often used to elicit a response. This is because high-fat diets are linked to increased disease risk due to the interaction of fatty acids with intestinal immune cells promoting low-grade systemic inflammation[50]. The postprandial time-period is generally considered 6-12 hours. In many studies that measure postprandial inflammation, the mean timeframe to draw blood samples was 5.7 hours after a meal. This was a sufficient time-period to observe changes in cytokine markers, CRP measurements are shown to be more accurate in a 24-hour window[51].

Postprandial values of commonly measured inflammatory markers in response to a high fat meal differ depending on the marker. In a study using healthy individuals of young (20-25 yrs.) and older (60-75 yrs.) ages measuring inflammation markers TNF-α, IL6, and IL1β after a high-fat meal at fasting, 2, and 4 hours postprandially, IL1β and TNF-α did not significantly change after the high-fat meal (p =0.336) but IL6 did significantly increase from fasting at hour 4 (p =

0.007). Age did not significantly impact the response. The magnitude of mean response for IL6 and TNF-α ranged from 0-20 pg/mL and was measured with a multiplex magnetic bead assay (Milliplex MAP Kit Human Cytokine Magnetic Bead Panel Assay, Millipore, Billerica, MA, USA)[52]. Another study using younger active (18-35 yrs.) and older active and inactive (>60 yrs.) subjects measured IL6, Il10, IL17A, IL23, TNF-α, GMCSF, IFNγ, and IL1β for 6 hours after a high fat meal. Inflammatory marker measurement also took place with a multiplex bead assay. In response to the high-fat meal, all markers significantly decreased from fasting ($p < 0.05$) and age/activity level grouping had an effect on IL23 and IL10 in those young active subjects had significantly higher fasting values than older active and inactive subjects. TNF-α, IL17A, IL10, IL6, IL1β, and IFNγ mean values ranged from 0-20pg/mL, while IL23 mean values ranged from 200-1200 pg/mL and GMCSF mean values ranged from 100-600 pg/mL[53]. Between these two studies, it shows that inflammatory response to high-fat meals is variable and can be influenced by age and even physical activity level.

In response to mixed-meals, postprandial inflammation also can vary. In the PREDICT study, IL6 was measured in healthy subjects for a total of 6 hours and two separate mixed-meal interventions (breakfast and lunch). Other assessments, such as a gut microbiome analysis, anthropometrics, and measurement of postprandial glucose and triglycerides were also done. Postprandial IL6 response was increased by an average of 190% from fasting in 94% of subjects after the breakfast meal and continued to increase after the lunch meal in 71% of subjects. This response was correlated with subject adiposity, but not glucose and triglyceride values. Fasting IL6 values were weakly correlated to fasting glucose and triglyceride values. Subjects fasting IL6 values ranged from 0-3 mmol/L[54]. While the majority of subjects in this study had an

increase in inflammation after the mixed meals, not all did, which speaks to inter-subject variability when measuring inflammation response to the same meal.

The postprandial inflammation response is dependent on many factors beginning with the type of nutrients ingested and followed by receptor binding activity and hormonal responses. This is influenced by the underlying metabolic health of the individual. One of the main signaling mediators of postprandial inflammation is nuclear factor kappa-B (NF-kB).

NF-kB is a transcription factor shown to be activated during the digestion of certain nutrients. This activation carries out the initiation of gene expression of some pro-inflammatory cytokines in the nucleus of cells. The type of nutrient ingested has the largest impact on the signaling of NF-kB. Amino acids have been shown to reduce NF-kB signaling, having anti-inflammatory benefits. Immune cells in the gut, like MALT and GALT interact directly with amino acids through numerous protein specific kinases. For example, protein kinase B (PKB) (also known as Akt) is an amino acid specific kinase needed in the PI3K/Akt inflammation signaling pathway. PI3K/Akt inhibits NF-kB signaling, disrupting the production of proinflammatory cytokines. This is important for mediation of intestinal inflammation. PI3K/Akt is a diverse pathway that plays a role in inflammation as well as protein synthesis[55,56]. Glutamate is a particular amino acid that interacts with immune cells in a proinflammatory matter via glutamate specific receptors on immune cells like T lymphocytes, triggering inflammatory responses by making proinflammatory cytokines and recruiting macrophages[57].

Fats, particularly SFA, on the other hand promote NF-kB activation through interaction with toll-like receptor 4 (TLR4). SFA are typically a component of Lipid A in lipopolysaccharides (LPS). When this interacts with TLR4, it stimulates inflammation. Conversely, unsaturated fatty

acids in the Lipid A portion of LPS has been shown to stop this ligand binding TLR4 and therefore a following inflammatory response[58,59]. There are many other things that can activate inflammation signaling pathways, one of them being reactive oxygen species.

Since metabolic processes are upregulated in the postprandial state to metabolize nutrients, there is a natural increase in reactive oxygen species (ROS) production. When this continues in excess without the counteraction of redox reactions, ROS interrupt metabolic function and feeds into pro-inflammatory pathway, like the activation of NF-kB as well as lipid peroxidation and protein carbonylation[60]. Oxidative stress, defined as an imbalance between ROS and the body's ability detoxify these reactive species[61]. ROS are formed during metabolic processes in mitochondria, for instance electrons from the electron transport chain reacting with oxygen to produce a superoxide anion.

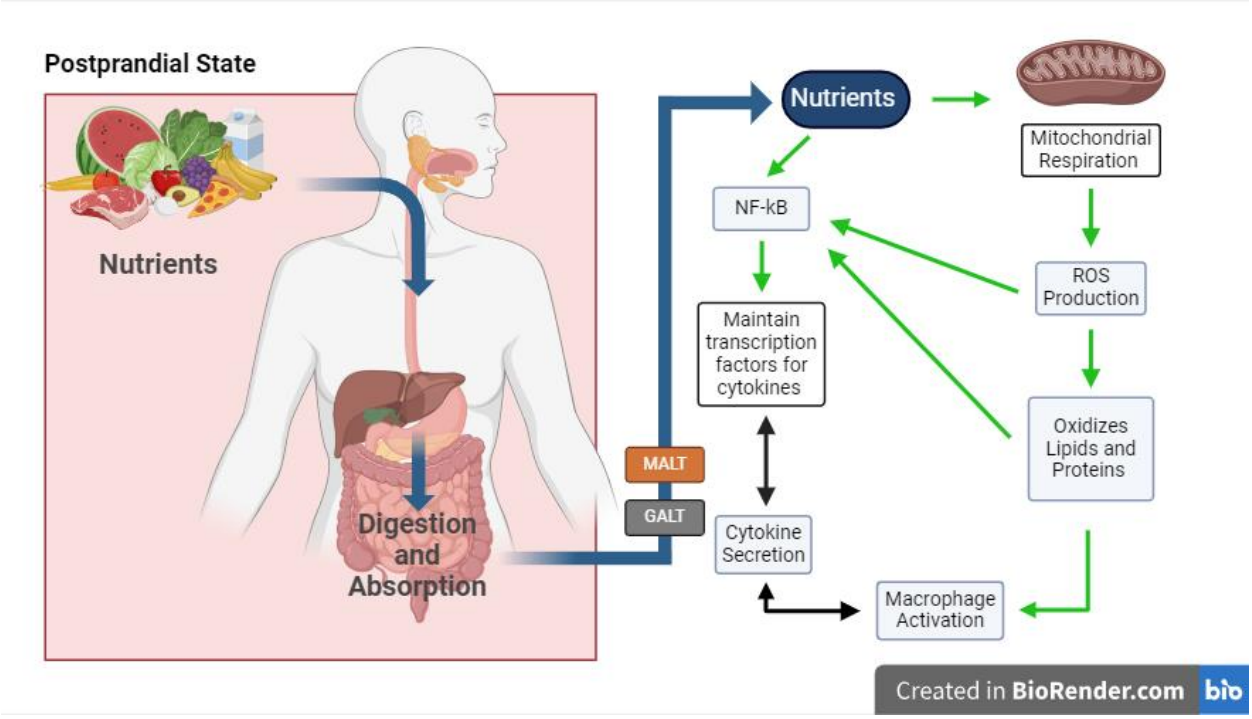
Oxidized lipids and proteins act as signaling molecules for immune cells to further the postprandial inflammation response. Lipid peroxidation is the interaction of cells membrane phospholipids with ROS, oxidizing the unsaturated fatty acid chains. This forms hydroperoxidized lipids (like prostaglandin 2 from oxidation of arachidonic acid) and reactive aldehydes that have been implicated in disease progression by promoting inflammation[62]. Protein carbonylation is the oxidation of amino acid side chains by ROS as well as products of lipid peroxidation. When peptide bonds are broken via oxidation, the hydrogen atoms on the sidechain group are removed. The alkyl radical formed after this removal then reacts with oxygen to form an alkoxyl-peroxide radical. This radical formation is what causes oxidative damage to cells and other structures, leading to inflammation and poor health[8,63]. Lipid oxidization can also occur via enzymatic lipid oxidization. This reaction is mediated by lipid oxygenase. For this enzyme to be

activated, it must react with ferrous iron. The rate at which this enzyme works is also dependent on the amount of enzyme present. When activated, the enzyme removes hydrogen from a polyunsaturated fatty acid (PUFA) to form a peroxyl radical. Peroxyl radical removes hydrogen from an unsaturated fatty acid, finally forming an alkyl radical. As in the autooxidation process, alkyl and peroxyl radicals create a cycle of removing hydrogens from fatty acids to create more radicals[64]. These oxidative processes occur inside cell membranes.

Immune cells that are the target of postprandial metabolites include but are not limited to macrophages. Macrophages are immune cells that originate in bone marrow and respond to immune system stimuli. There are two types of macrophages, M1 and M2. M1 cells are known to produce pro-inflammatory cytokines like TNF-α, which recruit other immune cells such as T-cells, and IL-1β which stimulates insulin production. M2 cells are diverse but generally work to combat pro-inflammatory pathways by promoting tissue repair and producing anti-inflammatory cytokines[65,66]. Macrophages have two types of receptors, scavenger and Toll-like. These receptors can bind with ligands from metabolites like oxidized lipids and oxidized proteins. The macrophage then produces cytokines, such as IL1-beta and IL-6, in response to these binding stimuli[67]. The type and amount of nutrients ingested will control this postprandial response. Prolonged time in this state has been linked to metabolic disease progression due to the constant activity of macrophages and corresponding cytokines[68]. Figure 3 depicts this pathway. While internal signaling is one of the main contributors to the progression of inflammation, body composition (especially visceral adiposity) plays a role in baseline risk for enhanced inflammation.

Visceral adiposity has also been cited as a factor that influences fasting inflammation levels and postprandial responses. Visceral adipose tissue, located around organs like the intestines, liver, and heart, contains T-lymphocytes which recruits macrophages and initiates an immune response that produces more pro-inflammatory cytokines[69]. This contributes to elevated fasting inflammation levels and altered postprandial response in individuals particularly with elevated visceral adiposity measured by a waist circumference above 40 inches in men and 35 inches in women[70]. As mentioned earlier, the type of nutrients ingested in a meal play a role in the magnitude of the inflammatory response. Insights into nutrient composition of beef indicates the possibility for a unique relationship between beef consumption and postprandial inflammation.

Figure 3. Postprandial Inflammation.



After the digestion and absorption of ingested nutrients, undergo mitochondrial respiration. This generates reactive oxygen species (ROS) which contribute to the oxidation of lipids and proteins. Oxidized lipids and proteins bind to receptors on macrophages, stimulating the secretion of cytokines as well as stimulating the NF-kB pathway. The transcription of cytokines, like TNF-α, is controlled by the NF-kB pathway that is also stimulated by feeding. Created with BioRender.com

Inflammation and Beef Consumption

Consumption of red meat has been associated with a potential to increase risk for diabetes, cancer, and cardiovascular disease. The cited risks pertaining to unprocessed red meat are seen in diets containing an additional 100-120g of red meat each day[71]. Nutritional components of beef in general can have anti and pro inflammatory properties. Branched chain amino acids (BCAA), leucine, isoleucine, and valine are essential amino acids present in beef and other protein containing foods. It has been shown by Van Hecke et al. that the majority of protein oxidation occurs in the colonic phase of digestion of red meat[72]. While protein is

oxidized during digestion and metabolism of BCAA's, specifically leucine, have been shown to decrease proinflammatory nitric oxide production and expression of IL-6[73]. In contrast, a proinflammatory glycan, N-glycolylneuramic acid, derived specifically from red meat products, has been shown to promote inflammation by interacting with existing antibodies to contribute to chronic inflammation[74].As stated previously, the fatty acid composition from ingested dietary sources also has an impact on inflammation. N-3 fatty acids, a form of unsaturated fatty acid, have anti-inflammatory properties while saturated fatty acids incite inflammation by mimicking the endotoxin activity of lipopolysaccharides. The compilation of fatty acids research does depict contradicting information regarding specific fatty acid types[75]. The essential micronutrient iron is abundant in meat products. Metals are a known catalyst of hydroperoxide decomposition, therefore making meat products an especially sensitive target to radical ROS formation.

Most literature is focused on red meat consumption in general, not beef specifically. In response to red meat consumption, plasma CRP values were measured in 2314 women between the ages of 55-62 and compared to self-reported habitual red meat intake. Higher red meat intake (both processed and unprocessed) was associated with higher CRP levels ($p < 0.05$)[76]. In response to beef consumption in specific, inflammation has been measured in rodent models and human models acutely. In a study comparing the inflammation response (measured by plasma CRP) to lean and fat chicken and beef in rats after 14 days of consumption, CRP values were the greatest in rats who were fed lean beef[7]. In humans, specifically healthy male subject, TNF-α and IL6 were measured in response to different beef thermal processing methods (pan fried or sous vide). After 4 hours, IL6 did not differ between processing method and mean TNF-α decreased or remained at baseline[77]. Lastly, there is a human randomized crossover study comparing

postprandial CRP, IL6 and TNF-α values in 10 healthy male and female subjects after a single meal of kangaroo meat or wagyu beef. From fasting to 2 hours after the meal, all three inflammation markers increased significantly from fasting ($p < 0.001$). Wagyu beef inflammation response was lower compared to kangaroo meat[78].

When cattle-feeding style is incorporated as a variable in postprandial inflammation research, research is currently limited to animal models. In mice, prostaglandin E_2 (PGE2) was measured in response to a two-week diet of range-fed or feedlot fed beef. PGE2 is a pro-inflammatory mediator produced from arachidonic acid. Levels of PGE2 were significantly greater in the mice who received feedlot fed beef compared to range-fed beef[79].

Summary

Beef products from grass and conventional feeding styles appear to differ in nutritional components because of the production techniques utilized. This is predicted to have downstream impacts on the nutritional composition of products sourced from these agricultural techniques[80]. Protein and fat composition of food in particular influence acute postprandial inflammation response via the oxidation of the compounds and reaction with macrophages and toll-like receptors that incite the production of cytokines. Cytokines are signaling proteins that function on a spectrum of pro and anti-inflammatory. Beef products have specific characteristics that will impact postprandial inflammation response, like BCAA content and fatty acids makeup, that is directly linked to the feeding style used to produce meat products. It is important to understand how different foods impact postprandial inflammation because this response is directly linked to CVD and metabolic disease risk and progression.

CHAPTER THREE

IMPACT OF CATTLE FEEDING-STYLE ON BEEF AND HUMAN POSTPRANDIAL INFLAMMATION

Contribution of Authors and Co-Authors

First Author: Meghan L. Spears
Contributions:
Data collection and analysis
Statistical analysis
First draft
Final edits

Second Author: Emily Peterson
Contributions:
Data collection
Project Conceptualization

Third Author: Jane Boles
Contributions:
Conventional beef acquisition
Cutting and preparation of intervention meals
Blinding of intervention materials
Expertise in meat science
Edits of final draft

Fourth Author: Mary P. Miles
Contributions:
Principal Investigator
Project Conceptualization
Acquisition of funding
Edits of first and final draft

Manuscript Information

Meghan L. Spears, Emily Peterson, Jane Boles, Mary P. Miles

American Society of Nutrition- The Journal of Nutrition: Nutrition and Disease

Status of Manuscript:
☒ Prepared for submission to a peer-reviewed journal
☐ Officially submitted to a peer-reviewed journal
☐ Accepted by a peer-reviewed journal
☐ Published in a peer-reviewed journal

Introduction

Diet is one of the key factors influencing chronic inflammation and the development of chronic diseases such as cardiovascular disease (CVD) and metabolic syndrome[81]. Particularly, the habitual consumption of red meat, like beef, in Western diets has been linked to increased risk for developing type 2 diabetes mellitus (T2DM), CVD, and the promotion of systemic inflammation[7,82]. A closer look at beef consumption suggests that the cattle feeding method may impact these health implications. Grass-fed feeding style (GRA) differs from conventional techniques (CON) in that grass-fed cattle spend their entire lifespan grazing pasture while conventional feeding style offers cattle a mainly grain/high-energy feed diet. It is known that grass-fed methods have a greater content of polyunsaturated fatty acids (PUFA), vitamins, minerals, and phytonutrients compared to conventional feeding methods, which has greater mono-unsaturated fatty acid (MUFA) content[83–85]. These nutritional components have been linked to the attenuation of inflammation, which can decrease risk for chronic disease[81,86]. Understanding the postprandial inflammatory response to beef from different cattle feeding methods can bridge the gap between nutritional components and health impacts in consumers.

When considering the impact of dietary habits on an individual's risk for developing chronic diseases, the acute postprandial response to a meal is important to understanding the link between acute responses and long-term impacts[87]. An increase in inflammatory markers and mediators, such as lipopolysaccharides (LPS), leukocytes, and tumor necrosis factor-α (TNF-α) is expected after a meal due to the metabolism of carbohydrates, fats, and protein[59]. The magnitude of this response may impact chronic inflammation state. Prolonged elevation of proinflammatory mechanisms contributes to a state of low-grade inflammation, which can hinder

metabolic processes like cellular signaling and gene expression, therefore increasing an individual's risk for metabolic related diseases[88].

A meal mainly comprised of fat and protein, like beef, impacts postprandial inflammation through the oxidation of lipids and protein, producing reactive oxygen species (ROS) which enhance proinflammatory gene expression[60,89].The type of lipids and amino acids in a meal combined with other potentially anti-inflammatory nutrients may attenuate this response and alter the production of pro-inflammatory components. The cattle feeding methods of beef are expected to influence this by affecting the nutritional components of beef products.

Current literature is limited to measuring postprandial inflammation in response to general red meat intake, where higher red meat intake is strongly associated with high levels of inflammatory markers, like c-reactive protein (CRP)[76]. There is a small base of literature comparing cattle-feeding style, but this is limited to mouse models. In a mouse model comparing an inflammatory marker, prostaglandin E2, after two weeks of a diet containing range-fed or feedlot-fed beef, inflammation was greater in the feedlot fed beef diet than the range-fed beef diet[79]. This study aims to fill the current gap by measuring postprandial inflammation in response to cattle-feeding style in human subjects.

The purpose of this study was first to investigate the acute inflammation response to beef in humans. It was hypothesized that there will be an increase in postprandial inflammation following a beef meal. Secondly, the purpose of this study was to evaluate the possible differences that cattle-feeding style (GRA and CON) may have on the postprandial inflammation response. It was hypothesized that there will be a difference in postprandial inflammation response between GRA and CON. There are some potential differences that may favor lower

inflammation in GRA; however, many factors in beef have the potential to mediate inflammation and the net effect of differences between feeding methods are not known.

Methodology

Study Population

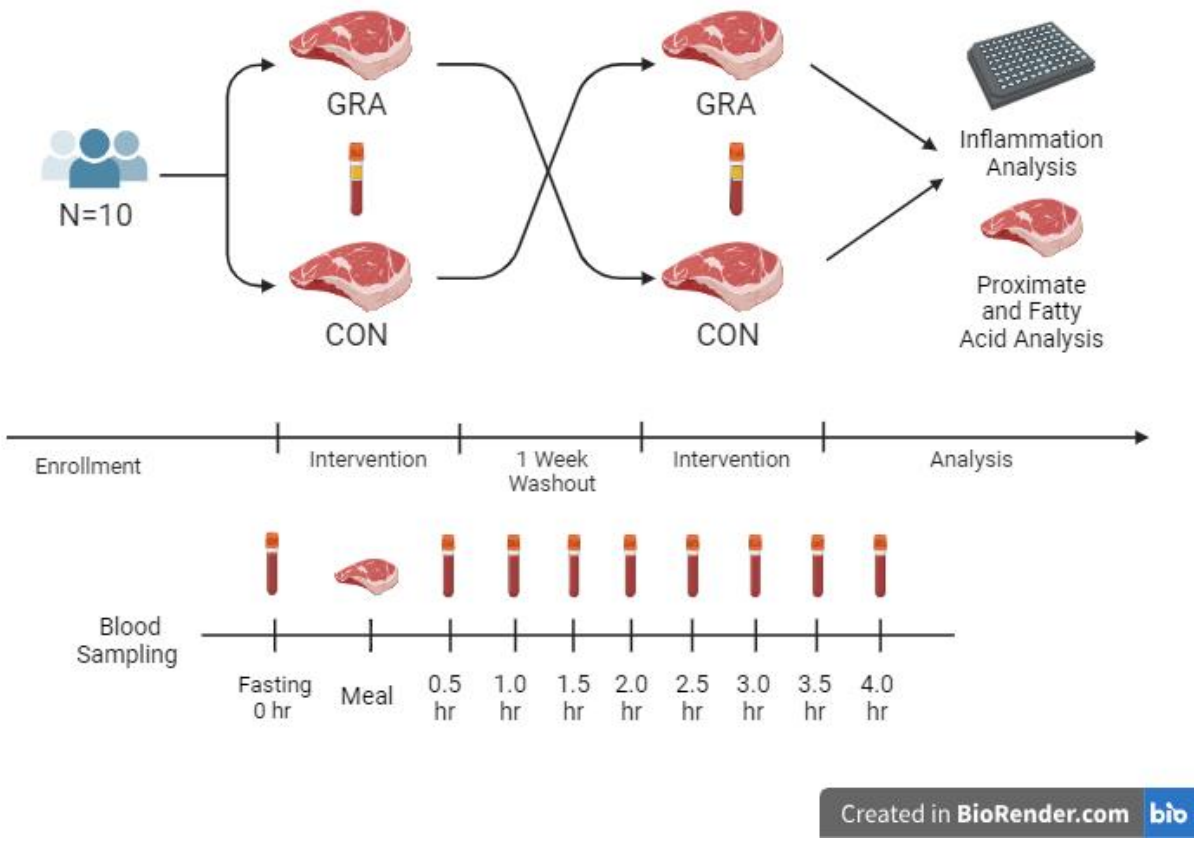
Participants were recruited in Bozeman, MT from August 2022-March 2023 by word of mouth on a rolling basis. Inclusion criteria included age being between 18 and 45 years old with a body mass index (BMI) of 18-27 kg/m^2. Participants were excluded if they had preexisting conditions including diabetes, allergy to red meat, gall bladder condition, or taking medication to lower cholesterol, blood pressure, and inflammation. Screening of potential participants was conducted using REDCap (version 13.10.6)[90]

Experimental Design

The study was a double-blind crossover randomized clinical trial with two conditions, GRA and CON beef. Participants came to the Nutrition Research Laboratory on three occasions for testing. The first visit consisted of an informed consent review followed by a general health questionnaire, and anthropometric measurements. Informed consent was reviewed verbally in-person, followed by written informed consent obtained from all participants prior to participation. This study was prospectively registered with ClinicalTrial.gov (NCT05460754). The second and third visits occurred at least 7 days apart and included the dietary intervention with half-hourly blood collection for 4 hours postprandially. Inflammatory markers were measured from fasting and hourly samples. See Figure 4 for summary of study design. The nutritional composition of GRA and CON were measured using proximate analysis and fatty

acid analysis. Cytokines were measured using serum samples extracted from hourly blood collection.

Figure 4. Study Intervention Timeline.



Randomization

Participants were randomly assigned the order in which steaks from GRA and CON were administered. Steaks were blinded with a three-digit code written on the vacuum sealed package by a member of the research team not involved in data collection or analysis. Researchers, volunteers and clinical staff aiding in data collection were blinded from condition assignments at visits 2 and 3.

Anthropometrics

Anthropometrics were measured at visit 1. Participants were assessed for BMI, absolute and relative fat mass/fat free mass, resting energy expenditure, whole body muscle, torso muscle, and visceral adipose with segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany). Waist circumference was measured in triplicate with an anthropometric measuring tape.

Dietary Intervention and Blood Sample Collection

The dietary intervention followed the cross-over study design. All participants received a steak from each condition (GRA and CON). The order in which participants received steaks at visits 2 and 3 was determined by the randomization protocol. During participation, beginning 72 hours before visit 2 and ending at the completion of visit 3, participants were instructed not to consume any red meat for this period of time and particularly avoid fatty fish the day before visit 2 and 3. The diet the day before visit 2 was then replicated by participants before visit 3 to improve comparability.

Participants were instructed to arrive at visit 2 and 3 having fasted for 12 hours and to not have participated in strenuous activity or the consumption of alcohol for 24 hours prior to visits. An intravenous catheter was inserted in the antecubital vein upon arrival by a phlebotomist or medical doctor. Blood samples were collected into serum separating vacutainer tubes (BD Vacutainer, Frankline Lakes, NJ, USA) after a 3 mL waste withdrawal. Once sample was collected, a sterile saline flush was given. The fasting blood sample was taken 15 minutes after catheter insertion. Postprandial blood samples were collected every 30 minutes for 4 hours beginning 30 minutes after participant began eating the study steak. Samples were allowed to

clot for 15 min prior to centrifugation (1200RPM, 15 minutes) to separate serum. The serum was then aliquoted and frozen at -80°C for future analysis.

Steak Preparation

Organic, grass-fed beef strip loin was sourced from B Bar Ranch Big Timber, MT and conventionally fed beef choice strip loin was sourced from a local purveyor. All steaks were cut from the longissimus lumborum muscle. Two cows per condition were used to source strip loin cuts. Steaks were matched for marbling between conditions and then vacuum sealed in stored at -20ºC until needed for participants. All steaks were removed from the freezer and thawed in a refrigerator between 24-48 hours before the planned time of consumption. Pre-cooking weight of the raw steak was recorded. Steaks were cooked on a clamshell grill with a thermometer (ThermoWorks THS-313-158 probe with Therma Waterproof THS-232-101) inserted into the thickest part of the meat until the thermometer read 70ºC. Weight was recorded immediately after taking off the grill. The steak was then wrapped in foil and rested for 5 minutes. After 5 minutes of rest, the cooked steak was cut to 0.375 lbs. (6 oz.) and served to participants.

Proximate and Fatty Acid Analysis

Percent moisture and ash, as well as crude protein (%) and crude fat (%) were analyzed using Association of Official Agricultural Chemists (AOAC) methods 950.46, 923.03. 983.23 and 992.15[91–93]. and ash as well as crude protein and fat (described at percentage) Fatty acid analysis followed established protocols utilizing gas chromatography[94]. Tests were performed in duplicate with two samples from each GRA and CON.

Cytokine Analysis

To assess inflammatory response at fasting and postprandially, the concentration of eight cytokines were measured in pg/mL at hourly timepoints using a high-sensitivity multiplex assay (MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel Immunology; Millipore, Billerica, MA, USA) on a Bio-Rad Luminex. Included cytokines were granulocyte macrophage colony stimulating factor (GMCSF), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukins (IL) IL-1β, IL-23, IL-17, IL-10, and IL-6. Serum samples from fasting and hourly timepoints were run in duplicate. A coefficient of variability between duplicate samples above 15% indicated need to re-run the sample. The average coefficient of variation for each cytokine ranged from 2 to 12%. Hourly timepoints were used for inflammation analysis to coincide with the literature related to postprandial inflammation[95].

Statistical Analysis

Net area under the curve (AUC) was calculated for each cytokine (GMCSF, IFN-γ, TNF-α, IL-1β, IL-23, IL-17, IL-10, and IL-6) by subtracting the area below baseline from the area above baseline[96]. A one-sample Wilcox test was used to determine if the average AUC for each cytokine was significantly greater than zero in response to beef as well as cattle-feeding style. Normal distribution of the AUC data was assessed with the Shapiro-Wilk test. To achieve normally distributed AUC data, data was transformed using orderNorm from the bestNormalize package in R Studio when appropriate. Simple linear modeling and ANOVA were used to compare transformed AUC between conditions. Two-way repeated measures ANOVA on inflammatory response (fasting (0), 1, 2, 3, and 4 hours) used to determine the effect of an interaction of time and condition, as well as time alone. The repeated measures accounted for

were time and subject. The maximal change (maxchange) in cytokine values after fasting (maximum cytokine value reached between hours one and four (CMAX) – fasting value) was calculated for each cytokine and then compared to zero using a one-sample t-test with and without consideration for condition. The time postprandially at which this maximal value between hours one and four (CMAXtime) occurred was also recorded. One-sided P value of < 0.05 was considered statistically significant. Analysis was done in RStudio (V.4.3.2).

Results

Characteristics of Participants

Between August 2022 and March 2023, a total of 18 men and women were assessed for eligibility by completing a REDCap survey to assess eligibility criteria. Of the 18, 17 met eligibility criteria and were invited to participate in the study. Two participants were denied entrance into the study, even if eligibility criteria were met, when the number of participants per sex was previously met (5 male, 5 female). Of the 15 participants who were entered into the study, 4 dropped out during visit 2 or 3 due to inability to maintain intravenous catheter integrity and one voluntarily dropped out due to conflicting commitments. Final participant (n=10) characteristics for those participants successfully completing both conditions are depicted in Table 2.

Table 2. Final participant characteristics.

	Age (years)	**BMI (**kg/m^2**)**	Waist circumference (cm)	**VAT (L)**
All **N=10**	26.6 ± 5.8	24.44 ± 2.20	82.33 ± 8.32	0.78 ± 0.73
Female **N=5**	24.8 ± 5.2	23.99 ± 2.38	76.77 ± 7.34	0.34 ± 0.09
Male **N=5**	28.4 ± 6.3	24.89 ± 2.16	87.88 ± 4.97	1.22 ± 0.85

Values are presented as mean ± standard deviation (SD). BMI; body mass index, VAT; visceral adipose tissue.

Proximate and Fatty Acid Analysis

Percent moisture, ash, protein, and fat results from the proximate analysis are described in Table 3.

Table 3. Proximate analysis results.

Feeding style	% Moisture	% Ash	% Protein	% Fat
GRA (n=2)	70.3 ± 1.3	0.9 ± 0.0	22.7 ± 0.6	7.3 ± 0.1
CON (n=2)	70.9 ± 0.2	0.9 ± 0.0	20.8 ± 0.2	7.6 ± 0.1

Values are presented as the average % ± sample SD for two samples run in duplicate from GRA and CON.

Fatty acids analysis results are described in Table 4. The omega name and common name of listed fatty acids can be found in Table A1.

Table 4. Fatty acid analysis results.

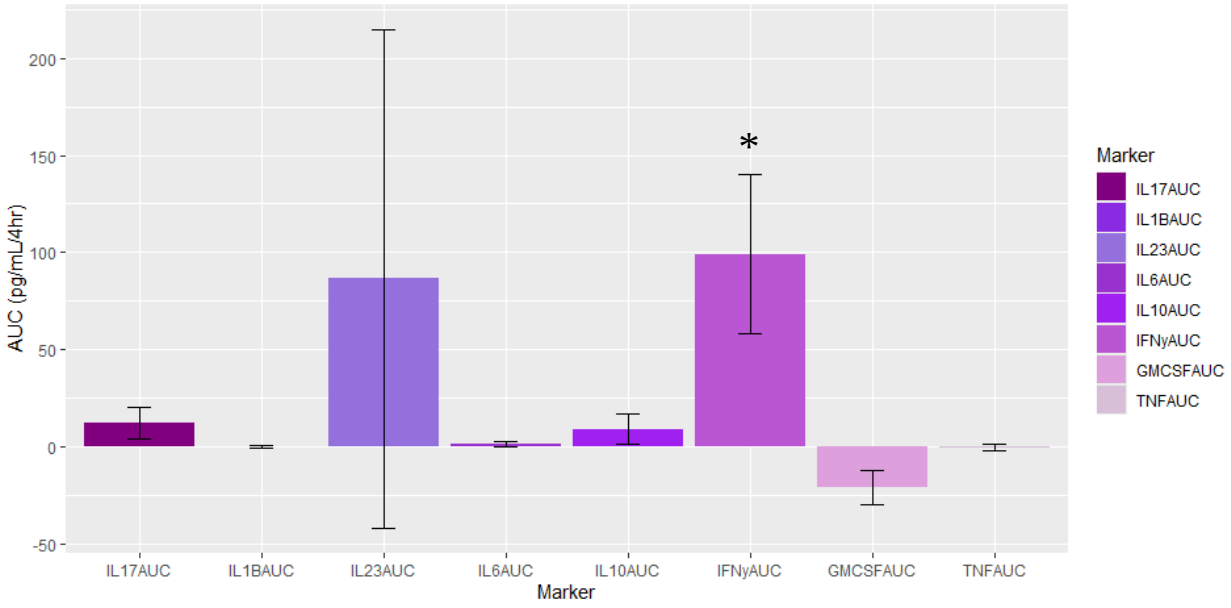
Feeding Style	Individual fatty acids (g/100g)								
	Saturated fatty acids (SFA)								
	Total	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0	C24:0	
GRA (n=2)	41.8 ± 7.9	4.7 ± 0.2	2.4 ± 0.1	21.1 ± 0.1	1.1 ± 0.1	12.3 ± 0.4	0.0 ± 0.0	0.1 ± 0.0	
CON (n=2)	39.8 ± 7.4	4.8 ± 0.2	2.1 ± 0.3	19.4 ± 1.3	1.1 ± 0.1	12.3 ± 0.9	0.0 ± 0.0	0.1 ± 0.0	
	Monounsaturated fatty acids (MUFA)								
	Total	C14:1	C16:1	C17:1	C18:1c9	C18:1c11	C20:1	t-vaccenic	
GRA (n=2)	40.7 ± 13.5	0.6 ± 0.1	3.4 ± 0.1	0.9 ± 0.0	34.2 ± 0.4	1.6 ± 0.0	0.1 ± 0.1	2.9 ± 0.2	
CON (n=2)	38.8 ± 13.0	0.4 ± 0.1	2.9 ± 0.3	0.9 ± 0.1	33.0 ± 2.0	1.6 ± 0.1	0.0 ± 0.0	2.5 ± 0.6	
	Polyunsaturated Fatty Acids (PUFA)								
	Total	C18:2	C18:3	C18:2c9t11	C18:2t10c12	C20:2	C20:4	C20:5	C22:6
GRA (n=2)	4.5 ± 1.2	3.5 ± 0.2	0.0 ± 0.1	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CON (n=2)	5.2 ± 1.4	4.1 ± 0.5	0.0 ± 0.1	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0

Values are presented as the percentage of total fatty acids (average ± sample SD) for two samples run in duplicate from GRA and CON. Total is the sum of all individual fatty acids by saturation and feeding style ± sample SD.

Postprandial Inflammation

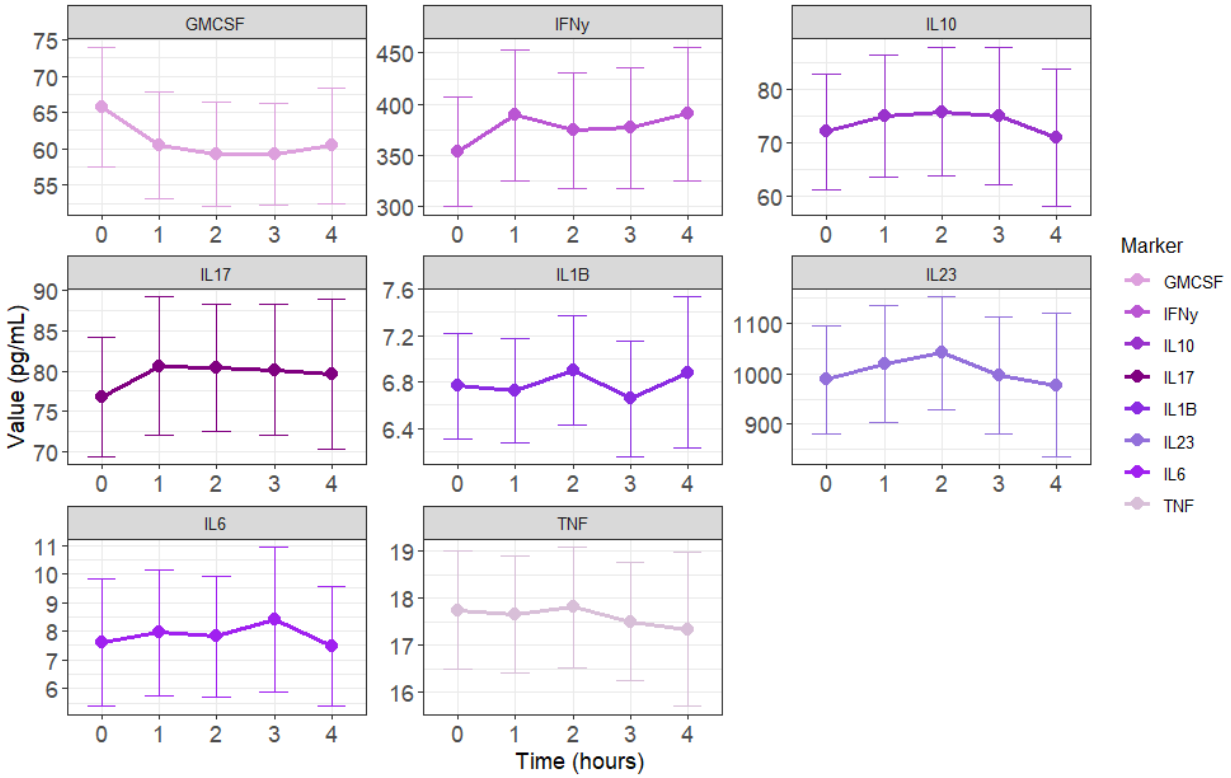
Response to Beef. Without consideration for cattle-feeding style, the average AUC response was only significantly greater than 0 in IFNγ ($p < 0.05$) (Figure 53). Time did not have a significant impact on response to beef ($p > 0.05$) (Figure 6) however, the maxchange was significantly greater than zero for all eight cytokines ($p < 0.05$). CMAXtime favored hour three for 5/8 cytokines (Table 5).

Figure 5. AUC Response to Beef.



Bar graph of the mean AUC response for each cytokine with standard error (se) bars (n=20). When compared to 0 (no response), IFNγ mean AUC was greater than 0 ($p < 0.05$). *Indicates mean AUC significantly greater than zero.

Figure 6. Time course Response to Beef.



Line graph depicting the mean response to beef at fasting (0), 1, 2, 3, and 4 hours postprandially (n=20). Time did not significantly impact the response. Cytokines are shown on their original scale, statistical analysis was done on transformed values.

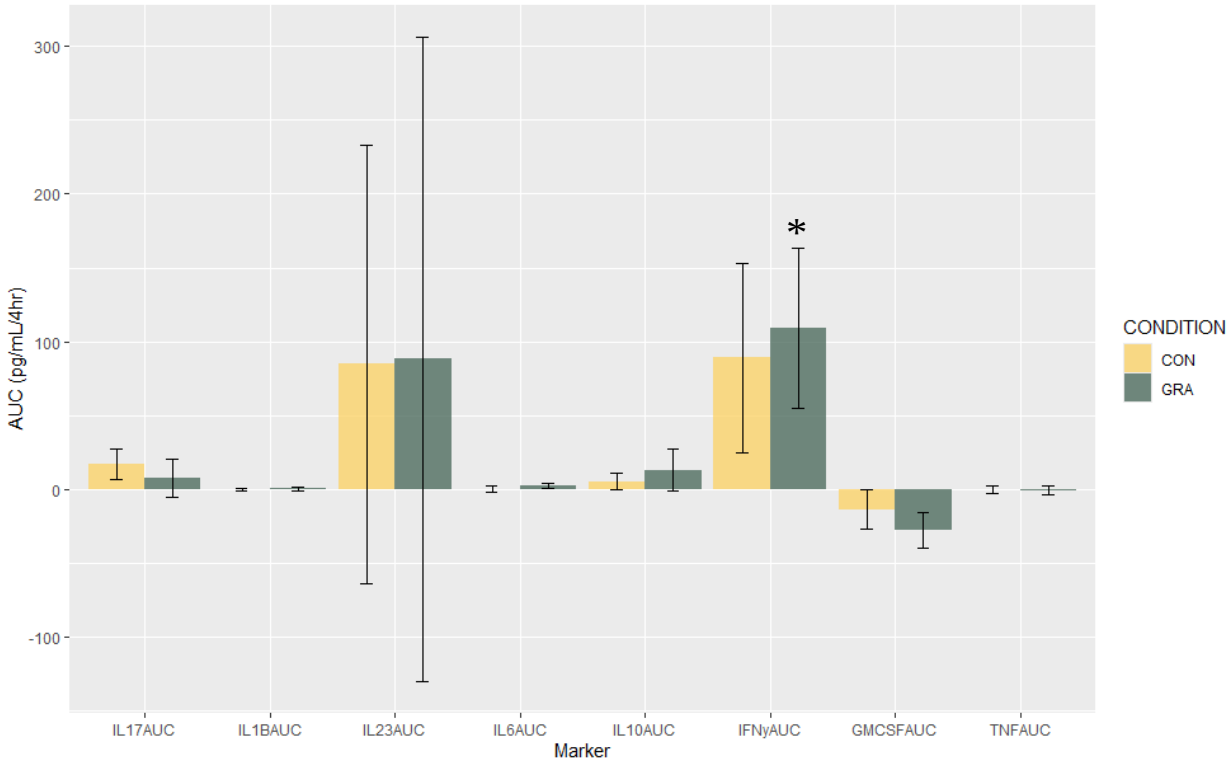
Table 5. Maxchange and CMAXtime response to beef.

	IL23	IL17	IL10	IL6	IFNγ	TNF-α	IL1β	GMCSF
Maxchange (pg/mL)	*223.38 ± 236.94	*15.60 ± 15.10	*11.83 ± 15.05	*1.66 ± 2.17	*82.25 ± 82.73	*3.446 ± 5.08	*1.42 ± 2.05	*5.62 ± 12.68
CMAXtime (Hour)	2	3	2	3	3	2	3	3

Maxchange= Mean ± sd (n=20). CMAXtime= mean hour rounded to nearest whole number (n=20). *Indicates significantly greater than 0.

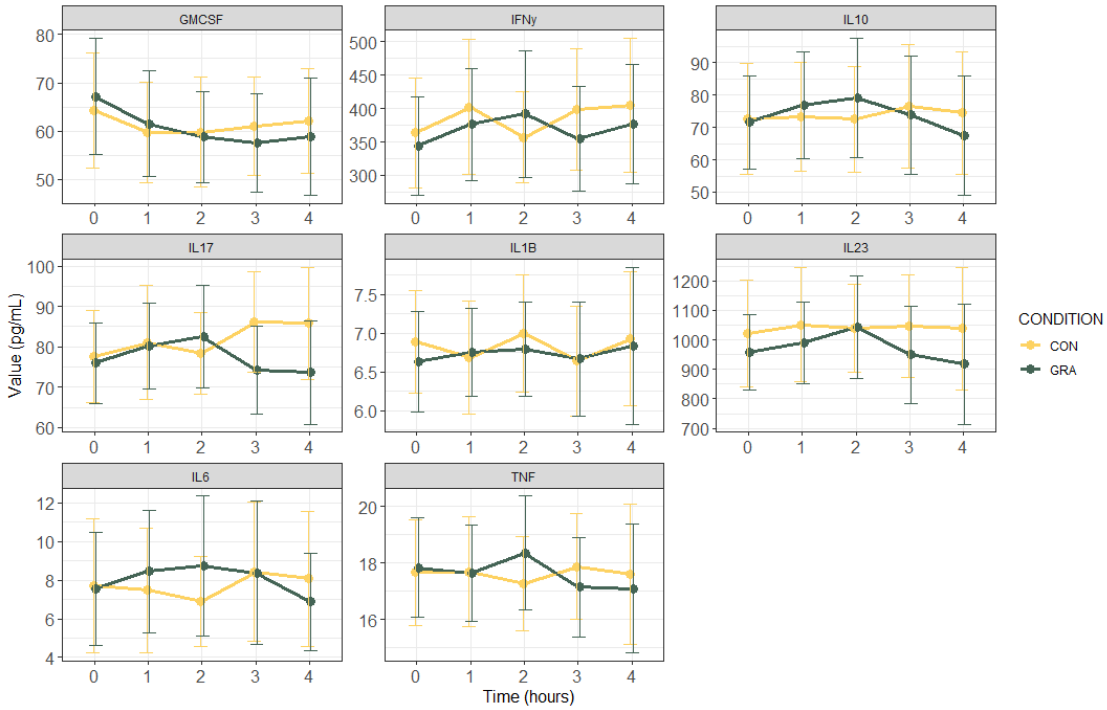
Response to Cattle-Feeding Style. After accounting for cattle-feeding style, there were no differences in AUC in each of the eight cytokines between GRA and CON ($p > 0.05$). Within CON, all mean cytokine AUC were not significantly greater than zero ($p > 0.05$) (Figure 7). Within GRA, all mean cytokine AUC were not significantly greater than zero ($p > 0.05$), except for IFNγ mean AUC ($p < 0.05$) (Figure 7). After accounting for condition and time, two-way repeated measures ANOVA showed that neither had significant impact on postprandial inflammation. Accounting for time only in conditions separately, time did not have a significant impact on inflammation response (Figure 8). The Maxchange within GRA and CON was significantly greater than 0 for all by one cytokine in GRA (Table 6). Between GRA and CON, the maxchange was not significantly different (Table 6). CMAXtime was evenly distributed between hours two and three within GRA and CON (Table 6).

Figure 7. AUC Response of GRA and CON.



Bar graph of the mean AUC response for each cytokine with standard error (se) bars (n=20). AUC of each cytokine was not significantly different between conditions ($p > 0.05$). IFNγ within GRA was significantly greater than zero ($p < 0.05$). *Indicates AUC significantly greater than zero ($p < 0.05$).

Figure 8. Postprandial Inflammation Time Course.



Change in inflammatory marker concentration (pg/mL) from fasting, up to four hours postprandially (GRA; n=10, CON; n=10). Time did not significantly impact cytokine response between conditions or within conditions separately ($p > 0.05$). Cytokines are displayed on the original scale. The statistical analysis was completed on normalized variables.

Table 6. Maxchange and CMAXtime Response to GRA and CON.

	IL23	IL17	IL10	IL6	IFNγ	TNF-α	IL1β	GMCSF
	CON							
Maxchange (pg/mL)	*243.26 ± 213.87	*16.00 ± 15.05	*10.95 ± 12.66	*1.22 ± 1.81	*82.75 ± 101.27	*3.47 ± 4.65	*1.28 ± 1.84	*7.32 ± 13.96
CMAXtime (Hours)	2	3	2	3	3	2	2	3
	GRA							
Maxchange (pg/mL)	*203.51 ± 316.95	*15.21 ± 16.35	*12.71 ± 17.79	*2.10 ± 2.51	*81.76 ± 64.75	*3.42 ± 5.73	*1.56 ± 2.33	3.93 ± 11.76
CMAXtime (Hours)	3	2	2	2	3	2	3	2

Maxchange= Mean ± sd. CMAXtime= mean hour rounded to nearest whole number. *Indicates significantly greater than 0.

Discussion

This study is the first of its kind to measure the human inflammatory response to beef and different cattle-feeding styles. Key findings include a significant increase in postprandial inflammation in response to beef, but no difference in inflammation response between grass-fed beef and conventional beef. The beef used in this study was matched for marbling before analysis, resulting in beef that did not differ in fatty acid composition. When using matched beef from grass-fed and conventional cattle feeding styles, the postprandial inflammation response does not differ in healthy subjects.

The results of the proximate and fatty acid analysis showed minimal difference between the composition of GRA and CON beef meat. To achieve a comparable comparison of meat samples from GRA and CON, samples used for proximate and fatty acid analysis were matched for marbling. These results differ from research done by Leheska et al., analyzing nutrient

composition of grass-fed and conventional feeding styles on beef strip steaks using proximate analysis and fatty acid gas chromatography analysis. In this study the moisture percent was significantly greater in grass-fed beef and fat % was significantly greater in conventional beef. Both protein and ash percent were higher in conventional, but not significantly[4]. In our findings, all four proximate analysis measures differed by 1-1.9% between GRA and CON. In Leheska et al., the fatty acid composition between grass-fed and conventional beef showed significant difference between total SFA and MUFA composition, but not total PUFA composition[4]. Our findings showed no more than a 2% difference between total SFA and MUFA composition and less than a 1% difference in PUFA composition between GRA and CON. Without exact knowledge of the raising techniques of the conventional beef used in our study, it is difficult to say for certain whether the results of our study showing minimal differences between cattle-feeding style is indicative of all feeding styles in the grass-fed and conventional techniques.

In concurrence with our hypothesis, postprandial inflammation did increase from fasting in response to beef. By showing that the mean AUC response of IFNγ and peak inflammation response for all cytokines (GMCSF, IFN-γ, TNF-α, IL-1β, IL-23, IL-17, IL-10, and IL-6) were significantly increased, it demonstrates that the response to beef rose above fasting values within the four-hour postprandial window. These findings are congruent with current knowledge on the impact of short-term beef consumption on inflammation in rodents. Van Hecke et al. used multiple measures of inflammation (TMAO, CRP, and oxidative stress parameters) and found that after 14 days of ad libitum consumption of beef, increased inflammation in rats[7]. Our findings contribute to the current knowledge gap pertaining to the impact of the inflammation response to beef in human subjects.

Our hypothesis that postprandial inflammation between GRA and CON would be different was refuted. The AUC response between GRA and CON was not significantly different for any of the eight cytokines measured. This shows that the overall response to GRA and CON did not differ. Within conditions, only IFNγ GRA AUC was significantly increased, indicating an influential impact of GRA beef on IFNγ inflammation response. The largest cytokine response did not significantly differ between conditions, but the largest inflammation response of each cytokine within condition was significantly increased. This indicates that while peak cytokine response did not differ between conditions, each condition independently showed a significant inflammation response. This supports the first key finding that there is an increased inflammation response to beef by confirming that the independent GRA and CON responses, that were used to make up the response to beef, are also significantly greater than zero. Currently, there is literature noting the possible health implications associated with studies measuring nutritional composition differences between these cattle feeding styles[80,83]. Our findings add to this literature base by combining nutritional composition analysis and measurement of human responses. Based on the proximate and fatty acid analysis results of our study, it is not surprising that we did not observe significant differences between GRA and CON on inflammation response. Since the beef was matched for marbling, it resulted in a similar fatty acid composition between grass-fed and conventional beef. This is different than current literature comparing fatty acid composition between grass-fed and conventional beef. In general, grass-fed beef has greater N-3 fatty acid content and less SFA content compared to grain-fed beef[83]. Nutritional components, like SFA and N-6 PUFA's, are known to be related to increase inflammation response in humans. SFA have potential proinflammatory effect by promoting TLR4 signaling

which stimulates the transcription of cytokines like TNF-α[97]. N-6 PUFA's have potential proinflammatory effect by promoting eicosanoid production[98]. Since the GRA and CON beef used in our study were similar in SFA composition and N-6 PUFA's (arachidonic and linoleic acid), our inflammation results are expected. Studies measuring human postprandial inflammation in response to cattle-feeding style are still needed with beef not matched for marbling.

The use of a healthy population of men and women in this study was done to observe postprandial inflammation without the possible influence of factors such as obesity and metabolic health. Since this study is one of the first measuring the impact of beef and cattle-feeding style in human subjects, the use of healthy individuals sets a baseline. Postprandial inflammation response between healthy and obese/overweight subjects has been studied. In both male and female subjects, the inflammation response to a high-fat meal was significantly greater in an obese population when compared to a healthy populatoin[99,100]. Metabolic health also has been shown to impact postprandial responses in combination with measures of obesity. Metabolically healthy, but overweight, subjects respond better to high-fat meal challenges than metabolically unhealthy and overweight subjects. A "better" response was considered lower AUC values of postprandial glucose and insulin[101]. With this knowledge that metabolic health and anthropometrics impact postprandial inflammation, the results of this study can be interpreted as the inflammation response to beef and cattle-feeding style without extraneous variables. Further research is needed to understand the impact of beef and cattle-feeding styles in populations other than healthy adults.

A limitation of this study is the disparity in information between cattle lifestyle of the GRA and CON conditions. The GRA cattle were directly sourced from a local ranch in Big Timber, MT in which the individual in charge has given us full detail of the feeding style and lifestyle of the cow used to source meat for this study. The CON condition choice striploin beef was sourced from a local (to Bozeman, MT) purveyor in which there is a lack of knowledge of the specific source, and rather information was derived from the label. The reason for this sourcing of the CON meat was to mimic the options a consumer would have when choosing meat not labeled organic grass-fed. While this is beneficial to mimicking realistic consumer scenarios, the lack of information about the CON meat was sourced from limits direct comparison to the GRA condition. A strength of this study is that it measures the human postprandial response to GRA and CON beef, rather than comparing the nutritional components of the meat alone as done in research, like Leheska et al.[4]. There are current studies discussing postprandial inflammation in beef and/or meat, but to our knowledge this is the first reporting of the impact of grass-fed organic and conventionally raised beef on humans[102].

In conclusion, there is a postprandial inflammation response to consuming a single meal of beef, but cattle-feeding style (specifically grass-fed and conventional) does not significantly impact this response. This information is important to build knowledge around how cattle-feeding styles impact consumer health.

CHAPTER FOUR

CONCLUSION

To provide more information about the health implications of varying beef types, this study investigated the acute inflammatory response to beef in humans and evaluated the possible differences that cattle-feeding styles may have of postprandial inflammation. The inflammatory response to a single meal is indicative of how postprandial inflammation can impact long-term metabolism and health. Chronic low-grade inflammation is one of the leading causes of disease like CVD and metabolic syndrome[11,12]. Diet impacts inflammation in many ways, depending on the nutrients from food and how those interact with immune cells and other processes in the body. If a diet habitually contains foods that promote inflammation, this can contribute to chronic low-grade inflammation. With growing attention to cattle-feeding practices in the consumer beef industry, it is important to understand how a diet containing beef may impact consumer health. There is currently a knowledge gap regarding the impact of beef consumption from differing cattle-feeding styles on humans that this studied aimed to fill.

Grass-fed and conventional beef used in this study was relatively similar in nutritional composition, measured by proximate analysis and fatty acid composition. It was also shown that acute beef consumption increased inflammation response, measured by cytokines (GMCSF, IFN-γ, TNF-α, IL-1β, IL-23, IL-17, IL-10, and IL-6), increased from fasting values in a healthy population. Cattle-feeding style (grass-fed and conventional) did not impact this inflammation responses differently. These inflammation results were not surprising considering the differences between nutritional composition of grass-fed and conventional beef used were small. While these results were measured acutely, they are important for informing future research on how chronic

beef consumption could impact consumer health. More research is needed to measure and understand chronic effects of consuming beef and consider how different cattle-feeding styles may play a role chronically.

REFERENCES CITED

1. *Back to Grass: The Market Potential for U.S Grassfed Beef*.; 2017.

2. Fairfield Market Research. Grass-Fed Beef Market.

3. Choi SH, Park S. Fatty Acid Composition of Grain and Grass-Fed Beef and their Nutritional Value and Health Implication. *Food Sci Anim Resour*. 2022;42(1). doi:10.5851/kosfa.2021.e73

4. Leheska JM, Thompson LD, Howe JC, et al. Effects of conventional and grass-feeding systems on the nutrient composition of beef. *J Anim Sci*. 2008;86(12):3575-3585. doi:10.2527/jas.2007-0565

5. Daley CA, Abbott A, Doyle PS, Nader GA, Larson S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr J*. 2010;9(1):1-12. doi:10.1186/1475-2891-9-10/TABLES/4

6. Reynolds CM, Draper E, Keogh B, et al. A Conjugated Linoleic Acid-Enriched Beef Diet Attenuates Lipopolysaccharide-Induced Inflammation in Mice in Part through PPARg-Mediated Suppression of Toll-Like Receptor 4 1-3. *The Journal of Nutrition Nutritional Immunology*. doi:10.3945/jn.109.113035

7. Van Hecke T, Jakobsen LMA, Vossen E, et al. Short-term beef consumption promotes systemic oxidative stress, TMAO formation and inflammation in rats, and dietary fat content modulates these effects. 2016;7:3760. doi:10.1039/c6fo00462h

8. Lund MN, Heinonen M, Baron CP, Estévez M. Protein oxidation in muscle foods: A review. doi:10.1002/mnfr.201000453

9. Estévez M, Xiong Y. Intake of Oxidized Proteins and Amino Acids and Causative Oxidative Stress and Disease: Recent Scientific Evidences and Hypotheses. *J Food Sci*. 2019;84(3):387-396. doi:10.1111/1750-3841.14460

10. Chai W, Morimoto Y, Cooney R V, et al. Dietary Red and Processed Meat Intake and Markers of Adiposity and Inflammation: The Multiethnic Cohort Study. Published online 2017. doi:10.1080/07315724.2017.1318317

11. Sharma P. Inflammation and the Metabolic Syndrome. Published online 2011. doi:10.1007/s12291-011-0175-6

12. Deboer MD. Obesity, systemic inflammation, and increased risk for cardiovascular disease and diabetes among adolescents: A need for screening tools to target interventions. doi:10.1016/j.nut.2012.07.003

13. Sin DD, Paul Man SF. Why Are Patients With Chronic Obstructive Pulmonary Disease at Increased Risk of Cardiovascular Diseases? *Circulation*. 2003;107(11):1514-1519. doi:10.1161/01.CIR.0000056767.69054.B3

14. Diet and Inflammation; Diet and Inflammation. Published online 2010. doi:10.1177/0884533610385703

15. Margioris AN. Fatty acids and postprandial inflammation. *Curr Opin Clin Nutr Metab Care*. 2009;12(2):129-137. doi:10.1097/MCO.0B013E3283232A11

16. Atmaca G. Antioxidant Effects of Sulfur-Containing Amino Acids. Yonsei Medical Journal. Published 2004. Accessed November 3, 2022. https://eymj.org/Synapse/Data/PDFData/0069YMJ/ymj-45-776.pdf

17. What is “grass fed” meat? Accessed October 26, 2022. https://ask.usda.gov/s/article/What-is-grass-fed-meat

18. Klopatek SC, Marvinney E, Duarte T, Kendall A, Yang X (Crystal), Oltjen JW. Grass-fed vs. grain-fed beef systems: Performance, economic, and environmental trade-offs. *J Anim Sci*. 2022;100(2). doi:10.1093/jas/skab374

19. Capper JL. Is the Grass Always Greener? Comparing the Environmental Impact of Conventional, Natural and Grass-Fed Beef Production Systems. *Animals*. 2012;2:127-143. doi:10.3390/ani2020127

20. Krusinski L, Sergin S, Jambunathan V, Rowntree JE, Fenton JI. Attention to the Details: How Variations in U.S. Grass-Fed Cattle-Feed Supplementation and Finishing Date Influence Human Health. *Front Sustain Food Syst*. 2022;6. doi:10.3389/fsufs.2022.851494

21. Field CJ, Robinson L. Dietary Fats. doi:10.1093/advances/nmz052

22. Iqbal J, Hussain MM. Intestinal lipid absorption. *Am J Physiol Endocrinol Metab*. 2009;296(6):E1183. doi:10.1152/AJPENDO.90899.2008

23. Klop B, Proctor SD, Mamo JC, Botham KM, Cabezas MC. Understanding Postprandial Inflammation and Its Relationship to Lifestyle Behaviour and Metabolic Diseases. *Int J Vasc Med*. 2012;2012:11. doi:10.1155/2012/947417

24. Hoofnagle AN, Heinecke JW. Lipoproteomics: Using mass spectrometry-based proteomics to explore the assembly, structure, and function of lipoproteins. *J Lipid Res*. 2009;50(10):1967-1975. doi:10.1194/jlr.R900015-JLR200

25. Tu-Sekine B, Raben DM. Lipid Signaling. *Encyclopedia of Cell Biology*. 2016;1:194-200. doi:10.1016/B978-0-12-394447-4.10023-9

26. Garcia C, Andersen CJ, Blesso CN. The Role of Lipids in the Regulation of Immune Responses. Published online 2023. doi:10.3390/nu15183899

27. Martinsen TC, Fossmark R, Waldum HL. Molecular Sciences The Phylogeny and Biological Function of Gastric Juice-Microbiological Consequences of Removing Gastric Acid. Published online 2019. doi:10.3390/ijms20236031

28. Isaacs LL. Pancreatic Proteolytic Enzymes and Cancer: New Support for an Old Theory. *Integr Cancer Ther*. 2022;21. doi:10.1177/15347354221096077

29. Mellanby BJ. THE SECRETION OF PANCREATIC JUICE.

30. Ozorio L, Mellinger-Silva C, Cabral LMC, Jardin J, Boudry G, Dupont D. The Influence of Peptidases in Intestinal Brush Border Membranes on the Absorption of Oligopeptides from Whey Protein Hydrolysate: An Ex Vivo Study Using an Ussing Chamber. doi:10.3390/foods9101415

31. Ziv E, Moi¨ M, Bendayan M. Intestinal Absorption of Peptides Through the Enterocytes. *Microsc Res Tech*. 2000;49:346-352. doi:10.1002/(SICI)1097-0029(20000515)49:4<346::AID-JEMT3>3.0.CO;2-B

32. Ruth MR, Field CJ. The immune modifying effects of amino acids on gut-associated lymphoid tissue. *J Anim Sci Biotechnol*. 2013;4(1):1-10. doi:10.1186/2049-1891-4-27/TABLES/1

33. Ward W, Richardson A. Effect of age on liver protein synthesis and degradation. *Hepatology*. 1991;14(5):935-948. doi:10.1002/hep.1840140529

34. Liver protein synthesis in physiology and in disease states : Current Opinion in Clinical Nutrition & Metabolic Care. Accessed April 1, 2024. https://journals.lww.com/co-clinicalnutrition/abstract/2002/01000/liver_protein_synthesis_in_physiology_and_in.9.aspx

35. Prinsen BHCMT, De Sain-Van Der Velden MGM. Albumin turnover: Experimental approach and its application in health and renal diseases. *Clinica Chimica Acta*. 2004;347(1-2):1-14. doi:10.1016/j.cccn.2004.04.005

36. Lee DY, Kim EH. Therapeutic Effects of Amino Acids in Liver Diseases: Current Studies and Future Perspectives. *J Cancer Prev*. 2019;24(2):72-78. doi:10.15430/jcp.2019.24.2.72

37. Rui L. Energy metabolism in the liver. *Compr Physiol*. 2014;4(1):177-197. doi:10.1002/cphy.c130024

38. Kany S, Tilmann Vollrath J, Relja B. Molecular Sciences Review Cytokines in Inflammatory Disease. doi:10.3390/ijms20236008

39. Foster JR. The functions of cytokines and their uses in toxicology.

40. Zhang JM, An J. Cytokines, Inflammation and Pain. doi:10.1097/AIA.0b013e318034194e

41. Zenobia C, Hajishengallis G. Basic biology and role of interleukin-17 in immunity and inflammation. Published online 2015. doi:10.1111/prd.12083

42. Korta A, Kula J, Gomułka K. The Role of IL-23 in the Pathogenesis and Therapy of Inflammatory Bowel Disease. *Int J Mol Sci*. 2023;24(12). doi:10.3390/ijms241210172

43. Lee KMC, Achuthan AA, Hamilton JA. GM-CSF: A promising target in inflammation and autoimmunity. *Immunotargets Ther*. 2020;9:225-240. doi:10.2147/ITT.S262566

44. Subramanian Iyer S, Cheng G. Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. Published online 2012.

45. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. doi:10.1101/cshperspect.a016295

46. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. *Cytokine Growth Factor Rev*. 2011;22(4):189-195. doi:10.1016/j.cytogfr.2011.10.001

47. Jang DI, Lee AH, Shin HY, et al. The role of tumor necrosis factor alpha (Tnf-α) in autoimmune disease and current tnf-α inhibitors in therapeutics. *Int J Mol Sci*. 2021;22(5):1-16. doi:10.3390/ijms22052719

48. Zhang J. Yin and yang interplay of IFN-γ in inflammation and autoimmune disease. *Journal of Clinical Investigation*. 2007;117(4):871-873. doi:10.1172/JCI31860

49. G Mills KH. IL-17 and IL-17-producing cells in protection versus pathology. doi:10.1038/s41577-022-00746-9

50. Duan Y, Zeng L, Zheng C, et al. Inflammatory Links Between High Fat Diets and Diseases. *Front Immunol*. 2018;9(NOV):2649. doi:10.3389/FIMMU.2018.02649

51. Herieka M, Erridge C. High-fat meal induced postprandial inflammation. *Mol Nutr Food Res*. 2014;58:136-146. doi:10.1002/mnfr.201300104

52. Milan AM, Pundir S, Pileggi CA, Markworth JF, Lewandowski PA, Cameron-Smith D. Comparisons of the Postprandial Inflammatory and Endotoxaemic Responses to Mixed Meals in Young and Older Individuals: A Randomised Trial. doi:10.3390/nu9040354

53. Emerson SR, Sciarrillo CM, Kurti SP, Emerson EM, Rosenkranz SK. High-Fat Meal–Induced Changes in Markers of Inflammation and Angiogenesis in Healthy Adults Who Differ by Age and Physical Activity Level. *Curr Dev Nutr*. 2019;3(1):nzy098. doi:10.1093/CDN/NZY098

54. Mazidi M, Valdes AM, Ordovas JM, et al. Meal-induced inflammation: postprandial insights from the Personalised REsponses to DIetary Composition Trial (PREDICT) study

in 1000 participants. *Am J Clin Nutr*. 2021;114(3):1028-1038. doi:10.1093/AJCN/NQAB132

55. RENl W k, Zhu XP, Liu G, Peng Y y. *GLUTAMINE ON INTESTINAL INFLAMMATION: A MECHANISTIC PERSPECTIVE*. Vol 11.; 2013.

56. Chen S, Huang J, Liu T, et al. PI3K/Akt signaling pathway mediates the effect of low-dose boron on barrier function, proliferation and apoptosis in rat intestinal epithelial cells. *Scientific Reports* |. 123AD;14:393. doi:10.1038/s41598-023-50800-2

57. Pacheco R, Gallart T, Lluis C, Franco R. Role of glutamate on T-cell mediated immunity. *J Neuroimmunol*. 2007;185(1):9-19. doi:10.1016/J.JNEUROIM.2007.01.003

58. Ehlers K, Brand T, Bangert A, Hauner H, Laumen H. Postprandial activation of metabolic and inflammatory signalling pathways in human peripheral mononuclear cells. *British Journal of Nutrition*. 2014;111(12):2167-2175. doi:10.1017/S0007114514000208

59. Meessen ECE, Warmbrunn M V., Nieuwdorp M, Soeters MR. Human postprandial nutrient metabolism and low-grade inflammation: A narrative review. *Nutrients*. 2019;11(12). doi:10.3390/nu11123000

60. Huang C, Zhang Y. *Oxidative Stress: Human Diseases and Medicine*. Springer Singapore; 2021. doi:10.1007/978-981-16-0522-2

61. Sohal RS, Weindruch R. Oxidative Stress, Caloric Restriction, and Aging. *Science*. 1996;273(5271):59. doi:10.1126/SCIENCE.273.5271.59

62. Nam TG. Lipid Peroxidation and Its Toxicological Implications. *Toxicol Res*. 2011;27(1):1-6. doi:10.5487/TR.2011.27.1.001

63. Suzuki YJ, Carini M, Butterfield DA. *Protein Carbonylation*. www.liebertpub.com

64. Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants 2019, Vol 8, Page 429*. 2019;8(10):429. doi:10.3390/ANTIOX8100429

65. Ross EA, Devitt A, Johnson JR. Macrophages: The Good, the Bad, and the Gluttony. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.708186

66. Dror E, Dalmas E, Meier DT, et al. Postprandial macrophage-derived IL-1β stimulates insulin, and both synergistically promote glucose disposal and inflammation. *Nat Immunol*. 2017;18(3):283-292. doi:10.1038/ni.3659

67. Libby P. Inflammatory Mechanisms: the Molecular Basis of Inflammation and Disease. *Nutr Rev*. 2007;65(suppl_3):S140-S146. doi:10.1111/J.1753-4887.2007.TB00352.X

68. Chawla A, Nguyen KD, Goh YPS. Macrophage-mediated inflammation in metabolic disease. doi:10.1038/nri3071

69. Kintscher U, Hartge M, Hess K, et al. T-lymphocyte Infiltration in Visceral Adipose Tissue. *Arterioscler Thromb Vasc Biol*. 2008;28(7):1304-1310. doi:10.1161/ATVBAHA.108.165100

70. Metabolic Syndrome | Johns Hopkins Medicine. Accessed February 26, 2024. https://www.hopkinsmedicine.org/health/conditions-and-diseases/metabolic-syndrome

71. Wolk A. Potential health hazards of eating red meat. *J Intern Med*. 2017;281(2):106-122. doi:10.1111/JOIM.12543

72. Van Hecke T, Bussche J Vanden, Vanhaecke L, Vossen E, Van Camp J, De Smet S. Nitrite Curing of Chicken, Pork, and Beef Inhibits Oxidation but Does Not Affect N-Nitroso Compound (NOC)-Specific DNA Adduct Formation during in Vitro Digestion. Published online 2014. doi:10.1021/jf4057583

73. Lee JH, Park E, Hyue •, et al. Anti-inflammatory and anti-genotoxic activity of branched chain amino acids (BCAA) in lipopolysaccharide (LPS) stimulated RAW 264.7 macrophages. doi:10.1007/s10068-017-0165-4

74. Samraj AN, Pearce OMT, Läubli H, et al. A red meat-derived glycan promotes inflammation and cancer progression. *Proc Natl Acad Sci U S A*. 2015;112(2):542-547. doi:10.1073/pnas.1417508112

75. Fritsche KL. The Science of Fatty Acids and Inflammation 1-3. doi:10.3945/an.114.006940

76. Ley SH, Sun Q, Willett WC, et al. Associations between red meat intake and biomarkers of inflammation and glucose metabolism in women. *Am J Clin Nutr*. 2014;99(2):352-360. doi:10.3945/AJCN.113.075663

77. Nuora A, Chiang VSC, Milan AM, et al. The impact of beef steak thermal processing on lipid oxidation and postprandial inflammation related responses. *Food Chem*. 2015;184:57-64. doi:10.1016/J.FOODCHEM.2015.03.059

78. Arya F, Egger S, Colquhoun D, Sullivan D, Pal S, Egger G. Differences in postprandial inflammatory responses to a “modern” v. traditional meat meal: a preliminary study. *British Journal of Nutrition*. 2010;104:724-728. doi:10.1017/S0007114510001042

79. Broughton KS, Rule DC, Handrich E. Prostaglandin E 2 production in mice is reduced by consumption of range-fed sources of red meat. doi:10.1016/j.nutres.2011.10.002

80. Klopatek SC, Xu Y, Yang X, Oltjen JW, Vahmani P. Effects of Multiple Grass-and Grain-Fed Production Systems on Beef Fatty Acid Contents and Their Consumer Health Implications. 2022;2:712-721. doi:10.1021/acsfoodscitech.2c00021

81. Ricordi C, Garcia-Contreras M, Farnetti S. Diet and Inflammation: Possible Effects on Immunity, Chronic Diseases, and Life Span. *J Am Coll Nutr*. 2015;34:10-13. doi:10.1080/07315724.2015.1080101

82. Wolk A. Potential health hazards of eating red meat. *J Intern Med*. 2017;281(2):106-122. doi:10.1111/JOIM.12543

83. Nogoy KMC, Sun B, Shin S, et al. Fatty Acid Composition of Grain- And Grass-Fed Beef and Their Nutritional Value and Health Implication. *Food Sci Anim Resour*. 2022;42(1):18-33. doi:10.5851/kosfa.2021.e73

84. Leheska JM, Thompson LD, Howe JC, et al. Effects of conventional and grass-feeding systems on the nutrient composition of beef. *J Anim Sci*. 2008;86(12):3575-3585. doi:10.2527/jas.2007-0565

85. van Vliet S, Provenza FD, Kronberg SL. Health-Promoting Phytonutrients Are Higher in Grass-Fed Meat and Milk. *Front Sustain Food Syst*. 2021;4:299. doi:10.3389/FSUFS.2020.555426/BIBTEX

86. Alfaddagh A, Martin SS, Leucker TM, et al. State-of-the-Art Review Inflammation and cardiovascular disease: From mechanisms to therapeutics. Published online 2020. doi:10.1016/j.ajpc.2020.100130

87. Meessen ECE, Warmbrunn M V, Nieuwdorp M, Soeters MR. Human Postprandial Nutrient Metabolism and Low-Grade Inflammation: A Narrative Review. doi:10.3390/nu11123000

88. Mazidi M, Valdes AM, Ordovas JM, et al. Meal-induced inflammation: postprandial insights from the Personalised REsponses to DIetary Composition Trial (PREDICT) study in 1000 participants. *American Journal of Clinical Nutrition*. 2021;114(3):1028-1038. doi:10.1093/ajcn/nqab132

89. Aleksandrova K, Koelman L, Rodrigues CE. Dietary patterns and biomarkers of oxidative stress and inflammation: A systematic review of observational and intervention studies. *Redox Biol*. 2021;42:101869. doi:10.1016/J.REDOX.2021.101869

90. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform*. 2019;95. doi:10.1016/j.jbi.2019.103208

91. Narayanan Nair M. Nutritional Composition of Novel Plant-Based Meat Alternatives and Traditional Animal-Based Meats. *Food Sci Nutr*. 2021;7(3):1-12. doi:10.24966/FSN-1076/100109

92. Cunniff P. Official methods of analysis of AOAC international University of San Carlos Tamil Nadu Veterinary and Animal Science. Published online 1995:1-2. Accessed March 28, 2024. https://search.worldcat.org/title/421897987

93. AOAC. Official methods of analysis of AOAC International. Latimer; WHGW, ed. Published online 2006:Method 999:11. Accessed March 28, 2024. https://search.worldcat.org/title/1085712083

94. Phillips KM, Ruggio DM, Howe JC, et al. Preparation and characterization of control materials for the analysis of conjugated linoleic acid and trans-vaccenic acid in beef. *Food Research International*. 2010;43(9):2253-2261. doi:10.1016/j.foodres.2010.06.012

95. Emerson SR, Kurti SP, Harms CA, et al. Magnitude and timing of the postprandial inflammatory response to a high-fat meal in healthy adults: A systematic review. *Advances in Nutrition*. 2017;8(2):213-225. doi:10.3945/an.116.014431

96. Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L. Blood Glucose Area Under the Curve: Methodological Aspects. *Diabetes Care*. 1990;13(2):172-175. doi:10.2337/DIACARE.13.2.172

97. Ruiz-Núñez∗ B, Dijck-Brouwer DAJ, Muskiet FAJ. The relation of saturated fatty acids with low-grade inflammation and cardiovascular disease. Published online 2016. doi:10.1016/j.jnutbio.2015.12.007

98. Poli A, Agostoni C, Visioli F. Dietary Fatty Acids and Inflammation: Focus on the n-6 Series. *Int J Mol Sci*. 2023;24(5). doi:10.3390/ijms24054567

99. Manning PJ, Sutherland WHF, McGrath MM, De Jong SA, Walker RJ, Williams MJA. Postprandial Cytokine Concentrations and Meal Composition in Obese and Lean Women. *Obesity*. 2008;16(9):2046-2052. doi:10.1038/OBY.2008.334

100. Blackburn P, Després JP, Lamarche B, et al. Postprandial variations of plasma inflammatory markers in abdominally obese men. *Obesity*. 2006;14(10):1747-1754. doi:10.1038/OBY.2006.201

101. Badoud F, Lam KP, Perreault M, Zulyniak MA, Britz-Mckibbin P, Mutch DM. Metabolomics Reveals Metabolically Healthy and Unhealthy Obese Individuals Differ in their Response to a Caloric Challenge. Published online 2015. doi:10.1371/journal.pone.0134613

102. Arya F, Egger S, Colquhoun D, Sullivan D, Pal S, Egger G. Differences in postprandial inflammatory responses to a “modern” v. traditional meat meal: a preliminary study. *British Journal of Nutrition*. 2010;104:724-728. doi:10.1017/S0007114510001042

APPENDIX

FATTY ACID COMPOSITION

Table A1. List of Fatty Acids Identified in Fatty Acid composition Analysis. Includes omega name and common name of fatty acids.

Omega name	Common name
C12:0	Lauric acid
C14:0	Myristic acid
C16:0	Palmitic acid
C17:0	Heptadecanoic acid
C18:0	Stearic acid
C20:0	Arachidic acid
C24:0	Lignoceric acid
C14:1	Myristoleic acid
C16:1	Palmitoleic acid
C17:1	Heptadecenoic acid
C18:1c9	Oleic acid
C18:1c11	*cis*-Vaccenic acid
C20:1	Eicosenoic aicd
C18:2	Linoleic acid (n-6)
C18:3	Alpha-Linoleic acid (n-3)
C18:2c9t11	Conjugated linoleic acid
C18:2t10c12	Conjugated linoleic acid isomer
C20:2	Eicosadienoic acid
C20:4	Arachidonic acid (n-6)
C20:5	Eicosapentaenoic acid (EPA) (n-3)
C22:6	Docosahexaenoic acid (DHA) (n-3)
T-vaccenic	*trans*-Vaccenic acid