

Utah State University

DigitalCommons@USU

All Graduate Theses and Dissertations, Fall
2023 to Present

Graduate Studies

12-2023

The Effects of Agroecological Farming Systems on Human Health

Olivia Kathryn Mason

Utah State University, a02399458@usu.edu

Follow this and additional works at: <https://digitalcommons.usu.edu/etd2023>



Part of the [Human and Clinical Nutrition Commons](#)

Recommended Citation

Mason, Olivia Kathryn, "The Effects of Agroecological Farming Systems on Human Health" (2023). *All Graduate Theses and Dissertations, Fall 2023 to Present*. 11.

<https://digitalcommons.usu.edu/etd2023/11>

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations, Fall 2023 to Present by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



THE EFFECTS OF AGROECOLOGICAL FARMING SYSTEMS ON HUMAN
HEALTH

by

Olivia Kathryn Mason

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

of

MASTER OF SCIENCE

in

Nutrition Sciences

Approved:

Stephan Van Vliet, Ph.D.
Major Professor

Korry Hintze, Ph.D.
Committee Member

Robert Ward, Ph.D.
Committee Member

D. Richard Cutler, Ph.D.
Vice Provost of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2023

Copyright © Olivia Mason 2023

All Rights Reserved

ABSTRACT

The Effects of Agroecological Farming Systems on Human Health

by

Olivia Mason, Master of Science

Utah State University, 2023

Major Professor: Dr. Stephan van Vliet

Department: Nutrition Science

Background: There is a growing concern regarding current agricultural production systems on human and environmental health. Agroecological, or regenerative, farming techniques are aimed to provide sustainable “nature-based” solutions using multi-cropping, ley rotations, integrated crop-livestock systems, and/or adaptive grazing of livestock to put nutrients back into the soil, crops, and livestock. While some studies show a benefit to increased nutrient density in animal and plant foods for human consumption; there are no studies that have been performed analyzing the impact of agroecological farming practices on human health.

Objective: The goal of this work was to study the effects of agroecologically-produced foods vs. conventionally-made foods on human health. Both diets consisted of the same types of food in the same quantity, with the only difference being in the production style.

Design: The study followed a randomized crossover design and sixteen (n=16) middle-aged adults (mean \pm SD: age: 45 ± 7.60 y; BMI: 29.62 ± 3.15 kg/m²) consumed each diet for 44 days with a 2-week washout in between. All meals and snacks were provided by the research team at an energy level that promoted weight maintenance. Diets were

matched for caloric intake and nutrient levels. Blood samples were collected at the beginning and end of each intervention trial for biomarker profiling.

Results: While all participants improved glucose (reduction of ~ 5 mg/dl) and triglycerides (reduction of ~ 36.3 mg/dl) compared to their habitual diet, there were no significant differences glucose and lipid biomarkers between both diets (all $p > 0.05$). We found trend of a decrease in the inflammatory biomarker interleukin-6 following the regenerative diet (6.7 ± 11.3 pg/ml) vs. the conventional diet (13.7 ± 14.0 pg/ml) ($p = 0.07$), but not difference was found for serum amyloid A ($p = 0.41$).

Conclusions: This work indicates that consumption of a whole foods-based diet, as opposed to a Standard American Diet, improves biomarkers of human health compared—including glycemic and lipidemic markers—irrespective of food production method. There was a minor indication that food produced using agro-ecological practices can further benefit the inflammatory marker interleukin-6. Additional metabolic profiling and a large sample size may be needed to determine if different agricultural production practices have an appreciable effect on human health.

(75 pages)

PUBLIC ABSTRACT

The effects of agroecological farming systems on human health

Olivia Mason

There is a growing concern that the current farming techniques are producing less-nutrient dense soils and foods impacting human health. To improve the health of soils, people, and the plant, a growing number of farmers are using regenerative, or agroecological, farming practices. Some of these methods include multi-cropping (growing various plants on the same plot of land), ley systems (alternating between crops and livestock), and rotational grazing of livestock. Previous studies have found that regenerative farming systems have various benefits for the lands, crops, and animals, as well as increasing the nutrient density of foods.

The purpose of this study was to compare conventional farming practices to regenerative farming practices on human health markers. Sixteen participants completed the study, where they consumed both a regeneratively-produced diet and a conventionally-produced diet for forty-four days with a two-week washout period between the two diet periods. At four different timepoints, blood was drawn from participants to analyze biomarkers of health. Overall, there was no differences in glucose, lipid, and inflammatory levels after the diet. There was significant data indicating there were positive differences between participants' habitual diet and the whole-foods diet that both study diets were based upon. The work showed that there are positive health indications from consuming a whole-foods diet versus a typical standard American diet

rich in ultra-processed foods. Future work will include deep metabolic profiling of the blood and stool samples of people.

CONTENTS

	Page
Abstract.....	iii
Public Abstract.....	v
List of Tables.....	viii
List of Figures.....	ix
Introduction.....	1
Literature Review.....	4
Agroecology.....	4
Impact of farming techniques on nutritional composition.....	5
Crops.....	6
Livestock.....	10
Impact of farming techniques on human health.....	14
Methods.....	17
Results.....	28
Discussion.....	35
Conclusion.....	41
References.....	42
Appendices.....	62
Appendix A. Inclusion & Exclusion Criteria.....	63
Appendix B. <i>Human ELISA Kit Protocol</i>	64
Appendix C. <i>All food products used in study with corresponding nutrients</i>	66

LIST OF TABLES

	Page
Table 1: Participant characteristics at baseline.....	19
Table 2: Study Schedule.....	22
Table 3: Average dietary intake during the study period.....	29
Table 4: Average blood pressure following each diet.....	30
Table 5: Measured fasted inflammatory biomarkers and metabolic panel post regenerative and conventional diets.....	32
Table 6: Fasted lipid panel following each diet.....	34

LIST OF FIGURES

	Page
Figure 1: Study protocol.....	23
Figure 2: Participant sample menu.....	23
Figure 3: Study summary.....	27
Figure 4: Measured blood pressure before and after each diet period.....	31
Figure 5: Measured weight (kg) before and after each diet period.....	31
Figure 6: Measured fasted glucose levels before and after each diet period.....	33
Figure 7: Measured fasted triglyceride levels before and after each diet period.....	34

INTRODUCTION

There is a large growing concern about the impact of food production techniques on human health (Pörtner et al., 2022). There are indications that the micronutrients in our food supply has decreased by 25-30% compared to about seventy-five years ago (Thomas, 2007). A growing group of scientists suspects that this may be in part due to less nutrient-rich soils (Pilling & Hoffmann, 2020; Wall et al., 2015), which results in less nutrient-dense crops and animals eating those crops or forages grown on such soils. The current farming techniques may also contribute to the ongoing environmental struggle that in turn negatively impacts human health as well. If the current food systems continue to function as they are, it is predicted there will be increased rates of food-related diseases, such as diabetes, heart disease, stroke, and cancer, and increased environmental degradation as well (Clark et al., 2022).

There have been various studies conducted investigating the impacts of current versus agro-ecological farming techniques on soil, crop, ecosystem, and livestock nutrient levels. Agro-ecology is defined as an integrated approach that applies ecological principles (“nature-based solutions”) to farming (Wezel et al., 2014). Promising agroecological (also referred to as regenerative) farming practices include multi-cropping, ley rotations, integrated crop-livestock systems, and/or adaptive grazing of livestock. There have been numerous studies conducted comparing regenerative and conventionally-grown soils and crops. The results indicate agroecological techniques resulted in increased nutrient levels in both the soils and crops, which promotes biodiversity, and soil microbial activity (Albizua et al., 2015; Bender & van der Heijden,

2014; Duchene et al., 2017; Fenster et al., 2021; Hu et al., 2016; Montgomery & Biklé, 2022; Nabel et al., 2018; Sun et al., 2020; Verbruggen et al., 2011; Wagner et al., 2021).

With regards to livestock products comparing grass-fed and grain-fed animals, it has been found that grass-fed livestock contain plentiful phytochemicals, antioxidants, and omega-3 fatty acids (Benbrook et al., 2018; Carillo et al., 2016; Daley et al., 2010; Montgomery et al., 2022; Provenza et al., 2019; van Vliet et al., 2021). Meanwhile their grain-fed counterparts contained a higher total fat content, less anti-oxidants, more omega-6 fatty acids, and the animals displayed signs of metabolic dysfunction (Apaoblaza et al., 2020; Carillo et al., 2016; van Vliet et al., 2021). The relative comparative levels of nutrients found in meat from grass-fed and grain-fed animals have been observed in dairy products as well (Allothman et al., 2019).

There are limited studies relating regenerative farming practices to human health, but there have been some relating the micronutrients commonly found in agroecologically grown crops and livestock to health benefits. These include, vitamins B, E, and K, and phenolics (Montgomery et al., 2022) that are associated with chronic and neurological diseases (Del Rio et al., 2013; Provenza et al., 2019; Wang et al., 2014; Weisburger et al., 2009). Others have correlated a number of health problems to conventional farming techniques finding increased birth defects, intellectual disorders, and depression (Beard et al., 2014; Grandjean & Landrigan, 2014; Gunier et al., 2017; Horton et al., 2011; Rauh et al., 2015; Munger et al., 1997; von Ehrenstein et al., 2019).

There is increasing evidence that different farming techniques impact the nutrient levels of both livestock and crops. Due to this information, it may be possible that these

farming techniques also have an effect on human health, but there is a lack of research in this area, which indicates more studies need to be conducted to get more information.

LITERATURE REVIEW

Agroecology

The idea of agroecology began in 1943 with the Haughley Research Farm and Eve Balfour (Montgomery & Biklé, 2022). After plenty of research she documented in her book, *The Living Soil*, she advocated for (at that time) regenerative farming techniques to increase the health of soil and crops (Montgomery & Biklé, 2022).

Today, agroecology is known as the collection of sustainable practices seen in agriculture and various food systems (Bezner et al., 2021). From there, the term agroecology, or regenerative agriculture, is a broader term that encompasses many different objectives, such as economic, environmental, social, health, and cultural initiatives (Bezner et al., 2021). There are different principles involved, but some of the most common techniques include economic diversification, reduced input, nutrient recycling, improving biodiversity, and linking soil and animal health (Wezel et al., 2020). To date, the main agroecological aims are linked with food security, poverty relief, climate change, and biodiversity, but there is a growing body of data that indicates increased nutritional value may also be an important outcome of these practices (van Zutphen et al., 2022). Agroecology argues for a holistic approach and is aimed at increasing biodiversity and the nutritional value of crops and vegetation (van Zutphen et al., 2022). This in turn, may increase the health of animals, humans, and the environment.

It is known that there is a large problem of malnutrition throughout the world, with 1/3rd of people worldwide suffering from at least one micronutrient deficiency (Han et al., 2022). Some have postulated that by allowing for greater biodiversity through

agroecology and thus increased nutritional content, more of the population will obtain the proper nutrients in smaller amounts, allowing for decreased portions to acquire the same nutrients (Karas, 2023).

While there is limited data linking regenerative agriculture with increased nutrients, there are some studies (Feng et al., 2022; Hepperly et al., 2018; Montgomery et al., 2022) that have indicated that there is a positive correlation between agroecological practices and nutritional content (Bezner et al., 2021). Many of these studies do not use objective testing methods to properly evaluate the effect of the agroecological practice on human health (van Zutphen et al., 2022). With this being said, there is currently a gap of knowledge relating regenerative agriculture practices with human health.

Impact of farming techniques on nutritional composition

Some have suggested that current conventional farming practices may be one of the primary reasons for decreased nutrient content found in various crops, soils, and livestock (Davis et al., 2004). These systems prioritize calories instead of nutritional quality (Montgomery & Biklé, 2022). While the Green Revolution after World War II may have brought increased crop production, inorganic fertilizers/pesticides, industrialized farming equipment, etc., one of its consequences appears to be decreased nutrients found in soils and crops (John & Babu, 2021). This movement also separated livestock from other animals and vegetation, which led to a decline in nutrient content found in their meat and milk (van Vliet et al., 2021). Within the last twenty years or so, increasing research has been conducted investigating the changes in nutritional content in

various crops and livestock. Many of these studies indicate the declining numbers may be due to current farming techniques.

Crops

From 1950-1999, there have been nutritional declines, most notably in nutrients such as protein, calcium, phosphorus, iron, riboflavin, and ascorbic acid in consumer vegetables (Davis et al., 2004). Additionally, there is more evidence showing major declines in essential minerals like potassium, phosphorus, magnesium, calcium, iron, and copper in fruits/vegetables compared to the 1930s and 1940s (Mayer, 1997; Thomas, 2007). All of these studies compare pre-agriculture revolution to post indicating that modern agricultural practices could be attributed to these changes, although changes in cultivars and their impacts on nutritional quality cannot be excluded (Davis et al., 2004; Mayer 1997; Thomas, 2007).

Due to negative consequences of modern farming techniques, some farmers started reverting back to “traditional,” regenerative practices that promote biodiversity, soil and plant health, and sustainability (Montgomery & Biklé, 2022). Some of the most common regenerative practices for crops consist of intercropping, rotating crops, ley systems, cover-crops, manure fertilizers, etc. (Albizua et al., 2015; Duchene et al., 2017). There are countless benefits associated with them, such as decreased soil erosion, increased soil health, and increased soil organic matter (Montgomery & Biklé, 2022). Overall, these regenerative farming principles are important for supporting and nourishing the soil, which increases organic matter, life, and supports fertility (Montgomery & Biklé, 2022).

Regenerative farming techniques first target the soil and its microbes, and works its way up on the food chain (Fenster et al., 2021). While the soil and corresponding microbes are suggested to be most important for promoting healthy and nutrient-dense crops, it has also been stated that this part is often the most overlooked (Duchene et al., 2017; Montgomery et al., 2022). With that being said, about 1/3 of the world's total agricultural land has seen significant topsoil erosion and decreased fertility (Montgomery & Biklé, 2022). The topsoil is considered important because it acts as a "vehicle" for nutrients and other compounds to enter crops, livestock, and ultimately humans (Montgomery & Biklé, 2022). Agroecological farming practices aim to keep the soils heavily populated with diverse microbes to continuously cycle the nutrients and bring it into the crops. There are many different species of fungi, protists, and bacteria that are found in the soil (Frac et al., 2022; Geisen, 2021) that are thought to contribute to the nutritional quality of plants.

Another main concern with the conventional farming techniques is the use of synthetic fertilizers. They usually contain high levels of phosphorus and other substances that deplete the soils of proper microbe functioning, which can decrease nutrient levels for crops (Montgomery et al., 2022). There have been various studies conducted showing a decrease in microbe activity after being treated with conventional fertilizers (Lambert et al., 1979; Marschner & Dell, 1994; Ryan et al., 2008; White & Broadley, 2009; Zhang et al., 2012). From there, when the microbe activity is suppressed the crops planted in those soils have lower uptake levels of minerals, such as phosphorus, zinc, copper, iron, manganese, potassium, and more (Lambert et al., 1979; Marschner & Dell, 1994). Other studies have supplemented the soils with various strains of rhizobacteria and

cyanobacteria that increased protein content, micronutrient content (iron, manganese, and copper) and yield in their crops compared to conventional chemical fertilizers (Rana et al., 2012). Even in studies that did not add any microbes to the soil or any synthetic fertilizers, there were micronutrient increases in the crops while also having a high yield (Hepperly et al., 2018). This indicates that by allowing soil microbial activity to increase there are benefits in micronutrient content for the crops and potentially overall yield (Hepperly et al., 2018; Montgomery et al., 2022; Rana et al., 2012).

One popular regenerative farming technique is known as intercropping. This technique puts multiple types of crops on the same field, allowing for changes in root distribution and structure (Duchene et al., 2017). On the other hand, conventional farming participates in monoculture cropping that has a devastating impact on soil (Chai et al., 2021). Studies have seen increased crop productivity and soil nutrient availability in intercropped fields (Duchene et al., 2017; Hu et al., 2016). Along with this, there may be increases in crop production while having little negative impact on the environment (Hu et al., 2016). Many studies have investigated this system using cereal/legume intercropping (Duchene et al., 2017). Legumes are common crops and have been seen to manipulate nitrogen in ways that increase biomass production in crops (Nabel et al., 2018). Additionally, the ability to harness nitrogen is very important for the crops' growth and accumulation of nutrients. When too much nitrogen is provided in conventional fertilizers, it can decrease potassium, phosphorus, soluble protein, and sugar availability in soils and crops (Sun et al., 2020). Thus, by using natural nitrogen cyclers, such as legumes, with other crop species the right amount of nitrogen will be available in the soil to promote high nutrient crops (Nabel et al., 2018; Sun et al., 2020). In many

studies, researchers perform a number of regenerative farming techniques together to maximize the benefits.

A more in-depth study was performed looking at different regenerative techniques, such as no-till, cover crops, and diverse rotations, and comparing crop nutrient-levels and soil health on the regenerative farms to conventionally-produced crops and soil (Montgomery et al., 2022). Unlike conventional practices, no-tilling means that the soil is not turned and less disturbance occurs, which allows for less soil erosion (Elliott, 2022). From there, cover crops were utilized to further help slow erosion, increase soil health, decrease weeds, and increase biodiversity (Clark, 2015). Along with the cover crops, incorporating different crop rotations allows for increased crop yields while also improving soil health in the process (Hepperly et al., 2018; Wagner et al., 2021). The researchers found that not only did the regenerative farms have more organic soil matter and healthier soil, but the crops grown in these soils had substantially higher levels of beneficial various phytochemicals, vitamins, and minerals (Hepperly et al., 2018; Montgomery et al., 2022). The compounds that saw the greatest increase include, vitamin K, vitamin B1 & 2, carotenoids, phenolics, phytosterols, calcium, iron, and phosphorus (Montgomery et al., 2022). The conventionally-grown crops had higher values of cadmium, nickel, and sodium, which are known to be detrimental to human health (Montgomery et al., 2022).

Another study utilized crop rotations and ley systems to compare conventional and regenerative farming techniques. Ley system refers to the practice of alternating consumer crops and livestock vegetation for the purpose to increase biodiversity and improve soil health (Albizua et al., 2015). It was found that the soils/fields treated with

ley had the highest microbial content, specifically in arbuscular mycorrhizal fungi (AMF), compared to those that used conventional farming techniques (Albizua et al., 2015). This is important because AMF has shown to decrease nutrient leaching, plant stress, and the need for supplemental phosphate fertilizers, while increasing nutrient uptake (Albizua et al., 2015; Bender & van der Heijden, 2014; Verbruggen et al., 2011). This data indicates that using regenerative farming techniques has beneficial effects that result in not only improving the environment, but also providing nutrient-dense crops.

From there, another study was conducted that looked at the differences in soil nutrients in regenerative and conventionally-farmed almonds. The regenerative farms, using practices such as, utilizing cover crops, no-till, maintaining natural vegetation inhabitants, and planting hedgerows, had soils containing significantly more phosphorus, calcium, and sulfur (Fenster et al., 2021). From there, they also contained more total nitrogen, carbon, and microbial activity which supports plants and allows them to take in nutrients (Fenster et al., 2021). Then when looking at the nutrient level of the almonds themselves, it was seen that the regenerative-farmed almonds contained higher levels of magnesium than their conventionally-raised counterparts (Fenster et al., 2021). This study supports the usage of regenerative techniques to improve human health and ecological health (Fenster et al., 2021).

Livestock

While consumer crops' nutrient levels have declined over time, so have nutrients obtained through livestock products (van Vliet et al., 2021). Studies have found that there are significant declines in micronutrients seen in beef from 1940-2002, such as

magnesium, calcium, iron, copper, and zinc (Thomas, 2007). While each animal product was lacking in different minerals/ micronutrients, it was apparent there were significant declines in the main livestock products and dairy byproducts (chicken, turkey, beef, pork, milk, cheese) (Thomas, 2007). Many scientists are attributing more confined and intensive rearing practices to the nutrient differences seen in today's meat (Thomas, 2007; van Vliet et al., 2021).

There are a number of studies that support the claim that regenerative practices, specifically pasture-based grazing systems, produce more nutrient-dense livestock. One study examined the differences in phytonutrients between grass-fed and grain-fed cows. It was evident that those livestock that were consuming a variety of vegetation in the pastures contained more health-promoting phytonutrients than those that solely consumed grain-based feed (van Vliet et al., 2021). Phytochemicals are important as some are characterized to have anti-inflammatory, anti-carcinogenic, and cardioprotective properties (van Vliet et al., 2021). Other studies compared grass-fed and grain-fed cows and found that not only did the grass-fed beef have lower total fat content, but they also contained more n-3 polyunsaturated fatty acids (Carrillo et al., 2016; Provenza et al., 2019), which are known for their health-promoting effects (Mason et al., 2020) including triglyceride and very low-density lipoprotein lowering effects. Meanwhile, the grain-fed meat contained more n-6 polyunsaturated fatty acids (Carillo et al., 2016; Nogoy et al., 2022), which in high amounts, may be considered detrimental for human health (Simopoulos, 2016). Another study supported the notion of increased inflammation-causing agents in conventional meat rather than in its traditionally-raised counterpart (Arya et al., 2010). There are indications that inflammation is responsible for many

disorders, such as heart disease, cancer, arthritis, autoimmune and neurodegenerative (Provenza et al., 2019; Simopoulos, 2020).

There have been more studies conducted looking at the overall differences in fatty acid (FA) and antioxidant composition between conventionally-raised and regenerative beef (Garcia et al., 2008; Montgomery et al., 2022; Nogoy et al., 2022; Provenza et al., 2019). Since the omega-3 fatty acid, alpha linolenic acid (ALA) is essential for functioning, but the body cannot synthesize some of them itself they must be obtained through the diet (Daley et al., 2010). Additionally, there are benefits to ingesting ALAs products, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) directly (Swanson et al., 2012). The fatty acids that have seen to be consistently higher in grass-fed beef are omega-3s, which have been shown to have a positive effect on the prevention of numerous neurological disorders (Daley et al., 2010; Duckett et al., 1993; Hibbeln, 1998; Laugharne et al., 1996; Montgomery et al., 2022; Nogoy et al., 2022; Provenza et al., 2019; Stoll et al., 1999; Wood & Enser, 1997; Yehunda et al., 1996). The grass-fed beef showed to have a lower total amount of fat, less cholesterol-raising FA, increased levels of total conjugated linoleic acid (CLA), trans vaccenic acid (TVA), precursors to vitamin A and E, glutathione and superoxide dismutase (Daley et al., 2010; Davis et al., 2022; Decalzo et al., 2005; Duckett et al., 2009; Garcia et al., 2008; Montgomery et al., 2022; Nogoy et al., 2022). Antioxidants, such as glutathione and superoxide dismutase, as well as CLA and TVA have been correlated with cancer-fighting abilities (Bauman & Lock, 2006; Daley et al., 2010). CLA and TVA are also associated with reducing inflammation, atherosclerosis, diabetes onset, and overall good human health (Kritchevsky et al., 2000; Montgomery et al., 2022; Pariza & Cook et al., 2000; Steinhart

et al., 2003). The increased levels of vitamin A precursors, most notably, beta-carotene that is necessary for proper vision, bone growth, reproduction, cell division, and cell differentiation (Scott, 1994). Vitamin E is important to fight off free radicals produced during metabolism that could damage the cells, as well as prevent coronary artery disease and enhance the immune system (Lonn & Yusuf, 1997).

Along with these claims, it has been shown that pasture-raised animals tend to have overall darker meat, which can be indicative of efficient metabolic processes (Apaoblaza et al., 2020). More specifically, the grass-fed meat contained more mitochondrial-based oxidative enzyme content, less glycolytic enzymes, and produced less lactate than the grain-fed beef, which in turn demonstrates the negative impact the grain-based diet has on energy metabolism (Apaoblaza et al., 2020). Some of these declines found in the grain-fed cows correspond to metabolic dysfunction provoked by defective oxidative metabolism, which is associated with increased visceral fat and insulin resistance in muscles (Nisoli et al., 2007). The effects associated with metabolic disease indicate the cows consuming conventional grain feed are more at risk for serious health conditions compared to free-range cows, which could have an effect on humans if consumed.

Studies also compared regenerative dairy products with their conventional equivalents. The results showed the same nutrients in meat that were found in great amounts were also increased in dairy products compared to the conventional products (Alothman et al., 2019). This means the grass-fed dairy products contained significantly higher levels of omega-3 polyunsaturated FAs and conjugated linoleic acid, while having lower levels of omega-6 FAs (Alothman et al., 2019). Additionally, there were higher levels of vaccenic acid found in regenerative dairy products as well. Overall, there was a

lower milk fat content from the cow's feeding on the pastures, which is consistent with the regenerative beef fat content (Alothman et al., 2019; Chillard et al., 2007; Liu et al., 2016). It is stated that the difference between the nutrient levels is due to a high supply/deliver of nutrients (from the diverse vegetation) and fatty acids to the mammary gland that ultimately produces the milk (Bargo et al., 2006; Villeneuve et al., 2013). Along with this, there are higher amounts of various micronutrients that were found in the regeneratively produced dairy products, such as beta-carotene, terpenes, lutein, vitamin A & E, and phytol (Agabriel et al., 2007; Alothman et al., 2019; Che et al., 2012; Coppa et al., 2011; Lucas et al., 2005; O'Callaghan et al., 2016). These results are, again, consistent with those comparing regenerative and conventional meat products.

Impact of farming techniques on human health

It has been stated that the children today are expected to live shorter and less healthy lives than their parents; this is the first generation in the nation's history to do that (Montgomery & Biklé, 2022). There are studies showing that there is positive correlation between mineral/trace element deficiencies and mental illnesses (Thomas, 2007). Along with that, many physical diseases, such as childhood leukemia, obesity, cardiovascular disease, arthritis, infertility, etc. can be linked to insufficient micronutrient consumption (Thomas, 2007).

It has also been found that one in three people globally are deficient in micronutrients (McGuire, 2015). Many researchers believe that by consuming nutrient-dense crops/livestock products that there would be decrease in these deficiencies and, therefore, improves human health (Bouis & Saltzman, 2017; DeMoura et al., 2013).

Vitamins/minerals and phytochemicals have been found to have many health-promoting properties, most notably with preventing cancers, chronic diseases, and more (Del Rio et al., 2013; Provenza et al., 2019; Wang et al., 2014; Weisburger et al., 2009). Antioxidants are commonly found in polyphenols and terpenoids that may be more abundant in regeneratively-produced goods. These molecules are usually associated with cancer prevention and treatment, but they also have been seen to fight the progression and onset of many metabolic and neurological diseases (Hahn et al., 2020; Wang et al., 2014). Along with this, these polyphenols have been shown to fight against inflammation that is present in many chronic diseases, such as obesity and atherosclerosis (Del Rio et al., 2013; Zanotti et al., 2015). The contributing forces of both the anti-inflammation and antioxidant properties of these molecules allow them to be powerful opposition to many diseases (Del Rio et al., 2013; Zanotti et al., 2015).

Along with the numerous health benefits from micronutrients, there are health concerns due to the pesticides and other synthetic chemicals used in conventional farming. There are studies that found that pregnant mothers and infants exposed to pesticides, specifically glyphosate, chlorpyrifos, diazinon, malathion, avermectin, and permethrin, were at higher risk of developing or having a child with intellectual disabilities (Grandjean & Landrigan, 2014; Gunier et al., 2017; Horton et al., 2011; Rauh et al., 2015; Munger et al., 1997; von Ehrenstein et al., 2019). Along with this, the exposure to pesticides also increased the risk of various birth defects (Bell et al., 2001; Croen & Shaw, 2001; Damgaard et al., 2006; Garry et al., 2002; Munger et al., 1997; Winchester et al., 2009). From there, pesticide usage has been associated with increased

rates of depression (Beard et al., 2014; Besler & Stallones, 2008; Farahat et al., 2003; Salvi, 2003).

While there are fewer studies showing the impacts of consuming regeneratively produced crops/livestock on human health there is some evidence that there are benefits to nutrient density that should be explored further. Thus, the goal of this work was to perform the first ever randomized controlled trial comparing the consumption of plant and animal foods produced using regenerative vs. conventional practices on lipid, inflammation, and glycemic markers of middle-aged adults at risk of metabolic disease. We hypothesized that the regenerative diet would have additional benefits of biomarkers of health compared to the regenerative diet.

METHODS

Participants and ethical approval

The study was approved by the Institutional Review Board at Utah State University, registered on ClinicalTrials.gov (NCT05575258), and conformed to standards for the use of human participants in research outlined in the seventh revision of the Declaration of Helsinki. Participants were recruited from Cache Valley, Utah. Interested participants were pre-screened by telephone, using a scripted list of questions to identify individuals that may be eligible for study entry. Suitable candidates were provided a REDCap® (Research Electronic Data Capture) survey link to complete asking for further details about their health, dietary habits, and sleep habits. Responses were reviewed by two study staff members (all members completed necessary CITI training) and potential eligible candidates were contacted to schedule a consent/screening visit. The pre-screening was utilized to confirm the participant met most of the inclusion/exclusion criteria before the subject was scheduled for a consent meeting. It gave interested subjects an opportunity to ask additional questions about the study to see if they were interested in moving forward with a consent/screening visit. The phone screen saved time and resources for the participant and research staff.

Eligible participants were between 35-60 years old with a body mass index between 25-35 kg/m² and a stable weight for the last 3 months prior to starting the study (loss or gain <4%). Volunteers must have also met criteria regarding hemoglobin A1C (HbA1C ≤ 6.4%) and fasting plasma glucose concentration (<126 mg/dl). Participants were excluded if they had diagnoses of active malignancy, congestive heart failure,

diabetes mellitus, chronic obstructive pulmonary disease or any inflammatory diseases. Volunteers did not meet the qualifications if they were using antibiotics or antibiotics within the last 60 days. The full list of inclusion/exclusion criteria can be found in *Appendix A*.

All participants were informed about the experimental procedures, purpose of the study, and any potential risks prior to giving written consent. It was mandatory for all subjects to attend a consent session conducted by a trained member of the research team to present the details of the study. Interested participants completed the informed consent process privately with study staff. Participants were given up to sixty minutes to read the consent and ask questions. The research team contacted the participant three days after the initial consent visit to see if the subject came to a decision about participating. No study procedures took place prior to obtaining written consent. Twenty-two people were enrolled in the study. Of those twenty-two, three dropped out and three were dismissed from the study due to compliance issues. Sixteen (n=16) middle-aged adults (mean \pm SD: age 46 ± 7.4 y) completed the study and their characteristics are presented in Table 1.

Table 1: Participant characteristics at baseline ($n=16$)

Variable		
Age (y)	45.00	± 7.60
Weight (kg)	93.27	± 22.69
BMI	29.62	± 3.15
Systolic BP (mmHg)	120.40	± 14.50
Diastolic BP (mmHg)	73.72	± 9.60
Fasting Glucose (mg/dL⁻¹)	91.63	± 5.78
Hb1AC	5.60	± 0.34
Triglycerides	148.00	± 69.47

Data are mean ± SEMs.

Experimental design and diets

The study employed a randomized cross-over design to compare an agroecological vs. conventional sourced diet and determine the effects on inflammation (interleukin-6 and serum amyloid a) and metabolic biomarkers (glycemic and lipoprotein profiles). Diet randomization was performed by computerized random-number (1 or 2) generation and participants did not learn which diet they were on until the study was complete. Both diets were administered for 44 days with a 14-day washout period between them. For 7 days prior to their first intervention, participants were asked to keep a 7-day food log and record all the food and beverages they consumed with corresponding portion sizes. This information was processed in REDCap® by a registered dietician to provide insight into self-reported habitual caloric intake. This data

was used to calculate the maintenance caloric needs for their menus. Table 2 shows a detailed study schedule. During the last 7 days of their 14-day washout prior to their second dietary intervention, participants were asked to replicate the same 7-day habitual diet they consumed prior to their first dietary intervention. This was done to standardize habitual diets prior to each intervention. For the first 7 days of their washout, participants were told to restart their habitual diet, but were not given a 7-day food log to replicate. This was done to give participants a mental break from the study and promote compliance. A schematic overview of the study design is provided in Figure 1.

The interventional diets (agro-ecological and conventional) were designed by a registered dietician (Jennifer Cloward, RD) and approved by the Principal Investigator (Stephan van Vliet, PhD). The agro-ecological diet was produced first and the conventional diet was subsequently designed to mimic the agro-ecological diet in terms of meals and foods. Produce and meats for the agro-ecological diet were provided by the Greenacres farm (Cincinnati, OH), while a few remaining items/snacks (rice, frozen fruit, and bread) were purchased from brands that used agro-ecological practices and/or were labeled Regenerative Organic Certified. The food given in the conventional diet included non-organic produce and conventional meat and milk (no indication of organic, grass-fed, or pasture-raised) from local grocery stores. All food that participants consumed during the 7-week periods were stored in the food-grade fridges and freezers in the Center for Human Nutrition Studies (CHNS) Metabolic Kitchen and food boxes were prepared by the research team and provided to participants every 4 days. The two menus were matched calorically and were based on nutrition labels, the foods were not tested for nutrients itself. The dietician ensured both diets provided weight-maintenance diets with

an approximate macronutrient distribution of 20% protein, 30% fat, and 50% carbohydrate based on each individual's daily energy requirement (Harris-Benedict Equation) (Harris & Benedict, 2018). A sample menu given to participants is seen in Figure 2. Both diets were provided as 4-day rotating menus and participants picked up their meals bi-weekly from CHNS. This allowed the research team to interact face-to-face with the participant, address any issues, and further ensure compliance. Participants were also given diet-specific condiments at the beginning of each diet. Participants were instructed not to consume any other foods than what was provided by the research team. They were also told to log their consumption of all meals and snacks using custom-built daily logs in REDCap, and indicate the amount of food consumed. Participants were asked to return any unconsumed foods back to the research staff. We considered >85% of all provided meals consumed by the participant as an acceptable compliance rate.

Table 2: Study schedule

ACTIVITY/VISIT	WEEK	DURATION
PHONE SCREEN A scripted phone screen was performed to determine pre-eligibility prior to consent.	1	15 min
CONSENT A one-on-one consent visit was conducted privately with staff either in-person or via Zoom.	1	1 hr.
SCREENING This visit took place in the morning after an overnight fast and had the following procedures: informed consent, screening blood draw (including serum pregnancy test for women of child-bearing potential), health history, height and weight, and screening questionnaires regarding food intake, health, and sleep.	1	2 hr.
BASELINE VISIT DIET 1 This visit took place in the morning after an overnight fast. Participants were asked to complete a fasted blood draw and questionnaires, and brought in urine and stool samples that were collected using at-home collection kits.	2	1 hr.
INTERVENTION DIET 1 Participants were randomized to consume one of the following sourced diets: agroecological or conventional. They followed this nutrition pattern for 44 days. During this time, participants came to the CHNS building twice a week to pick up food.	2-9	1 hr. per week
POST VISIT DIET 1 On the last day of diet 1, participants were asked to complete a fasted blood draw and questionnaires, and brought in urine and stool samples that were collected using at-home collection kits.	9	1 hr.
WASHOUT PERIOD Participants consumed self-selected (habitual) diets for 14 days. There was no involvement from research team.	10-11	none
BASLINE VISIT DIET 2 This visit took place in the morning and had a fasted blood draw. Participants brought in stool and urine samples that were collected using at-home collection kits.	11	1hr.
INTERVENTION DIET 2 Participants started their second nutritional intervention (agroecological or conventional sourced diet depending on the diet consumed during the first intervention). During this time, participants came to the CHNS building twice a week to pick up food.	11-17	1 hr. per week
POST VISIT DIET 2 On the last day of diet 2, participants were asked to complete a fasted blood draw and questionnaires, and brought in urine and stool samples that were collected using at-home collection kits.	17	1 hr.

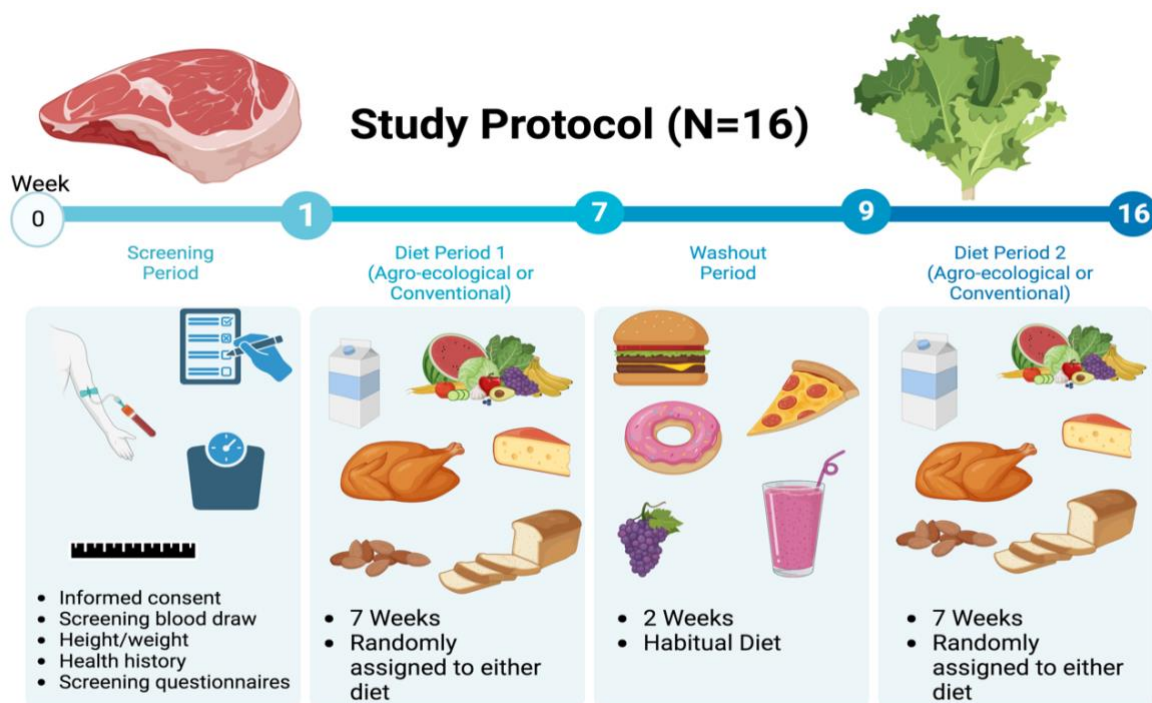


Figure 1: Participants were randomly assigned to either the agro-ecological or conventional diet for 7 weeks with a 2-week washout in between them. All meals and snacks were provided by the research team an energy level that promoted weight maintenance.

	Meal:	Ingredients:
Breakfast	Yogurt w/ granola and berries	125 g yogurt, 28 g granola
	Multi grain english muffin	1 english muffin
		70 g blueberries
Lunch	Grilled chicken cobb salad	Lettuce mix, tomatoes
	Garlic Bread	Spinach, onion, cucumber
		3 oz Grilled Chicken, 1 hardboiled egg, slice of bread
Dinner	Ground pork hash*	3.5 oz ground pork, russet potato, onion
	Steamed carrots	Steamed carrots
	Rice	55 g dry rice
Snacks	Fruit	140 g fruit
	Chocolate	30 g chocolate
	Rice Cake	2 Rice cakes

Figure 2: Participants were given menus and the corresponding portioned food. They were to record the amount that was consumed and return any of the extra food.

Food production, distribution, and analysis

All regenerative produce and meat were sourced from Greenacres Farm. Greenacres provided about 75% of the agroecological food products using regenerative techniques, such as ley rotations, cover crops, and no till. They have their animals graze on four specific plots (quadrants) of land and the animals (cattle, sheep, and chickens) remain on those quadrants for two-years. Following those two years, crops were then planted on that land. After the crops were harvested, the farm staff planted cover crops to fix nitrogen and improve nutrient recycling in the soil. Livestock were raised and foraged on the pastures and were routinely switched between various fields to maintain animal and land health. The aims of these measures were to increase organic matter, fertility, and sustainability.

Green Acres shipped the vegetables and meats to CHNS to be stored until given to participants. All other foods found on the agroecological menus were obtained through partnerships with companies with trusted regenerative methods. These companies consist of Sol Simple, Seal the Seasons, Pecan shop, Maple Hill Creamery, Alter-eco, and Lundberg. They provided frozen/dried fruit, nuts, grains, dairy products, and chocolate. The equivalent conventional food products were purchased from local grocery stores (Lee's and Smith's). A full list of provided foods can be found in *Appendix C*.

Research staff received, packaged, and distributed the food to participants. Upon arrival, vegetables and meat were placed in a refrigerator and other nonperishable foods were placed in the CHNS kitchen. Each food item was weighed and packaged appropriately following the given quantities listed on the participants' menus. All food items were weighed using grams, excluding the meat products, which were in oz. The

participants' food was prepared one-two days prior to the pickup to ensure freshness. The participants came to CHNS biweekly to receive their food for the next four days.

Participants were instructed to record their food intake via food logs and upload them to redcap. The research team compiled and analyzed their food consumption using excel sheets. The nutrients of each food item recorded on the food log was calculated and broken down into nutrients, such as protein, fat, carbohydrates, fiber, cholesterol, sodium, and sugar. The average nutrient breakdown in each diet is shown in figure 3.

Sample collection

Following a 12 hour overnight fast, participants visited the Center for Human Nutrition Studies (Utah State University) at 5 timepoints, screening, baseline visit diet 1 (day 1 of diet 1), post visit 1 (day 44 of diet 1), baseline visit diet 2 (day 1 of diet 2), and post visit diet 2 (day 44 of diet 2). In the screening visit, height (cm) was measured using a wall-mounted stadiometer (SECA Model 26419000009) without shoes. Weight (kg) was measured without shoes, sweaters, or coats using a digital scale (Cardinal Detecto Model 758C). After 15 minutes of resting in a chair, blood pressure was obtained using an automated blood pressure monitoring system (Omron BP5250) with the cuff on the participants left upper arm. Blood pressure was obtained three times at one-minute intervals and the average of the readings was recorded. Immediately thereafter, fasting blood draws were completed via venipuncture by trained phlebotomists (including Olivia Mason). 15 mL of blood was collected from a vein in the participant's arm (antecubital fossa). Blood samples were sent to LabCorp (Logan, UT) and analyzed for HbA1C, glucose, a basic metabolic panel (BMP), and a lipid panel. The subsequent visits (diet

baselines and post diet visits) consisted of obtaining 15 mL of fasted blood was taken from participants' arm or hand for inflammatory biomarker assays that were analyzed by the research staff. These blood samples were spun immediately for plasma and serum isolation (3000 rpm x 15 min), and were stored at -80° C until further analysis of blood inflammatory markers. Concentrations of plasma inflammatory and metabolic biomarkers—interleukin-6 (IL-6) and serum amyloid A (SAA) — were determined in duplicate using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (ab178013, Abcam, Cambridge, UK). Detailed instructions of ELISA kits seen in the *Appendix B*. When participants fully completed the study trained phlebotomists obtained 15 mL of fasted blood to send to LabCorp to compare post-study HbA1C, glucose, tests included in a basic metabolic and lipid panel to these levels recorded in the beginning of the study. A complete summary of the methods in this study is summarized in Figure 3.

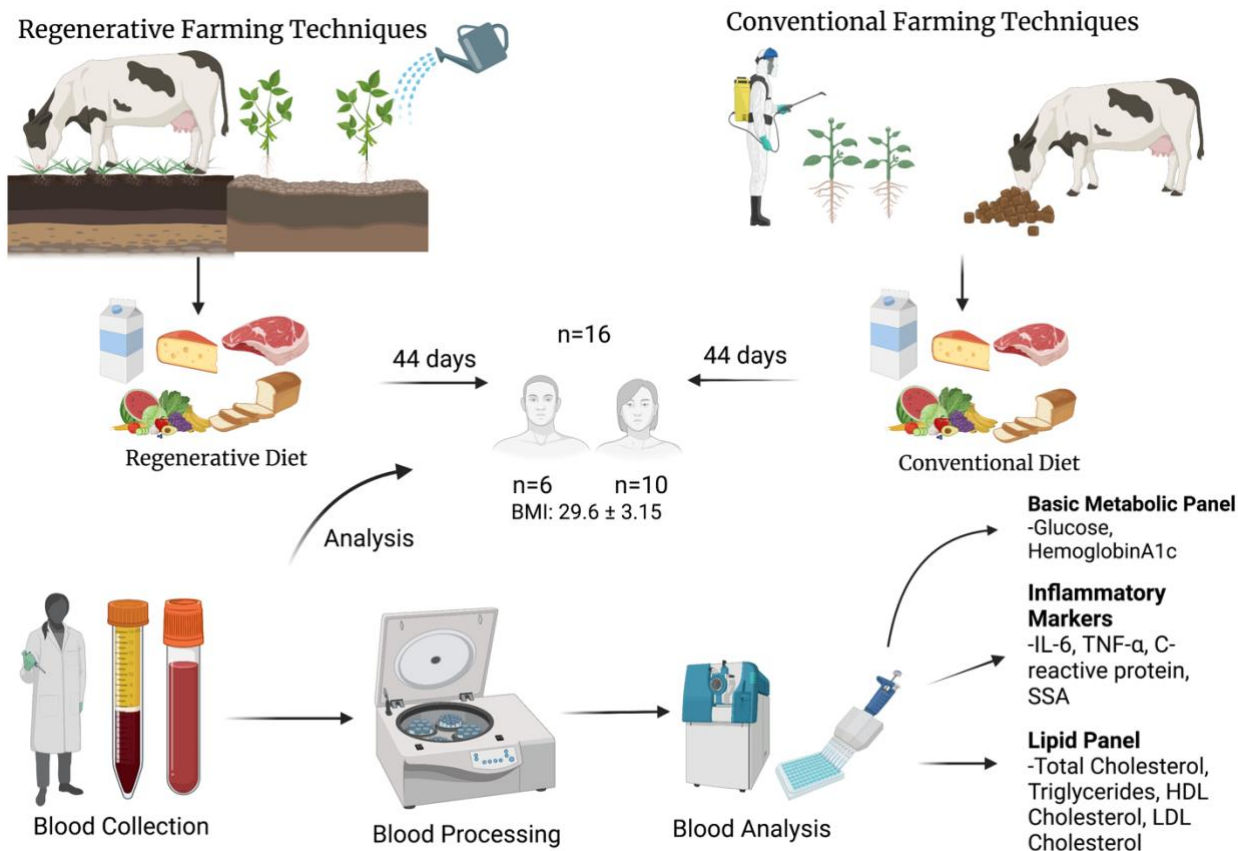


Figure 3: Sixteen participants completed the study. Six participants began on the regenerative diet and ten participants started on the conventional diet. Both groups continued on this diet for forty-four days and then was given a two-week washout and then completed the opposite diet for forty-four days. At five timepoints blood was taken from the participants for analysis.

Statistics

A within-subject crossover design was used for this study. All data is expressed as mean \pm SD's and paired, two-sided t-tests were used to compare the diet groups. Two-sided t-tests were analyzed using the arrays from regenerative and conventional to evaluate an accurate p-value. Significance was declared at $p < 0.05$. All statistical analysis was performed using IBM SPSS Statistics (version 28, Chicago, IL) unless otherwise designated.

RESULTS

Food intake, weight, and blood pressure

Nutrient intakes of both the regenerative and conventional diets are reported in Table 3. Average daily intake of calories, protein, and sodium over the 44-day period were similar between both diets (all $p > 0.05$); however, participants consumed significantly less carbohydrates and fiber in the conventional diet compared to the regenerative diet. It is important to note that these were determined from the label of each individual food and several of the regenerative grain products contained slightly more fiber contributing to the overall carbohydrate intake. To ensure real-world applicability we matched food for gram amounts provided (e.g., 50 grams of oatmeal on each diet) as opposed to matching on nutritional composition exactly. Consumption of calories and specific nutrients were observed in the participants' first diet (either regenerative or conventional) and menus for the second diet were created so specific calorie and food amounts would match the first diet.

Table 3: Average dietary intake during the study period (44 days each)

Nutrients	Regenerative (n=16)	Conventional (n=16)	p-value
Caloric Intake (kcal/d)	1,693 ± 256	1,665 ± 264	0.28
Protein (g/d)	111 ± 78	110 ± 73	0.74
Protein (%/d)	22 ± 1.5%	25 ± 8%	--
Carbohydrates (g/d)	173 ± 30	162 ± 28	0.01**
Carbohydrates (%/d)	40 ± 3.5%	40 ± 2%	--
Sugar (g/d)	54 ± 6	52 ± 9	0.2
Fiber (g/d)	27 ± 4.7	21 ± 4	>0.001**
Fat (g/d)	65 ± 12	63 ± 9	0.23
Fat (%/d)	36 ± 3%	36 ± 3%	--
Saturated Fat (g/d)	29 ± 4	28 ± 4	0.09
Sodium (mg/d)	1229 ± 220	1181 ± 169	0.21

*Data was analyzed with a paired samples t-test. Significantly different ($P < 0.05$).

The macronutrient ratios provided in the study diets differ with the average American. Protein intake in the regenerative and conventional diets made up 22% and 25% of total energy intake, respectively, and is higher than the average American adult consumption (about 15% of total energy intake) (Lieberman et al., 2020).

Table 4: Average blood pressure following each diet

Blood Pressure	Post Regenerative (<i>n</i> =16)	Post Conventional (<i>n</i> =16)	p-value
Systolic (mmHg)	111.75 ± 10.80	115.31 ± 15.62	0.23
Diastolic (mmHg)	71.75 ± 7.46	75.31 ± 11.11	0.14
Weight (kg)	80.84 ± 10.41	79.98 ± 10.62	0.14

*Data was analyzed with a paired samples t-test. Significantly different ($P < 0.05$).

This is explained as the registered dietitian planned for protein intake to be around 20% of total energy intake to fit diet guidelines and to ensure that we could provide enough animal sourced foods to also test their effects on biomarkers of health. Carbohydrate consumption is about 48% of total energy intake in adult Americans (Lieberman et al., 2020) and the participants in our study, on average, consumed about 41% of carbohydrates out of their total energy intake. The planned consumption was 50%, but the participants did not meet this level due to the nutrient-dense diet they were following. Nonetheless, the protein intakes are well within the Acceptable Macronutrient Distribution Range (AMDR) of protein, which is 10-15%. While the carbohydrates are lower than the recommended AMDR of 45-65%, the participants consumed the low levels in their first diets, so the menus were matched same amount of nutrients would be consumed. It was more important for the participants to have consistent eating habits on both diets, rather try to increase levels to meet standards. Blood pressure and weight decreased from the start of both diets but there was no difference between diets seen in Figures 4 and 5.

Blood Pressure

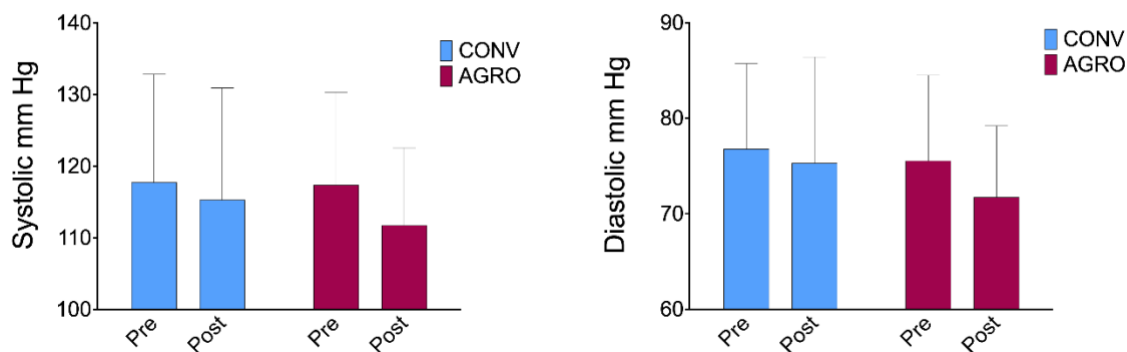


Figure 4: Measured blood pressure before and after each diet period.

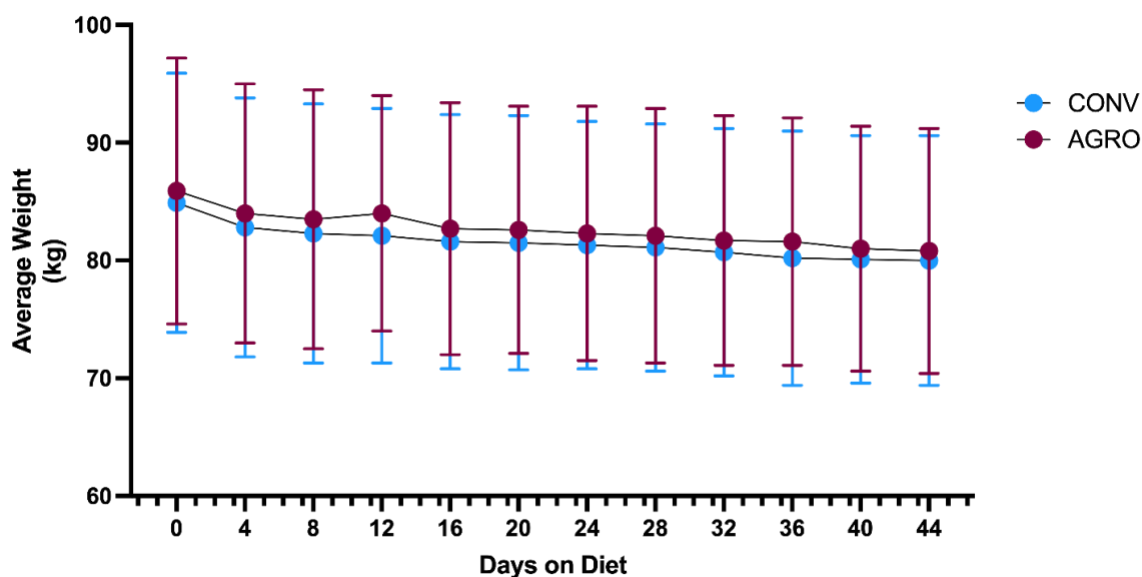


Figure 5: Measured weight (kg) before and after each diet period

Inflammatory and metabolic biomarkers

All inflammatory and metabolic biomarkers are reported in Table 5. There were no statistically significant ($p < 0.05$) differences in the inflammation or metabolic biomarkers tested between the regenerative and conventional diets following each regimen. However, a trend was observed for IL-6 ($p = 0.07$), which trended to be lower in

the regenerative group. There was a statistically significant decrease in blood glucose (mg/dL) following both the conventional (p-value= 0.025) and regenerative diet (p-value = 0.027) as seen in figure 6. This indicates the diet as whole contributed more to the blood glucose level than the farming practice that produced the food.

Table 5: Measured fasted inflammatory biomarkers and metabolic panel post regenerative and conventional diets

Inflammatory Biomarkers & Basic Metabolic Panel	Post Regenerative (n=16)	Post Conventional (n=16)	p-value
IL-6 (pg/mL)	6.8 ± 11.4	13.8 ± 14.0	0.07
SAA (ng/mL)	339 ± 109.2	329.9 ± 139.8	0.42
Glucose (mg/dL)	84.9 ± 7.8	87.1 ± 6.3	0.30
Hemoglobin A1c (%)	5.4 ± 0.2	5.5 ± 0.3	0.59
Blood Urea Nitrogen (mg/dL)	14.3 ± 3.8	14.8 ± 3.6	0.54
Creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.1	1.00
GFR (mL/min)	100.5 ± 21.2	96.7 ± 12.7	0.40
BUN/Creatinine Ratio	16.9 ± 4.2	17.5 ± 4.2	0.53
Sodium (mmol/L)	140.7 ± 2.4	139.0 ± 2.0	0.08
Potassium (mmol/L)	4.4 ± 0.3	4.8 ± 8.9	0.31
Chloride (mmol/L)	104.5 ± 1.7	103.2 ± 3.2	0.12
Calcium (mmol/L)	9.3 ± 0.4	9.2 ± 0.3	0.52
Carbon Dioxide, Total (mmol/L)	23.0 ± 2.1	21.7 ± 5.6	0.44

*Data was analyzed with a paired samples t-test. Significantly different ($P < 0.05$).

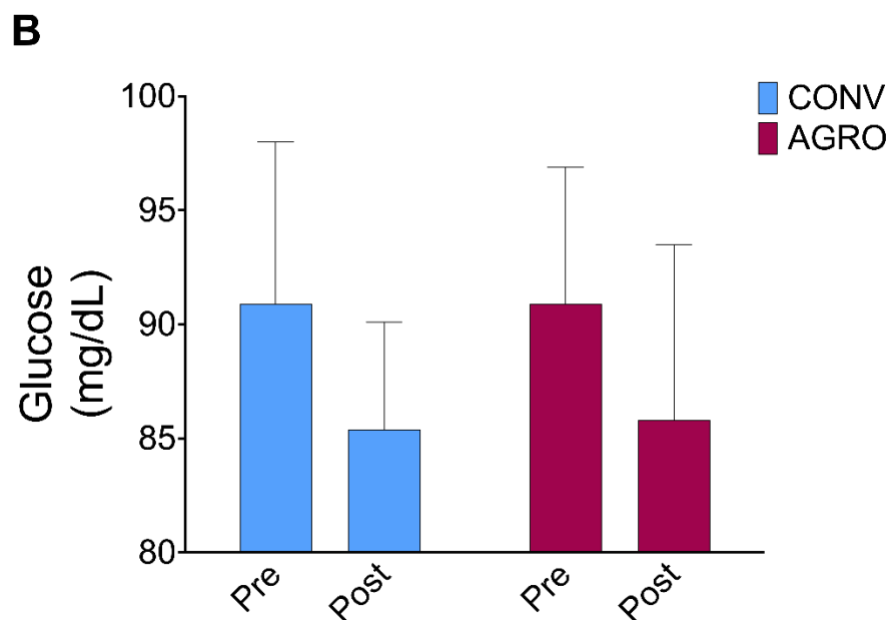


Figure 6: Measured fasted glucose levels before and after each diet period

Lipoprotein Profile

Results of the lipid panel is reported in Table 6. There were no statistically significant ($p < 0.05$) differences in lipid profiles tested between the regenerative and conventional diets following each regimen. There was a significant change in the triglyceride level (mg/dL) after both the agroecological (p -value = 0.032) and conventional (p -value = 0.031) diet. This is another indication that whole foods-based diet may play a larger role in health benefits than farming method.

Table 6: Fasted lipid panel following each diet

Lipid Biomarkers	Post Regenerative (<i>n</i> =16)	Post Conventional (<i>n</i> =16)	p-value
Cholesterol, Total (mg/dL)	181.4 ± 36.7	178.9 ± 40.9	0.60
Triglycerides (mg/dL)	107.9 ± 51.8	105.1 ± 41.2	0.20
HDL Chol (mg/dL)	45.0 ± 10.1	46.1 ± 9.2	0.47
VLDL Chol (mg/dL)	19.9 ± 8.7	19.5 ± 6.7	0.58
LDL Chol (mg/dL)	116.5 ± 33.4	113.3 ± 36.7	0.63

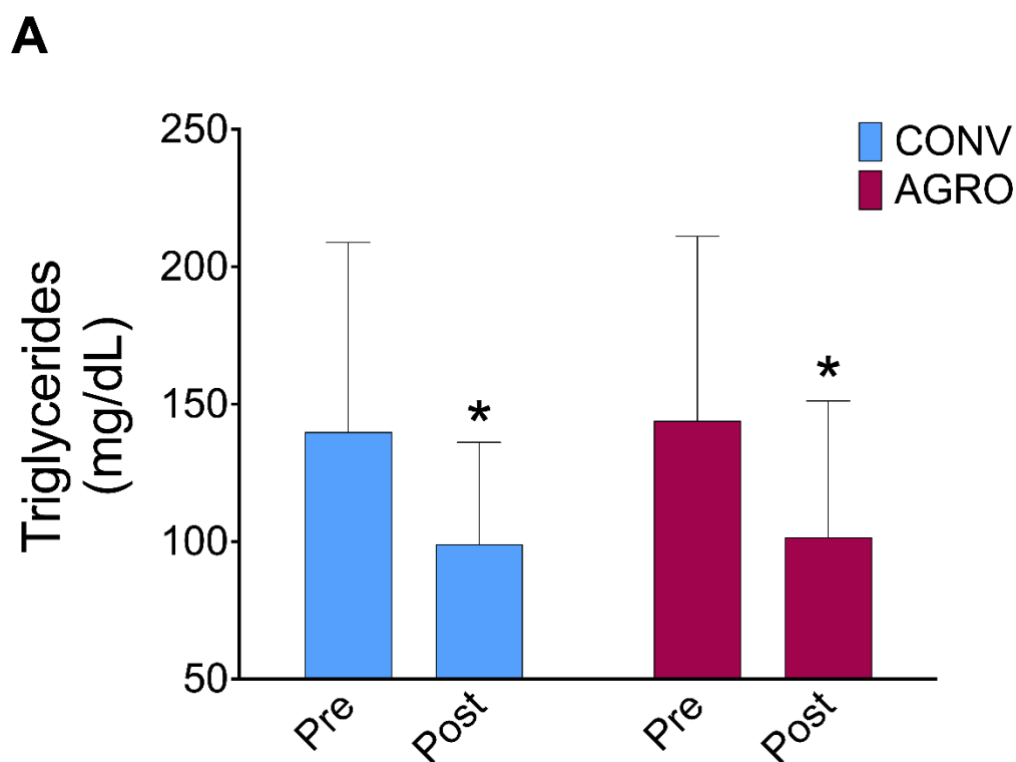


Figure 7: Measured fasted triglyceride levels before and after each diet period

DISCUSSION

The goal of this randomized cross-over trial was to study whether the production methods of similar diets, matched for energy/macronutrient intake and food groups, differentially impacted biomarkers of metabolic health. Participants were randomly assigned to either an agroecological diet or conventional diet for 44 days followed by a 2-week washout. Following the washout, they consumed the alternate diet for another 44 days. All meals and snacks were provided by the research team at an energy level that encouraged weight maintenance; however, given the nutrient-density of the diet (whole food based), most participants were not able to consume that level of energy and caloric intake averaged 1,693 and 1,665 calories on the regenerative and conventional diet, respectively. Diets were matched by food quantity and caloric index to decrease chances of any other influences on the results but were based on given nutrition labels.

Throughout the data, there are no indications that there are any differences between regeneratively-produced and conventionally-made foods on measured inflammatory biomarkers, metabolic and lipid panels, blood pressure, and weight, except for a trend ($p=0.07$) for interleukin-6, suggesting some indication for an anti-inflammatory effect of the regenerative diet. Future work will have to be performed using metabolomics profiling to study whether markers of metabolism and oxidative stress were altered as a result. Nonetheless, the main finding of the study was that the consistent whole-foods diet participants consumed in both diets had positive impacts on their health, specifically on their triglyceride and glucose levels, blood pressure, and weight, which were all decreased.

With this being said, it is likely that the participants were consuming more ultra-processed food prior to the study. It is known that the average American gets 66% of their total calories from ultra-processed foods (Martinez Steele et al., 2016; Juul et al., 2022). The nature of a whole-foods diet is the consumed food is minimally processed and is very nutrient-dense. Additionally, these foods are based on whole grains, meat, dairy, fruit, and vegetables. This means that regardless of the diet they were consuming they were likely ingesting more nutrient-dense foods than they probably were prior to the study and able to eat less while receiving proper nourishment. This was reflected in their caloric intake and health markers. Every participant in the study was unable to maintain their starting caloric intake. There are indications that this is due to the high nutrient content of the study diet compared to their normal diet as participants indicating they could not consume all food provided due to feelings of fullness. Along with caloric intake, there was statistically significant evidence that the participants got healthier overall while following either diet during the study. There are various sources of data supporting this claim. First, as a whole, the participants lost about five kilograms on each diet. Despite creating menus for participants to achieve maintenance weight, the participants could not consume all the food provided for them. Due to this, the second diet they were involved in was calorically and food-matched to their first to ensure they were eating the same foods in the same quantities. The lower carbohydrate percent can be attributed to this as well. In the United States, the average fiber intake is around 16 g/day (Quagliani & Felt-Gunderson, 2016), while the participants consumed about 27g and 21g during the regenerative and conventional diets, respectively.

As noted, the AMDR recommends that 45-65% of total energy consumption comes from carbohydrates, but due to the nutrient-dense foods provided they were unable to achieve this recommendation. Along with weight, participants blood pressure decreased as well. Blood pressure is heavily influenced by weight loss. Studies have indicated that there is a linear association between body weight and blood pressure (Sabaka et al., 2017; Sharabi, 2004; Staessen et al., 1988). On average, 4.5 kg difference in body weight correlates with a 4-mmHg difference in systolic blood pressure, which was also consist in our results (Sabaka et al., 2017). There were also differences seen in the fasted blood panels performed. Specifically, the largest decreases seen throughout the study were in blood glucose and triglycerides. As the participants ate less food and lost body fat, it is likely this had a positive impact on blood glucose. Since triglycerides are the main form of fat in the body, the decrease in this test shows that not only were the participants losing weight, but some of that weight loss was in fat. A decrease in fat in the body will help decrease insulin resistance in the body, which in turn decreases blood glucose (Kong et al., 2020). The insulin will be able to move the glucose into cells more efficiently and reduce the chances of glucose-related disorders, such as diabetes. This means that the reported measurements and blood tests show that overall the participants became healthier after each diet, but there were no significant differences between the two diets.

Inflammatory biomarkers, specifically interleukin-6 (IL-6) and serum amyloid A (SAA), were incorporated in the testing portion of this study. These biomarkers provide indications about the inflammation levels in the body, which is important as inflammation is correlated with many chronic diseases and provide an indication of overall health (Pahwa et al., 2022). IL-6 is a cytokine that works as a signaling agent to

communicate with other regions of the body concerning inflammation and immune responses (Unver & McAllister, 2018). Testing for this cytokine can provide indications concerning overall inflammation levels and may help with the diagnosis of rheumatoid arthritis, inflammatory bowel disease (IBD), type 2 diabetes, cardiovascular disease, and more (Unver & McAllister, 2018). The normal range of IL-6 in healthy individuals is between 0-43.5 pg/mL (Said et al., 2020). While the data did not reach statistical significance, we observed a trend for lower values of IL-6 after the regenerative diet compared to the conventional diet (51% lower). Lower levels of IL-6 may indicate lower risk of chronic metabolic disease in the future if the consumption of foods produced using regenerative practices was maintained (Unver & McAllister, 2018).

Alternatively, the absence of a statistical difference between the two diets, could be the result of the time-frame (7 weeks on each diet). There are conflicting results in studies providing the expected time frame for changes in IL-6 levels, with some studies suggesting 3-4 weeks and others up to six months is necessary to detect changes, which means the study may not have been long enough to detect significant changes (Fabbrini et al., 2015; Miller et al., 2006). This likely depends on the nature of the intervention and how drastic the dietary change is. SAA is another inflammatory biomarker, specifically an acute-phase protein that detects inflammation due to tissue injury or trauma (Husby et al., 1994). The test helps look at the overall quantity and severity of inflammation in the body (Sorić et al., 2021). The normal amount of SAA in healthy humans is under 3 mg/mL and the results of this study are well under that level (Sorić et al., 2021). There was no significant data to suggest there was a difference in this marker throughout the duration of the whole study or between the different diets.

One interesting data point seen in the results was the macronutrient differences between the diets. As previously stated, the diets are matched from the first to second for participants to eat the same foods in the same quantities, only differing in production style. On average, the participants consumed a lot less carbohydrates and fiber on the conventional diet versus the agroecological diet. The regeneratively produced products, specifically fruit, oatmeal, and bread products contained more fiber than the its conventionally-produced counterpart (*Appendix C*). The increase in fiber is likely the reason for the overall increase in carbohydrates in the regenerative data, as the carbohydrates were not calculated as net-carbohydrates, thus the fiber was not excluded. The discrepancy in nutrients may be attributed to inaccurate food labels, indicating incorrect relative macronutrients that can vary as much as 10-15% (Hanacek, 2022). Noteworthy is that the differences in carbohydrates between the two diets are within 15%.

The results of the study indicated that regenerative farming practices may not have benefits to human health, but there was still a lot of beneficial data provided. This was a controlled clinical trial, where all food was properly weighed and distributed to participants. All food was accounted for and consumption of all food was recorded by participants via food logs. The food logs indicated the type and quantity of food ingested. From there, participants were overall compliant and honestly self-reported any non-compliant food consumption. It was also controlled in that all regenerative food was sourced from Greenacres, a trusted farm with known procedures. This study provided a good starting point for the investigation of regenerative farming techniques and its effect on human health.

With this being said, there were some limitations in this study that could be altered for more studies in the future. The entire study was a little under two months long with each diet lasting only forty-four days, but there are indications that it may take longer to see effects in the blood. This means that future studies may include longer diet periods to see if the duration of the diet has impacts evident in blood tests. Along with this, sixteen participants completed this study so it would make the results more significant with a larger sample size, which will be pursued this upcoming year. Additionally, regenerative agriculture includes various farming techniques so it would be beneficial to conduct studies receiving regenerative food from different farms to see if there are differences on health based on different agricultural practices. The produces sourced from Greenacres were seasonal so there was some variation in this study based on the agroecological crops provided to research team. In future studies, having more consistent vegetables may make a difference in results. From there, this study heavily relied on nutritional food labels to provide information about relative macronutrients. Food labels are commonly incorrect so the diets in this study may not have been matched as closely as they could have been if all the food had their macronutrients tested. Future studies should test all macronutrients used in the study to minimize the chance of this error. Lastly, future studies may look to include people with different disease states. This study largely excluded people with chronic diseases, but perhaps regenerative farming may provide them with additional benefits not seen in the generally healthy population.

CONCLUSION

There were minor indications that there are benefits to human health when consuming a regeneratively-produced diet compared to a conventionally-made diet as illustrated by a trend for lower levels of IL-6. There was strong evidence that participants experience health improvements on both diets as illustrated by reductions in glucose and triglycerides, suggesting that a whole foods-based diet may be advantageous to human health. This research contributes to the growing body of literature that suggest that consumption of whole foods should be at the forefront of obesity and chronic disease prevention and possible treatment. This work also acts as a starting point for more research looking into the effects of regenerative agriculture on human health. Future work will include a larger sample size and more in-depth profiling (e.g., metabolomics, gut microbiota profiling, and nutritional assessment testing) to provide more insight into how different farming techniques may or may not impact human metabolic health.

REFERENCES

- Agabriel, C., Cornu, A., Journal, C., Sibra, C., Grolier, P., & Martin, B. (2007). Tanker milk variability according to farm feeding practices: Vitamins A and E, carotenoids, color, and terpenoids. *Journal of Dairy Science*, *90*(10), 4884–4896. <https://doi.org/10.3168/jds.2007-0171>
- Albizua, A., Williams, A., Hedlund, K., & Pascual, U. (2015). Crop rotations including Ley and manure can promote ecosystem services in conventional farming systems. *Applied Soil Ecology*, *95*, 54–61. <https://doi.org/10.1016/j.apsoil.2015.06.003>
- Allothman, M., Hogan, S. A., Hennessy, D., Dillon, P., Kilcawley, K. N., O'Donovan, M., Tobin, J., Fenelon, M. A., & O'Callaghan, T. F. (2019). The “grass-fed” milk story: Understanding the impact of pasture feeding on the composition and quality of Bovine Milk. *Foods*, *8*(8), 350. <https://doi.org/10.3390/foods8080350>
- Apaoblaza, A., Gerrard, S. D., Matarneh, S. K., Wicks, J. C., Kirkpatrick, L., England, E. M., Scheffler, T. L., Duckett, S. K., Shi, H., Silva, S. L., Grant, A. L., & Gerrard, D. E. (2020). Muscle from grass- and grain-fed cattle differs energetically. *Meat Science*, *161*, 107996. <https://doi.org/10.1016/j.meatsci.2019.107996>
- Arya, F., Egger, S., Colquhoun, D., Sullivan, D., Pal, S., & Egger, G. (2010). Differences in postprandial inflammatory responses to a ‘modern’ v. traditional meat meal: A preliminary study. *British Journal of Nutrition*, *104*(5), 724–728. <https://doi.org/10.1017/s0007114510001042>

Bargo, F., Delahoy, J. E., Schroeder, G. F., Baumgard, L. H., & Muller, L. D. (2006).

Supplementing total mixed rations with pasture increase the content of conjugated linoleic acid in milk. *Animal Feed Science and Technology*, *131*(3-4), 226–240.

<https://doi.org/10.1016/j.anifeedsci.2006.04.017>

Bauman, D. E., & Lock, A. L. (2006). Conjugated linoleic acid: Biosynthesis and

nutritional significance. *Advanced Dairy Chemistry*, *3*(2), 93–136.

https://doi.org/10.1007/0-387-28813-9_3

Beard, J. D., Umbach, D. M., Hoppin, J. A., Richards, M., Alavanja, M. C. R., Blair, A.,

Sandler, D. P., & Kamel, F. (2014). Pesticide exposure and depression among male private pesticide applicators in the Agricultural Health Study. *Environmental Health Perspectives*, *122*(9), 984–991. <https://doi.org/10.1289/ehp.1307450>

Health Perspectives, *122*(9), 984–991. <https://doi.org/10.1289/ehp.1307450>

Bell, E. M., Hertz-Picciotto, I., & Beaumont, J. J. (2001). A case-control study of

pesticides and fetal death due to congenital anomalies. *Epidemiology*, *12*(2), 148–

156. <https://doi.org/10.1097/00001648-200103000-00005>

Benbrook, C. M., Davis, D. R., Heins, B. J., Latif, M. A., Leifert, C., Peterman, L.,

Butler, G., Faergeman, O., Abel-Caines, S., & Baranski, M. (2018). Enhancing the fatty acid profile of milk through forage-based rations, with nutrition modeling of

Diet Outcomes. *Food Science & Nutrition*, *6*(3), 681–700.

<https://doi.org/10.1002/fsn3.610>

Bender, S. F., & van der Heijden, M. G. A. (2014). Soil biota enhance agricultural

sustainability by improving crop yield, nutrient uptake and reducing nitrogen

leaching losses. *Journal of Applied Ecology*, 52(1), 228–239.

<https://doi.org/10.1111/1365-2664.12351>

Beseler, C. L., & Stallones, L. (2008). A cohort study of pesticide poisoning and depression in Colorado Farm residents. *Annals of Epidemiology*, 18(10), 768–774.
<https://doi.org/10.1016/j.annepidem.2008.05.004>

Bezner Kerr, R., Madsen, S., Stüber, M., Liebert, J., Enloe, S., Borghino, N., Parros, P., Mutyambai, D. M., Prudhon, M., & Wezel, A. (2021). Can agroecology improve food security and nutrition? A Review. *Global Food Security*, 29, 100540.
<https://doi.org/10.1016/j.gfs.2021.100540>

Bouis, H. E., & Saltzman, A. (2017). Improving nutrition through biofortification: A review of evidence from harvestplus, 2003 through 2016. *Global Food Security*, 12, 49–58. <https://doi.org/10.1016/j.gfs.2017.01.009>

Carrillo, J. A., He, Y., Li, Y., Liu, J., Erdman, R. A., Sonstegard, T. S., & Song, J. (2016). Integrated metabolomic and transcriptome analyses reveal finishing forage affects metabolic pathways related to beef quality and animal welfare. *Scientific Reports*, 6(1). <https://doi.org/10.1038/srep25948>

Chai, Q., Nemecek, T., Liang, C., Zhao, C., Yu, A., Coulter, J. A., Wang, Y., Hu, F., Wang, L., Siddique, K. H., & Gan, Y. (2021). Integrated farming with intercropping increases food production while reducing environmental footprint. *Proceedings of the National Academy of Sciences*, 118(38).
<https://doi.org/10.1073/pnas.2106382118>

- Che, B. N., Kristensen, T., Nebel, C., Dalsgaard, T. K., Hellgren, L. I., Young, J. F., & Larsen, M. K. (2012). Content and distribution of phytanic acid diastereomers in organic milk as affected by feed composition. *Journal of Agricultural and Food Chemistry*, *61*(1), 225–230. <https://doi.org/10.1021/jf304079r>
- Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., & Doreau, M. (2007). Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology*, *109*(8), 828–855. <https://doi.org/10.1002/ejlt.200700080>
- Clark, A. (2015). *Cover Crops for Sustainable Crop Rotations*. Sustainable Agriculture Research and Education.
- Clark, M., Springmann, M., Rayner, M., Scarborough, P., Hill, J., Tilman, D., Macdiarmid, J. I., Fanzo, J., Bandy, L., & Harrington, R. A. (2022). Estimating the environmental impacts of 57,000 food products. *Proceedings of the National Academy of Sciences*, *119*(33). <https://doi.org/10.1073/pnas.2120584119>
- Coppa, M., Martin, B., Pradel, P., Leotta, B., Priolo, A., & Vasta, V. (2011). Effect of a hay-based diet or different upland grazing systems on milk volatile compounds. *Journal of Agricultural and Food Chemistry*, *59*(9), 4947–4954. <https://doi.org/10.1021/jf2005782>
- Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal*, *9*(1). <https://doi.org/10.1186/1475-2891-9-10>

- Damgaard, I. N., Skakkebaek, N. E., Toppari, J., Virtanen, H. E., Shen, H., Schramm, K.-W., Petersen, J. H., Jensen, T. K., & Main, K. M. (2006). Persistent pesticides in human breast milk and cryptorchidism. *Environmental Health Perspectives*, *114*(7), 1133–1138. <https://doi.org/10.1289/ehp.8741>
- Davis, D. R., Epp, M. D., & Riordan, H. D. (2004). Changes in USDA food composition data for 43 Garden Crops, 1950 to 1999. *Journal of the American College of Nutrition*, *23*(6), 669–682. <https://doi.org/10.1080/07315724.2004.10719409>
- Davis, H., Magistrali, A., Butler, G., & Stergiadis, S. (2022). Nutritional benefits from fatty acids in organic and grass-fed beef. *Foods*, *11*(5), 646. <https://doi.org/10.3390/foods11050646>
- Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. 2013. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling* *18*(14):1818–1892
- De Moura, F. F., Miloff, A., & Boy, E. (2013). Retention of provitamin A carotenoids in staple crops targeted for Biofortification in Africa: Cassava, maize and sweet potato. *Critical Reviews in Food Science and Nutrition*, *55*(9), 1246–1269. <https://doi.org/10.1080/10408398.2012.724477>
- Descalzo, A., Insani, E., Biolatto, A., Sancho, A., García, P., Pensel, N., & Josifovich, J. (2005, May). Influence of pasture or grain-based diets supplemented with vitamin

E on antioxidant/oxidative balance of Argentine beef. *Meat Science*, 70(1), 35–44.
<https://doi.org/10.1016/j.meatsci.2004.11.018>

Duchene, O., Vian, J.-F., & Celette, F. (2017). Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A Review. *Agriculture, Ecosystems & Environment*, 240, 148–161. <https://doi.org/10.1016/j.agee.2017.02.019>

Duckett, S. K., Neel, J. P., Fontenot, J. P., & Clapham, W. M. (2009). Effects of winter stocker growth rate and finishing system on: III. tissue proximate, fatty acid, vitamin, and cholesterol content¹. *Journal of Animal Science*, 87(9), 2961–2970.
<https://doi.org/10.2527/jas.2009-1850>

Duckett, S. K., Wagner, D. G., Yates, L. D., Dolezal, H. G., & May, S. G. (1993). Effects of time on feed on beef nutrient composition. *Journal of Animal Science*, 71(8), 2079–2088. <https://doi.org/10.2527/1993.7182079x>

Elliott, S. (2022). *ARS scientist highlights till vs. no-till farming*. Agricultural Research Service.

Fabbrini, E., Yoshino, J., Yoshino, M., Magkos, F., Tiemann Luecking, C., Samovski, D., Fraterrigo, G., Okunade, A. L., Patterson, B. W., & Klein, S. (2015). Metabolically normal obese people are protected from adverse effects following weight gain. *Journal of Clinical Investigation*, 125(2), 787–795.
<https://doi.org/10.1172/jci78425>

- Farahat, T. M. (2003). Neurobehavioural effects among workers occupationally exposed to organophosphorous pesticides. *Occupational and Environmental Medicine*, 60(4), 279–286. <https://doi.org/10.1136/oem.60.4.279>
- Feng, C., Yi, Z., Qian, W., Liu, H., & Jiang, X. (2022). Rotations improve the diversity of rhizosphere soil bacterial communities, enzyme activities and tomato yield. <https://doi.org/10.1101/2022.06.22.497151>
- Fenster, T. L., Oikawa, P. Y., & Lundgren, J. G. (2021). Regenerative almond production systems improve soil health, biodiversity, and Profit. *Frontiers in Sustainable Food Systems*, 5. <https://doi.org/10.3389/fsufs.2021.664359>
- Fraç, M., Hannula, E. S., Belka, M., Salles, J. F., & Jedryczka, M. (2022). Soil mycobiome in Sustainable Agriculture. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.1033824>
- Garcia, P. T., Pensel, N. A., Sancho, A. M., Latimori, N. J., Kloster, A. M., Amigone, M. A., & Casal, J. J. (2008). Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Science*, 79(3), 500–508. <https://doi.org/10.1016/j.meatsci.2007.10.019>
- Geisen, S. (2021). The future of (soil) microbiome studies: Current limitations, integration, and perspectives. *MSystems*, 6(4). <https://doi.org/10.1128/msystems.00613-21>

- Grandjean, P., & Landrigan, P. J. (2014). Neurobehavioural effects of developmental toxicity. *The Lancet Neurology*, *13*(3), 330–338. [https://doi.org/10.1016/s1474-4422\(13\)70278-3](https://doi.org/10.1016/s1474-4422(13)70278-3)
- Gunier, R. B., Bradman, A., Harley, K. G., Kogut, K., & Eskenazi, B. (2017). Prenatal residential proximity to agricultural pesticide use and IQ in 7-year-old children. *Environmental Health Perspectives*, *125*(5), 057002. <https://doi.org/10.1289/ehp504>
- Han, X., Ding, S., Lu, J., & Li, Y. (2022). Global, regional, and national burdens of common micronutrient deficiencies from 1990 to 2019: A secondary trend analysis based on the global burden of disease 2019 study. *EClinicalMedicine*, *44*, 101299. <https://doi.org/10.1016/j.eclinm.2022.101299>
- Hahn, D., Shin, S. H., & Bae, J.-S. (2020). Natural antioxidant and anti-inflammatory compounds in foodstuff or medicinal herbs inducing heme oxygenase-1 expression. *Antioxidants*, *9*(12), 1191. <https://doi.org/10.3390/antiox9121191>
- Hepperly, P. R., Omondi, E., & Seidel, R. (2018). Soil regeneration increases crop nutrients, antioxidants and adaptive responses. *MOJ Food Processing & Technology*, *6*(2). <https://doi.org/10.15406/mojfpt.2018.06.00165>
- Hibbeln, J. R. (1998). Fish consumption and major depression. *The Lancet*, *351*(9110), 1213. [https://doi.org/10.1016/s0140-6736\(05\)79168-6](https://doi.org/10.1016/s0140-6736(05)79168-6)

- Horton, M. K., Rundle, A., Camann, D. E., Boyd Barr, D., Rauh, V. A., & Whyatt, R. M. (2011). Impact of prenatal exposure to piperonyl butoxide and permethrin on 36-month neurodevelopment. *Pediatrics*, *127*(3). <https://doi.org/10.1542/peds.2010-0133>
- Hu, F., Gan, Y., Chai, Q., Feng, F., Zhao, C., Yu, A., Mu, Y., & Zhang, Y. (2016). Boosting system productivity through the improved coordination of interspecific competition in maize/pea strip intercropping. *Field Crops Research*, *198*, 50–60. <https://doi.org/10.1016/j.fcr.2016.08.022>
- Husby, G., Marhaug, G., Dowtor, B., Sletten, K., & Sipe, J. D. (1994). Serum amyloid A (SAA): Biochemistry, genetics and the pathogenesis of AA amyloidosis. *Amyloid*, *1*(2), 119–137. <https://doi.org/10.3109/13506129409148635>
- John, D. A., & Babu, G. R. (2021). Lessons from the aftermaths of Green Revolution on food system and health. *Frontiers in Sustainable Food Systems*, *5*. <https://doi.org/10.3389/fsufs.2021.644559>
- Juul, F., Parekh, N., Martinez-Steele, E., Monteiro, C.A., and Chang, V.W. (2022). Ultra-processed food consumption among US adults from 2001 to 2018. *Am J Clin Nutr* *115*, 211-221. [10.1093/ajcn/nqab305](https://doi.org/10.1093/ajcn/nqab305)
- Karas, S. (2023). *Could Regenerative Agriculture Increase the Nutritional Quality of Our Food?* Center for Regenerative Agriculture and Resilient Systems – Chico State.

- Kong, D.-X., Xiao, Y.-xin, Zhang, Z.-X., & Liu, Y.-B. (2020). Study on the correlation between metabolism, insulin sensitivity and progressive weight loss change in type-2 diabetes. *Pakistan Journal of Medical Sciences*, 36(7).
<https://doi.org/10.12669/pjms.36.7.3027>
- Kritchevsky, D., Tepper, S. A., Wright, S., Tso, P., & Czarnecki, S. K. (2000). Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. *Journal of the American College of Nutrition*, 19(4).
<https://doi.org/10.1080/07315724.2000.10718950>
- Lambert, D. H., Baker, D. E., & Cole, H. (1979). The role of mycorrhizae in the interactions of phosphorus with zinc, copper, and other elements. *Soil Science Society of America Journal*, 43(5), 976–980.
<https://doi.org/10.2136/sssaj1979.03615995004300050033x>
- Larick, D. K., Hedrick, H. B., Bailey, M. E., Williams, J. E., Hancock, D. L., Garner, G. B., & Morrow, R. E. (1987). Flavor constituents of beef as influenced by forage- and grain-feeding. *Journal of Food Science*, 52(2), 245–251.
<https://doi.org/10.1111/j.1365-2621.1987.tb06585.x>
- Laugharne, J., Mellor, J., & Peet, M. (1996). Fatty acids and Schizophrenia. *Lipids*, 31(1), S163–S165. <https://doi.org/10.1007/BF02637070>
- Liebman, M., & Dyck, E. (1993). Crop rotation and intercropping strategies for weed management. *Ecological Applications*, 3(1), 92–122.
<https://doi.org/10.2307/1941795>

- Lieberman, H. R., Fulgoni, V. L., Agarwal, S., Pasiakos, S. M., & Berryman, C. E. (2020). Protein intake is more stable than carbohydrate or fat intake across various US demographic groups and international populations. *The American Journal of Clinical Nutrition*, *112*(1), 180–186. <https://doi.org/10.1093/ajcn/nqaa044>
- Liu, S., Zhang, R., Kang, R., Meng, J., & Ao, C. (2016). Milk fatty acids profiles and milk production from dairy cows fed different forage quality diets. *Animal Nutrition*, *2*(4), 329–333. <https://doi.org/10.1016/j.aninu.2016.08.008>
- Lucas, A., Rock, E., Chamba, J.-F., Verdier-Metz, I., Brachet, P., & Coulon, J.-B. (2005). Respective effects of milk composition and the cheese-making process on cheese compositional variability in components of nutritional interest. *Le Lait*, *86*(1), 21–41. <https://doi.org/10.1051/lait:2005042>
- Marschner, H., & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil*, *159*(1), 89–102. <https://doi.org/10.1007/bf00000098>
- Martínez Steele, E., Baraldi, L.G., Louzada, M.L.D.C., Moubarac, J.-C., Mozaffarian, D., and Monteiro, C.A. (2016). Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study. *BMJ Open* *6*, e009892. [10.1136/bmjopen-2015-009892](https://doi.org/10.1136/bmjopen-2015-009892)
- Mason, R. P., Libby, P., & Bhatt, D. L. (2020). Emerging mechanisms of cardiovascular protection for the omega-3 fatty acid eicosapentaenoic acid. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *40*(5), 1135–1147. <https://doi.org/10.1161/atvbaha.119.313286>

- Mayer, A. M. (1997). Historical changes in the mineral content of fruits and vegetables. *British Food Journal*, 99(6), 207–211. <https://doi.org/10.1108/00070709710181540>
- McGuire, S. (2015). FAO, IFAD, and WFP. the state of food insecurity in the World 2015: Meeting the 2015 international hunger targets: Taking stock of uneven progress. rome: FAO, 2015. *Advances in Nutrition*, 6(5), 623–624. <https://doi.org/10.3945/an.115.009936>
- Miller, G. D., Nicklas, B. J., Davis, C., Loeser, R. F., Lenchik, L., & Messier, S. P. (2006). Intensive weight loss program improves physical function in older obese adults with knee osteoarthritis*. *Obesity*, 14(7), 1219–1230. <https://doi.org/10.1038/oby.2006.139>
- Montgomery, D. R., & Biklé, A. (2022). *What your food ate: How to restore our land and reclaim our health*. W W NORTON.
- Montgomery, D. R., Biklé, A., Archuleta, R., Brown, P., & Jordan, J. (2022). Soil Health and nutrient density: Preliminary comparison of regenerative and conventional farming. *PeerJ*, 10. <https://doi.org/10.7717/peerj.12848>
- Munger, R., Isacson, P., Hu, S., Burns, T., Hanson, J., Lynch, C. F., Cherryholmes, K., Van Dorpe, P., & Hausler, W. J. (1997). Intrauterine growth retardation in Iowa communities with herbicide-contaminated drinking water supplies. *Environmental Health Perspectives*, 105(3), 308–314. <https://doi.org/10.1289/ehp.97105308>

- Nabel, M., Schrey, S. D., Temperton, V. M., Harrison, L., & Jablonowski, N. D. (2018). Legume intercropping with the bioenergy crop *Sida Hermaphrodita* on marginal soil. *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/fpls.2018.00905>
- Nisoli, E., Clementi, E., Carruba, M. O., & Moncada, S. (2007). Defective mitochondrial biogenesis. *Circulation Research*, 100(6), 795–806. <https://doi.org/10.1161/01.res.0000259591.97107.6c>
- Nogoy, K. M., Sun, B., Shin, S., Lee, Y., Zi Li, X., Choi, S. H., & Park, S. (2022). Fatty acid composition of grain- and grass-fed beef and their nutritional value and health implication. *Food Science of Animal Resources*, 42(1), 18–33. <https://doi.org/10.5851/kosfa.2021.e73>
- O’Callaghan, T. F., Faulkner, H., McAuliffe, S., O’Sullivan, M. G., Hennessy, D., Dillon, P., Kilcawley, K. N., Stanton, C., & Ross, R. P. (2016). Quality characteristics, chemical composition, and sensory properties of butter from cows on pasture versus indoor feeding systems. *Journal of Dairy Science*, 99(12), 9441–9460. <https://doi.org/10.3168/jds.2016-11271>
- Pariza, M. W., Park, Y., & Cook, M. E. (2000). Mechanisms of action of conjugated linoleic acid: Evidence and speculation. *Proceedings of the Society for Experimental Biology and Medicine*, 223(1), 8–13. <https://doi.org/10.1046/j.1525-1373.2000.22302.x>
- Pilling, D., Bélanger, J., and Hoffmann, I. (2020). Declining biodiversity for food and agriculture needs urgent global action. *Nature Food* 1, 144-147.

Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M.

Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (2022). *Climate Change 2022: Impacts, Adaptation, and Vulnerability*. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp.,
doi:10.1017/9781009325844.

Prache, S., Cornu, A., Berdagué, J. L., & Priolo, A. (2005). Traceability of animal feeding diet in the meat and milk of small ruminants. *Small Ruminant Research*, 59(2-3), 157–168. <https://doi.org/10.1016/j.smallrumres.2005.05.004>

Provenza, F. D., Kronberg, S. L., & Gregorini, P. (2019). Is grassfed meat and dairy better for human and environmental health? *Frontiers in Nutrition*, 6.
<https://doi.org/10.3389/fnut.2019.00026>

Quagliani, & Felt-Gunderson. (2016, July 7). *Closing America's Fiber Intake Gap: Communication Strategies from a food and Fiber Summit*. American journal of lifestyle medicine. Retrieved April 24, 2023, from
<https://pubmed.ncbi.nlm.nih.gov/30202317/>

Rana, A., Joshi, M., Prasanna, R., Shivay, Y. S., & Nain, L. (2012). Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and

cyanobacteria. *European Journal of Soil Biology*, 50, 118–126.

<https://doi.org/10.1016/j.ejsobi.2012.01.005>

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D., & Whyatt, R.

(2015). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environmental Hazards and*

Neurodevelopment, 77–96. <https://doi.org/10.1201/b18030-6>

Ryan, M. H., McInerney, J. K., Record, I. R., & Angus, J. F. (2008). Zinc bioavailability

in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *Journal of the Science of Food and Agriculture*, 88(7), 1208–1216.

<https://doi.org/10.1002/jsfa.3200>

Sabaka, P., Dukat, A., Gajdosik, J., Bendzala, M., Caprnda, M., & Simko, F. (2017). The

effects of body weight loss and gain on arterial hypertension control: An observational prospective study. *European Journal of Medical Research*, 22(1).

<https://doi.org/10.1186/s40001-017-0286-5>

Said, E. A., Al-Reesi, I., Al-Shizawi, N., Jaju, S., Al-Balushi, M. S., Koh, C. Y., Al-

Jabri, A. A., & Jeyaseelan, L. (2020). Defining IL-6 levels in healthy individuals: A meta-analysis. *Journal of Medical Virology*, 93(6), 3915–3924.

<https://doi.org/10.1002/jmv.26654>

Salvi, R. M. (2003). Neuropsychiatric evaluation in subjects chronically exposed to

organophosphate pesticides. *Toxicological Sciences*, 72(2), 267–271.

<https://doi.org/10.1093/toxsci/kfg034>

- Scott, L. W. (1994, June 13). Effects of beef and chicken consumption on plasma lipid levels in hypercholesterolemic men. *Archives of Internal Medicine*, *154*(11), 1261–1267. <https://doi.org/10.1001/archinte.154.11.1261>
- Sharabi, Y. (2004). Susceptibility of the influence of weight on blood pressure in men versus women lessons from a large-scale study of Young Adults. *American Journal of Hypertension*, *17*(5), 404–408. <https://doi.org/10.1016/j.amjhyper.2003.12.012>
- Simopoulos, A. (2016). An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*, *8*(3), 128. <https://doi.org/10.3390/nu8030128>
- Simopoulos, A. P. (2020). Omega-3 fatty acids in growth and development. *Omega-3 Fatty Acids in Health and Disease*, 115–156. <https://doi.org/10.1201/9781003066453-6>
- Sorić Hosman, I., Kos, I., & Lamot, L. (2021). Serum amyloid A in inflammatory rheumatic diseases: A compendious review of a renowned biomarker. *Frontiers in Immunology*, *11*. <https://doi.org/10.3389/fimmu.2020.631299>
- Staessen, J., Fagard, R., & Amery, A. (1988). The relationship between body weight and blood pressure. *National Library of Medicine*, *2*, 207–217.
- Steinhart, H., Rickert, R., & Winkler, K. (2003). Identification and analysis of conjugated linoleic acid isomers (CLA). *National Library of Medicine*, *8*(8), 370–372.

- Stoll, A. L., Locke, C. A., Marangell, L. B., & Severus, W. E. (1999). Omega-3 fatty acids and bipolar disorder: A Review. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 60(5-6), 329–337. [https://doi.org/10.1016/s0952-3278\(99\)80008-8](https://doi.org/10.1016/s0952-3278(99)80008-8)
- Sun, J., Li, W., Li, C., Chang, W., Zhang, S., Zeng, Y., Zeng, C., & Peng, M. (2020). Effect of different rates of nitrogen fertilization on crop yield, soil properties and leaf physiological attributes in banana under subtropical regions of China. *Frontiers in Plant Science*, 11. <https://doi.org/10.3389/fpls.2020.613760>
- Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Advances in Nutrition*, 3(1), 1–7. <https://doi.org/10.3945/an.111.000893>
- Thomas, D. (2007). The mineral depletion of foods available to us as a nation (1940–2002) – a review of the 6th edition of McCance and Widdowson. *Nutrition and Health*, 19(1-2), 21–55. <https://doi.org/10.1177/026010600701900205>
- van Vliet, S., Provenza, F. D., & Kronberg, S. L. (2021). Health-promoting phytonutrients are higher in grass-fed meat and milk. *Frontiers in Sustainable Food Systems*, 4. <https://doi.org/10.3389/fsufs.2020.555426>
- van Zutphen, K. G., van den Berg, S., Gavin-Smith, B., Imbo, E., Kraemer, K., Monroy-Gomez, J., Pannatier, M., Prytherch, H., Six, J., Thoennissen, C., Winter, S., & Barjolle, D. (2022). Nutrition as a driver and outcome of agroecology. *Nature Food*, 3(12), 990–996. <https://doi.org/10.1038/s43016-022-00631-7>

Verbruggen, E., Kiers, E. T., Bakelaar, P. N., Rölting, W. F., & van der Heijden, M. G.

(2011). Provision of contrasting ecosystem services by soil communities from different agricultural fields. *Plant and Soil*, 350(1-2), 43–55.

<https://doi.org/10.1007/s11104-011-0828-5>

Villeneuve, M.-P., Lebeuf, Y., Gervais, R., Tremblay, G. F., Vuillemard, J. C., Fortin, J.,

& Chouinard, P. Y. (2013). Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. *Journal of Dairy Science*, 96(11),

7181–7194. <https://doi.org/10.3168/jds.2013-6785>

von Ehrenstein, O. S., Ling, C., Cui, X., Cockburn, M., Park, A. S., Yu, F., Wu, J., &

Ritz, B. (2019). Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-control study. *BMJ*, 1962.

<https://doi.org/10.1136/bmj.1962>

Wagner, S. E., Jin, V., & Schmer, M. (2021, October 25). *More Diverse Crop Rotations*

Improve Yield, Yield Stability and Soil Health. Nebraska Institute of Agriculture and Natural Resources. Retrieved March 20, 2023, from

<https://cropwatch.unl.edu/2021/more-diverse-crop-rotations-improve-yield-yield-stability-and-soil-health>

Wall, D.H., Nielsen, U.N., and Six, J. (2015). Soil biodiversity and human health. *Nature*

528, 69-76 Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P,

Kwun I, Shen C-L. 2014. Novel insights of dietary polyphenols and obesity.

Journal of Nutritional Biochemistry 25(1):1–18 DOI
10.1016/j.jnutbio.2013.09.001.

Weisburger, J. H. (1991). Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants, and carotenoids. *The American Journal of Clinical Nutrition*, 53(1). <https://doi.org/10.1093/ajcn/53.1.226s>

Wezel, A., Herren, B. G., Kerr, R. B., Barrios, E., Gonçalves, A. L., & Sinclair, F. (2020). Agroecological principles and elements and their implications for transitioning to sustainable food systems. A Review. *Agronomy for Sustainable Development*, 40(6). <https://doi.org/10.1007/s13593-020-00646-z>

White, P. J., & Broadley, M. R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist*, 182(1), 49–84.
<https://doi.org/10.1111/j.1469-8137.2008.02738.x>

Wood, J. D., & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving Meat Quality. *British Journal of Nutrition*, 78(1).
<https://doi.org/10.1079/bjn19970134>

Yang, A., Larsen, T. W., & Tume, R. K. (1992). Carotenoid and retinol concentrations in serum, adipose tissue and liver and carotenoid transport in sheep, goats and cattle. *Australian Journal of Agricultural Research*, 43(8), 1809.
<https://doi.org/10.1071/ar9921809>

Yehuda, S., Rabinovitz, S., Carasso, R. L., & Mostofsky, D. I. (1996). Essential fatty acids preparation (SR-3) improves alzheimer's patients quality of life. *International Journal of Neuroscience*, 87(3-4), 141–149.

<https://doi.org/10.3109/00207459609070833>

Zanotti I, Dall'Asta M, Mena P, Mele L, Bruni R, Ray S, Del Rio D. 2015.

Atheroprotective effects of (poly)phenols: a focus on cell cholesterol metabolism. *Food & Function* 6:13–31 DOI 10.1039/C4FO00670D.

Zhang, Y.-Q., Deng, Y., Chen, R.-Y., Cui, Z.-L., Chen, X.-P., Yost, R., Zhang, F.-S., & Zou, C.-Q. (2012). The reduction in zinc concentration of wheat grain upon increased phosphorus-fertilization and its mitigation by Foliar Zinc Application. *Plant and Soil*, 361(1-2), 143–152. <https://doi.org/10.1007/s11104-012-1238-z>

APPENDICES

Appendix A: Inclusion & Exclusion Criteria

Inclusion Criteria

- Age ≥ 35 and ≤ 60 years
- BMI ≥ 25 and ≤ 35 kg/m²
- Stable Weight in last 3 months (loss or gain <4%)
- Fasting plasma glucose concentration <126 mg/dl
- Hemoglobin A1C (HbA1C $\leq 6.4\%$)
- Speak and Understand English
- Stable medication/supplement use for 3 months prior to study

Exclusion Criteria

- Use of medications that are known to affect the study outcome measures (e.g. NSAIDs, corticosteroids) or increase the risk of study procedures (e.g. anticoagulants) that cannot be temporarily discontinued for this study
- Strict dietary patterns (e.g., vegan, keto)
- Consuming >14 alcoholic drinks per week
- Use of cigarettes (or other tobacco products) in last 3 months
- Engaged in high level of competitive exercise (e.g., iron man, marathons, powerlifting)
- Diagnoses of active malignancy, congestive heart failure, diabetes mellitus or chronic obstructive pulmonary disease
- Any inflammatory diseases (e.g., autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease, arthritis)
- Use of antibiotics in last 60 days
- Pregnant or planning to become pregnant in the next 5 months or lactating women
- Persons who are unable or unwilling to follow the study protocol or who, for any reason, the research team considers not an appropriate candidate for this study, including non-compliance with screening appointments or study visits

Appendix B: Human ELISA Kit Protocol

Ab100635 Human SAA ELISA Kit Protocol

All reagents were prepared according to kit instructions. Standards were created by adding 500 μL of Assay Diluent C into 300 ng/mL of stock standard. A dilution sequence was performed by moving 200 μL of the standard + Diluent C solution as illustrated in the kit instructions. The samples were diluted by adding 75 μL of Diluent C in 25 μL of sample. The standard solutions (1-8) were plated in duplicates in the first two columns of the plate. All materials and prepared reagents were equilibrated to room temperature. The samples were also plated in duplicates. 100 μL of standard and samples were pipetted into their appropriate wells. After plating, the wells were incubated at room temperature and shaken at 400 rpm. The solutions were discarded and washed 4x with 300 μL using a multi-channel pipette. Each All wash buffer was discarded between each wash and then the plate was inverted and blotted it against clean paper towels. 100 μL of Biotinylated SAA Detection Antibody was added to each well. After this step, the wells were incubated for an hour at room temperature with gentle shaking. After the hour, the solution was discarded and the wash routine was repeating. 100 μL of HRP-Streptavidin solution was added to each well and was incubated for 45 minutes at room temperature with gentle shaking. The solution was then discarded and the wash step procedure was performed. 100 μL of TMB one-step substrate reagent was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50 μL of stop solution was added to each well and then read at 600 nm.

Ab178013 Human IL-6 ELISA Kit Protocol

All reagents were prepared according to kit instructions. Standards were created by adding the indicated value of Sample Diluent NS from the bottle. A dilution sequence was performed by moving 150 μL of the standard + Sample Diluent NS solution as illustrated in the kit instructions. 50 μL of all sample and standard solutions were added to the appropriate wells. 50 μL of pre-made antibody cocktail was added to each well. The plate was sealed and incubated for an hour at room temperature on a plate shaker at

400 rpm. Each well was washed 3x with 350 μL of wash buffer. After the last wash the plate was inverted and blotted against clean paper towels to remove excess liquid. 100 μL of TMB Development Solution was added to each well and incubated for 10 minutes in the dark on a plate shaker at 400 rpm. After the 10 minutes, 100 μL of Stop Solution was added to each well. The plate was gently shaking at 400 rpm at room temperature for a minute. The plate was read at 600 nm.

