INFLUENCE OF COTTONSEED OIL

ON FAT OXIDATION IN STEADY STATE EXERCISE

by

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DEDICATION

This thesis is dedicated to everyone who has mentored, supported, and guided me along the way. To my incredible lab mates, your sheer drive and dedication have been truly inspiring. Working alongside you has given me new perspectives and enriched my understanding of our field. The countless hours we've spent together, whether troubleshooting experiments or celebrating successes, have made this journey both productive and memorable.

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With deepest gratitude,

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ABSTRACT

Impaired fat oxidation is a common issue in individuals with chronic disease such as obesity and cardiovascular disease. Nutrition interventions aimed at increasing fat oxidation capacity could potentially have clinical relevance in delaying the progression and/or preventing chronic disease. Dihydrosterculic acid (DHSA), a bioactive component in cottonseed oil (CSO), has previously been seen to increase fat oxidation in mice. However, it is unknown the effect of CSO on fat oxidation in humans. The purpose was to determine the impact of a diet rich in either cottonseed oil (CSO) or olive oil (OO), consumed at dosages of 60g or 30g per day for a duration of 28 days, on fat oxidation during a 30-minute steady-state exercise bout in a fasted state. **Methods:** Healthy adults (n=47, BMI<27 kg/m^2), aged 18 to 55, engaged in a 30-minute exercise test performed at 75% of relative VO2 max, pre- and post-dietary intervention. Over 28 days, participants were randomized to receive daily doses of 30g/day or 60g/day of either CSO or olive oil (OO). Using indirect calorimetry at 10-minute intervals during the exercise tests, fat oxidation in kilocalories per minute was calculated and summarized as area under the curve (AUC) at each time point. The shift in fat burning between pre- and post-intervention tests, were then computed. ANOVA was used to find differences between groups. **Results:** There were no significant changes in fat kcal/22 mins between all groups (p=0.43). Combining both CSO dosage groups revealed a trend increase in fat kcal/22 mins compared to OO (CSO: 5.89 ± 4.89 kcals/22 mins, OO: -7.71 ± 7.32 kcals/22 mins p=0.13). No differences were found between 30g and 60g dosages (p=0.51). Sexes showed a significant difference in change of fat oxidation, with females (8.69 ± 4.28 kcals/22 mins) exhibiting an increase and males (-9.71 ± 7.25 kcals/22 mins) a decrease (p=0.04). Change in fat percentage showed no significant differences between CSO and OO groups (p=0.98). **Conclusion:** These findings suggest that CSO may boost fat oxidation in humans, offering potential benefits for metabolic health.

CHAPTER ONE

INTRODUCTION

Development of the Problem

Introduction to Chronic Diseases

Chronic diseases, including cardiovascular diseases, diabetes, obesity, and other metabolic disorders, are leading causes of morbidity and mortality worldwide, with 73% of global deaths in 2022 attributed to these conditions.[1] Chronic diseases impose an economic burden on healthcare systems, individuals, and society, with the estimated cost of worldwide chronic disease to be $47 trillion by 2030.[3] Chronic diseases often lead to reduced quality of life due to the symptoms, complications, and limitations in daily activities. By focusing on prevention and management, effective interventions can help alleviate symptoms, improve functioning, and enhance the quality of life.[2] By addressing these diseases, overall health and the well-being of populations can improve.

Lipid Metabolism and Chronic Diseases

The dysregulation of lipid metabolism, characterized by decreased fat oxidation, contributes to the progression and severity of many chronic diseases, ultimately leading to conditions that affect the heart and blood vessels such as cardiovascular disease (CVD), type 2 diabetes, nonalcoholic fatty liver disease, and obesity in at-risk individuals.[4,5] Recognizing the significance of regulating lipid metabolism for health maintenance and disease prevention, particularly in conditions like CVD, type 2 diabetes, and obesity, could improve disease

management and prevention efforts through targeted interventions addressing impaired fat oxidation and advancing knowledge.

Dietary PUFAs and Fat Oxidation: Impact on Chronic Disease

Enhancing fat oxidation efficiency presents a promising avenue to potentially mitigate the symptoms and complications of obesity, cardiovascular disease, and type 2 diabetes, thereby reducing the overall burden of these chronic diseases.[6] Increasing fat oxidation can be done through various lifestyle modifications, including diet, exercise, and/or pharmacological interventions. Dietary interventions rich in polyunsaturated fatty acids (PUFAs) are being investigated as potential strategies for enhancing fat oxidation and mitigating chronic diseases like obesity, cardiovascular disease, and type 2 diabetes.[7] Despite being a common ingredient in unhealthy foods (chips, crackers, fries, doughnuts), cottonseed oil (CSO) is rich in polyunsaturated fatty acids (PUFAs) and linoleic acid, both of which have been recognized for their potential to favorably modify lipids, including lowering cholesterol levels. This is supported by previous research demonstrating the impact of CSO on lipid profiles, including decreasing low-density lipoprotein (LDL) and increasing high-density lipoprotein (HDL).[8,9]

Exercise and Its Relation to Fat Oxidation:

Fat oxidation is a fundamental metabolic process during exercise, particularly evident in steady-state, moderate-intensity activities where the body increasingly relies on stored fat for energy. During such exercises, the body shifts its primary energy source from carbohydrates to fats as exercise duration extends. This shift is facilitated by a series of metabolic adaptations and mechanisms that optimize fat utilization.

Initially, exercise induces the mobilization of triglycerides from adipose tissue through the action of hormone-sensitive lipase, resulting in the release of free fatty acids (FFAs) into the bloodstream. These FFAs are transported into muscle cells via transport proteins such as FAT/CD36 and fatty acid-binding proteins (FABPs). Within the mitochondria, FFAs undergo β-oxidation, a multi-step process that breaks down long-chain fatty acids into acetyl-CoA units. This process is critical for generating ATP, the primary energy source of the cell, and is supported by increased mitochondrial density and enzyme activity resulting from regular exercise. Enhanced mitochondrial capacity, improved fatty acid uptake, and increased lipolysis contribute to more efficient fat oxidation and overall energy expenditure during physical activity.

The rate of fat oxidation is influenced by exercise intensity and duration. During low- to moderate-intensity exercise, the body predominantly oxidizes fats, whereas higher intensities shift the reliance toward carbohydrates. Nonetheless, prolonged moderate-intensity exercise maintains elevated fat oxidation rates, which are crucial for endurance and weight management. Regular physical training induces several adaptations that further enhance fat oxidation, including increased mitochondrial density, improved enzyme activity, and enhanced insulin sensitivity. These adaptations collectively improve the ability of the body to utilize fat as an energy source more efficiently.

Dietary fats also play a significant role in modulating fat oxidation during exercise. For instance, PUFAs found in CSO and MUFAs from olive oil (OO) may impact fat metabolism differently. PUFAs, such as linoleic acid in CSO, can enhance fat oxidation by influencing lipid profiles and enzyme activity, while MUFAs, like oleic acid in OO, may improve metabolic

efficiency and insulin sensitivity. Understanding these interactions is crucial for optimizing dietary strategies to enhance fat metabolism and support overall health and performance.

Overview on Cottonseed Oil:

Derived from the seeds of cotton plants, CSO is used extensively in cooking, food processing, and industrial applications. It has gained attention due to its high content of PUFAs, primarily linoleic acid, which constitutes approximately 50-60% of its fatty acid composition. Linoleic acid, an omega-6 fatty acid, plays a crucial role in maintaining cellular membrane integrity and has been associated with beneficial effects on cholesterol levels. Research indicates that CSO may positively influence lipid profiles by lowering low-density lipoprotein (LDL) cholesterol and increasing high-density lipoprotein (HDL) cholesterol. This effect can contribute to improved cardiovascular health and reduced risk of heart disease. CSO is widely used across the globe due to its versatile properties. It is a common ingredient in processed foods, such as snacks, margarine, and baked goods. Its stability at high temperatures makes it a popular choice for frying and deep-frying. Beyond the kitchen, CSO is also utilized in the production of soaps, cosmetics, and as a carrier oil in various industrial applications. In countries like the United States, China, and India, cottonseed oil is frequently incorporated into diets due to its availability and cost-effectiveness. The use of the oil is particularly prevalent in regions where cotton farming is a major agricultural activity. This widespread application highlights its significance in both culinary and industrial contexts.

Role of Dihydrosterculic Acid (DHSA) in Fat Oxidation

Dihydrosterculic acid (DHSA) is a cyclopropane fatty acid that is a bioactive component found in CSO. DHSA is an inhibitor of stearoyl-CoA desaturase-1 (SCD1), an enzyme important

to fat oxidation. By inhibiting SCD1, DHSA potentially influences fat oxidation through complex mechanisms. Specifically, SCD1 inhibition reduces the conversion of saturated fatty acids to monounsaturated fatty acids (MUFAs), which are known to inhibit mitochondrial fatty acid oxidation. This inhibition can lead to an increase in the availability of saturated fatty acids for mitochondrial β-oxidation, which enhances fat oxidation.[10,11] Moreover, decreased SCD1 activity has been associated with upregulation of genes involved in fatty acid oxidation and thermogenesis, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and uncoupling protein 1 (UCP1), leading to enhanced lipid catabolism and energy expenditure.[11,12] Furthermore, DHSA-mediated SCD1 inhibition may promote the utilization of stored triglycerides via adipose tissue lipolysis, facilitating the release of fatty acids for mitochondrial oxidation and thereby further contributing to increased fat oxidation and energy production.[10,13] By inhibiting SCD1, DHSA could be a potential therapeutic approach for managing chronic diseases such as obesity, type 2 diabetes, and cardiovascular disease, all of which frequently involve impaired fat oxidation.

Research on Cottonseed Oil (CSO) Dosage

Further research is needed to establish the optimal dosage of CSO, considering insights from previous studies investigating its effects on lipid profiles and metabolic health. For instance, a study utilizing CSO at 30% of daily energy needs reported decreases in low-density lipoprotein (LDL), total cholesterol (CHOL), and triglycerides (TRIG).[8] Another study exploring lipid-modifying effects using 35% of daily energy needs found reductions in CHOL and LDL, along with increases in high-density lipoprotein (HDL).[9] In contrast, a different study administering 95g of CSO per day observed decreases in CHOL and LDL, but no significant

changes in TRIG.[14] Despite these findings, a clear dose-response relationship has not yet been established in the literature, and the upper and lower limits for the effects of CSO remain unknown. Individual differences in metabolism, dietary habits, and health status may influence the efficacy of CSO supplementation. According to USDA guidelines, oils rich in polyunsaturated fatty acids (PUFAs), like CSO containing significant amounts of linoleic acid, are beneficial for promoting heart health and overall well-being when consumed in moderation as part of a balanced diet. The chosen dosages of 30g and 60g of CSO in recent studies reflect practical considerations within this context, aiming to explore the potential metabolic benefits associated with higher intakes of PUFA-rich oils. By investigating fat oxidation during exercise under controlled conditions, the study seeks to elucidate how CSO supplementation may enhance metabolic flexibility and contribute to better management of chronic diseases. Therefore, conducting more research to assess sustained effects and potential adverse outcomes associated with different CSO dosages is necessary. Through further research on the dose-response relationship of CSO supplementation, lower and upper limits for CSO dosage can be established to optimize its therapeutic potential.

Differences between Cottonseed Oil and Olive Oil:

Cottonseed oil and OO differ significantly in their fatty acid compositions and metabolic effects, which influence their impact on fat oxidation. Cottonseed oil is notably rich in PUFAs, primarily linoleic acid, an omega-6 fatty acid. Linoleic acid has been associated with enhanced fat oxidation due to its ability to modulate metabolic pathways, such as SCD1. This inhibition reduces the conversion of saturated fatty acids to monounsaturated fatty acids, thereby increasing the availability of fatty acids for mitochondrial β-oxidation and potentially enhancing fat

oxidation rates. In contrast, OO is primarily composed of MUFAs, especially oleic acid. While MUFAs are beneficial for improving insulin sensitivity and lipid profiles, their impact on fat oxidation is generally less direct compared to PUFAs. MUFAs primarily enhance metabolic efficiency rather than directly affecting fatty acid availability for oxidation. Consequently, the distinct metabolic effects of PUFAs in CSO and MUFAs in OO highlight their differential impacts on fat metabolism and underscore the importance of their fatty acid profiles in influencing fat oxidation processes. Investigating how CSO/OO supplementation interacts with exercise offers an opportunity to understand their influence on metabolic flexibility and fat oxidation.[6,13]

Purpose

To determine the impact of a diet rich in either cottonseed oil or olive oil, consumed at dosages of 60g or 30g per day for a duration of 28 days, on fat oxidation during a 30-minute steady-state exercise bout in a fasted state.

Hypothesis

It is hypothesized that fat oxidation (kcals/min) will be greater in the CSO groups than the OO groups after the dietary intervention. It is also hypothesized that the larger dose (60g/day) will have greater fat oxidation than the smaller dose (30g/day).

H_0: $\mu_{OO}=\mu_{CSO}$
H_a: $\mu_{OO}<\mu_{CSO}$

H_0: $\mu_{30}=\mu_{60}$
H_a: $\mu_{30}<\mu_{60}$

Where, the notations of μ_{OO} and μ_{CSO} are the sample means of changes in fat oxidation (kcals/22 mins) in the olive oil group and the cottonseed oil group, respectively. For the second hypothesis, the notations of μ_{30} and μ_{60} are the sample means of changes in fat oxidation (kcals/22 mins) in the 30g/day dosage group and the 60g/day dosage group, respectively.

Implications

This study aims to better understand the relationship between CSO and fat oxidation in humans. Notably, the primary benefit attributed to CSO is its improvement of the lipid profile.[8,10] However, the mechanism by which DHSA in CSO influences fat oxidation in humans remains unclear. Investigating the impact of CSO on metabolism is crucial to assess its potential in disease management, particularly in metabolic disorders. Future research could investigate CSOs impact on fat oxidation in individuals with chronic diseases, thereby exploring its potential as a method of treatment for metabolic disorders.[15] This study could help lead to a potential treatment approach focused on enhancing the capacity to oxidize fatty acids, thereby presenting a new strategy for managing metabolic disorders.

Delimitations

1. The study was restricted to people between the ages of 18 and 55 years. Therefore, the findings of this study cannot be generalized to adults outside of this age range.
2. The study was restricted to healthy individuals with a BMI between 18.5 kg/m^2 and 27.0 kg/m^2.

3. The diet intervention was limited to only 28 days. Different results could have been seen with a longer intervention.
4. The exercise duration was limited to only 30 minutes. Fat oxidation could have been different if the exercise duration was increased.[6]
5. The exercise was completed at 75% of VO2 max. This is a higher intensity than what is optimal for high fat oxidation.[6,16]
6. There was no true control group in the study.

Limitations

1. The findings of this study cannot be generalized to an at-risk population with chronic disease or a population at high-risk for development of chronic disease population. Therefore, it is unknown if the results from this study will remain true in a population with chronic disease.

CHAPTER TWO

LITERATURE REVIEW

Fat Oxidation

Fat oxidation, the enzymatic breakdown of triglycerides and subsequent conversion to energy via mitochondrial metabolism, plays a pivotal role in human metabolism, impacting energy balance, metabolic flexibility, and endurance.[6] Serving as a primary energy source during low to moderate-intensity exercise and during caloric deficit, fat oxidation plays a role in maintaining energy balance, metabolic flexibility, and endurance.[6] Dysregulation of fat metabolism has been implicated in the pathogenesis of obesity, insulin resistance, type 2 diabetes, and cardiovascular disease, emphasizing the clinical significance of understanding and modulating fat oxidation pathways.[32] Despite its importance, the regulation of fat oxidation and its interplay with other metabolic pathways remains not fully understood. Thus, the mechanisms of fat oxidation hold promise for advancing our understanding of metabolic physiology and developing targeted interventions for optimizing metabolic health and metabolic disorders.[33]

Research comparing low-intensity, steady-state aerobic exercise with moderate-intensity interval training has elucidated significant differences in fat oxidation rates, emphasizing the role of exercise intensity in enhancing fat metabolism and post-exercise fat oxidation. For example, a study investigated the effects of low-intensity endurance exercise versus moderate-intensity continuous exercise on fat oxidation rates in young adults.[34] The randomized crossover study involved participants performing two exercise sessions: one at low intensity (40% of VO2 max) and the other at moderate intensity (60% of VO2 max), with fat oxidation rates assessed using indirect calorimetry. The results revealed that while both low-intensity and moderate-intensity

exercise increased fat oxidation rates during the exercise session, moderate-intensity exercise resulted in significantly higher fat oxidation rates compared to low-intensity exercise (1.5 ± 0.2 vs. 1.2 ± 0.2 g/min, $p < 0.05$) with total energy expenditure being controlled. Specifically, fat oxidation rates were approximately 20% higher during moderate-intensity exercise compared to low-intensity exercise. Moreover, post-exercise fat oxidation rates were significantly elevated following moderate-intensity exercise compared to low-intensity exercise, with fat oxidation rates remaining elevated during the post-exercise recovery period. These findings highlight the importance of exercise intensity in modulating fat oxidation rates and support the use of moderate-intensity continuous exercise as an effective strategy for enhancing fat metabolism and improving metabolic health, particularly in young adults.

Research comparing low-intensity, steady-state aerobic exercise with high-intensity interval training (HIIT) has shown that HIIT significantly enhances fat oxidation rates and prolongs post-exercise metabolic effects more effectively than moderate-intensity continuous training (MICT) in individuals with metabolic syndrome. For instance, a study investigated the effects of HIIT versus moderate-intensity continuous training (MICT) on fat oxidation rates in individuals with metabolic syndrome.[35] This randomized controlled trial involved participants performing either HIIT or MICT sessions three times per week for 16 weeks. Fat oxidation rates were assessed using indirect calorimetry during exercise sessions. The results demonstrated that while both exercise modalities increased fat oxidation rates during exercise, HIIT elicited significantly greater increases compared to MICT. Specifically, fat oxidation rates were 36% higher during HIIT sessions compared to MICT (1.2 ± 0.1 vs. 0.9 ± 0.1 g/min, $p < 0.05$).

Moreover, post-exercise fat oxidation rates remained elevated for a longer duration following HIIT compared to MICT, suggesting a prolonged metabolic effect.

Research has demonstrated that low-carbohydrate/high-fat (LCHF) and ketogenic diets significantly enhance fat oxidation rates during exercise compared to traditional high-carbohydrate diets, highlighting their potential benefits for weight loss and metabolic health. For instance, a study was conducted to compare the effects of a ketogenic diet with a traditional high-carbohydrate diet on fat oxidation rates during exercise.[36] They found that individuals following the ketogenic diet exhibited significantly higher rates of fat oxidation, with an increase from 0.5 ± 0.1 to 0.9 ± 0.1 grams per minute (g/min), compared to a slight decrease in the high-carbohydrate group from 0.6 ± 0.1 to 0.5 ± 0.1 g/min. Similarly, another study investigated the impact of LCHF diets on fat oxidation during submaximal exercise and reported increased fat oxidation rates in individuals adhering to the LCHF diet compared to those on a high-carbohydrate diet, with mean rates of 0.41 ± 0.14 versus 0.25 ± 0.11 grams per minute, respectively.[37] These findings align with a meta-analysis which concluded that low-carbohydrate diets lead to greater weight loss and increased fat oxidation compared to low-fat diets, with an overall effect size of 0.34 (95% CI, 0.17 to 0.51; $p < 0.001$).[38] Collectively, these studies emphasize the potential benefits of carbohydrate restriction, as seen in ketogenic and LCHF diets, in promoting fat oxidation rates, although individual variations and broader metabolic implications necessitate further research.

A study found that omega-3 fatty acid supplementation significantly increases fat oxidation during exercise and improves insulin sensitivity in healthy individuals compared to a placebo. A study aimed to investigate the effects of omega-3 fatty acid supplementation on fat

oxidation during exercise and insulin sensitivity in healthy individuals.[39] The study included 21 healthy adults who were randomly assigned to receive either omega-3 fatty acid supplements (containing 1.8 grams of eicosapentaenoic acid and 1.2 grams of docosahexaenoic acid) or placebo supplements daily for 12 weeks. The researchers measured fat oxidation during exercise using indirect calorimetry and insulin sensitivity using an oral glucose tolerance test. They found that omega-3 fatty acid supplementation significantly increased fat oxidation during exercise compared to placebo ($p < 0.05$). Additionally, omega-3 fatty acid supplementation significantly improved insulin sensitivity compared to placebo ($p < 0.05$). These findings suggest that omega-3 fatty acid supplementation may enhance fat metabolism and improve insulin sensitivity in healthy individuals.

Research emphasizes the critical role of fat oxidation in metabolic health, revealing that moderate-intensity exercise, low-carbohydrate/high-fat diets, and omega-3 fatty acid supplementation can significantly enhance fat oxidation rates and improve conditions such as obesity, insulin resistance, and cardiovascular disease. Despite its significance, the regulation of fat oxidation and its interaction with other metabolic pathways remains incompletely understood. Studies have shown that moderate-intensity exercise leads to higher fat oxidation rates compared to low-intensity exercise. Low-intensity exercise refers to physical activities performed at a heart rate of about 50-65% of your maximum, allowing for easy conversation without significant breathlessness. Examples include leisurely walking, gentle cycling, and restorative yoga. In contrast, moderate-intensity exercise elevates the heart rate to approximately 65-75% of the maximum, making conversation more challenging. This level of effort includes activities such as brisk walking, jogging, swimming at a steady pace, and cycling at a moderate speed.

Furthermore, investigations into the effects of dietary interventions, such as low-carbohydrate/high-fat (LCHF) diets and omega-3 fatty acid supplementation, have highlighted their potential to enhance fat oxidation rates and improve metabolic health. Collectively, these findings emphasize the importance of understanding the mechanisms of fat oxidation for advancing metabolic physiology and developing targeted interventions to optimize metabolic health and manage metabolic disorders.

Cottonseed Oil

Cottonseed oil is unique among oils due to its distinct composition, characterized by high levels of linoleic acid (18:2n6) and omega-6 polyunsaturated fatty acids (PUFAs), alongside a large portion of saturated fats. Linoleic acid plays an important role in the attenuation of cholesterol levels, thereby mitigating the risk of cardiovascular disease.[17] This beneficial effect is particularly significant given the well-established association between dyslipidemia and increased susceptibility to cardiovascular disorders.[18] Despite the presence of saturated fats in CSO, previous research has demonstrated a favorable effect on lipid profiles, indicating that CSO may have a positive impact on cardiovascular health.[8,9] CSO may emerge as a promising dietary intervention for the management of dyslipidemia and the prevention of cardiovascular disease.

Previously, it was believed that omega-6 PUFAs increased the production of proinflammatory eicosanoids.[19] However, subsequent studies have challenged this notion. Specifically, research conducted by Vaughan and colleagues contradicted this previous understanding by demonstrating that linoleic acid, a prominent omega-6 PUFA, singularly does not induce low-grade inflammation.[20] The researchers administered dietary interventions to six

groups of mice over a four-week period.[20] They were fed either saturated, monounsaturated, linoleic acid, or alpha-linolenic acid. Mice with diets rich in saturated fats or monounsaturated fats had elevated proinflammatory markers in both liver and adipose tissue. In contrast, mice receiving linoleic acid did not have significant changes in proinflammatory or procoagulant markers in any of the assessed tissues. The studies observations suggest that while linoleic acid may be needed for eicosanoid synthesis, its presence alone does not increase inflammation.[20] These new findings necessitate a reevaluation of the effect of omega-6 PUFAs on overall health.

Cottonseed oil has been recognized for its potential lipid-modifying effects, including cholesterol-lowering properties in previous research.[8,9] Researchers did a study on the effects of CSO and OO consumption over a 5-day period with fifteen healthy individuals.[8] They demonstrated reductions in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG). TC decreased from 148.40 ± 6.39 to 135.93 ± 6.31 mg/dL, LDL-C from 92.20 ± 5.57 to 78.13 ± 5.60 mg/dL, and TG from 80.11 ± 4.91 to 56.37 ± 5.46 mg/dL (all $P < .05$).[8] Similarly, in a separate 8-week dietary intervention with 30% of energy needs from either CSO or OO involving forty-three individuals with hypercholesterolemia, researchers found that CSO consumption led to significant reductions in fasting serum TC and LDL-C compared to OO, with greater reductions observed from baseline to week 8 (TC: -17.0 ± 3.94 mg/dL vs. -2.18 ± 3.72 mg/dL; LDL-C: -19.7 ± 3.94 mg/dL vs. -5.72 ± 4.23 mg/dL; $p < 0.05$ for both).[9] These findings demonstrate the potential of CSO to improve lipid profiles.

A study by Paton and colleagues demonstrated that diets enriched with CSO impaired desaturase activity and increased DHSA levels in male mice, suggesting that CSO may enhance PPARδ expression and promote fatty acid oxidation.[10] In the study male mice were fed chow or

CSO-, saturated fat (SFA)-, or linoleic acid (18:2)-enriched diets that were matched for macronutrient content for 4 weeks.[10] Fatty acid composition of the diets and livers revealed an impairment in desaturase activity and the presence of dihydrosterculic acid (DHSA) in the CSO-fed mice. The effect of DHSA on PPARδ and stearoyl-CoA desaturase-1 expression mimicked that of the CSO-fed mice. Taken together, these data suggest that DHSA from CSO may be an effective means to increase PPARδ expression with concomitant suppression of liver stearoyl-CoA desaturase-1 activity. In addition to muscle, the livers of mice fed CSO-enriched diets displayed a large increase in the PPAR*δ* coactivator PGC-1*α*, along with PPAR*δ* itself, and lactate dehydrogenase a or b isoform (LDHa/b) expression compared with all other diets. The protein expression changes in the liver strongly suggest that CSO provides metabolic adaptations that favor enhanced fatty acid oxidation and utilization.

The bioactive compound DHSA found in CSO has been identified as a potent inhibitor of SCD1, a key enzyme involved in lipid metabolism.[21] SCD1 catalyzes the desaturation of saturated fatty acids to monounsaturated fatty acids (MUFAs), thereby promoting lipid storage over oxidation.[22] Inhibition of SCD1 activity by DHSA may contribute to the reduction of hepatic lipid accumulation observed in mice, providing insight into the potential mechanism of action of CSO. In a study involving six mice fed different high-fat diets, including CSO, respiratory exchange ratio (RER) measurements revealed increased fat oxidation in CSO-fed animals compared to those fed saturated fatty acids (SFA). CSO-fed mice also exhibited elevated expression of peroxisome proliferator-activated receptor delta (PPARδ) in skeletal muscle, along with increased expression of PPARδ coactivator PGC-1α and lactate dehydrogenase isoforms in the liver.

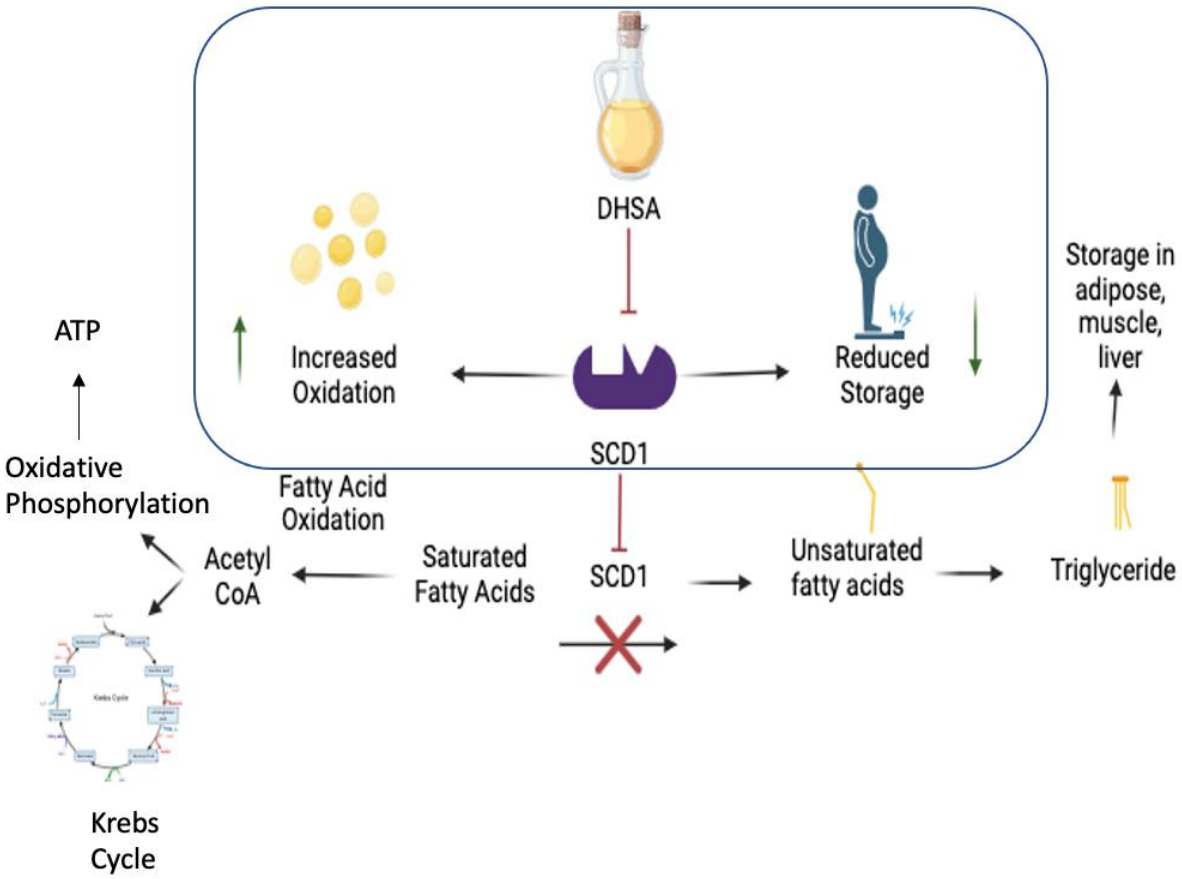

Figure 1: Potential Mechanism for CSO Increasing Fat Oxidation. DHSA, in CSO, inhibits SCD1.Inhibition of SCD1 through DHSA enhances fat oxidation and reduces body fat storage by preventing the conversion of saturated fatty acids into unsaturated fatty acids. These unsaturated fatty acids typically undergo conversion into triglycerides, which are stored in adipose tissue, muscle, and the liver. With inhibition, saturated fatty acids are directed towards fatty acid oxidation, where they are converted into acetyl-CoA. Subsequently, acetyl-CoA enters either the Krebs cycle or oxidative phosphorylation pathways to produce ATP. DHSA; dihydrosterculic acid, SCD1; stearoyl-CoA desaturase 1, Acetyl-CoA; acetyl coenzyme A, ATP; adenosine triphosphate.

Additionally, suppression of Δ9 desaturase activity, mediated by SCD1 inhibition, was evident in CSO-fed mice. These findings align with previous studies indicating that saturated fatty acids upregulate SCD1 expression, while n-6 PUFAs suppress lipogenic gene expression.[23] The combination of DHSA-mediated SCD1 inhibition and the regulatory effects of high PUFA

content in CSO on lipogenic and cholesterogenic gene expression positions it as a promising nutritional therapeutic for targeting cardiovascular health.[11,12] Understanding the role of DHSA provides a foundation for developing nutritional strategies to address metabolic disorders, with implications for future research in human subjects. This insight into the potential metabolic pathways emphasizes the observed health benefits of CSO in mice and identifies specific areas for further investigation in human populations.

Metabolic Adaptations to High-Fat Diet

Research indicates that high-fat diets, typically comprising 40-50% of daily calories from fat in a calorically balanced diet, significantly impact metabolic health by increasing cholesterol levels, impairing glucose tolerance, and reducing insulin sensitivity. High-fat diets aim to optimize fat metabolism in the body. According to a study, these diets typically consist of 60-65% fat, with protein and carbohydrates making up 15-20% each.[24] This macronutrient distribution is designed to shift energy reliance in the body from carbohydrates to fats, promoting enhanced fat utilization and greater energy efficiency. In a systematic review and meta-analysis, which included 15 randomized controlled trials and 2,000 participants, significant increases in total cholesterol (mean increase of 15 mg/dL, $p < 0.001$) and LDL cholesterol levels (mean increase of 10 mg/dL, $p < 0.001$) were observed in response to high-fat dietary interventions.[25] The high-fat diets in these studies typically constituted 40-50% of total daily calories from fat. The systematic review and meta-analysis show a clear link between high-fat diets and increased cholesterol levels, suggesting cardiovascular risks. In comparison, Gardner and colleagues' study provides insight into how high-fat diets can impair glucose tolerance and increase fasting glucose levels, pointing to metabolic risks. Gardner and colleagues conducted a longitudinal study with

100 participants over 12 months, assigning them to either high-fat or low-fat diets.[26] Glycemic control was assessed using oral glucose tolerance tests and fasting blood glucose measurements. The study found impaired glucose tolerance, with a mean increase in 2-hour postprandial glucose levels of 30 mg/dL ($p < 0.05$) and increased fasting glucose levels (mean increase of 15 mg/dL, $p < 0.05$) in individuals following a high-fat diet compared to those on a low-fat regimen. The high-fat diet in this study comprised approximately 45% of total daily calories from fat. Bellot and colleagues conducted a prospective cohort study involving 500 participants over 5 years, assessing lipid metabolism through regular blood lipid measurements.[27] They found persistent alterations in lipid metabolism, characterized by elevated total cholesterol (mean increase of 20 mg/dL, $p < 0.001$) and triglyceride levels (mean increase of 30 mg/dL, $p < 0.001$) in individuals with sustained high-fat intake. The high-fat diet in this study consisted of approximately 50% of total daily calories from fat. In a cross-sectional study involving 150 individuals with varying dietary fat intakes, insulin sensitivity was measured using frequently sampled intravenous glucose tolerance tests.[28] A significant negative correlation was found between high-fat intake and insulin sensitivity, with each 10% increase in dietary fat intake associated with a 5% decrease in insulin sensitivity ($p < 0.05$). Dietary fat intake varied among participants, ranging from 20% to 50% of total daily calories. This research emphasizes the profound impact of a high fat diet on metabolic health.

Recent studies on high-fat diets have revealed significant positive effects on metabolic health, including increased hepatic glucose production, alterations in insulin action, enhanced fat oxidation, and modulations in the gut microbiome. A study involving 26 young men investigated the short-term impact of high-fat overfeeding on hepatic and peripheral insulin action, revealing

significant increases in hepatic glucose production and fasting glucose levels in response to overfeeding.[29] Despite these changes, peripheral insulin action, muscle mitochondrial function, and gene expression related to oxidative phosphorylation remained unaffected. Interestingly, compensatory increases in insulin secretion were observed, potentially preceding the development of peripheral insulin resistance and other metabolic alterations associated with overfeeding. In a separate investigation, healthy slightly overweight men were subjected to saturated or polyunsaturated fat-rich diets for six weeks during weight maintenance.[30] This study revealed intriguing findings, including unchanged peripheral insulin sensitivity regardless of fatty acid type, increased fat oxidation potential in muscle, and alterations in hepatic insulin clearance, glucose production, de novo lipogenesis, and plasma triglyceride levels. Moreover, high-fat intake induced changes in the plasma proteome towards immune support and modulated the gut microbiome, particularly with PUFAs. Consistent findings were observed in mice subjected to eucaloric feeding of human PUFA and saturated fatty acid diets, which led to reduced hepatic triglyceride content and metabolic adaptations suggestive of decreased gluconeogenesis and lipogenesis.[31] These findings show the adaptiveness of the metabolic response to high-fat diets, suggesting the potential for effective fat disposal without adverse metabolic consequences.

A high-fat diet typically consists of a significant proportion of calories coming from fats, usually more than 30-40% of total daily caloric intake. These diets often emphasize sources of fat such as oils, nuts, seeds, fatty fish, and full-fat dairy products, while reducing carbohydrates and sometimes proteins. High-fat diets can vary in their composition, including ketogenic diets (very low in carbohydrates), moderate-fat diets, or those that focus on healthy fats from whole

foods. They are often studied for their effects on weight loss, metabolic health, and energy metabolism. These studies elucidate how high-fat diets enhance fat oxidation through the activation of intracellular sensors and signaling pathways, which can positively impact metabolic health by modulating fatty acid catabolism, mitochondrial function, and overall energy metabolism. However, while enhanced fat oxidation may improve lipid profiles, such as reducing LDL cholesterol and triglycerides, it is important to consider potential adverse outcomes. For instance, high-fat diets, particularly those rich in saturated fats, have been associated with impaired glucose tolerance and insulin resistance. This paradox arises because excessive fat intake can lead to the accumulation of fatty acids in tissues that are not efficient at oxidizing fats, potentially disrupting glucose metabolism. Consequently, despite improvements in lipid profiles, high-fat diets may exacerbate metabolic disturbances like impaired glucose tolerance, highlighting the need for a balanced approach in dietary interventions to manage chronic diseases effectively. Increased fat oxidation in response to a high-fat diet is primarily triggered by the abundance of dietary fats, particularly fatty acids derived from ingested triglycerides. Intracellular sensors such as fatty acid transporters (e.g., CD36) and nuclear receptors (e.g., PPAR-alpha) recognize elevated fatty acid levels as indicators of high-fat diet consumption. This recognition activates various signaling pathways, including PPAR-alpha and AMPK, which promote fat oxidation. Activation of PPAR-alpha leads to the upregulation of genes involved in fatty acid oxidation, such as CPT1 and Acyl-CoA dehydrogenase, enhancing the capacity for fat catabolism. AMPK activation inhibits fatty acid synthesis pathways while promoting fatty acid oxidation enzymes. Post-translational modifications further modulate enzyme activity, impacting fatty acid oxidation rates. Enhanced fat oxidation influences cellular

processes such as mitochondrial biogenesis, ATP production, and redox balance. Factors like nutrient availability, hormonal regulation, and exercise also modulate fat oxidation in response to a high-fat diet, highlighting the complex interplay of stimuli and signaling pathways involved in metabolic adaptations. Understanding these mechanisms is crucial for understanding the metabolic effects of high-fat diets on overall health.

Relevance of Ketogenic Diet Research to Clinical Populations

Research on ketogenic diets reveals significant effects on fat metabolism and exercise performance, which are highly relevant for clinical populations dealing with metabolic disorders. The ketogenic diet, which limits carbohydrate intake to less than 5% of total energy and increases fat consumption to 75-80%, fundamentally alters the energy substrate preference of the body, shifting from carbohydrates to fats.[67] This dietary approach has been linked to improvements in various clinical conditions, such as obesity, type 2 diabetes, and metabolic syndrome, where enhanced fat oxidation and metabolic efficiency are crucial.[68]

Several studies have demonstrated the benefits of ketogenic diets in improving body composition and metabolic health. For instance, Westman and colleagues conducted a study with individuals diagnosed with obesity and type 2 diabetes who adhered to a ketogenic diet.[69] Over a 24-week period, participants experienced a significant reduction in body fat, from 34.0% to 30.0%, and improved glycemic control, as evidenced by reduced HbA1c levels.[69] This study underscores the potential of the diet for effective weight management and metabolic improvement.

In another study, Volek and colleagues investigated the impact of a ketogenic diet on overweight men over 12 weeks. Participants on the ketogenic diet exhibited a 16% reduction in

body fat and significant improvements in insulin sensitivity.[70] This highlights the potential of the ketogenic diet in enhancing metabolic health and supporting weight loss, which can be particularly beneficial for individuals struggling with obesity and type 2 diabetes.

The impact of the ketogenic diet on exercise performance, particularly in clinical populations, is more nuanced. Burke and colleagues explored how a ketogenic diet affected elite race walkers and found that while fat oxidation rates during submaximal exercise increased, peak power output and aerobic capacity decreased by 7% and 6%, respectively.[71] This study indicates that while the ketogenic diet may enhance fat utilization, it could impair high-intensity exercise performance and aerobic capacity.

Similarly, Hulston and colleagues found that a high-fat diet reduced muscle glycogen availability and impaired glycolytic capacity, affecting high-intensity exercise performance negatively.[72] These findings suggest that while high-fat diets might benefit endurance activities by increasing fat oxidation, they could limit performance in activities requiring high-intensity effort due to decreased glycogen stores.

In contrast, Volek and colleagues demonstrated that endurance-trained individuals on a ketogenic diet for 24 weeks experienced a 15% increase in time to exhaustion during submaximal exercise.[73] This suggests improved metabolic efficiency and fat adaptation, potentially leading to better endurance performance over prolonged exercise sessions. The study highlights the potential of the ketogenic diet to enhance fat oxidation and endurance capacity, even in trained individuals.

Research also suggests that ketogenic diets may improve quality of life and physical function in clinical populations. Phinney and colleagues observed that individuals with type 2

diabetes on a ketogenic diet not only reduced body fat but also reported enhanced quality of life and physical function, including improved energy levels and reduced symptoms of metabolic syndrome.[74] This emphasizes the potential broader benefits of ketogenic diets beyond metabolic and body composition improvements.

Training, Diet, and Performance Across Exercise Intensities

Past research has shown that exercise intensity and dietary interventions significantly impact fat oxidation rates and performance, with moderate-intensity exercise and ketogenic diets leading to higher fat oxidation and improved metabolic responses compared to low-intensity exercise and high-carbohydrate diets. Studies have demonstrated physiological responses to varying exercise intensities, with low-intensity aerobic exercise enhancing endurance capacity and fat oxidation rates, while high-intensity interval training (HIIT) elicits rapid improvements in anaerobic power and metabolic adaptations. For instance, researchers conducted a randomized crossover study investigating the effects of low-intensity endurance exercise versus moderate-intensity continuous exercise on fat oxidation rates in young adults.[40] Their findings revealed that moderate-intensity exercise resulted in significantly higher fat oxidation rates compared to low-intensity exercise (1.5 ± 0.2 vs. 1.2 ± 0.2 g/min, $p < 0.05$), highlighting the importance of exercise intensity in modulating metabolic responses. Dietary interventions have also shown promise in optimizing performance outcomes, with research indicating the differential effects of macronutrients and specific dietary strategies on endurance, strength, and power performance. For example, researchers conducted a study comparing the effects of a exercise.[41] They found that individuals following the ketogenic diet exhibited significantly higher rates of fat oxidation, with an increase from 0.5 ± 0.1 to 0.9 ± 0.1 grams per minute (g/min), compared to a slight

decrease in the high-carbohydrate group from 0.6 ± 0.1 to 0.5 ± 0.1 g/min. Furthermore, studies exploring the interaction between exercise intensity and diet have provided insights into the metabolic responses to exercise under different dietary conditions, emphasizing the role of nutrient timing and substrate utilization in modulating performance.

Past studies indicate that varying exercise intensities lead to specific physiological adaptations, with endurance training enhancing mitochondrial density and oxidative capacity, high-intensity interval training (HIIT) improving neuromuscular function and muscle oxidative capacity, and high-intensity resistance training promoting muscle hypertrophy and strength gains. Endurance training induces increases in mitochondrial density and oxidative enzyme activity, enhancing aerobic capacity. Research demonstrates that endurance exercise leads to mitochondrial biogenesis and improvements in muscle oxidative capacity.[42] In their study, participants engaged in regular endurance training, resulting in a significant increase in mitochondrial content by 35-50% in skeletal muscle, alongside improvements in oxidative enzyme activity ($p < 0.05$). Notably, the study observed a 69% increase in citrate synthase activity, a marker of mitochondrial content, following endurance training ($p < 0.01$). High-intensity interval training (HIIT) elicits neuromuscular adaptations, such as increased motor unit recruitment and firing rates, enhancing force production and power output. A study found that HIIT significantly improved skeletal muscle oxidative capacity and maximal activity of mitochondrial enzymes compared to traditional moderate-intensity continuous training.[43] The research involved participants performing HIIT, leading to a 46% increase in muscle oxidative capacity, as measured by citrate synthase activity, compared to a 14% increase in the moderate-intensity continuous training group ($p < 0.001$). Additionally, resistance training at high

intensities induces muscle hypertrophy and strength gains through mechanisms like myofibrillar protein synthesis and neural adaptations. Research highlights the efficacy of high-intensity resistance training in promoting muscle hypertrophy and strength development.[44] Their meta-analysis revealed that high-intensity resistance training induced significant muscle hypertrophy, with effect sizes ranging from 0.49 to 0.67 for muscle cross-sectional area and 0.66 to 0.74 for strength gains ($p < 0.05$). These studies show physiological adaptations that occur in response to varying exercise intensities, ultimately optimizing performance across a spectrum of physical activities.

Research indicates that dietary strategies aimed at enhancing fat oxidation can improve athletic performance. For instance, a study found that adopting a high-fat, low-carbohydrate diet increased fat oxidation during exercise, potentially leading to improved endurance performance. In this study, elite race walkers consumed a diet comprising 68% fat and 15% carbohydrate for three weeks, resulting in a significant increase in fat oxidation rates during submaximal exercise compared to baseline levels ($p < 0.05$).[45] Similarly, a study demonstrated that supplementing with medium-chain triglycerides (MCTs) increased fat oxidation rates and prolonged time to exhaustion in athletes.[46] Participants consumed MCT oil as a supplement before exercise sessions, leading to a 20% increase in fat oxidation rates and a 25% increase in time to exhaustion compared to a control group ($p < 0.05$). Additionally, a study showed that omega-3 fatty acid supplementation enhanced fat oxidation during exercise and improved exercise performance measures.[47] Participants supplemented with omega-3 fatty acids exhibited a 15% increase in fat oxidation rates during exercise and improved exercise performance measures, such as a 10% increase in time to exhaustion or a 20% increase in distance covered, compared to

a placebo group ($p < 0.05$). These findings underscore the importance of prioritizing dietary fats, such as those from MCTs and omega-3 fatty acids, to optimize fat metabolism and athletic success.

Research indicates that high-fat diets, including ketogenic diets and pre-exercise high-fat meals, can significantly enhance endurance performance and increase fat oxidation during submaximal exercise in trained and recreationally active individuals. For instance, a study investigated the effects of a ketogenic diet, characterized by high fat intake and very low carbohydrate consumption, on endurance performance in trained cyclists.[41] Their randomized controlled trial with 20 participants demonstrated that after a 12-week ketogenic diet intervention, cyclists experienced significant improvements in endurance performance metrics, such as time to exhaustion during submaximal exercise ($p < 0.01$) and peak power output ($p < 0.05$), compared to baseline. Additionally, a study explored the impact of pre-exercise high-fat meals on substrate utilization and endurance performance in endurance-trained individuals.[48] Their crossover study with 12 participants revealed that consuming a high-fat meal two hours before exercise resulted in increased fat oxidation during submaximal exercise and improved endurance performance compared to a high-carbohydrate meal condition ($p < 0.05$). Furthermore, a study examined the effects of chronic high-fat feeding on exercise metabolism and performance in recreationally active individuals.[49] Their randomized controlled trial with 15 participants showed that six weeks of a high-fat diet led to increased fat oxidation during submaximal exercise ($p < 0.05$) and improved time trial performance compared to a control group consuming a standard diet ($p < 0.01$). These studies show the potential benefits of high-fat

diets for endurance performance under low to moderate exercise intensities, offering valuable insights for athletes and coaches seeking to optimize dietary strategies for endurance activities.

Fat Oxidation and Chronic Disease

Numerous seminal studies have established that impaired fat oxidation is closely associated with an increased risk of chronic metabolic conditions, including type 2 diabetes and cardiovascular diseases. A large-scale epidemiological study, spanning decades, consistently reveal an association between impaired fat oxidation and heightened risk for chronic metabolic conditions, including type 2 diabetes and cardiovascular diseases.[50] Analyses from these cohorts have demonstrated that individuals with lower rates of fat oxidation are at significantly higher risk of developing insulin resistance and metabolic syndrome. A study within these cohorts assessed the association between plasma fatty acid biomarkers and incident type 2 diabetes, finding that for each standard deviation increase in plasma saturated fatty acids, the risk of developing type 2 diabetes increased by 15% (95% CI 1.10-1.21), thus further emphasizing the importance of fat metabolism in metabolic health.[50]

A foundational study elucidated the critical role of the glucose-fatty acid cycle in metabolic regulation, demonstrating how dysregulated fat metabolism contributes to insulin resistance and obesity by impairing glucose utilization and promoting metabolic disorders such as type 2 diabetes.[51] Their research revealed the intricate interplay between glucose and fatty acid metabolism, highlighting how excess fatty acids can impair glucose utilization and promote insulin resistance, thereby contributing to the pathogenesis of type 2 diabetes and other metabolic disorders. Studies reported a significant positive correlation between plasma free fatty acids and

insulin levels in obese subjects ($r=0.72$, $p<0.001$), laying the groundwork for research into the molecular mechanisms underlying metabolic diseases.[51]

Research has shown that mitochondrial dysfunction, characterized by impaired oxidative capacity and reduced ATP production, plays a crucial role in the development of insulin resistance and metabolic syndrome, linking impaired fat oxidation with adverse metabolic outcomes. Studies conducted have demonstrated that dysfunctional mitochondria play a crucial role in the development of insulin resistance and metabolic syndrome, thereby linking impaired fat oxidation with adverse metabolic outcomes.[51] For instance, research has provided evidence of impaired mitochondrial oxidative capacity in skeletal muscle of obese individuals, with a 30% reduction in maximal mitochondrial ATP production rate compared to lean controls ($p<0.05$), highlighting the role of mitochondrial dysfunction in the pathogenesis of metabolic diseases.[52]

Recent clinical trials have elucidated significant findings regarding the impact of dietary macronutrient composition on fat oxidation and metabolic health.[53] For example, a randomized controlled trial comparing the effects of different diets on weight loss and metabolic outcomes, found that diets high in saturated fat and refined carbohydrates were associated with impaired fat oxidation and increased risk of metabolic syndrome.[53] Conversely, diets rich in unsaturated fats and complex carbohydrates promoted metabolic flexibility and improved health outcomes. Sacks and colleagues assigned individuals to the low-fat diet group experienced a 12.1% reduction in fat oxidation compared to a 4.6% reduction in the low-carbohydrate diet group ($p=0.04$), highlighting the differential effects of dietary macronutrients on fat oxidation and metabolic health.[53] Collectively, these studies form a robust body of evidence affirming the link between

fat oxidation and chronic disease pathogenesis, providing an understanding of the interplay between metabolic dysregulation and disease progression.

Recent studies have underscored the critical role of fat oxidation rates in metabolic health, revealing significant differences between lean and obese individuals, and highlighting associations with insulin resistance and risks for chronic conditions like type 2 diabetes and cardiovascular diseases. A study found that obese individuals had a significantly lower fat oxidation rate compared to lean individuals, with obese subjects exhibiting a mean fat oxidation rate of 0.52 ± 0.11 grams per minute at rest, while lean subjects had a rate of 0.72 ± 0.15 grams per minute.[61] During exercise, obese subjects showed a mean fat oxidation rate of 0.60 ± 0.13 grams per minute, whereas lean subjects had a rate of 0.92 ± 0.23 grams per minute. These differences were statistically significant ($p < 0.05$), indicating a disparity in fat oxidation capacity between lean and obese individuals. In a different study researchers reported a strong association between impaired fat oxidation and insulin resistance.[62] It was found that individuals with insulin resistance had significantly lower fat oxidation rates compared to insulin-sensitive individuals, with mean fat oxidation rates of 0.49 ± 0.08 grams per minute and 0.67 ± 0.09 grams per minute, respectively ($p < 0.01$).[62] This suggests that impaired fat oxidation is closely linked to insulin resistance, a key feature of metabolic syndrome and type 2 diabetes . In a separate study, data was from a cohort comprising thousands of participants over several years.[50] It was found that individuals with higher levels of specific plasma phospholipid saturated fatty acids, such as palmitic acid and stearic acid, had a significantly increased risk of developing type 2 diabetes. Every singular standard deviation increase in palmitic acid levels was associated with a 16% higher risk of type 2 diabetes (95% CI: 8-24%). Similarly, a prospective study involving

diabetic men and found that those with elevated lipoprotein(a) concentrations had a substantially higher risk of coronary heart disease compared to those with lower concentrations.[63] Specifically, individuals in the highest quartile of lipoprotein(a) levels had a 1.8-fold increased risk of coronary heart disease (95% CI: 1.1-3.0) compared to those in the lowest quartile. Furthermore, Fretts and colleagues investigated the association between plasma phospholipid saturated fatty acids and incident atrial fibrillation in the Cardiovascular Health Study cohort.[64] It was discovered that individuals with higher levels of certain saturated fatty acids, such as myristic acid and palmitic acid, had a significantly elevated risk of atrial fibrillation. For example, every one standard deviation increase in myristic acid levels was associated with a 13% higher risk of atrial fibrillation (95% CI: 1-27%).

Recent clinical trials investigating interventions aimed at enhancing fat oxidation have demonstrated significant benefits in improving metabolic health and reducing cardiovascular risk among individuals with type 2 diabetes and high-risk populations. For instance, a randomized controlled trial (RCT) focusing on individuals with type 2 diabetes, assessed the impact of an intensive lifestyle intervention (ILI) on cardiovascular outcomes.[65] Results from this trial showed significant weight loss and improvements in glycemic control in the ILI group compared to the control group, indicative of enhanced fat oxidation and metabolic health. Specifically, participants in the ILI group achieved an average weight loss of 8.6% versus 0.7% at year 1 and 6.0% versus 3.5% at year 4, along with mean reductions in HbA1c of 0.36% versus an increase of 0.01% at year 1, and 0.24% versus 0.08% at year 4. Similarly, another RCT, examined the effects of a Mediterranean diet supplemented with either extra virgin olive oil (EVOO) or mixed nuts on cardiovascular outcomes in high-risk individuals.[66] Findings revealed a significantly

lower incidence of major cardiovascular events in the Mediterranean diet groups compared to the control group, suggesting that dietary interventions rich in healthy fats may promote fat oxidation and reduce the risk of cardiovascular disease. Specifically, the hazard ratio for the primary endpoint (a composite of myocardial infarction, stroke, or cardiovascular death) was 0.70 (95% CI: 0.54-0.92) for the EVOO group and 0.72 (95% CI: 0.54-0.96) for the mixed nuts group, indicating a 30% reduction in cardiovascular events.

Summary

Cottonseed oil stands out as a unique source of omega-6 PUFAs, notably linoleic acid, with promising implications for metabolic health. Its bioactive compound, DHSA, functions by inhibiting stearoyl-CoA desaturase-1, suggesting a potential role in managing cardiovascular health through improved lipid profiles, modulation of inflammation, oxidative stress, and promotion of fat oxidation, which contributes to metabolic efficiency and may impact endurance performance. Previous studies have also demonstrated the positive effects of CSO on lipid profiles by reducing total cholesterol and LDL levels, further supporting its potential health benefit.[8-9,14]Additionally, insights from research, such as Shaw et al. (2019), highlight how high-fat diets can enhance fat oxidation and potentially improve endurance performance, underlining the importance of a balanced approach to dietary fat intake for bettering overall health. Previous research has not investigated the impact of CSO on fat oxidation during submaximal exercise in humans. Exploring its effects on metabolism could potentially pave the way for advancements in disease prevention and management.

CHAPTER THREE

THE IMPACT OF COTTONSEED OIL ON FAT OXIDATION COMPARED TO OLIVE OIL IN A HEALTHY POPULATION

Contribution of Authors and Co-Authors

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Manuscript Information

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Abstract

Impaired fat oxidation is common in chronic diseases such as obesity and cardiovascular disease. Nutrition interventions aimed at enhancing fat oxidation capacity may help prevent disease progression. Dihydrosterculic acid (DHSA), a bioactive component of cottonseed oil (CSO), has been shown to increase fat oxidation in mice, but its effects in humans are unclear. This study aimed to assess the impact of a diet rich in CSO or olive oil (OO), at doses of 30g or 60g per day for 28 days, on fat oxidation during a 30-minute steady-state exercise bout in a fasted state. Forty-seven healthy adults (BMI < 27 kg/m^2, ages 18-55) performed a 30-minute exercise test at 75% of relative VO_2 max, pre- and post-intervention. Participants were randomized to receive daily doses of 30g or 60g of either CSO or OO. Using indirect calorimetry at 10-minute intervals, fat oxidation in kilocalories per minute was calculated and summarized as area under the curve (AUC). ANOVA assessed differences between groups. No significant changes in fat oxidation (kcal/22 mins) were observed among groups ($p=0.43$). However, combining CSO doses showed a trend toward increased fat oxidation compared to OO (CSO: 5.89 ± 4.89 kcal/22 mins; OO: -7.71 ± 7.32 kcal/22 mins; $p=0.13$). Significant differences were noted between sexes: females showed an increase (8.69 ± 4.28 kcal/22 mins), while males showed a decrease (-9.71 ± 7.25 kcal/22 mins; $p=0.04$). Change in fat percentage showed no significant differences ($p=0.98$). These findings suggest CSO may enhance fat oxidation in humans, warranting further research on its mechanisms and long-term effects on metabolic health.

Introduction

Impaired fat oxidation is prevalent in individuals with chronic diseases such as obesity, type 2 diabetes, and cardiovascular disease, highlighting the urgent need for interventions that enhance fat oxidation capacity to delay disease progression and prevent onset.[1,2] Enhanced fat oxidation correlates with improved metabolic efficiency, greater insulin sensitivity, and regulated lipid levels, thereby reducing the risk of these chronic diseases.[1,8] Individuals with impaired fat oxidation face heightened risks of developing obesity, type 2 diabetes, and cardiovascular disease due to dysregulated energy metabolism and compromised lipid regulation.[1,9] Strategies aimed at enhancing fat oxidation represent a promising approach to mitigate the severity and progression of these conditions. By improving overall health outcomes and reducing the incidence of chronic diseases, such interventions can lead to significant cost savings in healthcare expenditures and enhance the quality of life for affected individuals.[2,7] Considering this context, cottonseed oil (CSO), rich in polyunsaturated fatty acids (PUFAs) like linoleic acid, holds promise as a dietary intervention to enhance fat oxidation.

Despite CSO being a common ingredient in foods that are often considered unhealthy (e.g., chips, crackers, fried food, doughnuts), it has shown promise in improving lipid profiles and reducing cardiovascular risk.[3] Recent research has emphasized the significance of understanding the roles of dietary fats, particularly unsaturated fats like omega-6 PUFAs found in CSO, in metabolic health.[4] While traditionally considered solely proinflammatory, recent studies suggest that the effects of omega-6 PUFAs may be context-dependent, warranting deeper exploration into their dietary significance.[4] Furthermore, CSO has shown promise in managing lipid profiles and reducing cardiovascular risk, potentially through mechanisms such as the

inhibition of stearoyl-CoA desaturase-1 (SCD1) by its bioactive component dihydrosterulic acid (DHSA).[5] Animal studies suggest that CSO may increase fat oxidation, highlighting its potential in improving cardiovascular health.[6] However, the specific impact of CSO on fat oxidation in humans remains uncertain, necessitating further investigation.

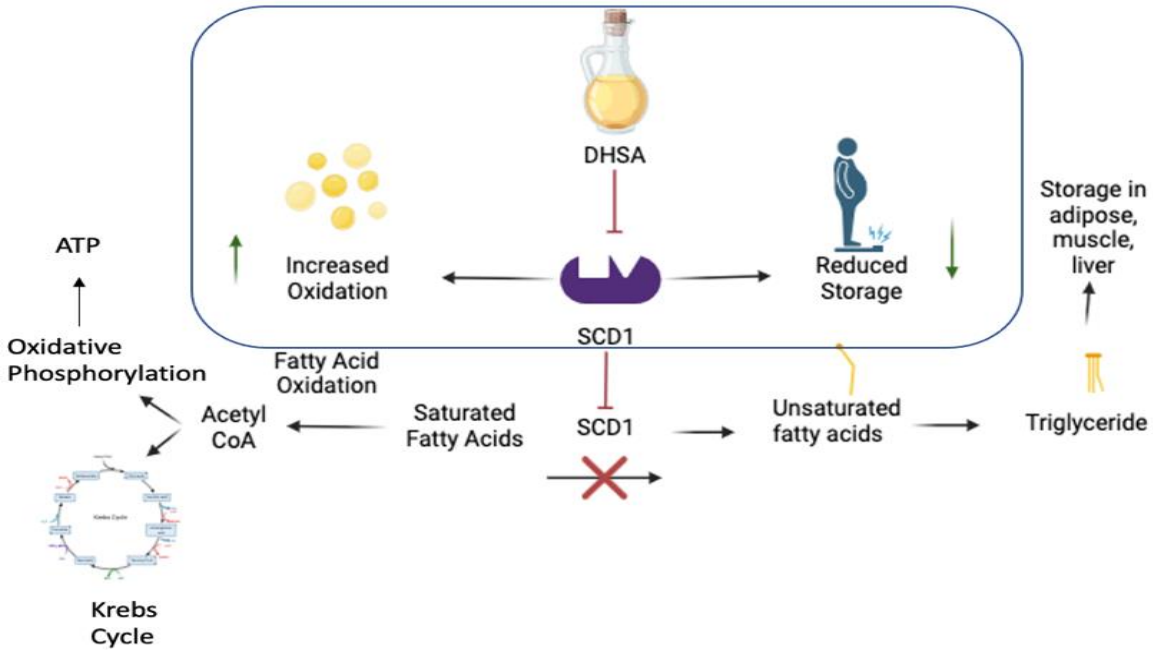

Figure 2: DHSA Mechanism. DHSA inhibits SCD1. Inhibition of SCD1 through DHSA enhances fat oxidation and reduces body fat storage by preventing the conversion of saturated fatty acids into unsaturated fatty acids. These unsaturated fatty acids typically undergo conversion into triglycerides, which are stored in adipose tissue, muscle, and the liver. With inhibition, saturated fatty acids are directed towards fatty acid oxidation, where they are converted into acetyl-CoA. Subsequently, acetyl-CoA enters either the Krebs cycle or oxidative phosphorylation pathways to produce ATP. DHSA; dihydrosterculic acid, SCD1; stearoyl-CoA desaturase 1, Acetyl-CoA; acetyl coenzyme A, ATP; adenosine triphosphate.

Examining the impact of CSO supplementation on fat oxidation during submaximal exercise provides a novel approach to investigating potential synergies or additive effects on metabolic health. Submaximal exercise is well-known for its ability to enhance fat oxidation and

promote metabolic flexibility, key elements in managing chronic conditions such as obesity, type 2 diabetes, and cardiovascular disease.[12] Investigating how CSO supplementation interacts with exercise offers an opportunity to understand its influence on metabolic flexibility and fat oxidation.[6,13] Using exercise to assess the impact of CSO on fat oxidation could lead to new strategies for mitigating the progression and severity of chronic diseases, offering potential benefits for individuals seeking to optimize their metabolic health.

Previous studies with CSO have utilized varying dosages, ranging from 30% [3] to 35%[10] of daily energy requirements, or specific amounts such as 95g[11] of CSO daily. However, a definitive dose-response correlation remains elusive, leaving both the upper and lower bounds of the impact of CSO is uncertain. Individual differences in metabolism, dietary behaviors, and health status may influence the efficacy of CSO supplementation. The USDA guidelines recommend daily consumption of oils ranging from 5 to 27 g. Dosages of both 30g./day and 60g./day can explore a range that aligns with dietary guidelines while also investigating increased benefits with a higher dosage. Further research is necessary to examine the effects and potential adverse outcomes linked to different CSO dosages, aiming to establish optimal lower and upper limits for CSO dosage and maximize its therapeutic potential in enhancing metabolic health.

Additionally, olive oil (OO), a widely used alternative rich in monounsaturated fatty acids, will be included as a comparison. Olive oil is well-documented for its beneficial effects on lipid profiles and cardiovascular health, and it is often recommended as a healthier dietary fat choice.[9-10] Comparing CSO and OO will provide insights into how different types of dietary oils impact metabolic health and fat oxidation. This comparison aims to guide dietary strategies for

chronic disease prevention and optimize the use of oils in promoting metabolic health. Through this research, we hope to advance our understanding of how CSO and OO contribute to fat oxidation and metabolic flexibility, ultimately informing dietary recommendations and interventions. Therefore, the purpose of this study is to determine the impact of a diet rich in either CSO or OO, consumed at dosages of 60g/day or 30g/day for 28 days, on fat oxidation during a 30-minute steady-state exercise bout in a fasted state. This research will help elucidate the effects of CSO on fat oxidation and contribute to the development of dietary strategies.

Methods

Population Characteristics

Apparently healthy adults between the ages of 18-55 years with a BMI < 27 kg/m^2 were recruited for the study. To be included, an individual had to have a healthy BMI between 18.5 kg/m^2 and 27 kg/m^2. The upper range of 27 kg/m^2 is chosen to encompass individuals who may still be healthy despite a higher BMI, recognizing that BMI alone does not account for variations in body composition, such as muscle mass. Although traditional guidelines define a healthy BMI as up to 24.9 kg/m^2, extending the upper limit to 27 kg/m^2 helps include those who maintain a healthy body fat percentage but have a higher BMI, while also considering research indicating increased mortality risk above this threshold. Participants were excluded from the study if they were taking medications to lower cholesterol, lipids, or inflammation, taking antioxidant supplements (Vitamin E or C or iron) at levels greater than 800 IU/day, had a gallbladder condition or had their gallbladder removed, took oral contraceptives regularly, smoked in the past 30 days, consumed alcohol in excess of 14 drinks per week, had an allergy to the study food, were pregnant or lactating, had been on a ketogenic or paleo diet in the past 6 weeks, were

planning to start a weight loss diet or change their exercise regimen, had diabetes, had physical mobility issues or were unable to walk on a treadmill, or had any other health conditions that would interfere with participation in the study.

This study was a double-blinded, randomized clinical trial with participants randomly assigned into 1 of 4 groups, either 30g/day or 60g/day dosage of either CSO or OO. Participants and researchers were blinded to group assignments. Block randomization was used to randomly assign participants into a group. Due to the double-blind study design, the participants were assigned to either red, blue, green, or yellow. Each of these colors corresponded to a different dosage (30g or 60g) and an oil (CSO or OO).

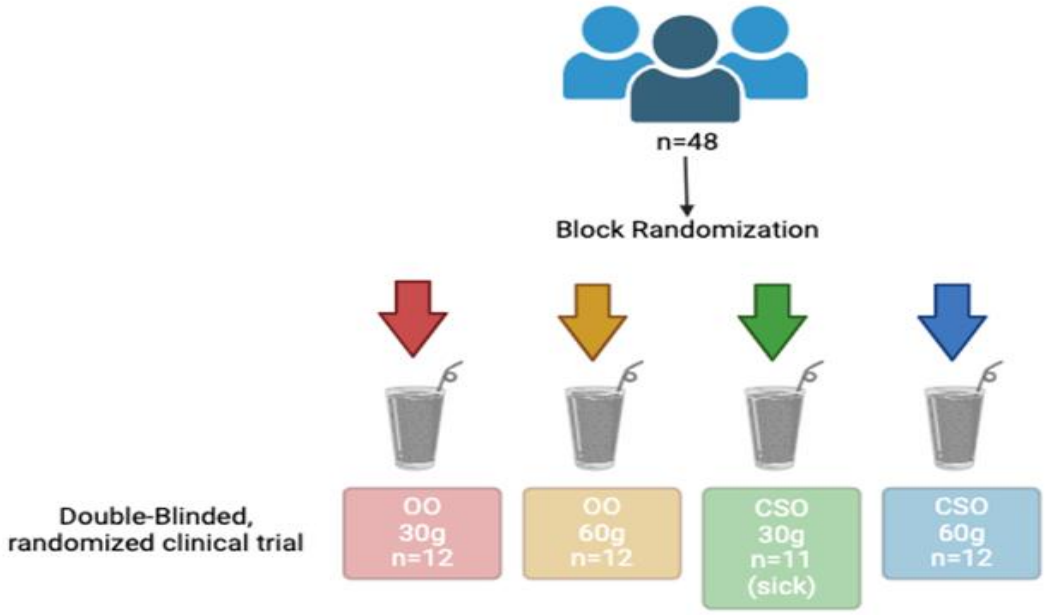

Figure 3: Block randomization of participants into each group.

Data collection occurred between August 2022 and October 2023, during which 12 participants per group, as predetermined, were recruited. This study included a 28-day dietary

intervention and three study visits including a screening visit (V1), a pre-diet visit (V2), and a post-diet visit (V3). The Institutional Review Board from Montana State University approved the study (IRB#2022-146), and written consent was obtained from all participants before beginning the study. Testing for participants took place in the Nutrition Research Lab at Montana State University. Subject consent and submaximal exercise test occurred during V1. Both V2 and V3 included a fasting blood collection, 30-minute exercise test, and a post-exercise blood collection. Participants completed a 28-day dietary intervention between V2 and V3. This trial was registered at clinicaltrials.gov as NCT05439590 on 6/30/22.

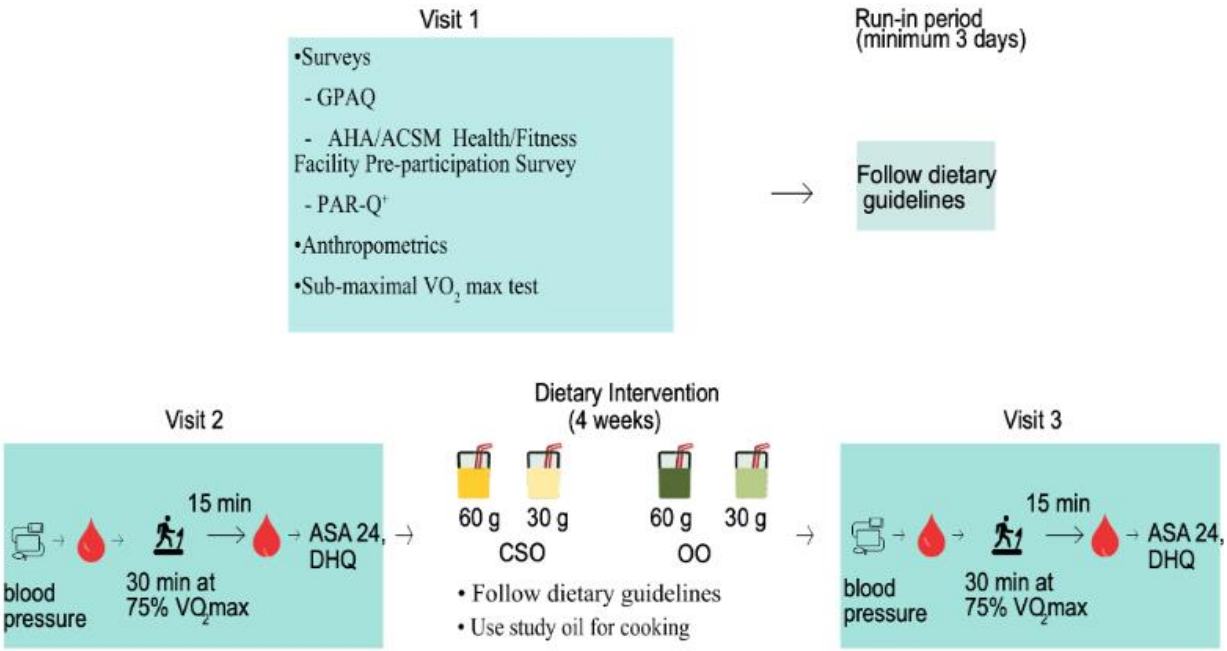

Figure 4: Study Design. Design credit: Prabina Bhattarai. GPAQ; general physical activity questionnaire, PAR-Q; physical activity readiness questionnaire, ASA 24; automated self-administered 24-Hour dietary assessment tool, DHQ; diet history questionnaire, OO; olive oil

Recruitment

Participants were recruited through email, social media advertisements flyers, newsletters, and word of mouth. Flyers were posted around the MSU campus and within approved locations in the community. Potential participants filled out a REDCAP study questionnaire to determine if they qualified for the study. The questionnaire included inclusion and exclusion criteria as well as contraindications for exercise.

Anthropometrics

All the anthropometrics were collected from participants at V1 and V3. Participants were told to come in fasted and to not have exercised in the three hours before testing. A segmental multi frequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany) was used to assess body weight, BMI, fat free mass, and fat mass. Waist circumference was measured using a standardized protocol to ensure accuracy and consistency. Participants were instructed to stand upright with their feet together and abdomen relaxed. A non-stretchable tape measure was placed around the bare abdomen at the level of the natural waist, which is typically located midway between the lowest rib and the top of the iliac crest. The measurement was recorded at the end of a normal expiration, with the tape snug but not compressing the skin. Measurements were taken three times, and the average value was used for analysis. Blood pressure was taken at the beginning of V2 and V3 after the participant had been seated for at least 15 mins. An automated measurement was taken in duplicate with the average used for data collection.

Submaximal Exercise Test

During V1, all participants completed a modified Bruce protocol on a treadmill to determine estimated VO2 max at their age-predicted maximum heart rate. The grade of the

treadmill (Woodway GmbH D-79576, Weil am Rhein, Denmark) was manually increased by 2% every stage, which lasted 2 minutes, until the participant reached 85% of their age-predicted maximal heart rate (APHR). The maximum grade on the treadmill was 14%, therefore, if participants had not met their 85% APHR, the speed was increased by 0.1 mph every 2 minutes until the 85% APHR was achieved. The modified Bruce Protocol used for this test can be found in Appendix A. Expired gasses were collected for analysis via a metabolic cart (ParvoMedics TrueMax 2400 Metabolic System, Sandy, Utah, USA). Heart rate data was collected during the test using the Polar Wearlink heart rate monitor (Kempele, Finland) and sent wirelessly to the metabolic cart. Heart rate (bpm) and VO2 (ml/kg/min) data from each participant were put into a linear regression model to predict VO2 max at the age-predicted maximal heart rate based on the equation presented by Tanaka, Monahan, and Seals. About 75% of VO2 max and corresponding HR was calculated to use for the exercise intensity for the second visit.

30-minute Exercise Test

During V2, participants completed a 30-minute exercise test of walking uphill at 75% of VO2 max, determined from submaximal exercise test, on the treadmill. Every 10 minutes of the exercise test, a 3-minute measurement on the metabolic cart was done to ensure the participant was exercising at 75% of VO2 max. Grade and speed of treadmill were adjusted to maintain 75% of participants predicted VO2 max. The grade and speed were written down for each minute on the exercise protocol sheet so that an identical exercise test could be performed during V3. The exact same protocol for the 30-minute exercise test was completed during V3.

Dietary Intervention

Participants began the diet intervention on the day of their V2 visit. They were asked to minimize or avoid fatty foods, ensuring that the only dietary fat they consumed came from the provided smoothies. The extra virgin olive oil (OO) used in the study was sourced from California Olive Ranch, while the cottonseed oil (CSO) was supplied by Cotton Incorporated. Each test oil underwent a fatty acid analysis. Participants were told to consume two doses of the smoothie per day for at least 28 days. The smoothies came in two flavors: chocolate and mango. Participants were randomly assigned to one of four smoothie groups, each containing either 30 grams of OO, 30 grams of CSO, 60 grams of OO, or 60 grams of CSO. Additionally, participants received a supplementary bottle of oil (either OO or CSO) based on their group assignment, which they used as a replacement for their regular cooking oil.

	Oil (g)	Bananas (g)	Mango extract (g)	Mangos (g)	Honey (g)	Chocolate powder (g)	Oat milk (g)	Weight (g)
60 g chocolate-smoothie	60	200	0	0	0	14	130	404
30 g chocolate-smoothie	30	289	0	0	60	21	130	530
60 g mango-smoothie	60	0	2	292	0	0	130	487
30 g mango-smoothie	30	0	2	310	80	0	130	555

Table 1: Ingredients in each smoothie.

Smoothies	Total energy (Kcal)	Total fat (g)	Total protein (g)	Total carb (g)	Total Dietary fiber (g)	Total MUFA (g)	Total MUFA (18:1) (g)	Total PUFA (18:2) (g)	Total PUFA (g)	Total ALA (g)	Total alpha-tocopherol (g)	N6:N3 (g)
60 g CSO mango	377.2	31	1.7	25.2	2.8	5.3	5.1	15.5	15.6	0.1	10.6	259.5
60 g CSO choc	403.3	31.4	2.7	30.5	3.6	5.3	5.1	15.5	15.6	0.1	10.6	259.5
60 g OO mango	372.4	31	1.7	25.2	2.8	20.7	20.3	2.52	2.7	0.2	0	13.9
60 g OO choc	398.5	31.4	2.7	30.5	3.6	20.8	20.3	2.52	2.7	0.2	0	13.9
30 g CSO mango	371.6	16	1.9	59.5	5.3	2.7	2.6	7.7	7.8	0.03	5.3	259.5
30 g CSO choc	414.1	16.9	3.8	68.6	5.1	2.7	2.6	7.7	7.8	0.03	5.3	259.5
30 g OO mango	369.1	16	1.9	59.5	5.3	10.4	10.2	1.3	1.4	0.1	0	13.9
30 g OO choc	411.7	16.9	3.8	68.6	5.1	10.4	10.2	1.3	1.4	0.1	0	13.9

Table 2: Macro- and micro- nutrient composition of smoothies.

Diet Adherence

Participants filled out a compliance form (found in appendix A) for how many smoothies they consumed per day. Participants came to the lab to pick-up smoothies either weekly or biweekly. Participants were asked to minimize or avoid consumption of foods with higher fat content from the screening visit to the post-diet visit. These foods included fried food, oil that was not given to them during the study, nuts, oil, seeds, fatty meat, sausages, fatty fish, cheese, full fat milk, eggs (yolk), butter, and omega-3 fatty acid supplements. If participants did consume these foods, they were asked to write them down in the smoothie compliance form mentioned previously. Participants were asked to maintain their habitual physical activity and habitual diet otherwise.

Diet History Questionnaire (DHQ):

The participants completed a habitual diet intake collected using the validated diet history questionnaire (DHQ III). The DHQ analyzes different food groups and amounts consumed from the previous month. Participants completed the online survey during visit 2 and visit 3. Questions addressed both dietary frequency and portion size. Total fat intake in gram amounts were analyzed for both pre and post dietary intervention.

Statistical Analysis

Statistical analyses were performed in RStudio (R version: 2023.12.1+402). Box plots were generated to visually inspect the distribution and potential outliers in the data, as presented in Figures 1 and 2. To compare the total kilocalories (kcals) burned and the average VO2 during the 30-minute exercise test pre- and post-intervention, paired t-tests were conducted. This test

was chosen because it allows for the comparison of two related samples, determining if there is a statistically significant difference in the means.

For the analysis of fat oxidation in kilocalories (kcals), fat oxidation rates were measured and summarized as area under the curve (AUC) at three specific time points: 8-10 minutes, 18-20 minutes, and 28-30 minutes. Given that data collection commenced at minute 8 and concluded at minute 30, the total duration of data collection is 22 minutes. Consequently, the fat oxidation is reported in kcals per 22 minutes. Delta change, representing the difference in fat burning between pre- and post-intervention tests, for each dietary group (CSO 60g, CSO 30g, OO 60g, and OO 30g) were calculated. The changes were then compared using repeated measures ANOVA to assess differences across the groups. This approach was used to account for within-subject variability and to determine if there were any significant effects of the dietary interventions. When combining the dosages, an independent t-test was used to compare the changes in AUC fat kcals between the combined CSO and OO groups.

To examine the effect of dosage on fat oxidation, the CSO and OO groups were combined into equal dosages of 30g and 60g. An independent t-test was again used to compare the changes between these two dosage groups. Potential sex differences in fat oxidation were explored by comparing the delta change in AUC fat kcals/22 mins between males and females using an independent t-test. To further analyze the percentage change in fat oxidation, the delta change in average fat (%) from pre- to post-intervention was compared across dietary groups using repeated measures ANOVA. Trends and differences were similarly explored for combined CSO and OO groups, different dosages, and between sexes. For all statistical tests, a p-value of less than 0.05 was considered statistically significant. Trends (p-values between 0.5 and 0.05

were noted to highlight potential differences. All statistical results, including means, standard deviations, and p-values, are reported in the corresponding figures and tables.

Results:

Subjects:

A total of 47 healthy participants completed the full study. These individuals were randomized to one of the meal interventions. There were 15 subjects who withdrew from the study following visit two. A total of 47 apparently healthy individuals (mean ± SD); age (29.9 ± 11.06 years); BMI (23.03 ± 1.95 kg/m^2) completed the 28-day intervention. A total of 25 of the 47 participants completed one to two days extra of the intervention smoothies due to scheduling issues on day 28 of the dietary intervention.

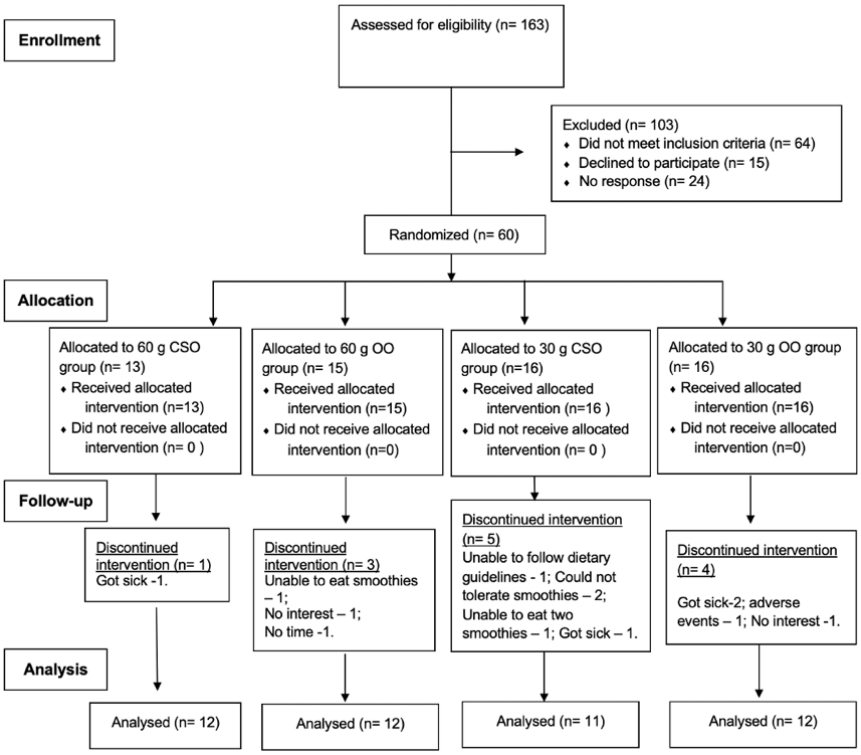

Figure 5: A consort diagram of the study design and enrollment. One hundred and sixty-three individuals completed the initial eligibility screening. Sixty individuals, who met the requirements were randomly assigned to 60g CSO, 60g OO, 30g CSO, or 30g of OO experimental groups. CSO, Cottonseed oil and OO, Olive oil

	Dietary oil	Treatment	Sex (F/M)	Age (years)	Height (cm)	Weight (kg)	Fat free mass (kg)	Fat mass (kg)	Waist circumference (cm)	VO_2Max (ml/kg/min)
Pre-Intervention	Cotton Seed Oil	60 g	7/5	29.9±12.3	172±7.40	66.1±8.69	52.5±9.18	13.5±5.5	76.6±7.3	47.8±9.90
		30 g	3/9	30.6±12.1	175±9.60	71.6±12.3	53.3±11.3	18.3±3.73	79.9±8.89	41.2±8.30
	Olive Oil	60 g	5/7	30.4±11.5	173±9.29	68.5±8.0	51.4±8.54	17.1±6.55	77.8±8.26	45.0±12.2
		30 g	9/3	34.9±12.9	176±8.25	74.0±9.50	60.4±8.93	13.6±2.95	78.7±7.0	53.3±7.50
Post Intervention	Cotton Seed Oil	60 g	7/5	29.9±12.3	172±7.40	66.3±9.30	52.5±9.48	13.9±5.35	72.3±15.5	47.8±9.90
		30 g	3/9	30.6±12.1	175±9.6	71.7±12.6	53.3±11.4	18.5±3.74	72.6±15.7	41.2±8.30
	Olive Oil	60 g	5/7	30.4±11.5	173±9.29	68.9±7.70	51.2±8.20	17.7±6.40	70.9±18.7	45.0±12.2
		30 g	9/3	34.9±12.9	176±8.25	75.2±9.30	60.1±8.97	13.8±3.45	70.7±19.4	53.3±7.50

Table 3: Participant characteristics pre and post dietary intervention (n=47). Grouped by assigned dietary intervention. BMI, body mass index.

	Dietary oil	Treatment	Sex (F/M)	Crossover Point (%)	VO2 (ml/kg/min)	HR (bpm)	RER (units)	Avg fat (%)	Avg CHO (%)	Avg fat (kcals/min)	Avg CHO (kcals/min)
Pre Intervention	Cotton Seed Oil	60 g	7/5	52.74±15.58	35.0±7.45	159±11.3	0.90±0.04	21.12±16.71	50.15± 32.72	3.38± 2.04	8.02± 2.99
		30 g	3/9	51.97±14.19	28.99±3.20	156±9.8	0.895±0.04	24.22±19.41	43.47±30.45	3.36±1.07	7.01±2.54
	Olive Oil	60 g	5/7	51.91±15.08	32.11±9.60	160±13.7	0.907±0.04	19.30±17.59	40.84±32.84	3.53± 2.34	7.47±2.43
		30 g	9/3	51.52±8.07	39.5±6.37	154±12.6	0.906±0.02	21.53±13.51	50.75±29.56	4.16± 1.29	10.35±2.27
Post Intervention	Cotton Seed Oil	60 g	7/5	NA	34.8±6.87	151±30.6	0.906±0.05	19.07±16.39	50.15±32.72	3.68± 1.87	7.72±3.52
		30 g	3/9	NA	28.72±4.69	153± 11.6	0.888±0.03	24.81±19.5	43.47± 30.45	3.60±1.24	9.45±9.88
	Olive Oil	60 g	5/7	NA	32.64±6.95	154±14.2	0.898±0.05	14.96±16.35	40.84±32.84	3.49±1.71	7.56±2.66
		30 g	9/3	NA	38.7±6.44	151±10.9	0.917±0.03	21.66±13.70	50.75±29.56	3.58± 0.78	10.6±3.23

Table 4: Participant exercise characteristics pre and post dietary intervention, grouped by assigned dietary intervention (n=47). RPE; rate of perceived exertion, HR; heart rate, RER; respiratory exchange ratio, Avg fat; average fat, Avg CHO; average carb.

Total Kcals and VO2 for 30-min
Exercise Test Pre and Post Intervention

The exercise test before and after the intervention was conducted with the same speed and incline for each participant, ensuring the intensity of the exercise was consistent. Results showed similar calories burned during both the pre- and post-intervention tests (PRE: 104.29 ± 4.89 kcals, POST: 102.34 ± 4.73 kcals, p = 0.77), indicating that the exercise intensity remained constant. Additionally, there was no significant difference in VO2 between the pre- and post-intervention tests (PRE: 33.89 ± 0.95 ml/kg/min, POST: 33.86 ± 0.9 ml/kg/min, p = 0.1), suggesting the same effort levels throughout both exercise tests. These findings suggest that the exercise test maintained the same intensity before and after the intervention, supporting the study methods reliability.

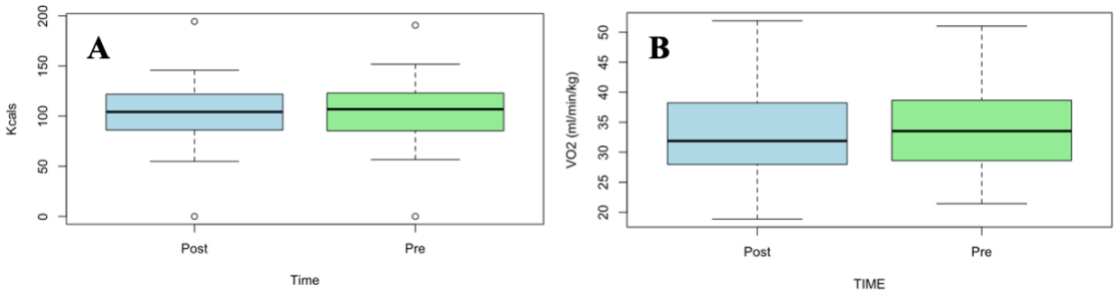

Figure 6: Box plots comparing pre and post total kcals and average VO2. A: Total kcals burned during 30-min exercise test pre and post intervention. B: Average VO2 during 30-min exercise test pre and post intervention. No differences between pre and post for either total kcals or average VO2 ($p>0.05$). Pre, Pre intervention and Post, Post intervention.

Diet History Questionnaire (DHQ)

Comparing Fat Intake by Group:

An ANOVA indicated a significant effect of treatment on change in total fat intake among the groups (CSO 30g, CSO 60g, OO 60g, and OO 30g, p=0.000113). Post hoc Tukey HSD tests showed that the change in total fat intake was significantly higher in the CSO 60g group (35.66 ± 8.09 g) compared to the CSO 30g group (1.38 ± 7.48 g), with a mean difference of 34.28 g (p=0.0242), and higher in the OO 60g group (35.55 ± 7.79 g) compared to the CSO 30g group, with a mean difference of 34.17 g (p=0.0247). No significant difference was found between the OO 30g group (-10.85 ± 8.73 g) and the CSO 30g group, with a mean difference of -12.23 g (p=0.78). The OO 30g group had significantly lower change in total fat compared to both the CSO 60g group, with a mean difference of -46.50 g (p=0.0009), and the OO 60g group, with a mean difference of -46.40 g During the intervention, fat intake, measured as a percentage of total caloric intake from fat, varied significantly across the different groups. The group receiving CSO 30g had an average fat intake of 31.02% ± 1.88%. This value is below the 35% threshold, which is commonly used to indicate a high-fat diet. In contrast, the group consuming CSO 60g exhibited a markedly higher average fat intake of 46.86% ± 3.18%. The olive oil groups demonstrated even greater fat intake: OO 60g had an average fat intake of 49.80% ± 3.19%, while OO 30g had an average of 35.24% ± 2.33%. Both OO groups exceeded the 35% threshold for a high-fat diet, with the OO 60g group having the highest percentage of calories from fat. (p=0.0010). No significant difference was observed between the OO 60g and CSO 60g groups, with a mean difference of -0.10 g (p=1.0000). These findings indicate that both the CSO 60g and OO 60g treatments significantly increased change in total fat intake compared to the CSO 30g

treatment, while the OO 30g treatment significantly decreased change in total fat intake compared to the CSO 60g and OO 60g treatments.

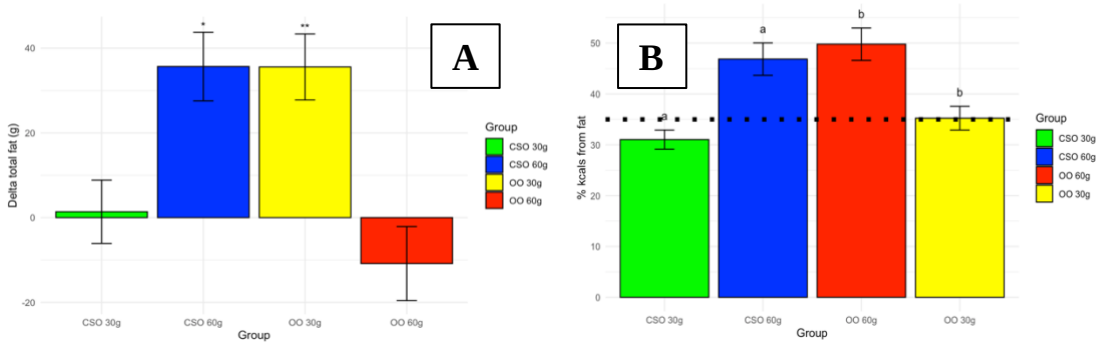

Figure 7: Fat intake during the study by group. A: Change in total fat (g) intake from pre to post intervention. *= p<0.05, **= p<0.01. B: Percent of calories from fat intake during the intervention. Anything above the black line indicates a high fat diet, with fat intake exceeding 35%. a=p<0.01 , b=p<0.01 . 60g; Olive Oil 60g dose group, OO 30g; Olive Oil 30g dose group, CSO 60g; Cotton Seed Oil 60g, CSO 30g; Cotton Seed Oil 30g.

Fat Oxidation (kcals) Comparing CSO 60g,
CSO 30g, OO 60g, and OO 30g:

In the 30-minute exercise test, there were no significant differences observed in the change in AUC of fat kcals/ 22 mins from pre to post intervention across the dietary groups. The change in AUC average of fat kcals/ 22 mins for OO 30g was -11.97 ± 9.29 kcals/ 22 mins, for CSO 60g it was 6.83 ± 7.63 kcals/ 22 mins, for OO 60g it was -3.07 ± 11.78 kcals/ 22 mins, and for CSO 30g it was 4.87 ± 6.32 kcals/22 mins (p = 0.43). Both CSO dosages are slightly increased and OO dosages are decreased.

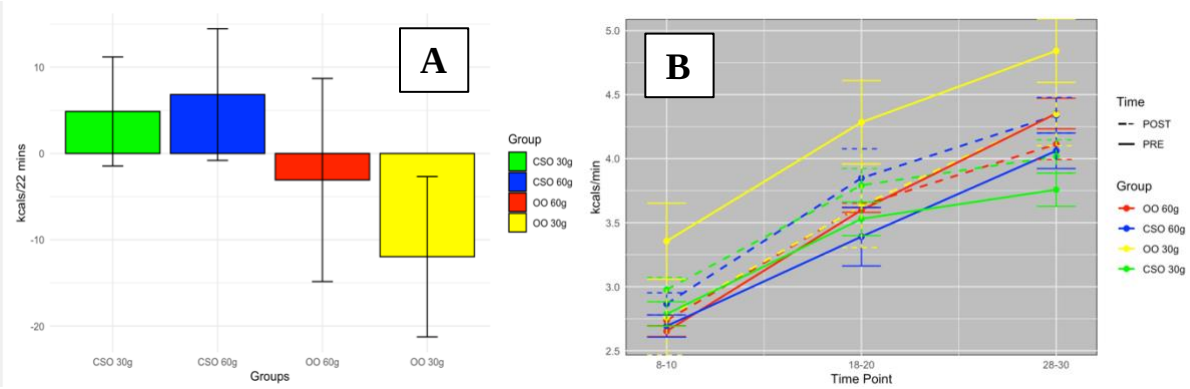

Figure 8: Change in fat kcals/22 mins from pre to post intervention. A: Change in AUC for fat kcals/22 mins from pre to post dietary interventions for each dietary group. B: Pre and post dietary intervention fat kcals/min during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins. OO 60g; Olive Oil 60g dose group, OO 30g; Olive Oil 30g dose group, CSO 60g; Cotton Seed Oil 60g, CSO 30g; Cotton Seed Oil 30g.

Fat Oxidation (kcals) Comparing CSO and OO:

When both dosage groups were combined, CSO had a non-significant trend of a potential increase in change AUC fat kcals/22 mins from pre to post intervention compared to OO which had a potential decrease in change (CSO: 5.89 ± 4.89 kcals/ 22 mins, OO: -7.71 ± 7.32 kcals/ 22 mins, p=0.13). This potential can be explored further in future research.

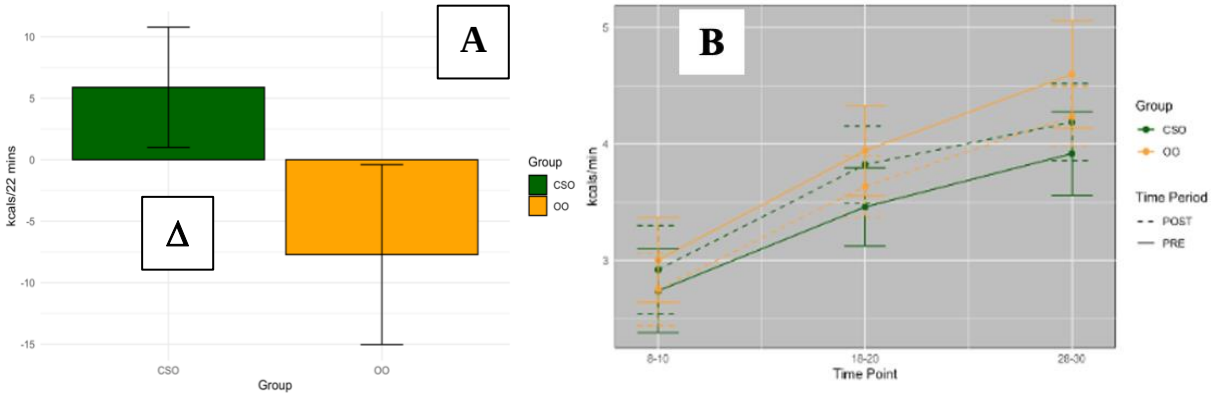

Figure 9: Change in fat kcals/22 mins from pre to post intervention in CSO and OO. A: Change in AUC for fat kcals/22 mins from pre to post dietary interventions for CSO and OO. B: Pre and post dietary intervention fat kcals/min during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins. OO, olive oil and CSO, cottonseed oil. ∇ = trend, p=0.13.

Fat Oxidation (kcals) Comparing 30g and 60g:

To look at the effect of dosage on fat oxidation the two different oils groups were combined into equal dosages. There were no differences found between 30g dosage and 60g dosage (p=0.51). The 60g dosage had a minimal increase in change AUC fat kcals/ 22 mins while 30g dosage had a minimal decrease (60g: 2.09 ± 6.81 kcals/ 22 mins, 30g: -3.91 ± 5.87).

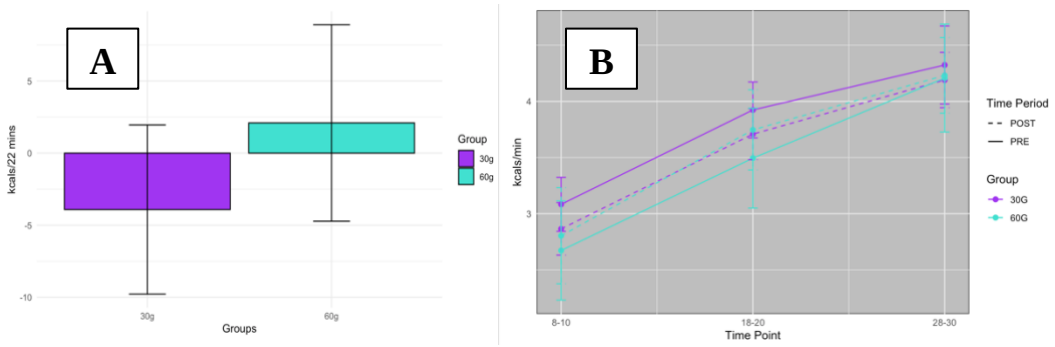

Figure 10: Change in fat kcals/22 mins from pre to post intervention in 30g and 60g.A: Change in AUC for fat kcals/22 mins from pre to post dietary interventions for 30g and 60g. B: Pre and post dietary intervention fat kcals/min during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins. 30g, 30g dosage including both CSO 30g and OO 30g and 60g, 60g dosage including both CSO 60g and OO 60g.

Sex Differences in Fat Oxidation (kcals):

We examined the change in fat oxidation between sexes for both pre and post intervention to determine if sex impacted fat oxidation. There was a significant difference between sexes ($p=0.04$) in change AUC fat kcals/ 22 mins with an increase in change in AUC of 8.69 kcals/ 22 mins ± 4.28 for females and a decrease in change in AUC of -9.71 ± 7.25 kcals/22 mins for males.

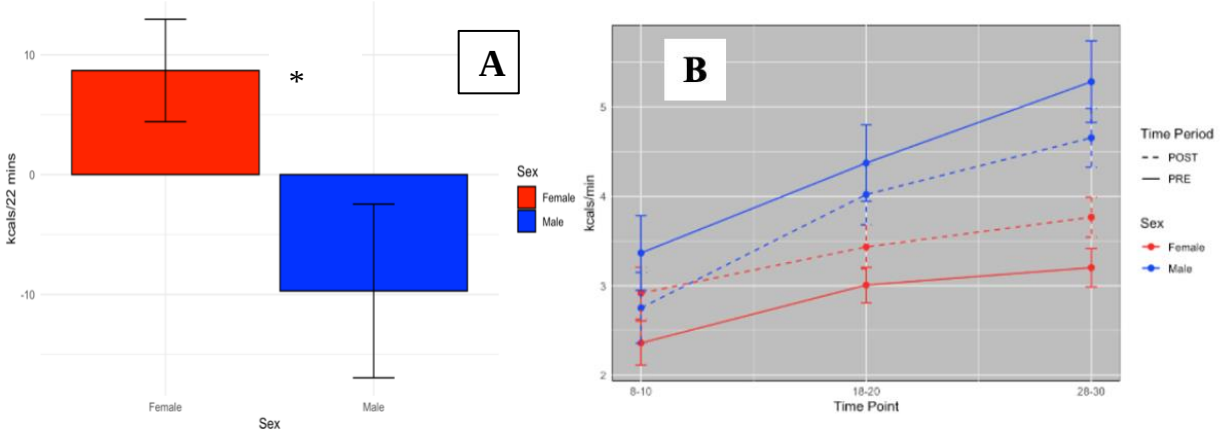

Figure 11: Change in fat kcals/22 mins in males vs females from pre to post intervention. A: Change in AUC for fat kcals/22 mins from pre to post dietary interventions for males and females. B: Pre and post dietary intervention fat kcals/min during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins. *= significant difference between groups, p=0.04.

Fat Oxidation (%) Comparing CSO 60g,
CSO 30g, OO 60g, and OO 30g:

There were no differences found between dietary groups in delta change of average fat (%) from pre to post intervention (p= 0.85). The change of average of fat (%) for OO 30g was -2.02 ± 3.37 %, for CSO 60g it was -0.16 ± 3.28 %, for OO 60g it was 2.55 ± 4.45 %, and for CSO 30g it was 0.70 ± 3.48 %.

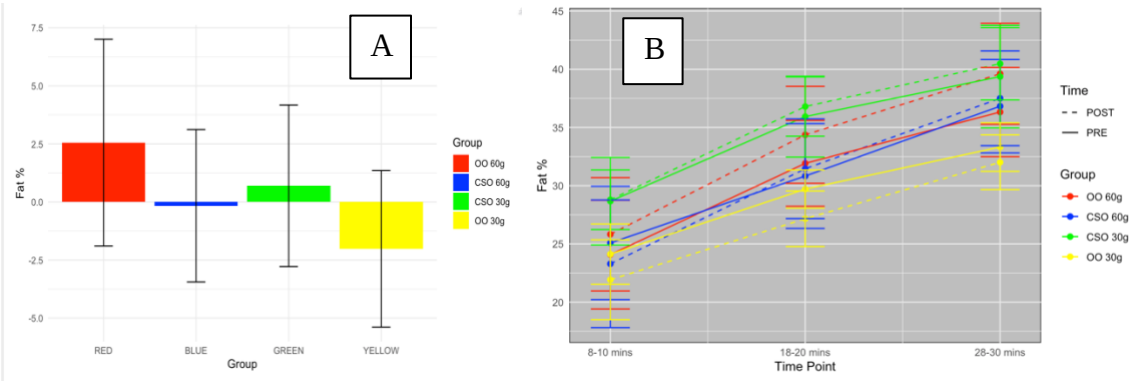

Figure 12: Change in fat (%) from pre to post intervention in all groups. A: Change in fat (%) from pre to post dietary interventions for each dietary group. B: Pre and post dietary intervention fat (%) during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins. OO 60g; Olive Oil 60g dose group, OO 30g; Olive Oil 30g dose group, CSO 60g; Cotton Seed Oil 60g, CSO 30g; Cotton Seed Oil 30g.

Fat Oxidation (%) Comparing CSO and OO:

There were no differences in CSO or OO fat % change from pre to post intervention (p=0.98). When both dosage groups were combined, both CSO and OO had a minimal increase in change of fat (%) from pre to post intervention (0.25± 2.33 % vs 0.17 ± 2.75 %).

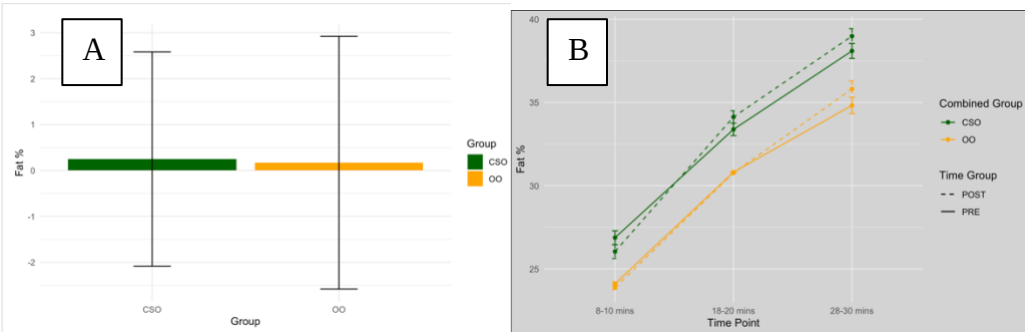

Figure 13: Change in fat (%) from pre to post intervention in CSO and OO.A: Change in fat % from pre to post dietary interventions for CSO and OO. B: Pre and post dietary intervention fat kcals/min during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins. OO, olive oil and CSO, cottonseed oil.

Fat Oxidation (%) Comparing 30g and 60g:

There were no differences found between 30g dosage and 60g dosage (p=0.85). The 60g dosage had a minimal increase in delta change for fat (%) while 30g dosage had a minimal decrease (60g: 1.14 ± 2.70 %, 30g: -0.72 ± 2.38).

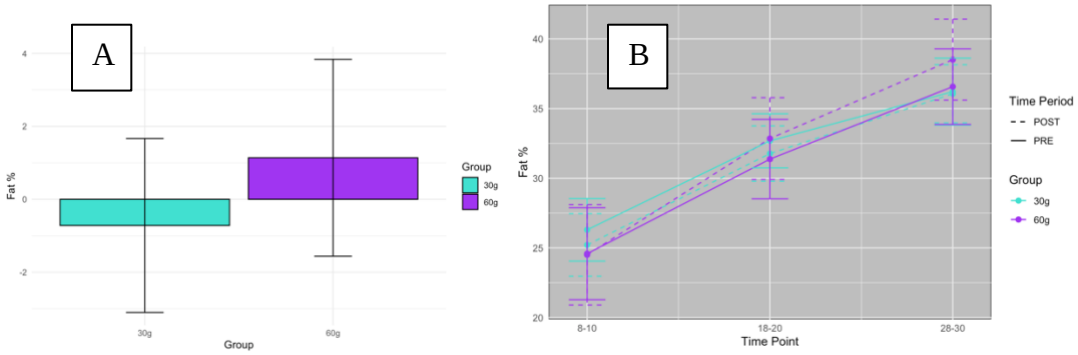

Figure 14: Change in fat (%) from pre to post intervention in 30g and 60g. A: Change in fat (%) from pre to post dietary intervention for 30g and 60g. B: Pre and post dietary intervention fat (%) during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins. 30g, 30g dosage including both CSO 30g and OO 30g and 60g, 60g dosage including both CSO 60g and OO 60g.

Sex Differences in Fat Oxidation (%):

There were no differences between sexes in change for fat (%) for females and males (1.53 ± 2.87 % vs -1.29 ± 3.29%, p=0.26).

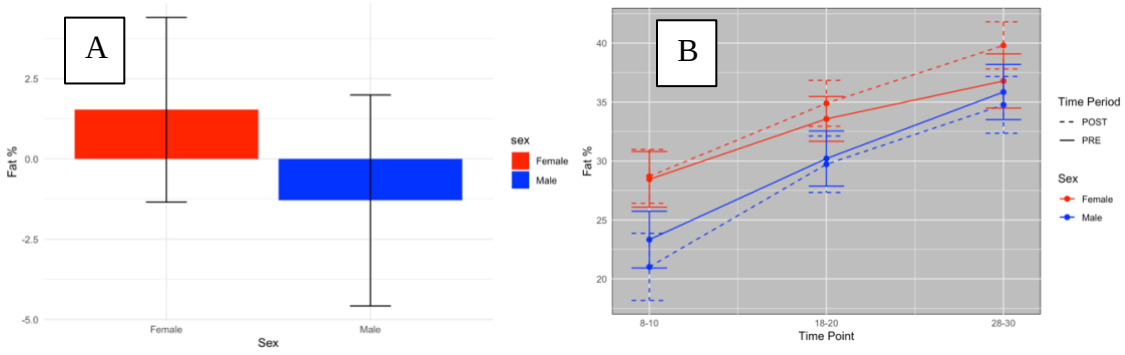

Figure 14: Change in fat (%) in males and females from pre to post intervention. A: Change in fat (%) from pre to post dietary interventions for males and females. B: Pre and post dietary intervention fat (%) during the 30-min exercise test at time points: 8-10 mins, 18-20 mins, 28-30 mins.

Discussion

The purpose of this study was to determine the impact of a diet rich in either CSO or OO, consumed at dosages of 60g or 30g per day for a duration of 28 days, on fat oxidation during a 30-minute steady-state exercise bout in a fasted state. It was hypothesized that fat oxidation (kcals/min) would be greater in the CSO groups than the OO groups after the dietary intervention. It was also hypothesized that the larger dose (60g/day) will have greater fat oxidation than the smaller dose (30g/day). Delta change from PRE to POST was used to determine the difference in fat oxidation between the dietary groups. This is significant because it addresses the potential metabolic benefits of CSO, which is rich in PUFAs, in contrast to OO. The study also explores potential sex-specific responses to CSO supplementation and discusses its mechanistic pathways related to fat oxidation. These aspects emphasize the relevance of the research to understanding dietary interventions for enhancing metabolic health and potentially preventing metabolic disorders. While previous studies have explored the effects of different dietary fats on metabolic health in animals and humans, the specific impact of CSO on fat

oxidation during exercise in healthy individuals had not been thoroughly investigated before this study. By addressing this gap, the study extends the existing literature and provides insights into the metabolic effects of CSO, thereby contributing to a better understanding of how dietary fats influence metabolic pathways in humans.

Cottonseed oil showed a trend towards enhancing fat oxidation during exercise. This aligns with previous research on PUFAs and their potential metabolic benefits. The study also highlights the mechanistic pathways through which CSO, particularly its PUFA content and specific compounds like DHSA, may influence fat oxidation, providing context for its observed effects.[24] This finding extends the insights from previous animal studies, which suggested potential metabolic benefits of CSO, particularly in enhancing fat oxidation in mice.[6] In human participants, research has demonstrated that diets high in PUFAs, like those found in CSO, can increase fat oxidation rates during exercise.[21] For instance, a study found that a diet enriched with PUFAs led to higher fat oxidation and improved metabolic flexibility in endurance athletes.[26] Another study by Smith and colleagues showed that PUFA supplementation increased the rate of fat oxidation during moderate-intensity exercise in recreationally active adults.[27] Additionally, research by Jones and colleagues indicated that higher dietary intake of PUFAs was associated with increased basal fat oxidation and lower respiratory quotient in physically active individuals.[28] The presence of specific compounds like DHSA in CSO has been shown to activate PPARα, a nuclear receptor that regulates genes involved in fatty acid oxidation.[21] This mechanism is supported by findings from a study, that demonstrated that DHSA supplementation in humans enhanced the expression of PPARα target genes, leading to increased fat oxidation during exercise.[29] Similarly, a review by Li and colleagues discussed the role of PUFAs in

modulating lipid metabolism through PPARα activation, emphasizing the potential of dietary PUFAs to enhance fat oxidation.[30] This study uniquely explores the impact of CSO on fat oxidation during exercise in human participants, thereby contributing to a better understanding of how dietary fats influence metabolic pathways in humans. This is consistent with emerging evidence suggesting that the fatty acid composition of oils, particularly those rich in polyunsaturated fatty acids like CSO, can significantly influence metabolic responses relevant to obesity and cardiovascular health. For instance, a meta-analysis concluded that PUFA-rich diets are associated with enhanced fat oxidation and improved metabolic health markers across diverse populations.[31] Additionally, a study reported that individuals consuming higher amounts of linoleic acid, a key PUFA in CSO, exhibited greater fat oxidation rates and improved exercise performance.[32]

There was no significant difference in the effects on fat oxidation for the 30g/day and 60g/day doses, suggesting that lower doses might be as effective as higher doses in promoting fat metabolism during exercise. There may be a ceiling effect playing a role as well. These finding contrasts with some hypotheses that higher doses might yield greater metabolic benefits but aligns with practical considerations of optimizing dosage for efficacy and safety in dietary interventions.[11] This is supported by another study, which found that moderate doses of PUFAs were sufficient to elicit significant metabolic benefits without the adverse effects sometimes associated with higher doses including gastrointestinal issues.[15]

The lack of a dose-response increase in fat oxidation may be due to a ceiling effect with CSO supplementation, which is one possible explanation for the observed lack of response. Beyond a certain threshold, 30g per day in this case, increasing the dosage may not add any

metabolic benefits in terms of enhancing fat oxidation during exercise. A study found that omega-3 PUFA supplementation improved fat oxidation in athletes, the effects plateaued at a certain dosage, suggesting a similar ceiling effect as observed in the present study.[25] The mechanisms underlying the dose-response relationship of CSO on fat oxidation likely involve the activation of PPARs by PUFAs which play a crucial role in fat oxidation.

Regarding the dietary intervention being a high fat diet, results indicate that participants consuming 60 grams of either CSO or OO were on a high-fat diet, while those consuming 30 grams of the same oils had fat intake levels that did not classify their diets as high-fat. According to the Acceptable Macronutrient Distribution Range (AMDR), which recommends that fat constitute 20% to 35% of total caloric intake, the higher fat intake levels observed in the 60-gram groups exceed this upper limit, categorizing their diets as high-fat.[76] In contrast, the fat intake levels in the 30-gram groups were below or just at this threshold, suggesting these diets did not reach the high-fat classification. The metabolic response to higher fat intake involves adaptations that enhance the ability of the body to oxidize fat more efficiently, which can influence overall metabolic health and lipid metabolism.[75] These adaptations may lead to improved metabolic efficiency in processing dietary fat. However, it is crucial to consider these effects within the broader context of overall dietary patterns and the duration of high-fat intake. Long-term adherence to high-fat diets may have implications for cardiovascular health and metabolic function, potentially impacting lipid profiles and overall health outcomes. Therefore, while increased fat intake can enhance fat oxidation, the long-term effects and health implications of sustained high-fat diets warrant further investigation. Future research should explore how

varying levels of dietary fat impact fat oxidation and overall health to provide clearer insights into the metabolic consequences of different fat intake levels.

Interestingly, sex differences observed in fat oxidation trends, with females showing increased delta change in fat kcal/22 mins compared to males, suggest a potential sex-specific response to CSO supplementation. This finding is present in existing literature indicating that sex hormones and metabolic responses to dietary interventions may interact in complex ways.[16] For example, a study highlighted that estrogen could enhance lipid oxidation during exercise, which might explain the effects observed in female participants in our study even though menstrual cycle and hormones associated with it were controlled for in this study.[17]

Our findings show a trend towards increased fat oxidation with CSO supplementation, which aligns with several previous studies on polyunsaturated fatty acids (PUFAs). For instance, a study found that supplementation with omega-3 fatty acids, another type of PUFA, significantly enhanced fat oxidation during exercise in athletes.[18] This similarity suggests that the fatty acid profile of CSO, which is rich in PUFAs, might play a crucial role in its potential metabolic benefits. Conversely, our results differ from studies examining saturated fats. For example, a study indicated that saturated fat consumption did not significantly impact fat oxidation rates during exercise.[19] This disparity highlights the importance of fatty acid composition in dietary oils and their metabolic effects.

The observed effects of CSO on fat oxidation could be attributed to several mechanisms. First, CSO is rich in PUFAs, particularly linoleic acid, which has been shown to enhance fat metabolism by modulating enzymes involved in fat oxidation.[20] PUFAs are known to activate peroxisome proliferator-activated receptors (PPARs), which play a key role in lipid

metabolism.[21] Second, PUFAs have been linked to improved insulin sensitivity.[22] Enhanced insulin sensitivity can facilitate greater fat oxidation during exercise, as insulin plays a crucial role in regulating energy substrate utilization. Third, studies suggest that PUFAs can improve mitochondrial function and biogenesis.[23] Enhanced mitochondrial capacity can increase the ability of the body to oxidize fat during prolonged exercise. DHSA has been identified as a compound in CSO that can increase fat oxidation. DHSA has been shown to inhibit stearoyl-CoA desaturase-1 (SCD1), an enzyme involved in lipid synthesis and storage. By inhibiting SCD1, DHSA reduces the synthesis of monounsaturated fats, promoting the oxidation of stored fats.[24] It can enhance the expression of genes involved in mitochondrial biogenesis and function, thereby improving the efficiency of mitochondrial oxidative pathways.[16] This dual action of DHSA on inhibiting lipid synthesis and enhancing mitochondrial function shows its potential as a metabolic enhancer for increasing fat oxidation.

It is important to note that our study was conducted with healthy individuals, and the results may not be the same in at-risk clinical populations. Individuals with metabolic conditions such as obesity, diabetes, or cardiovascular diseases might respond differently to CSO supplementation. Their altered metabolic states and associated comorbidities could influence the efficacy and safety of CSO in enhancing fat oxidation. For instance, a study found that omega-3 supplementation significantly improved fat oxidation in individuals with metabolic syndrome, suggesting that the benefits of PUFA-rich oils might be increased in those with metabolic disorders.[16]

Several limitations of this study should be considered when interpreting the findings. Firstly, the study was conducted with healthy individuals, and the results may not generalize to

populations with metabolic conditions such as obesity, cardiovascular disease, or diabetes. These groups might exhibit different responses to CSO supplementation due to their varying health statuses. Secondly, while efforts were made to control for confounding factors such as sex hormones, there may still be unmeasured variables influencing fat oxidation outcomes, such as genetic predispositions, dietary variations, sleep patterns, stress levels, and overall physical activity. Thirdly, the study duration of 28 days provides insights into short-term effects but does not capture long-term impacts or potential cumulative benefits of CSO supplementation. Additionally, the study design was not a crossover study, which limits the ability to compare directly within participants and could introduce variability due to inter-individual differences. Additionally, the sample size, while sufficient for detecting significant effects, may limit the ability to detect smaller differences or interactions that could be relevant in larger, more diverse populations. Future research addressing these limitations could provide a more comprehensive understanding of the potential benefits of CSO for diverse populations and over longer durations.

Based on the findings of this study, there are many different paths for future research to increase our understanding of the impact of CSO on fat oxidation and metabolic health. Longitudinal studies are warranted to look at the sustained effects of CSO over extended periods, elucidating whether initial benefits persist and any potential cumulative effects. Further exploration of dose-response relationships is crucial to determine the optimal CSO dosage that maximizes fat oxidation while considering individual metabolic variability. Mechanistic studies should delve into the underlying pathways through which CSO influences fat metabolism, including gene expression analyses. Comparative research with other polyunsaturated fatty acid-rich oils could provide insights into the relative efficacy of CSO in enhancing fat oxidation.

Ensuring the safety and tolerability of CSO supplementation across various populations and doses will be crucial for establishing evidence-based guidelines. Integrating CSO into comprehensive nutritional strategies aimed at improving metabolic health presents significant potential for future research efforts. Given the adverse effects associated with high fat intake and the concern that CSO is often presented in less healthy food products, it is possible that there were adverse effects not captured by the study design.

Additionally, the complexity of real-world dietary patterns means that CSO may be consumed alongside other components that could influence its overall impact. Factors such as the quality of the diet, the presence of other fats, and the form in which CSO is consumed might all affect its safety profile and health outcomes. Furthermore, since CSO is sometimes used in processed foods with high levels of other additives or unhealthy ingredients, it is important to consider these potential confounding factors. Therefore, further research is needed to evaluate the effects of CSO in more varied dietary contexts and to identify any potential interactions or cumulative effects that could emerge with long-term use. Ensuring a thorough assessment of these variables will be crucial in determining whether the benefits of CSO supplementation outweigh any potential risks.

Integrating CSO into comprehensive nutritional strategies aimed at improving metabolic health is a potential for future research efforts. By addressing these research priorities, future studies can build upon current findings to potentially guide effective strategies for disease prevention and management. Incorporating CSO into dietary strategies aimed at improving metabolic health could involve replacing less healthy fats with CSO in cooking or meal preparation. This can be part of a balanced diet that includes a variety of nutrient-dense foods

and emphasizes whole grains, lean proteins, fruits, and vegetables. Individuals interested in consuming CSO for its potential health benefits should consider moderate consumption within established dietary guidelines.

In conclusion, this study provides evidence that dietary supplementation with CSO can potentially enhance fat oxidation during steady-state exercise in healthy individuals. The lack of significant difference between the 30g and 60g dosages suggests that a moderate intake may be sufficient to achieve metabolic benefits. The study also highlights the importance of considering sex-specific responses to dietary interventions. Although CSO is commonly found in unhealthy processed foods, its potential health benefits have yet to be fully appreciated. This study emphasizes its role in promoting fat oxidation, suggesting that incorporating CSO into dietary strategies could be beneficial for metabolic health. However, more research is needed to fully understand the true influence of CSO on fat oxidation and its long-term impact on metabolic health. By recognizing the advantages of CSO, we can better appreciate its potential to contribute to disease prevention and overall health improvement. While the findings are promising, further research is needed to explore the long-term effects, optimal dosages, and the applicability of these results to populations with metabolic conditions.

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CHAPTER FOUR

CONCLUSION

The goal of this study was to investigate how a 28-day dietary intervention of either OO or CSO at dosages of 30g or 60g per day, impacted fat oxidation in a healthy population. This research study is critical as it lays the foundation for understanding whether CSO can enhance fat oxidation in humans, a critical aspect for both clinical and athletic populations. Improving fat oxidation is paramount for enhancing metabolic efficiency, which plays a key role in energy balance, weight management, and metabolic health.

Efficient metabolism enables the body to efficiently utilize substrates, promote energy balance by matching energy intake with energy expenditure. This helps an individual maintain a healthy body weight and avoid excessive weight gain or loss. This metabolic efficiency minimizes excess adipose tissue storage and optimizes nutrient utilization for energy production. For individuals with obesity and/or metabolic syndrome, enhancing metabolic efficiency can significantly impact energy utilization and fat storage reduction.

Furthermore, efficient metabolism facilitates the switch between carbohydrates and fats based on energy demands, supporting overall metabolic health. It also reduces the risk of insulin resistance and related metabolic disorders like type 2 diabetes. Optimal mitochondrial function, important for energy production and cellular respiration, is closely tied to efficient metabolism.

The cardiovascular benefits of efficient metabolism include mitigating risk factors associated with metabolic disorders such as dyslipidemia, hypertension, and inflammation. In clinical settings, improving metabolic efficiency aids in adapting to changes in energy demands and metabolic stressors, crucial for managing metabolic disorders. This makes it a needed key

focus in clinical populations for prevention, management, and treatment of metabolic disorders and related health conditions.

In the current study it was found that CSO did increase fat oxidation compared to OO with there being a trend between higher fat oxidation with the 60g/day dosage compared to the 30g/day dosage. This is the first study to look at increasing fat oxidation with CSO in humans and more research is necessary to make conclusions especially with clinical populations. Future studies should replicate this current study with a larger and more diverse population to further support the reliability of the results and increase the validity of the current study. Investigating this dietary intervention with a clinical population could allow for further findings regarding the impact of CSO on increasing fat oxidation and metabolic efficiency in a population with metabolic abnormalities.

Future research studies should look at performing a crossover study comparing the effect of both OO and CSO on each participant. Researching the impact of dietary intervention duration could also be useful to see if the duration of the intervention has an impact on fat oxidation. Different dosages could also be of interest in future research. Some of the participants in the current study had gastrointestinal issues potentially because of the dietary intervention. Perhaps different dosages could lessen the gastrointestinal issues and could potentially have different effects on fat oxidation. Different exercise intensities, as a percentage of VO2 max, in future research could also impact fat oxidation. In conclusion, future research studies should employ crossover designs to compare both OO and CSO, investigate the influence of dietary intervention duration and dosages on fat oxidation and gastrointestinal issues, and explore various exercise intensities to further explore and understand their impact on fat metabolism.

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APPENDICES

APPENDIX A

BRUCE MODIFIED PROTOCOL

Visit 1
Date:_____ ID: _____

Nutrition Research Lab
CSO Submaximal Graded Exercise Test Data Sheet

Age-predicted heart rate max (APHRmax) Calculation

1. Age = _____
2. APHRmax = 208 bpm – (0.7 × $\frac{\text{.....}}{\text{Age}}$) = ...bpm
3. 85% of APHRmax = 0.85 × $\frac{\text{.....}}{\text{APHRmax}}$ = ... bpm

- 5-Minute warm at participant selected pace

  Start of test time: _____

TREADMILL PROTOCOL						
Stage	**Time***	**Grade %**	**Speed**	**HR**	**RPE**	**Notes**
1	0:00-2:00	0				
2	2:00-4:00	2				
3	4:00-6:00	4				
4	6:00-8:00	6				
5	8:00-10:00	8				
6	10:00-12:00	10				
7	12:00-14:00	12				
8	14:00-16:00	14				
9	16:00-18:00	14				
10	18:00-20:00	14				
11		14				

Table 5. Bruce Modified Protocol

APPENDIX B

DIETARY COMPLIANCE FORM

Participant ID:
Date:

Daily Compliance Check (Week 1)

*Fill out compliance form daily □□

Date (M/D/Y)	Day #	How much smoothies did you consume on each day?					Comments
		1 full container	1 and ½ containers	Less than 1 and ½ containers	More than 1 and 1/2 containers	2 full containers	Please specify if you consumed anything that was not recommended by our research team. Refer to the dietary guidelines provided below. (Ex: 1 serving of nuts)
	1						
	2						
	3						
	4						
	5						
	6						
	*7						

*Please visit the lab for the smoothie's pick-up before day 7. Remember to bring back this compliance form, you may also take a picture of the form instead of bringing back a paper copy.

Table 6: Dietary Compliance Form

APPENDIX C

SUBJECT CONSENT FORM

SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH AT
MONTANA STATE UNIVERSITY

Study Title: Investigating the Potential Anti-Inflammatory Benefits of Different Dietary Oils

Investigators: Mary Miles, Ph.D.; Morgan Chamberlin; Prabina Bhattarai; [1]Alexandria Lotstein

Health & Human Development
Montana State University, Bozeman, MT
Phone: (406) 994-5001
mmiles@montana.edu

Summary:
You are being asked to volunteer as a participant in a research study of how different dietary oils influence inflammation and inflammation-related markers in the body. Many different metabolic markers such as triglyceride, cholesterol, and glucose, and thousands of different inflammation markers will be measured in the blood. These substances are produced by your immune system and chemical reactions in your body (metabolites) or the microorganisms such as bacteria that are in your digestive system. One of the many things we will measure in the blood is called "bile acids". Bile acids are compounds made from cholesterol and amino acids (from proteins) that help with breaking down fat in your intestine during digestion. Other substances that we will measure assess how well cells of the body are protected from metabolic stresses that may contribute to aging or the development of disease, e.g., a molecule called an isoprostane. Isoprostanes are formed when cells are stressed or damaged. Antioxidants are natural or manmade dietary components that protect the cells of your body from substances produced when cells are stressed, such as might occur during exercise. An exercise test is a healthy way to assess how well antioxidant's function.

The types and quantities of carbohydrates, fat, protein, fiber, and antioxidants in foods can influence the number of bile acids that enter your gut during digestion. We are specifically interested in how different dietary oils affect isoprostanes, bile acids, inflammation, metabolism, and other health measures.

Purpose: The purpose of this study is to determine how certain dietary oils may affect bile acids in the blood, isoprostanes in the blood after exercise, inflammation, and metabolic health of healthy individuals.

Specifically, we are asking the following questions:

1. How do specific dietary oils affect bile acids in the blood?
2. How do specific dietary oils affect isoprostanes and related molecule levels in the blood after exercise?
3. How do specific dietary oils change inflammation, metabolism, and other health measures?

If we learn how different dietary oils affect different health-related markers in the blood and how that may influence our health, then we can use that information to do more research to improve the health of people in future studies.

APPROVED MSU 06/12/2023
IRB #2022-146

Figure 15: Subject Consent From