UNIVERSIDADE DE SÃO PAULO
Faculdade de Ciências Farmacêuticas
Programa de Pós-Graduação em Ciência dos Alimentos
Área de Bromatologia

Effects of passion fruit (*Passiflora tenuifila* Killip) intake on eutrophic and obese subjects: Lipidomic approach.

Laila Guimarães Zeraik Cardoso

Dissertação para obtenção do Título de Mestre

Orientadora: Profª. Drª. Neuza Mariko Aymoto Hassimotto

São Paulo
2022

UNIVERSIDADE DE SÃO PAULO
Faculdade de Ciências Farmacêuticas
Programa de Pós-Graduação em Ciência dos Alimentos
Área de Bromatologia

Effects of passion fruit (*Passiflora tenuifila* Killip) intake on eutrophic and obese subjects: Lipidomic approach.

Laila Guimarães Zeraik Cardoso

Versão corrigida da Dissertação conforme resolução CoPGr 6018.

Dissertação para obtenção do Título de Mestre

Orientadora: Profª. Drª. Neuza Mariko Aymoto Hassimotto

São Paulo
2022

Laila Guimarães Zeraik Cardoso

Effects of passion fruit (*Passiflora tenuifila* Killip) intake on eutrophic and obese subjects: Lipidomic approach.

Comissão Julgadora
da
Dissertação para obtenção do Título de Mestre

Prof. Dr.
orientador/presidente

_____
1o. Examinador

_____
2o. Examinador

_____
3o. Examinador

_____
4o. Examinador

São Paulo, _____ de _____ de 202_

Ficha Catalográfica elaborada eletronicamente pelo autor, utilizando o programa desenvolvido pela Seção Técnica de Informática do ICMC/USP e adaptado para a Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP

Bibliotecária responsável pela orientação de catalogação da publicação:
Marlene Aparecida Vieira - CRB - 8/5562

C268e

Cardoso, Laila Guimarães Zeraik
Effects of passion fruit (Passiflora tenuifila Killip) intake on eutrophic and obese subjects: Lipidomic Approach / Laila Guimarães Zeraik Cardoso. - São Paulo, 2022.
88 p.

Dissertação (mestrado) - Faculdade de Ciências Farmacêuticas da Universidade de São Paulo. Departamento de Alimentos e Nutrição Experimental - Programa de Pós-Graduação em Ciência dos Alimentos.
Orientador: Hassimotto, Neuza Mariko Aymoto

1. lipidômica. 2. proantocianidinas. 3. fibras. 4. compostos bioativos. I. T. II. Hassimotto, Neuza Mariko Aymoto , orientador.

“First passion, then training”

Letters to a young scientist, E.O. Wilson

À minha mãe, Iara, que não mede esforços para garantir meu acesso à educação, independente da definição, e quem mais torce por mim.

À minha irmã, Lívia, que caminha comigo e partilha da minha essência.

À bisavó Venturosa, o início de tudo.

À minha avó, Wilma, e tias-avós, Valda e Vanira, a quem devo o apoio em meus estudos.

Aos meus tios, Airton e Yacy, e meus primos, Lucas e Gabriel, que firmam o alicerce de duas gerações como família.

Ao meu amigo, Kendi, parte dessa jornada.

À minha orientadora, Profª. Dra. Neuza, sempre disposta a oferecer as oportunidades em seu alcance.

Dedico.

AGRADECIMENTOS

À Profª Dra. Neuza Hassimotto, minha orientadora, pelos ensinamentos acadêmicos e não acadêmicos, pela confiança no desenvolvimento do projeto, pelas conversas e companhia sempre agradáveis.

Ao Prof. Dr. Franco Lajolo, pela oportunidade de trabalhar ao seu lado neste e outros projetos, e os aprendizados que começaram anos antes do desenvolvimento desta dissertação.

Ao Dr. Victor Castro-Alves e equipe do *MTM-Research Center* da Universidade de Örebro, pela colaboração, instrução e supervisão em meus experimentos e análises de dados, pelo incentivo.

Aos colegas do "sushibar", Camille, Eric, Isa Gouvea, Isa Duarte, Layanne, Renata e Vaneska, Julia e Felipe, com cuja ajuda sempre pude contar.

Aos Professores do laboratório de Química e Bioquímica de Alimentos, sempre disponíveis e prestativos.

Às técnicas do laboratório de Química e Bioquímica de Alimentos, Lúcia Justino, Luciene Lauer e Tânia Shiga, pelos treinamentos e assistência.

Aos funcionários do Departamento de Alimentos e Nutrição Experimental.

Ao *Food Research Center* (FoRC).

Aos amigos que cultivo desde o início da minha jornada na FCF-USP: F.R.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 88887.673622/2022-00

RESUMO

CARDOSO, L. G. Z. **Effects of passion fruit (*Passiflora tenuifila* Killip) intake on eutrophic and obese subjects: Lipidomic approach.** 2022. 88f. Dissertação (Mestrado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2022.

A obesidade e o sobrepeso são preocupações resultam em alterações metabólicas que se acumulam como fatores de risco para o desenvolvimento a longo prazo das principais doenças crônicas não transmissíveis. Dentre essas alterações, a dislipidemia um importante fator de risco para doenças cardiovasculares (DCV), expressa em níveis plasmáticos elevados de triacilgliceróis, colesterol e das lipoproteínas de baixa densidade (VLDL, LDL), e níveis diminuídos da lipoproteína de alta densidade (HDL). *Passiflora tenuifila* Killip é uma espécie de maracujá nativa da região Centro-Oeste brasileira, e é uma boa fonte de proantocianidinas e fibras alimentares. As proantocianidinas são compostos fenólicos com reportados efeitos na melhora do perfil de lipoproteínas, traduzida como a relação LDL/HDL. As fibras são fermentadas pela microbiota intestinal e produzem ácidos graxos de cadeia curta (AGCC), metabólitos também envolvidos na regulação do metabolismo energético.. Assim, a lipidômica não-*target* é aplicada como ferramenta exploratória neste estudo: uma intervenção de 30 dias consecutivos de ingestão de *P. tenuifila* na forma de farinha liofilizada em indivíduos eutróficos e obesos. O consumo do maracujá promoveu aumento da produção fecal de acetato, AGCC importante na modulação do metabolismo lipídico; a redução do percentual de gordura corporal em todos os indivíduos; e redução do colesterol total (CT) para os indivíduos com CT > 130 mg/dL. A análise lipidômica do plasma detectou 44 lipídios estatisticamente relevantes, independentemente do IMC, após a intervenção. Considerando a população do estudo com CT alterado, foi observada uma redução de glicerofosfolipídios, classe de lipídios estudada pelo seu envolvimento em DCV. Assim, a ingestão de *P. tenuifila* contribui para a melhora nos marcadores de risco cardiovascular e atua no metabolismo lipídico. Estes efeitos podem ser decorrentes de sinergia entre os diversos compostos bioativos do fruto. Ainda, outros estudos são necessários para identificar mecanismos relacionados a ação dos bioativos da *P. tenuifila* e estes podem ser mais bem direcionados pela lipidômica.

Palavras-chave: lipidômica, maracujá-alho, *Passiflora tenuifila* Killip, fibras, proantocianidinas, obesidade, doenças cardiovasculares.

ABSTRACT

CARDOSO, L. G. Z. **Effects of passion fruit (*Passiflora tenuifila* Killip) intake on eutrophic and obese subjects: Lipidomic approach.** 2022. 88f. Dissertation (Masters' degree) – School of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2022.

Obesity and overweight result in metabolic changes that build up as risk factors for the development of the main non-communicable diseases. Among these alterations, dyslipidemia is an important risk factor for cardiovascular diseases (CDV) and is expressed in elevated plasma levels of triacylglycerols, cholesterol, and low-density-lipoprotein (LDL, VLDL) and decreased plasma levels of high-density lipoprotein (HDL). *Passiflora tenuifila* Killip is a native passion fruit species of the Brazilian Midwest region and is a good source of proanthocyanidins and dietary fibers. Proanthocyanidins are a class of phenolic compounds that are attributed with improving lipoprotein profile properties, translated as improved LDL/HDL ratio. Fibers are fermented by the gut microbiota and produce short-chain fatty acids (SCFA), also involved in the regulation of energetic metabolism.. A 30-consecutive-day-long intervention with lyophilized *P. tenuifila* flour was performed in eutrophic and obese subjects. Passion fruit ingestion increased fecal production of acetate, key SCFA in the modulation of lipid metabolism; reduced body fat percentage in all subjects; and reduced total cholesterol (TC) of subjects who presented basal CT > 130 mg/dL. After the intervention, plasma lipidomic analysis detected 44 statistically significant lipids, regardless of BMI. Considering the study population with altered TC, reduced levels of glycerophospholipids were observed, a lipid class studied for their involvement in CVD. The intake of *P. tenuifila* contributed to the improvement in cardiovascular risk markers and acts on lipid metabolism. These effects may be due to synergic action between the bioactive compounds in the fruit. Still, other studies are necessary to identify mechanisms related to the action of bioactives of *P. tenuifila*, which can be better directed by this lipidomic approach.

Keywords: lipidomics, passion fruit, *Passiflora tenuifila* Killip, fibers, proanthocyanidins, obesity, cardiovascular diseases.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AC	Abdominal circumference
ALT	Alanine transaminase.
AST	Aspartate transaminase;
AT	Adipose tissue
BF	Body fat
BMI	Body mass index
BW	Bodyweight
CE	Cholesterol ester
CPT-1	Carnitine-palmitoyl-transferase-1
FA	Fatty acid
FFA	Free fatty acid
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model assessment - insulin resistance
LDL	Low-density lipoprotein
LPC	Lysophosphatidylcholine
NAFLD	Non-alcoholic fatty liver disease
PAC	Proanthocyanidin
PC	Phosphatidylcholine
PVA	Phenyl-valeric-acids
PVL	Phenyl-valeric-lactones
SCFA	Short-chain fatty acids
SM	Sphingomyelin
TAG	Triacylglycerol
TG	Triglycerides (lipid nomenclature)
TC	Total cholesterol
VLDL	Very-low-density lipoprotein

SUMMARY

1. INTRODUCTION

1.1. Obesity and energetic metabolism

The World Health Organization (WHO) defines obesity and overweight as excess or abnormal fat accumulation that threatens normal physiology, being an important risk factor for developing non-communicable diseases (NCDs) such as *diabetes mellitus*, hypertension, cancer, cardiovascular diseases, as well as impairment in quality of life (WORLD HEALTH ORGANIZATION, 2021). Also, according to WHO, NCDs are responsible for 74% of all deaths worldwide (WORLD HEALTH ORGANIZATION, 2022c), with cardiovascular diseases being the leading cause of death among them (WORLD HEALTH ORGANIZATION, 2022a). It is estimated that a third of the global population is either obese or overweight, turning obesity into an important global health issue (CHOOI; DING; MAGKOS, 2019). In Brazil, the recent data available suggests that 55% of the population is overweight while 20% are obese according to the Body Mass Index (BMI) classification (ABESO, [s. d.]) The health costs incurred by adult obesity and its consequences on the Brazilian health system in 2018 reached 1.42 billion reais (NILSON *et al.*, 2020).

Obesity is characterized by the accumulation of lipids in adipose tissue (AT) caused by a positive energy balance. Generally, obesity is caused by the increasing supply of foods with higher energy density and the decrease in the consumption of fruits, vegetables, and physical activity (MARIATH *et al.*, 2007; WORLD HEALTH ORGANIZATION, 2021, 2022b). The increase in energy storage causes hypertrophy and hyperplasia of adipocytes and when adipocytes fail to cope with the lipid excess, they increase lipolysis and free fatty acid (FFA) liberation into circulation, which are then taken up by organs such as the muscle and the liver (CAO, 2014; SINGLA; BARDOLOI; PARKASH, 2010). Hypertrophy and hyperplasia of adipocytes also impair local blood flow, which, in time, leads to hypoxia of the AT, causing macrophage infiltration, inflammation, and oxidative stress (SINGLA; BARDOLOI; PARKASH, 2010; WU; BALLANTYNE, 2020). Current evidence endorses the association of increased plasma TAG, cholesterol, atherogenic lipoproteins, and endothelial dysfunction (MILLER *et al.*, 2005).

The main abnormalities in the plasma lipid profile of obese individuals are elevated triacylglycerol (TAG), cholesterol, low-density lipoprotein (LDL), increased ApoB-100 and lower levels of high-density lipoprotein (HDL).

This altered lipoprotein profile grants a higher risk of atherosclerosis and cardiovascular events (MILLER *et al.*, 2005). Such lipid modifications in obesity seem to result from the increase in very-low-density lipoprotein (VLDL) due to insulin resistance since there is a heightened influx of FFA to the liver because of the unrestrained lipolysis in the AT (CHOI; GINSBERG, 2011) and the poor glucose uptake that stimulates gluconeogenesis, seen that glycerol can be used as a substrate for this pathway, and lipolysis (NELSON; COX, 2014). Insulin resistance also impairs lipoprotein lipase activity, undermining VLDL clearance and contributing to the obesity dyslipidemia profile (MILLER *et al.*, 2005).

Out of the tissues in the human body, the adipose tissue is one of the most responsive to insulin activity, where this hormone promotes lipid storage and inhibits lipolysis in lower concentrations than the ones needed for glucose metabolism (KAHN; FLIER, 2000). A proposed cause for insulin resistance is ectopic fat accumulation due to unrestrained lipolysis in the AT, among other complex mechanisms discussed in the scientific literature. It is well described how glucose metabolism is impaired due to insulin resistance in obesity, while the antilipolytic effects and hepatic *de novo* lipogenesis caused by insulin are preserved. If unchanged, the insulin resistance will progress into type 2 diabetes (KAHN; FLIER, 2000; TONG *et al.*, 2022).

In physiological conditions, the circulating FA from TAG hydrolysis by the AT are transported to the liver and peripheral tissues and are used to produce energy. Inside the cells, they are metabolized to acyl-CoA and converted to acyl-carnitines by the enzyme carnitine-palmitoyl-transferase-1 (CPT1). Acyl-carnitines are transported to the mitochondrial matrix by the acyl-carnitine-translocase transporter present in the inner membrane of the mitochondria, and there, via β-oxidation, they form molecules of acetyl-CoA that will take part in the citric acid cycle or ketone formation (FABBRINI; SULLIVAN; KLEIN, 2010; NELSON; COX, 2014). (NELSON; COX, 2014). In compensation, malonyl-CoA, the first intermediate of the *de novo* lipogenesis, inhibits CPT-1 activity, hindering the entry of FA into the mitochondria and decreasing β-oxidation (NELSON; COX, 2014). An important consequence of obesity is the development of non-alcoholic fatty liver disease (NAFLD), an excessive amount of hepatic TAG due to an increase in *de novo* lipogenesis and uptake of circulating FA, a saturation of mitochondria or reduction in lipid β-oxidation, and diminished release of VLDL (FABBRINI; SULLIVAN; KLEIN, 2010). The *de novo* lipogenesis is responsible for less than 5% of the FA incorporated in the VLDL and is significantly enhanced in NAFLD, a condition that can evolve to inflamed liver with fibrosis, and, ultimately, cirrhosis (DONNELLY *et al.*, 2005).

Besides the energetic reservoir function, the adipose tissue is a recognized endocrine organ that communicates with other metabolic organs by producing substances that influence the energetic metabolism, known as adipokines (SINGLA; BARDOLOI; PARKASH, 2010). Among the main adipokines and adipocytokines are TNF-α, the first discovered, leptin, adiponectin, resistin, and interleukins such as IL-6 (CAO, 2014). Adiponectin is believed to exert protective effects against hyperlipidemia by regulating glycemia and FA catabolism and is decreased in obesity compared to eutrophic subjects (WOZNIAK *et al.*, 2009). Leptin is involved in the neural control of satiety and appetite, and its plasma levels are increased in obesity, a phenomenon known as leptin resistance (OBRADOVIC *et al.*, 2021). Resistin blocks leptin activity, is increased in obesity in a scenario known as leptin resistance, and might play an important part in NAFLD, with higher circulating levels being proportionally related to liver fat accumulation, but data is still controversial (COLICA; ABENAVOLI, 2018; MAXIMUS et al., 2020). These briefly presented metabolic changes summed with the metabolic changes in the AT and systemic inflammation are some of the main factors associated with the development of NCDs (FURUKAWA *et al.*, 2017; SINGLA; BARDOLOI; PARKASH, 2010; WOZNIAK *et al.*, 2009). The dyslipidemia of obesity can be affected by diet macronutrients in both negative and positive ways, with a high-fat and high-carbohydrate diet being associated with an unhealthier lipid profile, whereas the intake of complex carbohydrates and fibers can ameliorate plasma lipid levels, especially in metabolically unhealthy obese subjects (MILLER *et al.*, 2005).

Proper nutrition and physical exercise play an important role in preventing, treating, and managing obesity and its outcomes. Research in food bioactive compounds is a growing area and these very diverse substances, present mainly in fruit and vegetables, have been associated with multiple health effects.

1.2. The Passiflora genera

Brazil is known for its biodiversity and variety of tropical fruits. The country is the main global producer and exporter of passion fruit, the popular name given to *Passiflora* genera fruits, the most representative genera in the family Passifloraceae. According to Embrapa, there are over 500 native *Passiflora* species across the American continent, with approximately 145 within Brazilian territory (FALEIRO *et al.*, 2020). Despite the diversity of species, *Passiflora edulis'* fruit is the main species commercialized on a large scale in the food market, followed

by *Passiflora alata.* For cosmetics and pharmaceutical purposes, leaves of *P. alata* and *P. incarnata* are also exploited (EMBRAPA CERRADOS, 2016).

Popular knowledge and beliefs attribute several health effects to passion fruit species, such as sedative, anxiolytic, diuretic, and analgesic properties (EMBRAPA CERRADOS, 2016). Phenolic compounds, especially flavonoids, present in *Passiflora* species are studied to investigate the actual health potential of these fruits. Zeraik and colleagues reviewed the health benefits of bioactive compounds present in *Passiflora edulis* fo. *flavicarpa* O. Deg., *P. alata* Curtis and *P. edulis* fo. *edulis*, which contain flavonoids, fibers, alkaloids, and other compounds that exert antioxidant, hypoglycemic, hypotensive, hypolipidemic, and nutritional effects (ZERAIK *et al.*, 2010).

Intervention studies conducted in humans with *P. setacea* juice (250mL, only dose) administered to male overweight volunteers (n=12) resulted in decreased levels of insulin, insulin homeostatic model assessment for insulin resistance (HOMA-IR), and increased HDL compared to placebo (DUARTE *et al.*, 2022, 2020). Another study with *P. setacea* found that acute ingestion of the fruit by overweight men (n=15, single dose of 50g) decreased insulin, HOMA-IR, and increased IL-2 compared to placebo; while IL-6 and HOMA-IR increased in the placebo branch of the chronic ingestion study (n=9, 50g/day, 14 days) (DUARTE *et al.*, 2022). The observed results suggest an anti-inflammatory and antidiabetic effect that could be attributed to the phenolic compounds in the fruit.

In vivo studies with *Passiflora edulis* f. *flavicarpa* Degener juice administered by gavage to Wistar rats (n=8) for 28 days, twice a day (1,000 mg/kg), showed an increase in plasma HDL and a decrease in LDL and FFA concentrations compared to control (n=8). Concentrations of TBARS were also decreased in the treated group, suggesting positive effects on lipid peroxidation and lipidic profile (SOUZA *et al.*, 2012). In a study assessing the offspring of diabetic Wistar rats after intervention with *P. edulis* juice for 30 consecutive days (oral gavage, 0.58 g/kg once a day), lipid levels were decreased in the treatment group of diabetic mothers (n=15) compared to the untreated group of diabetic mothers (n=15), who received water. The offspring of non-diabetic (n=15) and diabetic mothers (n=15) who were treated with *P. edulis* juice presented significantly lower plasma concentrations of total cholesterol, TAG, LDL, and increased HDL (BARBALHO *et al.*, 2011). The great variety of passion fruit species and their phenolic content provide great opportunities for scientific investigation. In this sense, the EMBRAPA-Cerrados Passitec Network aims to integrate several areas of knowledge, foreseeing the full usage of native *Passiflora* in medicine and nutrition, and exploring Brazilian

biodiversity in a sustainable manner (EMBRAPA CERRADOS, 2003). Among the selected *Passiflora* species for their project, *Passiflora tenuifila* Killip was chosen due to its potential health benefits.

1.3. Passiflora tenuifila Killip, bioactive compounds, and health effects.

Passiflora tenuifila Killip is a native and wild species of the *Passiflora* genera present in Cerrado and Atlantic Forest biomes. The fruit is known by the local communities as garlic passion fruit due to its characteristic garlic-like aroma. It is small (approximately 5-7cm in diameter), and presents a yellow color in the ripe stage, with peel, pulp, and seeds being edible. Locals consume the entire fruit from the mature-green to the yellow-ripe stage (SANTOS *et al.*, 2021).

Figure 1: *P. tenuifila* Killip in different ripening stages

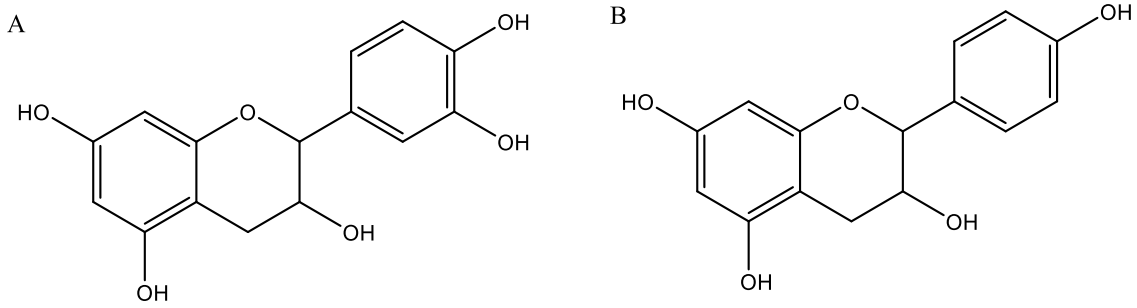
Source: Photo by Ana Maria Costa, Embrapa, Brazil.

Of the total phenolic compounds identified in *P. tenuifila*, from 84% to 88% are proanthocyanidins (PAC) dimers and monomers: [epi]catechin-[epi]catechin dimers (mean range from 108 to 215 mg/100g FW) and [epi]catechin-[epi]afzelechin dimers (93 to 148 mg/100g FW), with the latter being B-type proanthocyanidins. Procyanidin dimer digallate (A-type) and (+)-gallocatechin were also identified. The mean range of total PAC identified varies

from 267.6 to 383mg/100g FW. Considering flavonoids, apigenin and luteolin in C-glycosylated forms were found, with luteolin being the more abundant one (24.12 to 40.14 mg/100g) (SANTOS *et al.*, 2021). Apigenin and luteolin are flavonoids commonly found in the Passiflora genera (LUCAS-GONZÁLEZ *et al.*, 2022).

Proanthocyanidins are condensed tannins commonly found in cocoa, red wine, green tea, apples, nuts, and dark chocolate, among other vegetables. They consist of dimers, trimers, oligomers, and polymers of flavan-3-ols monomers with a wide range of polymerization degrees (WANG *et al.*, 2020). PAC and their flavan-3-ol monomers are the most consumed polyphenol in western diets, with ingestion estimated at around 300 mg/day (ZANOTTI *et al.*, 2015). A variety of health effects are attributed to these compounds, for instance, anti-inflammatory, immunostimulatory, chemopreventive, anti-diabetic, and cardioprotective properties (CAMPOS; STEHLE; SIMON, 2019). About the latter, PAC seem to have hypolipidemic effects that result in an improved LDL/HDL ratio (SALVADÓ *et al.*, 2015; SMERIGLIO *et al.*, 2017).

Figure 2: Flavan-3-ol components of PAC in *P. tenuifila*. A)(+/-)[epi]catechin, B) and (+/-)[epi]afzelechin (B).



Source: By the author.

Regarding the bioavailability of these compounds, it is estimated that 80-90% of ingested PACs reach the colon intact, where they undergo extensive metabolization by the gut microbiota (WANG *et al.*, 2020). Consequently, flavan-3-ol monomers are released, and a fraction is readily absorbed but most are metabolized to phenyl-y-valerolactones (PVLs) and phenyl-valeric acids (PVAs), as well as their further transformation into phenolic acids (SHANG *et al.*, 2017; SMERIGLIO *et al.*, 2017). With PVLs and PVAs being the bioavailable metabolites of PAC, they have been credited with hypolipemic and hypoglycemic properties,

among others, found in PAC interventional studies. In a randomized, controlled, crossover-chronic study, polyphenol-rich cocoa (7.5 g twice a day) and control interventions were administered in healthy (n=24) and moderately hypercholesterolemic (n=20) subjects for four consecutive weeks, considering epicatechin and its phase II metabolites and phase II derivatives of PVLs and PVAs as the main compounds derived from cocoa polyphenols. The observed effect was an increase in HDL and an improved cardiometabolic profile (MARTÍNEZ-LÓPEZ *et al.*, 2014). Still, there is a need for further investigation of the mechanisms by which PACs and their metabolites exert these effects with study designs that apply realistic doses of PAC and considers the microbiota metabolization of these polyphenols (SARRIÁ *et al.*, 2020).

P. tenuifila is also a good source of dietary fiber (DF), with insoluble fibers (IF) representing 99% of the total fiber content quantified in the EMBRAPA fruit batches (SANTOS *et al.*, 2021). DF consist of a relatively broad group of carbohydrate polymers (HOLSCHER, 2017), and some examples of IF are cellulose, hemicellulose, and lignin (LI; KOMAREK, 2017). A high-fiber diet is related to a reduced risk of cardiovascular diseases, hypertension, type 2 diabetes, improved lipidemia and glycemia, promotes regular gastrointestinal transit, and satiety (ANDERSON *et al.*, 2009). Furthermore, IF seem to have hypolipidemic effects by binding to bile acids, impairing cholesterol absorption (NIE; LUO, 2021; SÁNCHEZ; MIGUEL; ALEIXANDRE, 2012). Fibers that can be fermented by the gut microbiota can also act as prebiotics, favoring the proliferation of healthy bacteria and resulting in the production of short-chain fatty acids (SCFA) (ANDERSON *et al.*, 2009; MYHRSTAD *et al.*, 2020). From regulating intestinal permeability, energetic metabolism, and appetite, to presenting anti-inflammatory activity, the health effects related to SCFAs brought significant awareness to these molecules (DELEU *et al.*, 2021a).

1.4. Lipids and Lipidomics

Lipids are a rather broad set of organic molecules that exert multiple functions in living organisms: structural, energy storage, signaling, hormonal, the interface between environments, and so on. Many lipids function as messengers in our bodies, with their specific chemical structure enabling binding to receptors to exert a biological function, or to specific proteins so they can navigate through the circulatory system.

Despite many attempts, there is still no internationally agreed definition for what a lipid is, but a close one could be that lipids are fatty acids and fatty acids derivatives as well as molecules

that are related to these compounds biosynthetically or functionally, including those formed by condensed isoprene units (CHRISTIE, [s. d.]). The International Lipid Classification and Nomenclature Committee organizes lipids into eight categories: fatty acyls, glycerolipids (GL), glycerophospholipids (GP), sphingolipids (SP), saccharolipids (SL), polyketides (PK), prenol lipids (PR), and sterol lipids (ST). These categories are then subdivided into subclasses as exemplified in Table 1 (FAHY *et al.*, 2005).

Table 1 – Lipid classes and examples of subclasses.

Lipid class	Abbreviation	Examples of subclasses
Fatty acyls	FA	Fatty acids Eicosanoids Fatty alcohols, esters, and aldehydes
Glycerolipids	GL	Monoacylglycerols Diacylglycerols Triacylglycerols
Glycerophospholipids	GP	Phosphatidic acids Phosphatidylcholines Phosphatidylserines
Sphingolipids	SP	Ceramides Phosphosphingolipids Glycosphingolipids
Sterol lipids	ST	Sterols Steroids Secosteroids Bile acids
Prenol lipids	PR	Isoprenoids Quinones and hydroquinones Polyphenols
Saccharolipids	SL	Acylaminosugars Acyltrehaloses
Polyketides	PK	Macrolide polyketides Aromatic polyketides Peptide/polykeride hybrids

Source: LIPID MAPS®

Consistently, fatty acids could be considered the key component in lipid structures. They are aliphatic carbon chains with a variety of carbon numbers and a terminal carboxyl moiety. FA can be saturated, monounsaturated, or polyunsaturated, which confers different fluidity and oxidizing properties to these molecules. They can be classified into essential or non-essential FA based on the human capacity to produce them or the need to obtain them from dietary sources. Their free form is released from storage structures to produce energy on demand, but they are mostly found as a part of the other more complex lipids (CHRISTIE, 2022b).

Moving on to GP, triacylglycerols are the main representatives of the class, being mostly "inert" molecules that serve as energy storage. This "lack" of biological effect is crucial for its transportation throughout the body. TAG, however, are the main components of adipocytes, and the adipose tissue is now recognized as an important endocrine organ. Diacylglycerols (DAG) are formed as intermediates in TAG synthesis and breaking and can also act as signaling molecules. For instance, an increased concentration of DAG activates protein C kinase, a family of proteins that activate or deactivate other proteins by phosphorylation of certain amino acid residues, such as insulin receptors. Still, as is the case with monoacylglycerols, DAG are present in very low amounts in the organism (CHRISTIE, 2022b; KOLCZYNSKA *et al.*, 2020).

Glycerophospholipids are mainly known for their role in forming the bilayer structure of cell membranes. Ionic GP such as phosphatidylcholines, phosphatidylinositol, and phosphatidylserines are also precursors to signaling molecules like DAG, arachidonic acid, sphingomyelins, and endocannabinoids. They also maintain the cell membrane structure through electrostatic interactions with other components and enable vacuole formation, transport, and fusion. Many different GP species are involved in specific functions, such as bone structure, coagulation, mitochondrial function, and so forth (CHRISTIE, 2022b).

Regarding sphingolipids, sphingomyelins are the most abundant species. They too are key components in the cell membrane and important signaling molecules. Their FA components are usually long-chain FA (C>16), saturated or monounsaturated. Ceramides are precursors for complex sphingolipids and are involved in processes such as cell apoptosis and insulin secretion (CHRISTIE, 2022a; KIM; RAMACHANDRAN; WIERZBICKI, 2022).

For the sterols class, they comprise molecules with a structure based on the cyclopentanoperhydrophenanthrene skeleton. Cholesterol must be the most known species of the class due to its clinical importance in dyslipidemia and cardiovascular diseases and is the most abundant sterol in animals. Cholesterol is important for cell membrane functionally,

modulating its fluidity. In its free form, it is the precursor to vitamin D, steroid hormones, and bile acids. (CHRISTIE, 2022b).

Naturally, due to their diversity of structure and biological functions, lipids have been found to be associated with a plethora of human disease states or health statuses. With advances in science and engineering, new techniques such as the "omics", from the Latin "ome", meaning "mass or many", have been developed and applied in the most diverse areas of research, generating a handful amount of data. In this scenario, studies have reported associations between certain lipid classes and specific outcomes, such as between plasmatic lysophosphatidylcholines (LPC) and obesity in adolescence (WANG *et al.*, 2019), specific phosphatidylcholines (PC) and sphingomyelins (SM) associated with higher or lower body fat in obese and overweight adults (PAPANDREOU *et al.*, 2021), and lipids that correlate with stages of progression between NAFLD, non-alcoholic steatohepatitis (NASH) and liver fibrosis (MCGLINCHEY *et al.*, 2022).

Lipidomic analyses have also been applied to dietary intervention studies in health and disease, providing insights into the complex relationship between host characteristics, barriers, and responsiveness to food components. Insulin-resistant subjects (n=25) who were administered orange juice (400 mL) for 15 consecutive days showed a decrease in a large variety of TAG that was then correlated with plasma VLDL, total TAG, and blood pressure (SANTOS *et al.*, 2022). Also, a two-week-long period of orange juice intake (250 mL, twice a day) leads to an increase in short-chain acylcarnitines and a decrease in medium and long-chain acylcarnitines in healthy adult subjects (n=15, both sexes), demonstrating an effect on lipid metabolism (MOREIRA *et al.*, 2018). A high glycemic load diet compared to a low one in adult lean and obese subjects (n=80, both sexes) showed differences in plasma levels of lipids containing the C17:0 fatty acyl, with lower concentrations of these lipids associated with a low glycemic load diet after 28 days of intervention (DIBAY MOGHADAM *et al.*, 2020).

Within the described context, a lipidomic analysis is a set of techniques that allow lipid identification, quantification, and characterization in a biological system, and has the potential to detect metabolic changes at earlier stages than the ones measured by conventional analysis. Lipidomics can be used as a predictive tool for potential mechanisms of interaction between xenobiotic compounds and lipid metabolism (STANLEY *et al.*, 2017).

2. RATIONALE

Brazil is one of the largest producers of passion fruit. Of the many existing species of the genus Passiflora, few are commercially exploited. *Passiflora tenuifila* Killip is part of the local population's dietary habits and can be considered an important source of bioactive compounds like polyphenols and fibers. Available literature suggests that these substances have the potential to modulate the energetic metabolism, resulting in positive health outcomes.

Studies conducted by our research group demonstrated that consuming *P. tenuifila* for 30 consecutive days resulted in a lowering of LDL and total cholesterol in obese subjects with measures above the reference values (>130mg/dL and >190mg/dL, respectively) (SANTOS, 2020a) Chemical characterization of the fruit resulted in high content of PAC, a class of phenolic compounds studied, among other effects, for its influence on lipoprotein profile. The high DF content of *P. tenuifila* can be fermented by the gut microbiota and form SCFA. The main studied SCFA (acetate, butyrate, and propionate) are involved in intestinal barrier health and can influence energetic metabolism.

A lipidomic analysis enables to prospect hypotheses for xenobiotics' mechanisms of action. Thus, it is expected that the exploratory analysis contributes to the understanding of this complex food matrix in human health, indicating possible points of interaction between *P. tenuifila* and lipid metabolism.

This research project is linked to the doctoral thesis published by Dr. José Thiago do Carmo Santos, who led the work entitled "Metabolomic investigation of passion fruit intake (*Passiflora tenuifila*)" (SANTOS, 2020a). The project is also linked to the Passitec Network, coordinated by Dr. Ana Maria Costa (EMBRAPA-Cerrados).

3. OBJECTIVE

3.1. General objective:

To investigate the effects of *Passiflora tenuifila* Killip over the plasma lipidome and biochemical parameters of eutrophic and obese subjects.

3.2. Specific objectives:

Analyze the plasma lipidome of volunteers before and after the intervention.

Analyze volunteers' biochemical and anthropometrical parameters before and after the intervention.

Analyze short-chain fatty acids in fecal samples before and after the intervention.

4. MATERIAL AND METHODS

4.1. Passion fruit flour

EMBRAPA-Cerrados, in their experimental camp (Brasilia, Brazil), produced the *Passiflora tenuifila* Killip fruits and they were granted to the study by Dr. Ana Maria Costa, EMBRAPA and Passitec network researcher, to produce the lyophilized passion fruit flour. The physicochemical characterization of the fresh fruit batches, in both edible green and ripe stages, resulted in 11.62g/100g (FW) of DF, of which circa 99% are insoluble fibers, and PAC content ranged from 267 to 383 mg/100g (FW) (SANTOS, 2020b; SANTOS *et al.*, 2021). Approximately 80kg of frozen passion fruit were used to produce the lyophilized flour and their centesimal composition, as well as for the flour, are described in Table 2. The flour was produced by Sublimar produtos liofilizados LTDA – ME (Tatuí, SP, Brazil). The phenolic composition of the fruit batches, focused on the PAC and flavonoid content, is described in Table 3.

4.2. Study population

Originally, a total of 42 volunteers were recruited for the clinical trial conducted by Dr. José Thiago do Carmo Santos (SANTOS, 2020a). Volunteers represent both sexes and are between 18 and 59 years old and were either considered eutrophic (BMI < 24.9 Kg/m^2) or obese (BMI > 30.0 Kg/m^2) according to WHO guidelines (WORLD HEALTH ORGANIZATION, 2011a). No sample size planning or calculations were performed to determine the exact number of volunteers needed since the trial was a first screening study on the possible health effects of *Passiflora tenuifila* Killip, with no expected outcomes. For the same reason, a specific age range of adults or sex was not considered in this prospective study.

Inclusion criteria consisted of 1) no history of cardiovascular, hepatic, gastrointestinal, or kidney dysfunction; 2) non-alcoholic; 3) non-diabetic; 4) do not present any sort of infectious condition; 5) no current use of vitamins or mineral supplements, antibiotics, antacids, medication for constipation or diarrhea; 6) likes passion fruit; 7) women enrolled must not be pregnant, breastfeeding or be in current use of hormonal therapy due to menopause.

For this project, a total of 29 subjects (12 eutrophic and 17 obese) were selected from the original 42 due to sample availability of all parameters and analyses that were included in this study. The 29 volunteers are described in Table 4.

All procedures were approved by the Ethical Committee of the Dante Pazzanesi Cardiology Institute (CAAE 57619616.4.0000.0067) and were registered on the Brazilian Registry of Clinical Trials at https://ensaiosclinicos.gov.br/rg/RBR-1028cy7j (UTN code U1111-1264-7417) (SANTOS, 2020a).

Tabela 2: Centesimal composition of different batches of *Passiflora tenuifila* Killip (fresh fruit) and the lyophilized flour.

Centesimal composition	Batch 1		Batch 2		Batch 3		Passion fruit flour
	Green-mature	Ripe	Green-mature	Ripe	Green-mature	Ripe	
Moisture (%)	72.73 (0.52)	74.13 (0.50)	75.18 (1.14)	75.14 (0.63)	74.26 (0.63)	73.46 (0.34)	6.14 (0.35)
Ashes	1.32 (0.02)	1.09 (0.0)	1.14 (0.02)	1.42 (0.02)	1.00 (0.02)	1.13 (0.03)	4.41 (0.14)
Total Carbohydrates	20.83 (0.09)	18.94 (0.74)	18.46 (1.15)	16.25 (0.37)	19.86 (0.63)	18.65 (0.25)	64.67 (0.60)
Dietary Fiber	10.25	12.18	11.92	15.20	10.84	9.34	45.37
Soluble	0.03 (0.02)	0.09 (0.00)	0.5 (0.14)	0.07 (0.02)	ND	0.55 (0.04)	1.41 (0.40)
Insoluble	10.22 (0.51)	12.09 (1.03)	11.42 (1.41)	15.13 (2.12)	10.84 (1.69)	8.79 (1.51)	43.95 (2.47)
Proteins	2.36 (0.05)	2.58 (0.01)	2.13 (0.10)	2.70 (0.22)	2.09 (0.08)	2.44 (0.13)	9.84 (0.28)
Lipids	3.08 (0.05)	3.35 (0.05)	3.14 (0.01)	4.23 (0.07)	2.79 (0.08)	4.11 (0.03)	15.28 (0.00)
TEV (kcal)	120.35 (0.09)	116.20 (2.30)	110.41 (4.45)	114.27 (0.12)	112.92 (2.14)	121.48 (1.48)	434.85 (7.7)

Source: SANTOS, 2020b. Values are expressed in mean with standard deviation in parenthesis. Units expressed in g/100g.

Tabela 3: Flavonoid content of *P. tenuifila* fruits in two maturing stages.

	Batch 1		Batch 2		Batch 3	
	Green-mature	Ripe	Green-mature	Ripe	Green-mature	Ripe
Luteolin (total)	26.36	24.12	37.29	36.87	32.00	40.14
Apigenin (total)	6.82	7.32	18.62	11.08	8.33	11.14
Proanthocyanidin (total)	348.70	295.40	367.50	383.00	267.60	358.70
Pocyanidin dimer [a]	108.88 (14.14)	167.31 (3.68)	212.79 (9.15)	214.88 (21.95)	136.17 (8.22)	180.68 (39.29)
Proanthocyanidin dimer [b]	93.50 (11.55)	116.65 (2.17)	140.38 (6.15)	148.10 (20.86)	99.78 (7.99)	141.61 (26.83)
Procyanidin dimer digallate	6.97 (0.89)	6.06 (0.13)	9.35 (0.33)	11.33 (0.092)	8.95 (0.42)	10.55 (2.32)
(+)-gallocatechin	4.65 (0.23)	5.34 (0.31)	5.00 (0.20)	8.73 (0.47)	22.72 (2.76)	25.82 (7.36)

Source: SANTOS *et al.*, 2021. Values are expressed in mean with standard deviation in parenthesis. Units expressed in mg/100g. [a] epicatechin- epiafzelechin. [b] A-type.

4.3. Study design

4.3.1. Experimental design

The Clinical Trial was conducted by the School of Pharmaceutical Sciences at the University of São Paulo (FCF-USP) in partnership with Dante Pazzanese Cardiology Institute. Volunteers received individual packaging containing 5g of lyophilized *P. tenuifila* flour (an amount representative of approximately 3 units of the fruit) and were oriented to mix it with water and consume it after dinner. This is due to *P. tenuifila* being studied for its potential tremor-control effect, with sleepiness being a possible effect. The duration of the intervention was 30 consecutive days. Volunteers were oriented to restrict the intake of citrus fruits, foods containing citrus fruits, black and green tea, grapes, grape juice, and coffee during the 3 days prior to sample collection. There was no placebo or control group and volunteers acted as their own control for comparisons between before and after the intervention.

On the day before the start of the intervention, day 0, and on the 15^{th} and 30^{th} days after starting the intake of *P. tenuifila,* study volunteers attended an appointment at Dante Pazzanese Cardiology Institute's Ambulatory of Clinical Nutrition for sample collection considering a 10-hour fasting period. On the day before the appointments, they were given a standard meal for dinner, consisting of 150g of white rice, 150g of cube-diced chicken, and 100g of cooked green beans (456 kcal, 53g of protein, 46g of carbohydrates, 7g of lipids).

4.3.2. Sample collection

Blood for biochemical parameters was withdrawn considering a 10-hour-fasting. Blood samples were collected into tubes (16 mL) containing EDTA, fluoride (2 mL), and no anticoagulant (4 mL), and then centrifuged at 2.500 rpm / 15 min / 20°C to separate the plasma fraction. Aliquoted plasma was stored at -80°C until analysis. Fecal samples were collected by the volunteers themselves on the previous day, and they were oriented to keep samples under refrigeration until they were handed over during the sample collection appointments, after which they were immediately fractioned and stored at -80°C until analysis.

4.4. Biochemical and anthropometric parameters.

Fasting glucose and plasma lipid profiles (TC, LDL, HDL, VLDL, and triacylglycerols) were analyzed by the State Center for Clinical Analysis (CEAC), the laboratory responsible for clinical analysis at the Dante Pazzanese Cardiology Institute. Blood glucose, HDL, and TAG

were measured by dry colorimetric method, TC was assessed by colorimetric method, and LDL was obtained by the Friedwald equation. Insulin dosage was performed by the Clinical Laboratory Division of the University Hospital at the University of São Paulo (HU USP) using electrochemiluminescence. HOMA-IR values were calculated according to Matthews and collaborators by the equation: basal insulin x basal glycemia (mmol) / 22.5 (MATTHEWS *et al.*, 1985).

During the sample collection appointments, subjects were weighed and measured on days 0, 15, and 30 to obtain the following anthropometric information: body weight and height using a digital scale with a stadiometer (max 200kg, precision: 100g), abdominal circumference with an inelastic measure tape at the midpoint between the last rib and the ileal crest. Body composition was assessed using tetrapolar Bioimpedance apparatus (BIA 450 bioimpedance analyzer, Biodynamics Corporation, Washington, EUA). BMI was calculated according to the equation weight (kg) / height $(m)^2$ and cut-off points were set according to WHO (WORLD HEALTH ORGANIZATION, 2011a).

4.5. Untarget lipidomic analysis

4.5.1. Sample extraction and quality controls

Plasma lipidomics was performed according to a modified version of the Folch method, as described by Nygren and colleagues (NYGREN *et al.*, 2011). The following were added to 1.5-mL polypropylene tubes: 10µL of NaCl solution (0,9%); 120µL of the standard solution containing 2.5 µg/mL of the internal standards in $CHCl_3$:MeOH (2:1, v/v) (Table S1); and 10µL of plasma samples. Tubes were vortexed until achieving a milk-like appearance and incubated on ice for 30 min. Following incubation, samples were centrifuged at 9400 g (3 min, 4ºC) and 60µL of the lower phase was pipetted into 300 µL-glass vials with inserts already added with 60µL of $CHCl_3$:MeOH (2:1, v/v). In addition to the sample extracts, we also prepared procedure/extraction blanks, instrument blanks, pooled samples, *in-house*/intra-laboratory QC samples, and NIST QC samples (inter-laboratory QC). For pooled samples, 20µL of the lower phase from each sample was combined into an 8-mL glass vial, from which 60µL were aliquoted into 300 µL-glass vials with insets. Samples containing plasma collected from donors at Örebro University Hospital (Örebro, Sweden) were prepared as *in-house* QC samples, while NIST SRM 1950, a reference sample containing metabolites in frozen plasma developed in 2006 by the *National Institute of Standards and Technology* and the *National Institute of*

Diabetes and Digestive and Kidney Diseases was prepared to be used as QC between laboratories around the world.

The calibration solutions (Table S2) were prepared at concentrations ranging from 0.1 to 5 µg/mL and added of the internal standards in a concentration of 2.5 µg/mL.

4.5.2. Liquid chromatography coupled with high-resolution time-of-flight mass spectrometry (UHPLC-qToF-MS) analysis

Analysis was performed by ultra-high performance liquid chromatography coupled with high-resolution time-of-flight mass spectrometry (UHPLC-qToF-MS) analysis using a UHPLC 1290 Infinity system (Agilent, Santa Clara, USA) interfaced with a dual electrospray ion source to a 6545 qToF-MS system (Agilent). The column used was Acquity UPLC BEH C18, 2.1 mm x 100 mm, particle size 1.7 µm (Waters). Mobile phases were A) 10 mM ammonium acetate and 0.1% formic acid in water and B) 10mM ammonium acetate and 0.1% formic acid in acetonitrile/isopropanol (1:1). Gas temperature at the source was 193°C and sheath gas temp was 379°C. The injection volume was 1µL. Samples were run in positive mode and the software *MassHunter* (Agilent Technologies) was used for data collection and conversion to ".MZdata" format.

The calibration solution was analyzed at the start and at the end of the sample worklist, while acetonitrile/instrument blank was added every six samples; extraction/procedure blank was added at the beginning of the sample worklist and every 25 samples; pooled samples were added at the beginning, mid-run, and at the end of the worklist (three in total), while *in-house* QC sample and NIST sample were added at the beginning of worklist.

4.5.3. Data pre-processing and metabolite identification

Data pre-processing and peak annotation was performed using the software MZmine (version 2.53 and version 3), and Microsoft Excel was used for normalization and additional filtering. The pre-processing steps on MZmine included definition of noise level, construction of extracted ion chromatograms (ADAP chromatogram building), chromatogram deconvolution (i.e., integration of peaks in the extracted ion chromatograms), isotope peak grouping and filtering steps, which were applied to remove (a) features present in the void volume (RT < 0.5 min) as well as (b) features with relatively high precursor m/z (m/z > 400) at the beginning of the chromatogram (RT < 2 min) and (c) features with relatively low precursor m/z at the end of the chromatogram (RT > 6 min). Sample lists containing all the integrated

features were further aligned and precursor ions were identified by direct comparison with an *in-house library* (accurate m/z – RT pairs) of about 400 lipid species developed by the MTM-Research Centre at Örebro University (Örebro, Sweden).

The abovementioned steps resulted in a list of identified and unknown features and their respective peak areas in all samples and QCs. This sample list was then exported as a ".csv" file to be processed on Microsoft Excel. Peak areas of identified features were normalized using class-specific internal standards, while unknown compounds were normalized by the closest internal standard in retention time. Features with relatively high average peak area on procedure blanks (i.e., ratio sample/blank < 3) were excluded from the metadata. We also excluded features showing a %RSD > 30% on pooled samples. Finally, unknown features of interest defined by the statistical analysis were also tentatively annotated via MSMS data using online databases for MS spectra.

4.6. Short-chain fatty acids

4.6.1. Sample extraction

Short-chain fatty acids were analyzed using the method described by Masood *et al*. (2005) and Villalba *et al*. (2012) (GARCÍA-VILLALBA *et al.*, 2012; MASOOD; STARK; SALEM, 2005). Fecal samples were weighted and 1mL of phosphoric acid (0.5%) was added for every 0.1g of sample, which was immediately frozen at -20ºC. After thawing, fecal suspensions were vortexed for approximately 2 min and centrifuged for 10 min at 10.000 g, 4ºC. Then, 850µL of supernatant was recovered and another 850µL of ethyl acetate solution containing the internal standard 2-methyl-valeric acid were added to a microtube and centrifuged for 10 minutes at 10.000 g, 4ºC. Subsequently, 600µL of the organic upper phase were recovered and transferred to amber glass vials for gas chromatography analysis.

4.6.2. Gas chromatography analysis

Analysis was performed using gas chromatography (Hewlett–Packard 6890 - Agilent Technologies Inc., Santa Clara, USA). The chromatographic column employed was CPWAX 7747 (25 m x 0.32 µm x 0.25 µm i.d.). The temperature used in the injector was 250 °C. For the temperature program used, it was set a temperature ramp of 20 °C.min-1 of 50 °C to 180 °C, followed by a temperature ramp of 35 °C.min-1 to 200 °C and a final elevation of 50 °C/min to 250 °C, maintained for 4 min. The interface temperature between the chromatograph and the

mass selective detector was 280°C and the ionization technique employed was by electrons impact (70 eV) with the ion source temperature maintained at 230°C.

4.6.3. Data processing and metabolite identification

The software GC-MS Translator (Agilent Technologies) and MassHunter were used for raw data processing. Compounds were identified and quantified using NIST library and direct comparison with a standard solution (Sigma-Aldrich, Product ID CRM46975) containing acetic acid (CAS 64-19-7), formic acid (CAS 64-18-6), propionic acid (CAS 79-09-4), isobutyric acid (CAS 79-31-2), butyric acid (CAS 107-92-6), isovaleric acid (CAS 503-74-2), valeric acid (CAS 109-52-4), isocaproic acid (CAS 646-07-1), hexanoic acid (CAS 142-62-1), and heptanoic acid (CAS 111-14-8). Each peak was integrated and then normalized by IS (2-methyl-valeric acid).

4.7. Statistical Analysis

For the lipidomic analysis, multivariate and univariate analyses were performed using the online platform Metaboanalyst 5.0 (PANG *et al.*, 2021). Data was Log transformed and auto scaled before analysis. The volcano plots were set with a fold change threshold of 1.5 and a statistical significance of $p<0.05$. For the biochemical parameters, anthropometry, and short-chain fatty acids, hypothesis tests were conducted using the software Jamovi v. 2.2.5. For non-parametric data, the Wilcoxon W's test was applied for dependent variables and Mann Whitney's for independent variables. Parametric data were assessed by Student's t-test (dependent variables) and independent Student's t-test (independent variables). Normality was defined according to Shapiro-Wilk's normality test. Statistical significance was set to $p<0.05$. Graphic data representation was constructed using RStudio (2021.09.2 Build 382), ggplot2 package.

5. RESULTS

Originally, forty-two subjects (19 eutrophic and 23 obese) were enrolled in the clinical trial performed at the School of Pharmaceutical Sciences at the University of São Paulo. For this project, a total of 29 subjects (12 eutrophic and 17 obese) were selected due to the sample intersection and available data on biochemical and anthropometrical parameters and short-chain fatty acids. They are described in Table 4:

Table 4 – Characterization of subjects according to sex, age, biochemical and anthropometrical parameters on "day 0" of intervention.

Variable	All (n=29)	Eutrophic (n=12)	Obese (n=17)	p-value (Eutrophic vs Obese)
Sex				
Men	7	2	5	-
Women	22	10	12	-
Age (year)	38.6 (11.3)	30.7 (7.29)	44.2 (10.4)	-
Body weight (kg)	79.0 (20.95)	59.2 (7.11)	92.9 (15.38)	**<0.001**
BMI (kg.m2)	31.5 (7.28)	21.9 (1.52)	35.1 (3.78)	**<0.001**
Waist circumference (cm)	100 (18.58)	76.1 (2.84)	108.9 (11.23)	**<0.001**
Body fat (%)	33.6 (7.78)	26.7 (5.87)	38.0 (5.14)	**<0.001**
Glucose (mg/dL)	90.59 (12.93)	85.50 (9.92)	93.94 (14.00)	0.062
Insulin (mU/L)	11.93 (6.22)	7.50 (2.61)	15 (6.16)	**<0.001**
HOMA-IR	2.73 (1.60)	1.60 (0.64)	3.53 (1.60)	**<0.001**
Cholesterol (mg/dL)				
HDL	51.14 (17.11)	59.75 (19.83)	45.06 (12.13)	**0.022**
LDL	101.59 (28.90)	91.58 (32.71)	108.65 (24.46)	0.132
VLDL	20.55 (9.56)	16.17 (7.26)	23.65 (9.96)	**0.012**
Total	173.28 (38.75)	167.50 (48.78)	177.35 (30.81)	0.240
Triacylglycerol (mg/dL)	103.17 (47.77)	81.58 (35.94)	118.41 (50.09)	**0.012**
AST (U/L)	20.17 (10.13)	22.50 (13.27)	18.53 (7.18)	0.339
ALT (U/L)	35.31 (12.50)	30.92 (9.31)	38.41 (13.76)	0.120

BMI: body mass index; HOMA-IR: homeostatic model assessment-insulin resistance; LDL: low-density lipoprotein; HDL: high-density lipoprotein; AST: aspartate transaminase; ALT: alanine transaminase. *P*-value calculated using Mann-Whitney test (statistical significance set at $p<0.05$). Data expressed in mean values and standard deviation in parenthesis.

Subjects were divided into obese and eutrophic groups based on their BMI. The groups are statistically different in BMI, body weight, waist circumference, percentage of body fat, insulin, HOMA-IR, HDL, VLDL, and TAG, as expected considering the metabolic changes in obesity, and the obese are, on average, older than the eutrophic subjects. The mean values of TAG and LDL in the obese group, important indicators of obesity-associated dyslipidemia, are within the

reference values described by the Brazilian Guidelines for Dyslipidemia and Prevention of Atherosclerosis (FALUDI *et al.*, 2017). It is relevant to highlight that the LDL cut-off being considered is the one used for individuals classified with "low cardiovascular risk", implying LDL concentrations ≤ 130 mg/dL. Cardiovascular risk and desirable plasma concentrations for LDL are to be assessed by a specialized physician.

Fasting glucose and plasma insulin are within normal cut-off values. The Brazilian Diabetes Society sets a value of 2.71 for the HOMA-IR index cut-off in adults and the elderly (SOCIEDADE BRASILEIRA DE DIABETES, 2020), and out of the 29 subjects, 12 present HOMA-IR higher than 2.71, indicating insulin resistance. The mean HOMA-IR values for the obese group also indicate insulin resistance and the group presents a higher mean value of waist circumference than the cut-off appointed by WHO considering the risk of metabolic complications (WORLD HEALTH ORGANIZATION, 2011b).

5.1. Lipidomic analysis

The data processing steps resulted in metadata containing 58 samples (29 before intervention and 29 after intervention) and 921 features, with 339 (36.8%) of them identified using an *in-house* library developed at Örebro University (Örebro, Sweden). Of the 339 identified features, 14 are carnitines, 109 glycerolipids, 143 glycerophospholipids, 41 sphingolipids, 29 sterol lipids, 2 phosphatidylserines, and 1 retinol. Lipid abbreviations are written accordingly to LIPIDMAPS® recommendations, with the first two or three letters indicating lipid subclass and subsequent numbers indicating the FA chain size and saturation degree (i.e., LPC(14:0) is a lysophosphatidylcholine with a FA chain of 14 carbons and zero double bonds) (LIPIDMAPS, 2003).

All 921 features (i.e., identified and unknown features) were considered for an initial multivariate analysis. No clustering and grouping tendencies were observed considering the time of intervention and BMI separately or combined as covariates. In sequence, a paired univariate analysis was applied to identify a reduced number of important features among the 921 compounds, and the analysis pointed to 44 features of interest considering just the time of intervention (Table 5). These compounds were considered for further analyses and will be referred to as the volunteers' lipidome.

Table 5 – Description of the 44 features of interest identified by the univariate analysis (continues).

Feature	m/z	RT	Lipid Class
CE(16:0)	369,3509	9.22	Sterol
Fragment: CE signature ion (1)	369,3509	9.22	Sterol
Fragment: CE signature ion (2)	369,3507	9.77	Sterol
LPC(14:0)	468,3076	2.87	Glycerophospholipids
LPC(20:5)	542,324	2.77	Glycerophospholipids
LPC(22:6)	568,3386	2.92	Glycerophospholipids
PC(16:0/16:0)	734,569	6.48	Glycerophospholipids
PC(36:4)	782,569	5.65	Glycerophospholipids
PC(42:8)	875,6223	7.00	Glycerophospholipids
PC(O-36:5)	766,5738	6.13	Glycerophospholipids
PC(O-40:6)	820,6195	6.19	Glycerophospholipids
SM(d18:1/24:0)	815,6993	7.73	Sphingolipids
TG(18:2/18:2/18:2) or TG(18:3/18:2/18:1)	896,77	8.23	Glycerolipids
TG(48:0)	829,7243	9.12	Glycerolipids
TG(48:3)	818,7222	8.15	Glycerolipids
TG(54:6)	901,7248	8.23	Glycerolipids
TG(54:6)	896,7698	8.37	Glycerolipids
ID1077	854,6978	7.39	Unknown
ID1301	970,7843	7.93	Unknown
ID1867	896,5378	5.53	Unknown
ID1875	577,5183	9.38	Unknown
ID1881	989,6986	7.72	Unknown
ID191	902,585	6.58	Unknown
ID2013	612,3243	3.28	Unknown
ID2076	894,5615	6.87	Unknown
ID2164	272,1001	0.84	Unknown
ID2308	678,6748	8.28	Unknown
ID2582	718,5365	5.77	Unknown
ID2596	957,7855	8.61	Unknown
ID2618	906,6054	6.27	Unknown
ID310	316,2476	1.75	Unknown
ID3438	817,5626	5.92	Unknown
ID3460	909,5459	6.04	Unknown
ID373	795,5762	6.49	Unknown
ID3868	282,1194	0.64	Unknown
ID4620	132,0767	0.65	Unknown
ID464	644,5947	7.69	Unknown
ID4895	841,6417	6.66	Unknown
ID780	923,7089	7.8	Unknown
ID896	429,3719	5.47	Unknown
ID948	848,6518	6.87	Unknown

Table 5 – Description of the 44 features of interest identified by the univariate analysis (conclusion).

ID953	734,6236	6.78	Unknown
ID985	226,0948	0.83	Unknown
ID988	746,5679	6.6	Unknown

Abbreviations: CE, Cholesterol ester. PC, Phosphatidylcholine. LPC, Lysophosphatidylcholines. SM, sphingomyelin. TG, Triglycerides. ID followed by number: unknown feature. RT, retention time. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains. RT and m/z were obtained experimentally.

Next, the scores-plot of the PLS-DA analysis considering the 44 features, all individuals, no BMI classification, and the variable "time of intervention" resulted in a clear group separation (Figure 3). Principal component 1 (PC1) is accountable for 10.3% of variability, while PC2 represents 4.5%. Subjects before the intervention are plotted less distantly from each other while the intervention seemed to have pushed the scores plot into two possible groups considering PC1. The R2 and Q2 values show the power and accuracy of the predictive model (RUIZ-PEREZ *et al.*, 2020). The VIP score illustrates the important features detected in the PLS-DA for PC1. (Figure 4).

Figure 3: Scores plot between the selected Principal Components (PC) in PLS-DA analysis (the explained variances are shown in brackets) and cross-validation parameters.

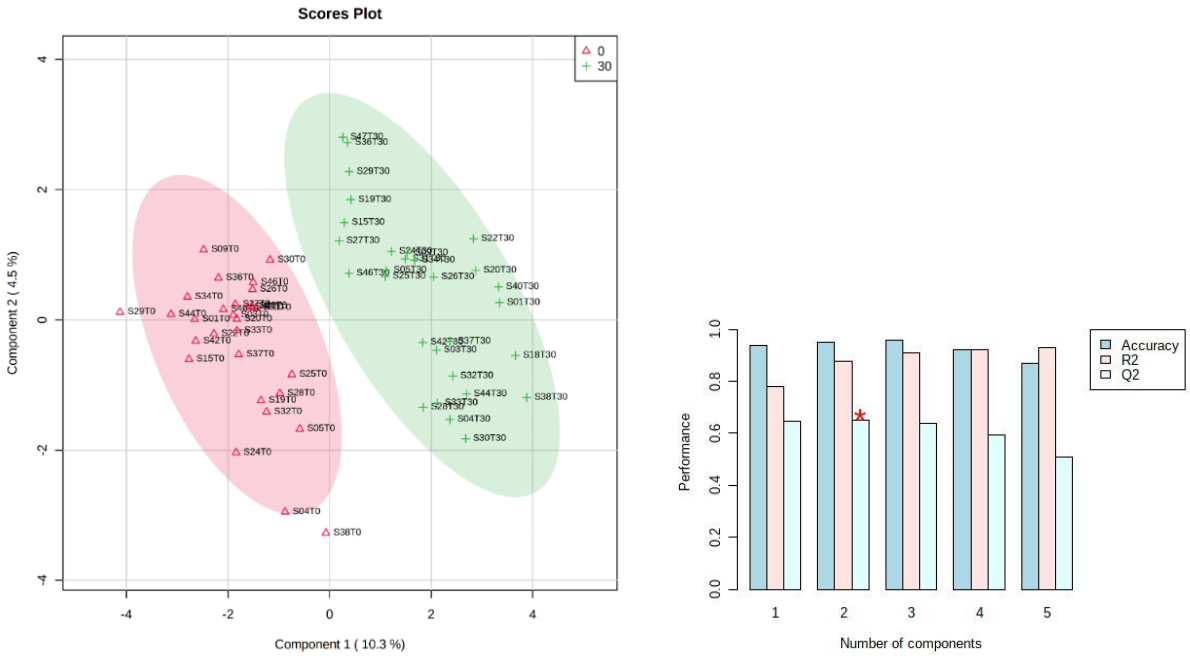
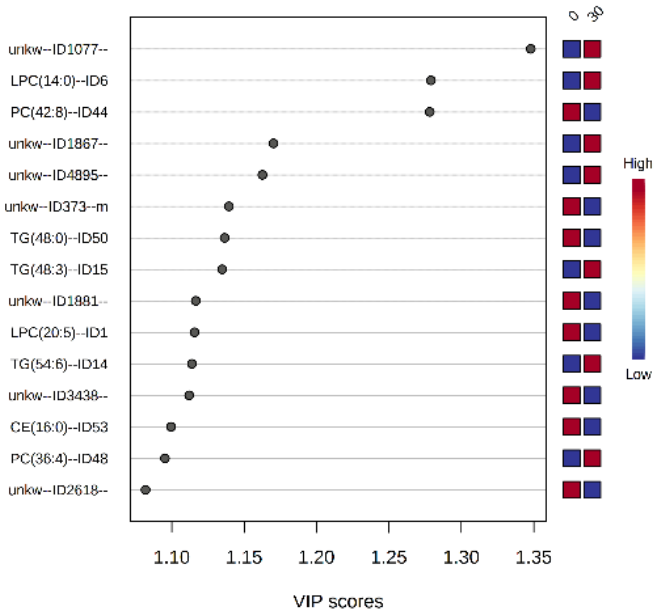


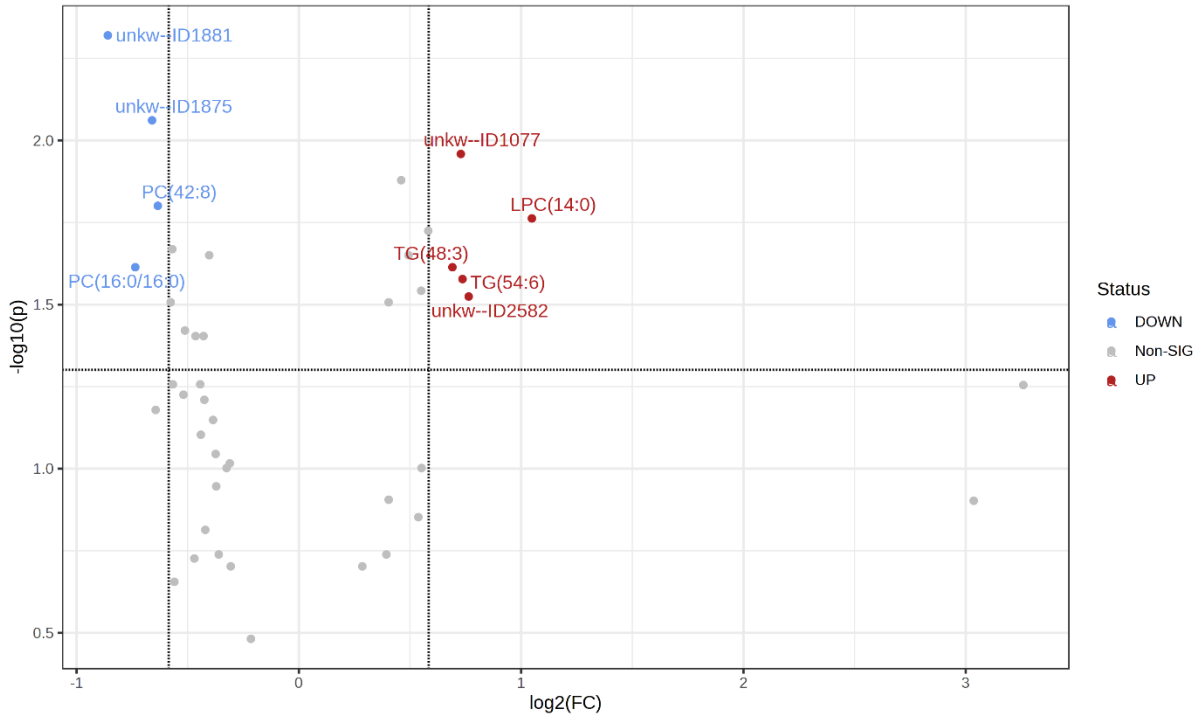
Figure 4: VIP score of important features identified by PLS-DA.



Abbreviations: CE, Cholesterol ester. PC, Phosphatidylcholine. LPC, Lysophosphatidylcholine. TG, Triglycerides. ID followed by number: unknown feature. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in time 0 or 30

The volcano plot (Figure 5) displays nine statistically different lipids considering the time of intervention (before vs. after, $p<0.05$) without separating subjects into eutrophic or obese. Increased levels are shown by a positive fold change value, whereas a decreased level is indicated by a negative fold change value. Increased features are ID1077, LPC(14:0), TG(48:3), TG(54:6), ID2582, and decreased features are ID1881, ID1875, PC(42:8), PC(16:0/16:0). These lipid species are, as expected, present in Figure 4. The top identified lipids separating subjects before and after the intervention are LPC(14:0), which is relatively increased on day 30, and PC(42:8), relatively decreased.

Figure 5: Important features selected by volcano plot.

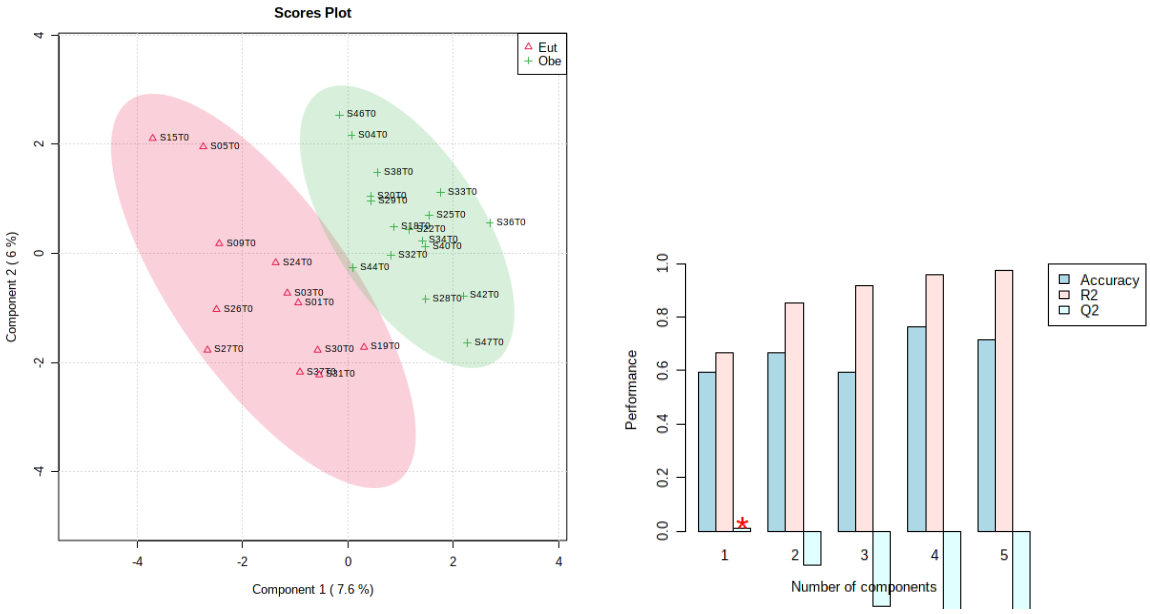


Abbreviations: PC, Phosphatidylcholine. LPC, Lysophosphatidylcholine. TG, Triglycerides. ID followed by number: unknown feature. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains. Both fold changes and p-values are log-transformed Both fold changes and p-values are log-transformed. The further its position away from the (0,0), the more significant the feature is.

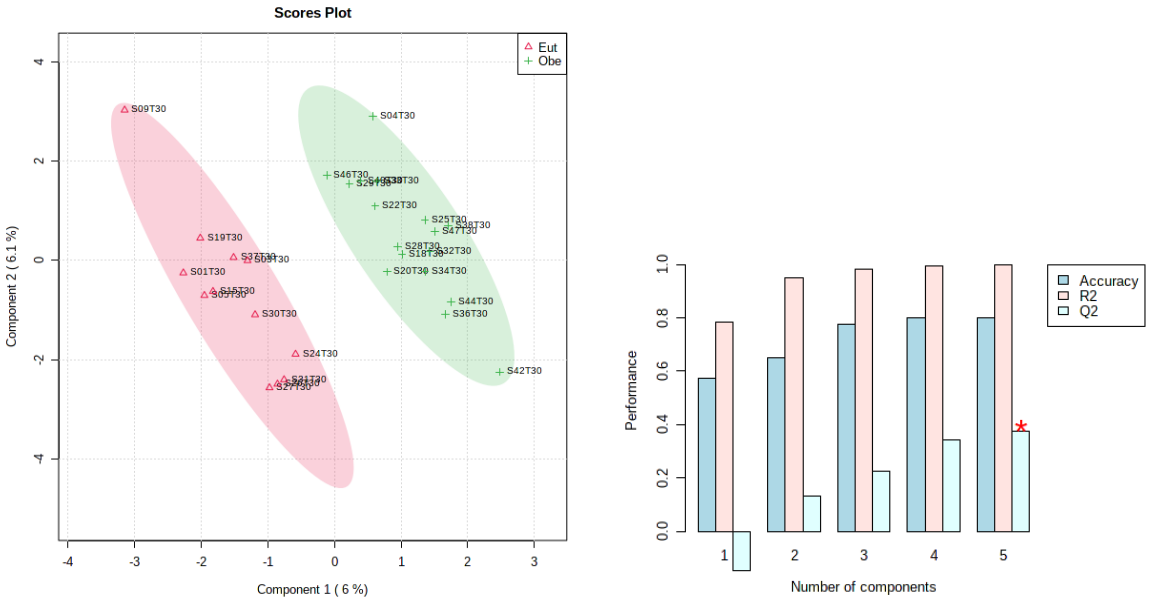
New grouping analyses were performed to understand if the eutrophic lipidome is different from the obese lipidome. PLS-DA shows separation between the groups considering their lipidome before and after the intervention (Figure 6). The eutrophic group on day zero is more scattered around the plot, suggesting subjects are less like themselves than the obese group when considering the basal lipidome. However, cross-validation for the separation model of the basal lipidome indicates the grouping might present overfitting. After the intervention, a PLS-DA analysis indicates subjects are closer between themselves when in the same group and groups more separated from one another, indicating that there is a difference in the way these groups respond to the intervention. Cross-validation of the model supports an improvement in the group separation in Figure 6B when compared to the grouping in Figure 6A. The main separating features for time 30 are displayed in Figure 7, however, the top features are still unidentified.

Figure 6: Scores plot between the selected Principal Components (PC) in the PLS-DA analysis for the eutrophic and obese subjects and cross-validation before and after the intervention.

A.

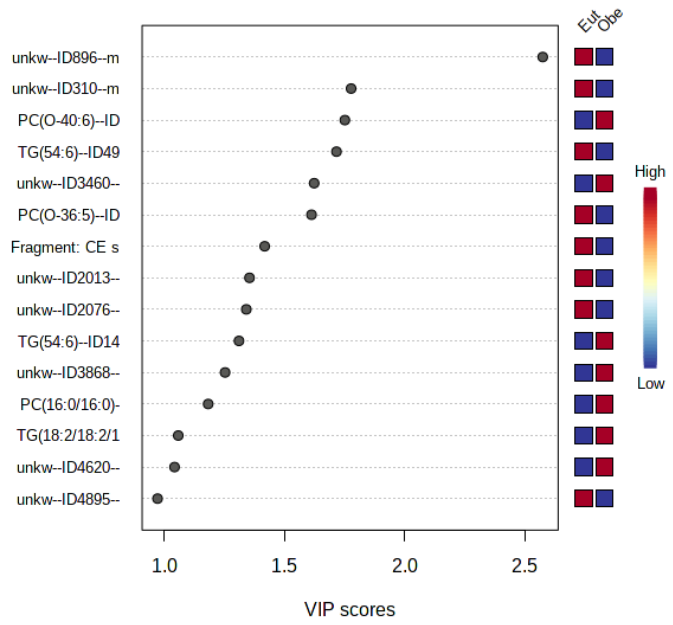


B.



A: day 0. B: day 30.

Figure 7: VIP Scores for features separating eutrophic and obese subjects after the intervention in the PLS-DA analysis.

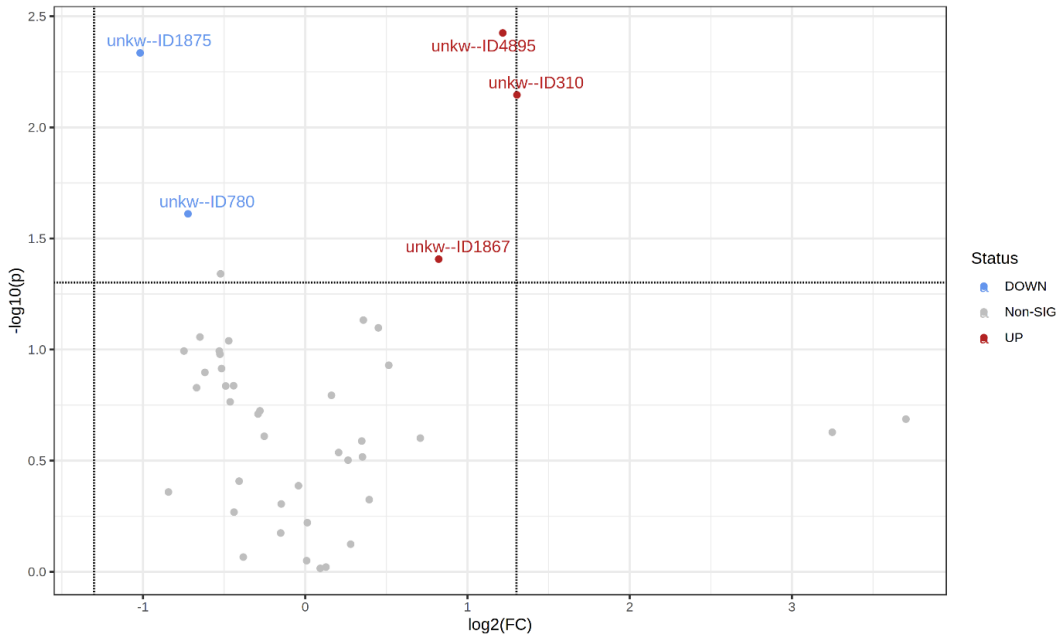


Abbreviations: CE, Cholesterol ester. PC, Phosphatidylcholine. LPC, Lysophosphatidylcholine. SM, sphingomyelin. TG, Triglycerides. ID followed by number: unknown feature. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains. A: Day 0, B: day 30.

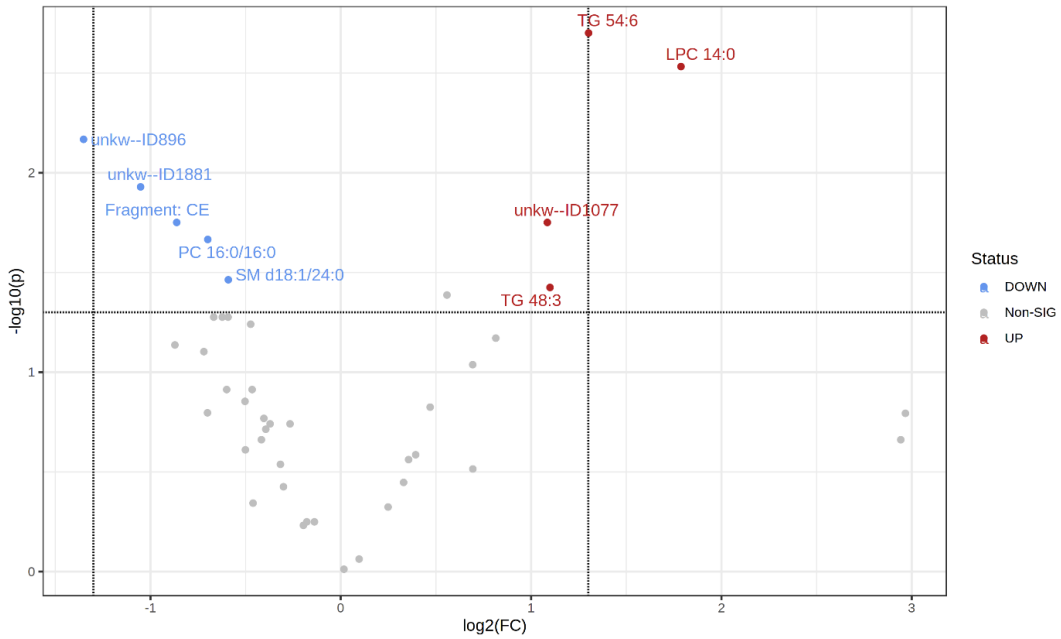
Volcano plots were built for eutrophic and obese subjects separately (Figure 8). Increased features for eutrophics are ID4895, ID310, and ID1867, all unknown, and decreased features are ID1875 and ID780, also unknown (Figure 8A). Compound ID1867 is the fourth feature of importance described in the VIP scores in Figure 4, contributing to the separation of groups in time considering all 29 subjects with no grouping by BMI. For the obese group (Figure 8B), nine important features were identified by the volcano plot. The features with increased levels after the intervention are TG(54:6), LPC(14:0), ID1077, and TG(48:3). Decreased levels are significant for ID896, ID1881, CE Fragment, PC(16:0/16:0), and SM(d18:1/24:0). All increased features for obese subjects and ID1881 (decreased) are present on Figure 4. ID1077 and LPC(14:0) are the top separating features of subjects throughout the time of intervention regardless of their BMI.

Figure 8: Important features indicate by the volcano plot for eutrophic (A) and obese (B) subjects.

A.



B.

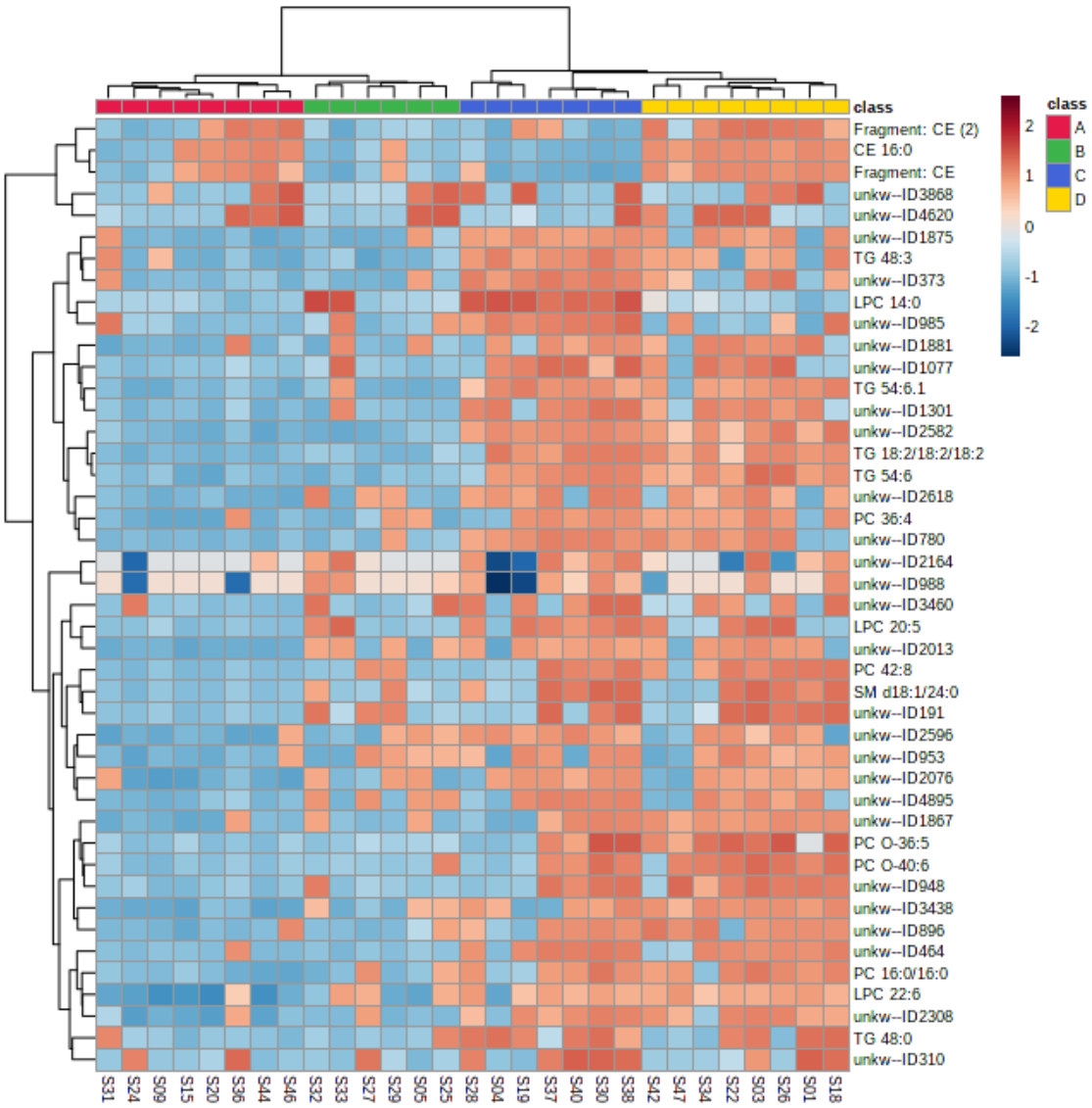


Abbreviations: CE, Cholesterol ester. PC, Phosphatidylcholine. LPC, Lysophosphatidylcholine. SM, sphingomyelin. TG, Triglycerides. ID followed by number: unknown feature. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains. Fold change threshold (x) was set to 1.5 and t-tests threshold (y) 0.05. Both fold changes and p-values are log-transformed. The further its position away from the (0,0), the more significant the feature is.

Understanding that there is a difference between volunteers' lipidome before and after the intervention, a heatmap was built considering the delta values of the plasma lipidome resulting in two defined profiles as indicated in Figure 9. To the left, a predominance of negative delta values (blue shades) for the features, whereas to the right, a predominance of positive delta values is observed (red shades). The division of subjects into eutrophic and obese groups seemed to have no influence on the clusterization. Based on the clusters formed, subjects were named into four groups, A, B, C, and D, regardless of their BMI. Groups A and D represent the extremes of the heatmap, and groups B and C represent the middle section.

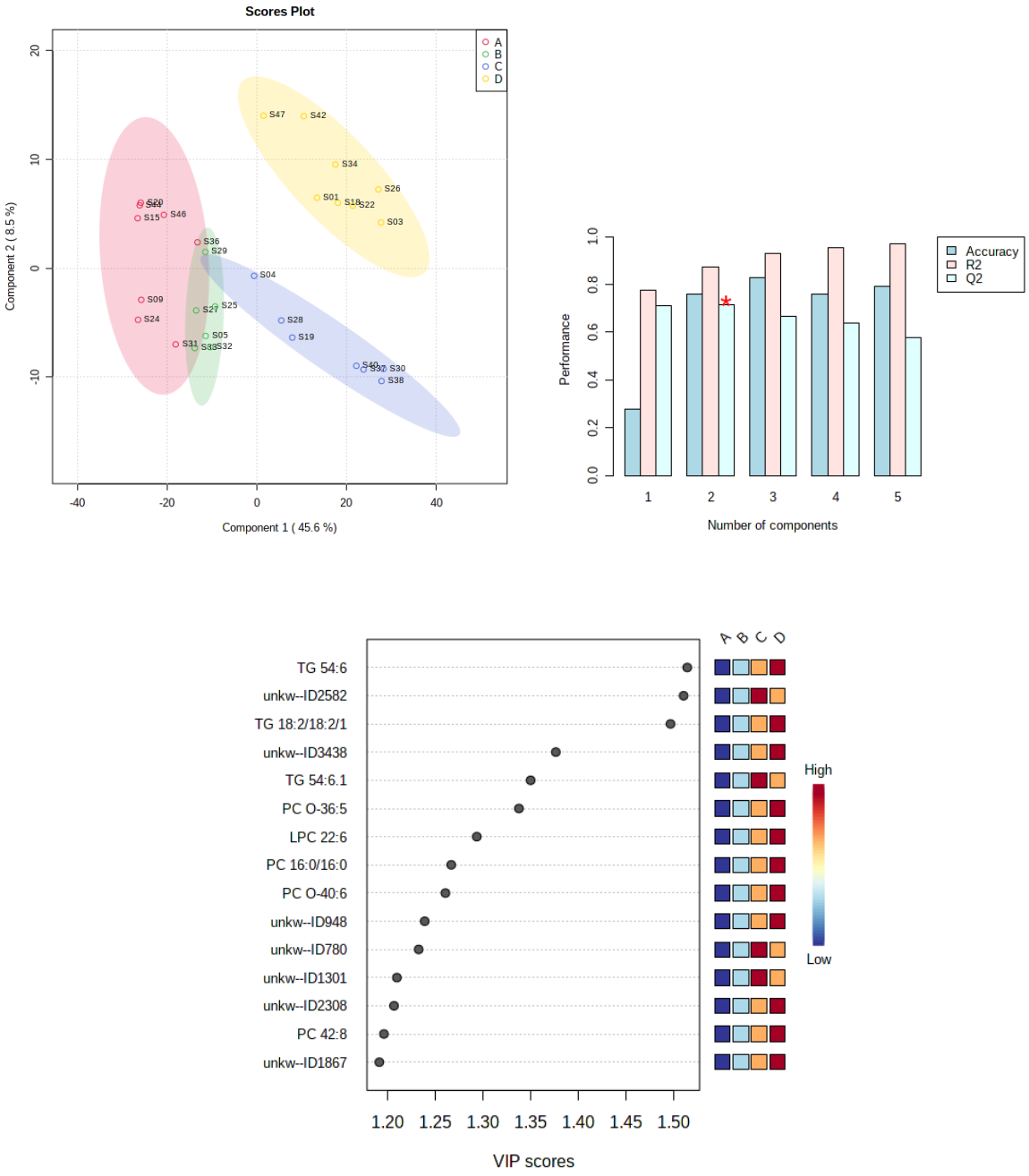
Then, a PLS-DA for these groups was performed to provide insights into whether this group separation is valid, and if yes, which features are pushing their separation (Figure 10). Group B overlaps a little bit with group A and could be considered a transition group. Group C is distinctly separated. Despite the clear separation between groups C and D, groups A and D are the most different from each other according to PC1 positioning. Groups C and D, within the side of higher delta values for the lipidome, present higher values for TG and PC species, mostly unsaturated, than the other extremity of the heatmap (Groups A and B).

Figure 9: Heatmap of delta values for the plasma lipidome.



Abbreviations: CE, Cholesterol ester. PC, Phosphatidylcholine. LPC, Lysophosphatidylcholine. SM, sphingomyelin. TG, Triglycerides. ID followed by number: unknown feature. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains.

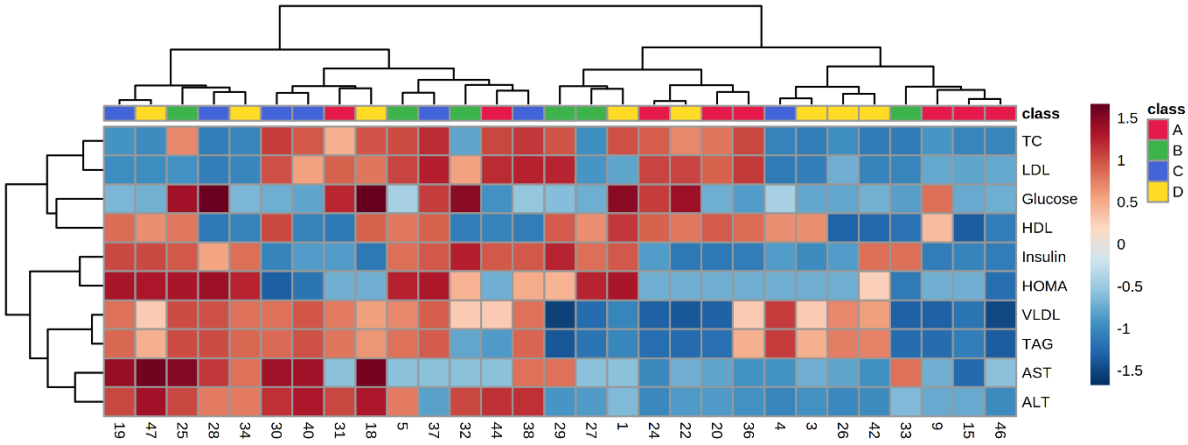
Figure 10: PLS-DA analysis of the groups A, B, C, and D, cross validation parameters and VIP score for the important features identified.



Abbreviations: PC, Phosphatidylcholine. LPC, Lysophosphatidylcholine. TG, Triglycerides. ID followed by number: unknown feature. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains.

Since the BMI appeared not to influence the clusterization in Figure 9, the biochemical parameters were tackled to investigate potential patterns for groups A, B, C, and D. Figure 11 tells us that this particular group division does not influence the clusters formed for biochemical parameters. Subjects with lower delta values for biochemical parameters are grouped to the right half of the heatmap. Out of all 29 subjects, seven present basal TC > 130mg/dL, above reference values, and five of them are positioned to the right half of the heatmap.

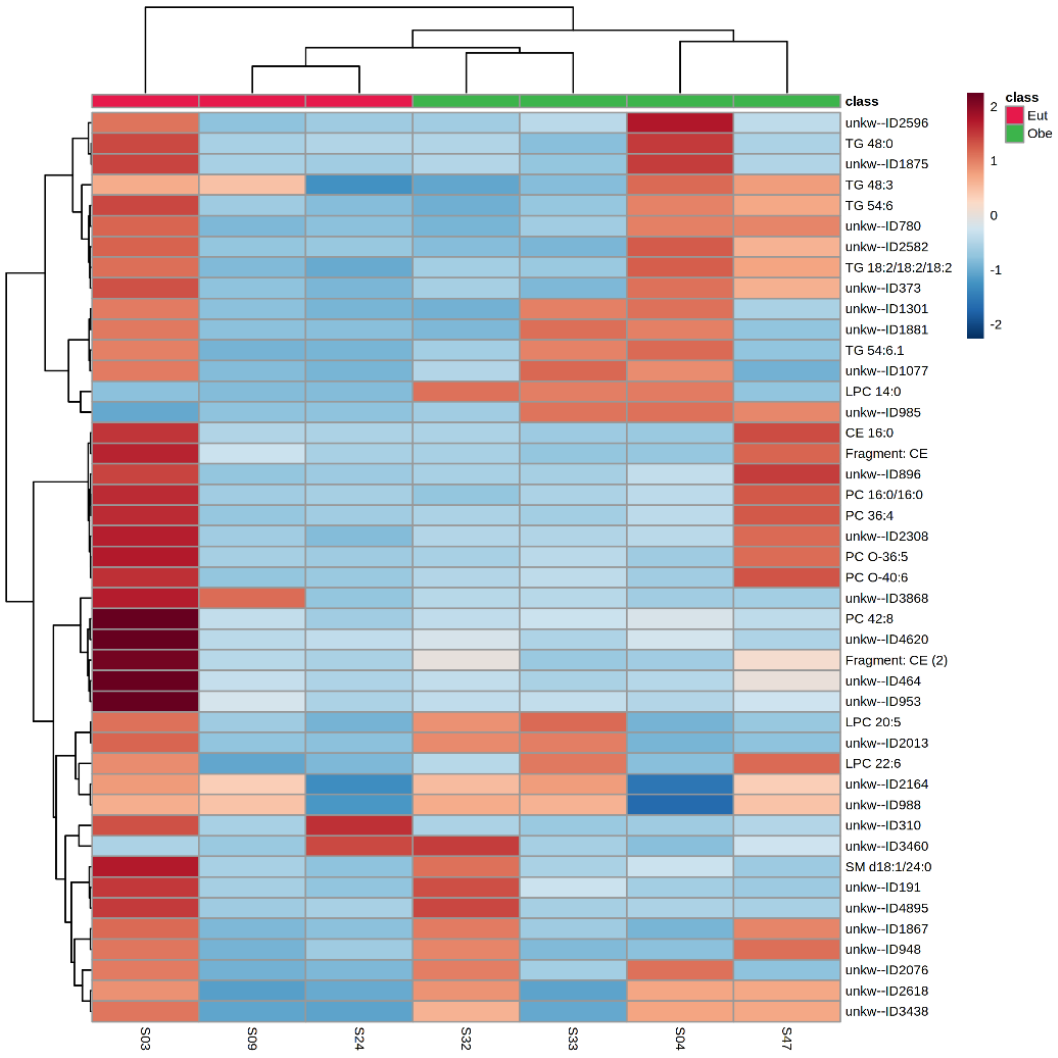
Figure 11: Delta values for biochemical parameters for groups A, B, C, or D.



HOMA-IR: homeostatic model assessment-insulin resistance; LDL: low-density lipoprotein; HDL: high-density lipoprotein; AST: aspartate transaminase; ALT: alanine transaminase.

Then, based on the heatmap of Figure 11, the delta values for the lipidome of the seven subjects with basal TC above reference values (three eutrophic and four obese) were plotted on another heatmap for investigation (Figure 12). Subject 03 is eutrophic and behaves differently when compared to other volunteers in either group, eutrophic or obese. Subjects 9, 24, 32, and 33 a clustered together and belong to groups A or B, groups that present relatively lower levels of PC and TG species. Five of the seven subjects with altered TC present a rather homogeneous behavior for lipidome features within the middle cluster (Figure 12), among which are a considerable number of PCs considering the identified features.

Figure 12: Delta values for the lipidome of subjects with basal TC > 190 ng/dL.



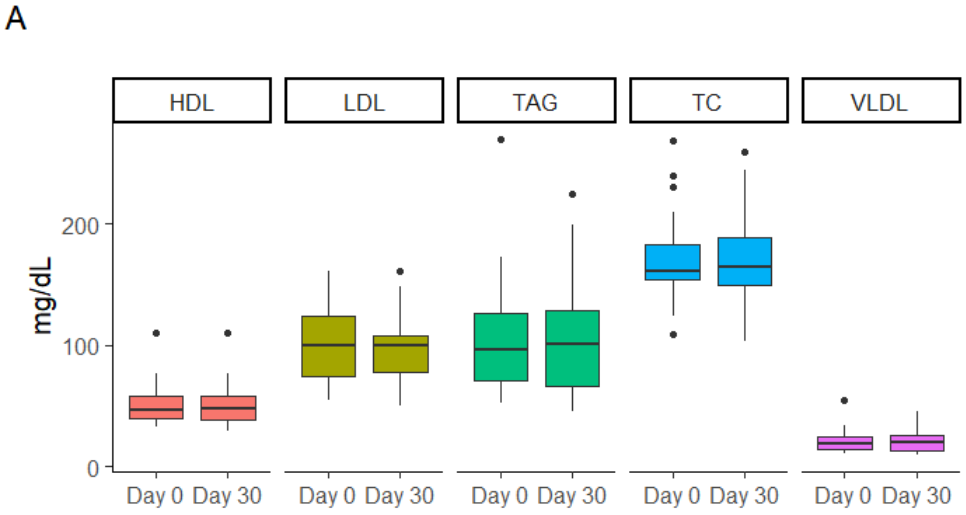
Abbreviations: CE, Cholesterol ester. PC, Phosphatidylcholine. LPC, Lysophosphatidylcholine. SM, Sphingomyelin. TG, Triglycerides. ID followed by number: unknown feature. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains.

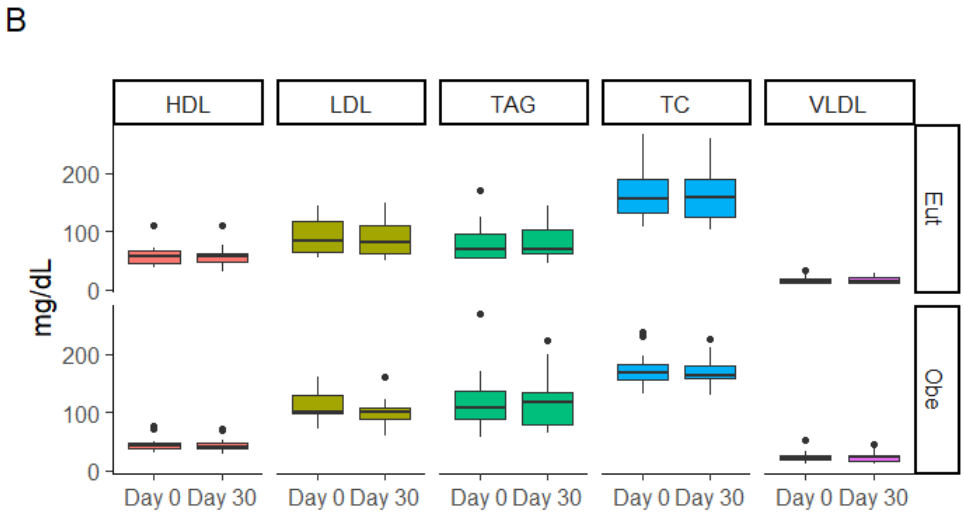
5.2. Biochemical parameters.

Biochemical parameters were assessed before and after the intervention considering all subjects and then separating the eutrophic and obese groups. No statistically significant difference was found for fasting blood glucose, insulin, HOMA-IR, and hepatic enzymes. The same comparison strategy was applied to assess the subjects' lipidic profile before and after the intervention. There is no statistical difference between TC, LDL, HDL, VLDL, or TAG before and after the intervention for all individuals or when separating them into eutrophic and obese. However, there is a noticeable decrease in the dispersion around the median displayed in the boxplot for LDL in all volunteers (Figure 13A) and in the obese group (Figure 13B).

Of the 29 subjects, seven (three eutrophic and four obese) presented plasmatic TC concentrations ≥ 190 mg/dL, the reference value proposed by the Brazilian Guidelines for Dyslipidemia and Prevention of Atherosclerosis (FALUDI *et al.*, 2017). When assessing these individuals only, there is a statistically significant decrease in TC values considering the time of intervention, with a p-value of 0.029 (Figure 14). Of these subjects, only 2 present HOMA-IR values indicative of insulin resistance, one eutrophic and one obese. A decrease in body-fat percentage is also observed for these subjects (p = 0.015). Five of them present waist circumference above reference values and 4 have body fat percentages ≥ 30% (BOILEAU, 1993).

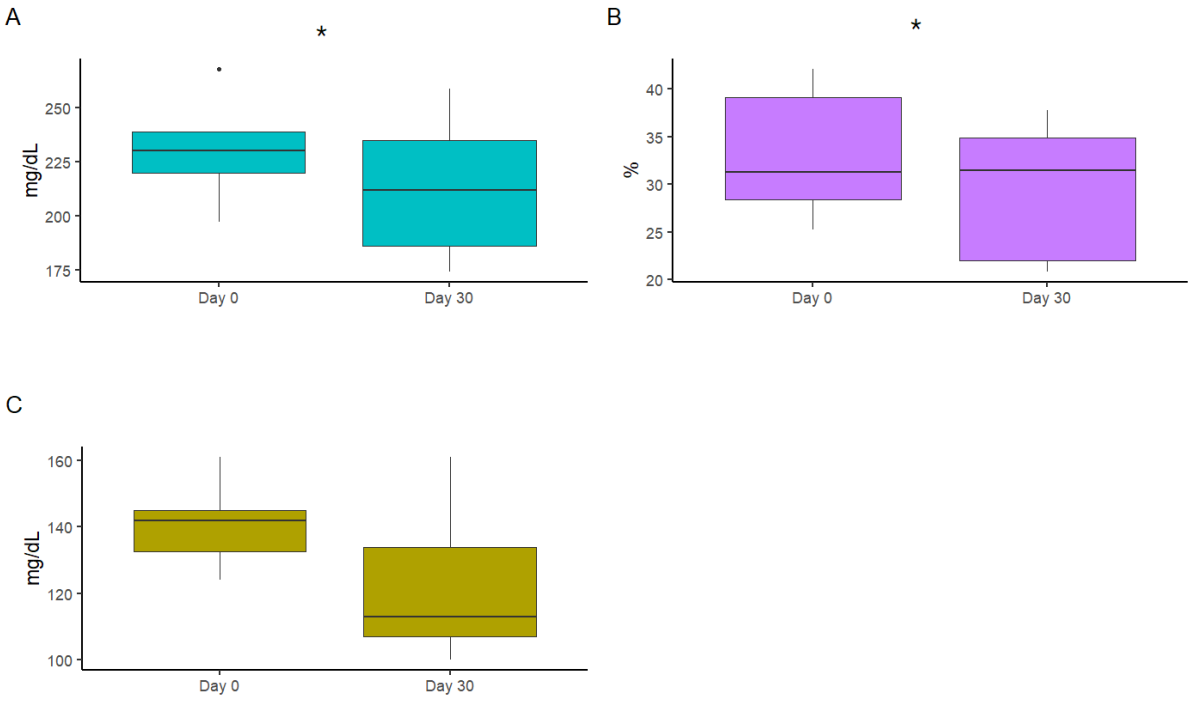
Figure 13: Lipid parameters before and after the intervention. All individuals (A) and grouped by their nutritional status (B).





P-values calculated either by Student's t-test or Wilcoxon W rank. Normality assessed by Shapiro-Wilk. Abbreviations: High-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), total cholesterol (TC), and triacylglycerol (TAG).

Figure 14: TC (A), Body fat percentage (B) and LDL (C) for subjects with altered basal TC (n=7).



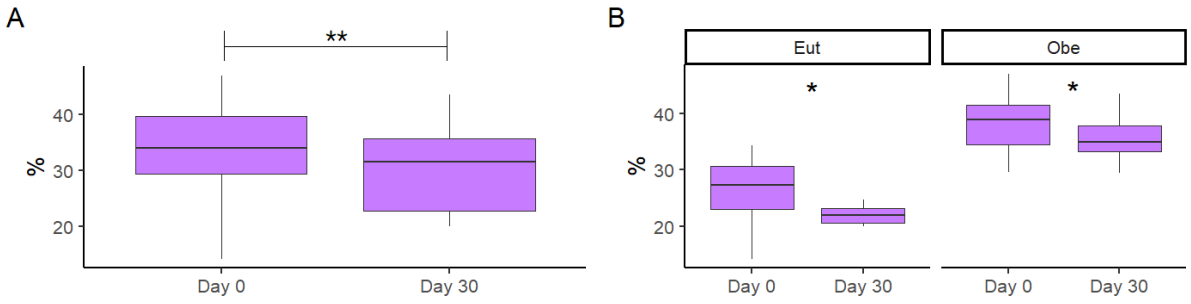
Asterisks indicate statistical significance (* = $p < 0.05$) according to Student's t-test. Normality assessed by Shapiro-Wilk test.

According to the American Heart Society, waist circumference is a more accurate risk factor for cardiovascular diseases than BMI (AMERICAN HEART ASSOCIATION, 2018). No other biochemical parameters are statistically different for this fraction of the study population. Six of the seven subjects with altered TC present altered basal LDL concentrations (≥ 130 mg/dL). There is a decrease in their LDL levels as indicated by the boxplot (Figure 14 C), however, it is not statistically significant (p=0.054).

5.3. Anthropometry

Subjects were measured for anthropometrical parameters, and, for this project, the abdominal circumference, percentage of body fat, BMI, and body weight were evaluated. Comparing all subjects, before and after the intervention, there is a statistically significant decrease in body fat percentage ($p<0.001$) (Figure 15A). After stratifying volunteers by the nutritional estate, this decrease remained statistically different in the eutrophic group ($p=0.015$) and the obese group ($p=0.016$) (Figure 15B). No significant changes were observed in body weight, BMI, and abdominal circumference.

Figure 15. Body fat percentage before and after the intervention. A: All subjects. B: grouped by their nutritional status.



Asterisks indicate statistical significance (** = $p < 0.01$, * = $p < 0.05$) according to Student's t-test. Normality assessed by Shapiro-Wilk test.

5.4. Short-chain fatty acids

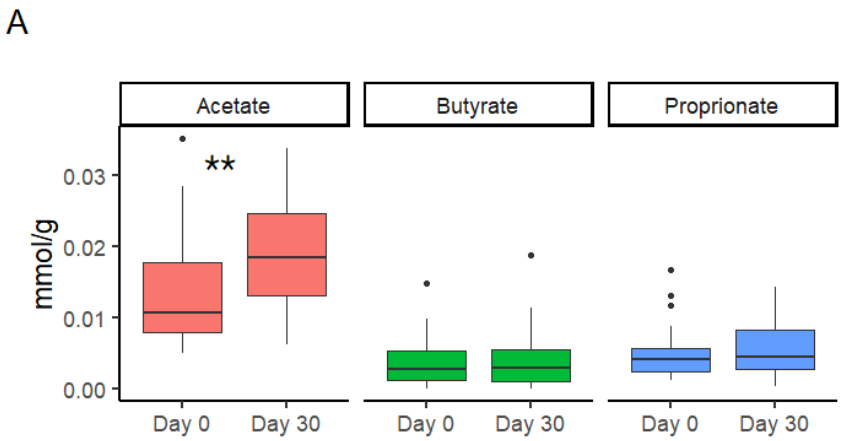
Data on short-chain fatty acids were obtained for 28 of the 29 subjects previously selected for this project due to sample availability. Of the 28 subjects, 11 are eutrophic and 17 are obese.

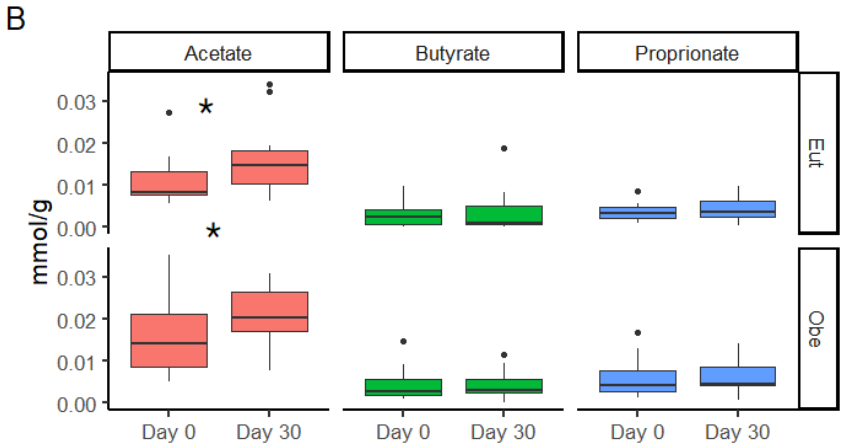
The main SCFA produced by gut microbiota metabolism were analyzed in fecal samples. Comparing the eutrophic group with the obese group before and then after the 30 days of the study, the statistical analysis showed that the groups are not different in fecal SCFA amounts regardless of the time of intervention. However, the paired analysis resulted in a significant increase in acetate ($p<0.001$) after the intervention (Figure 16A). This is maintained in the stratified analysis for both groups, obese ($p=0.025$) and eutrophic ($p=0.012$) (Figure 16B). For butyrate and propionate, no significant changes were observed.

A Spearman rank analysis between the variation of the short-chain fatty acids and biochemical parameters showed no statistically significant correlation between the acetate and TC variables (rho = 0.567, $p>0.05$).

Sample intersection between the volunteers who presented plasmatic TC ≥ 190 mg/dL and SCFA consists of six subjects (five obese and one eutrophic) since there was no fecal sample available for subject 9. Subjects either presented no variation in acetate or a positive delta value (Figure 17). A correlation analysis between the variables resulted in rho=0.37 and p=0.497, therefore, not statistically significant.

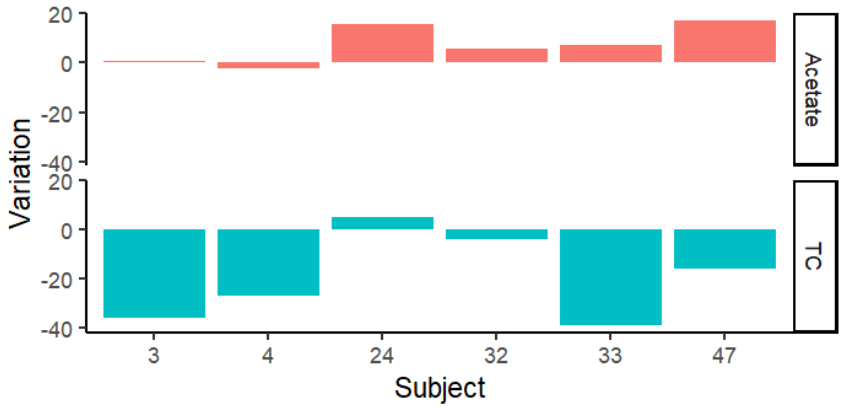
Figure 16. Main short-chain fatty acids before and after the intervention. A: All subjects. B: Grouped by nutritional status (B).





Asterisks indicate statistical significance (** = $p < 0.01$, * = $p < 0.05$) according to Student's t-test. Normality assessed by Shapiro-Wilk test.

Figure 17. Acetate (nmol/g) and TC (mg/dL) variation for subjects with basal TC ≥ 190 mg/dL.



6. DISCUSSION

Circulating lipids are modulated by an individual's diet, thus their relevance in nutritional science. Current existing tools for lipidomic analysis allows the identification and quantification of a large range of lipid species, making interpretation of the results rather challenging. Having narrowed down the number of features considered for the statistical analyses, it was possible to describe the volunteers' lipidome within lipid identification limitations, indicating differences in features and separation tendencies considering the time of the intervention.

After inferring that there was a change in the lipidome after the ingestion of passion fruit, further investigation of the lipids aimed to perceive if the eutrophic and obese groups responded differently to the intervention, which, based on the PLS-DA analyses, they did. The increased features in the volcano plot for obese subjects are all present in the VIP scores that separate all subjects before and after the intervention, with two of them being the top-rated features (ID1077 and LPC (14:0)). This suggests that the obese contribute more to this separation, which could be due to 1) there being slightly more obese subjects in the study than eutrophics, or 2) obese being more responsive to the intervention. It has been argued that dietary interventions with functional foods or bioactive compounds and their food matrices could benefit those who present an altered metabolism, such as obese or insulin-resistant subjects (SANTOS *et al.*, 2022; SANTOS, 2020a).

Despite the eutrophics and obese subjects presenting different responses to the intervention, the heatmap in Figure 6 shows us that there are two distinct graphical representations in terms of responsiveness to *P. tenuifila*, one that represents an increase and another representing a decrease in features, and they are not based on BMI. The PLS-DA analysis of the then attributed group classification into A, B, C or D confirms this different behavior, with groups C and D presenting higher relative levels of TG and PC species.

When investigating groups (A, B, C, and D) biochemical parameters, no clear group-dependent behavior was identified. However, most of the subjects with altered basal TC were placed in the same group of the heatmap on Figure 10 and belong, in majority, to groups A and B. Separating only the seven subjects with elevated basal TC, it is possible to observe (Figure 11) a cluster of decreased features with a fair number of PCs, disregarding subject 03, who behaves as an outlier according to heatmap clustering in MetaboAnalyst and is isolated from

cluster grouping. These subjects also presented a decrease in plasma TC when measured by conventional biochemical analysis.

Alongside ceramides, PCs and LPCs have been investigated for their potential association with cardiovascular diseases. Data from patient studies gathered by Carvalho *et al*. indicate that most LPC species seem to be negatively correlated with chronic heart conditions, whereas LPC 20:3 and 20:4 seem to be positively correlated. Esterified PC (16:0) is positively correlated with acute cardiovascular conditions (CARVALHO; CHAVES-FILHO; YOSHINAGA, 2022). PC 16:0/16:0 is decreased in obese subjects and in the analysis considering all individuals. Serna *et al.* found that PC, PE, LPC, and LPE species were lower in individuals with ideal to intermediate scores for cardiovascular diseases when compared to individuals with scores representative of higher risks (RIVAS SERNA *et al.*, 2021).

In addition, phosphatidylcholines are the main phospholipid in lipoproteins, representing up to 70% of this lipid class. It has been reported that impairment in biosynthesis or in the bioavailability of PCs reduces the liberation of VLDL in the liver and circulating VLDL with a lower amount of PCs are cleared at a faster rate compared to regular VLDL (COLE; VANCE; VANCE, 2012).

Even though they seem to be potential markers of both poor and good cardiovascular health, the role of glycerophospholipids in CDV is still to be elucidated, since the literature is controversial and varied. The saturation degree and the number of carbons in the alkyl chain are important characteristics of the activity of these lipid species. This is interestingly demonstrated by Eichelmann *et al*, who associate lipid classes with CDV risk, and the same class can present higher or lower associated risk depending on their FA chain composition (EICHELMANN *et al.*, 2022). The enzymatic pathways involved in lipid metabolism add further complexity to understanding their role in the pathology of CDV. For instance, PCs are secreted from the liver in VLDL, converted into LPCs, and then converted back into PC by specific enzymatic routes and the activity of these enzymes can alter the glycerophospholipid concentrations in the circulation (LAW *et al.*, 2019).The limitation of the number of unannotated features, which are quite significant, impaired the tentative to investigate more lipid species associated with BMI. In a study to investigate obesity-related lipids and the changes it causes on the lipidome, Pikó and colleagues found that the four lipid species PE P-16:0/20:3, TG 20:4_33:1, TG 2:6_36:4, TG 18:3_33:0 were positively associated with obesity, while three lipid species were negatively associated with the condition (Hex-Cer 18>1;O2/22:0, LPC 18:2, PC 18:2, PC 18:1_18:1) (PIKÓ *et al.*, 2021). In addition, they reported that certain

lipid classes were significantly increased in the plasma of obese and overweight subjects when compared with lean subjects. TGs, DGs, PEs, and SMs plasmatic concentrations increased with BMI, while LPCs and HexCer were lower in obese subjects (PIKÓ *et al.*, 2021).

Besides the dense variety of lipid structures, current research that aims to understand how specific lipids are involved in overweight, obesity, and NCD is controversial due to the variety of study designs and methods employed throughout the lipidomic workflow, the interindividual variability, and how different diets result in a different abundance of lipid species. A diet that is abundant in saturated fat will result in an increase of saturated lipid species and the contrary is also valid, with changes in the lipidome being acute or chronic (CASTRO-ALVES; OREŠIČ; HYÖTYLÄINEN, 2022).

Considering the biochemical parameters, the eutrophic subjects of this study can be considered healthy. On the other hand, the obese subjects present high mean values for HOMA-IR, an indicator of insulin resistance and a higher risk for NCDs and metabolic diseases compared to eutrophics or subjects within cut-off values for this parameter. According to Blüher (2020), even obese subjects considered metabolically healthy, with preserved insulin sensitivity, still present an important higher risk of type 2 diabetes than lean healthy subjects (BLÜHER, 2020). Despite other passion fruit species reporting effects of improving insulin sensitivity (DUARTE *et al.*, 2022, 2020), no alterations in glycemic profile were found for *P. tenuifila*.

A study published by the American Heart Society, which followed more than 500.000 subjects, suggests that the waist circumference and the waist-hip ratio are better predictors of myocardial infarction than the BMI, with a higher ratio associated with a higher risk of myocardial infarction in women (PETERS; BOTS; WOODWARD, 2018). The *Brazilian Longitudinal Study of Adult Health* (ELSA-Brasil) also found that a higher lower limb fat percentage compared to trunk fat is associated with a lower risk of developing cardiovascular events in 10 years (DE OLIVEIRA *et al.*, 2022). In this way, a decrease in total body fat percentage observed in subjects after the intervention with *P. tenuifila* for 30 consecutive days could indicate a decrease in CVD risk. However, data on body fat distribution is not available. This result could also be due to eventual alterations in subjects' diet during the month-long intervention. No changes in other anthropometrical parameters were observed.

Furthermore, dyslipidemia is another important metabolic factor for CVD risk or protection, mainly translated as alterations in the plasmatic TC, HDL, and LDL. The role of LDL in the

atherogenic process, resulting in cardiovascular diseases, is well elucidated: a higher plasmatic concentration of LDL means that LDL particles are in the blood for a longer period, facilitating their accumulation in the subendothelial space, where they are oxidized. This process happens in the combined scenario of increased plasma LDL, oxidative stress, and inflammation, all present in obesity (SIQUEIRA; ABDALLA; FERREIRA, 2006). Inversely, HDL levels are associated with a lower risk of cardiovascular events, with its main role being the transport of cholesterol from peripherical tissues to the liver. The decrease in TC observed in this work, considering subjects with altered basal TC, can be associated with a decreased risk for cardiovascular events, being a relevant outcome.

The most abundant phenolic compound in *P. tenuifila* are PAC. Polymerized forms of PAC are poorly absorbed throughout the digestive tract and most of it will reach the colon intact. There, they are metabolized by the gut microbiota, resulting in phenyl-γ-valerolactones and phenyl-valeric acids, which are then absorbed and bioavailable to exert biological effects (MENA *et al.*, 2022). Regarding the evidence in humans, epidemiological studies, metanalysis, and clinical trials have reported the reducing TC and LDL effects and an overall improved lipoprotein profile for foods rich in flavan-3-ol monomers and oligomers (KHAN; MUKHTAR, 2007; ZANOTTI *et al.*, 2015; ZHENG *et al.*, 2011).

In vitro and *in vivo* studies have demonstrated the positive effects of PAC in lipid metabolism. In a study to evaluate the hypolipidemic effects of PAC-rich GSPE (gavage, 250 mg/kg body weight *vs* lard oil 2.5 mL/ kg body weight) in Wistar rats and *in vitro* models, a decrease in hepatic and plasmatic TAG and cholesterol were observed, as well as repressing of miRNA-122 and miRNA-33, genes involved in lipid metabolism (BASELGA-ESCUDERO *et al.*, 2012). PAC modulation of sterol-regulatory element binding proteins 1c (SREBP-1c) and PPARα have been demonstrated in Caco-2/15 cells, with positive outcomes such as improved fatty acid β-oxidation and diminished lipogenesis, which seems to result from improved insulin sensitivity (KOUDOUFIO *et al.*, 2021). HepG2 cells treated with metabolites originating from the serum of GSPE-fed mice presented lowered free cholesterol (52%), lowered cholesterol ester (39%), and lowered TAG (72%) when compared to control (GUERRERO *et al.*, 2013). Quesada and colleagues studied the lipid-lowering effects of PAC in mice treated with GSPE (250 mg/kg body weight) and the TAG reduction after treatment was attributed to a decrease in VLDL and chylomicron in a time-dependent manner, suggesting that GSPE blocked the secretion of these lipoproteins. In addition, CPT-1 activity assessment in this work indicated elevated FA oxidation after administration of GSPE (QUESADA *et al.*, 2012).

Different degrees of polymerization and different positions where ligands can bind to the complex of flavan-3-ols result in a great variety of structures and biological effects. The diversity of PAC structures within a food matrix of interest adds further complexity to the scenario, affecting standardization, reproducibility, and elucidation of the mechanisms of action. In an in vivo study, diabetic mice were fed 200 mg/kg per day of cinnamon extracts rich in either type B or type A proanthocyanidins for 4 weeks, and both resulted in lowered fasting glucose and higher concentrations of serum insulin, but only type B rich extract exerted effects on lipids, lowering plasmatic TAG (CHEN *et al.*, 2012).

Besides structure variability, interindividual variability in proanthocyanidin metabolism is another complex factor when studying the effects of these compounds. Since the actual bioavailable compounds are the gut microbiota derivative metabolites of proanthocyanidins, PVLs and PVAs, variations in the gut microbiome composition are one of many factors that may affect the results observed in the available literature. To further investigate and understand the interindividual variability associated with PAC, attempts to stratify subjects by metabotypes are upcoming strategies with controversial but promising results for these specific bioactives (CORTÉS-MARTÍN *et al.*, 2019; MENA *et al.*, 2022).

Luteolin is the main flavonoid present in *P. tenuifila*, mainly in C-glycosylated forms, and their amount varied from 24.12 to 40.14 mg/100g FW in the analyzed batches of passion fruit (SANTOS *et al.*, 2021). Luteolin is reported to have positive effects on glucose metabolism and inflammation, investigated as an antidiabetic agent (AL-ISHAQ *et al.*, 2019). Apigenin is the second flavone class more abundant in *P. tenuifila*, also found as C-glycosides (6.82 – 18.62 mg/100g in the batches characterized). Likewise, apigenin studies point to effects on glucose metabolism (AL-ISHAQ *et al.*, 2019; SANTOS *et al.*, 2021).

The doctoral project conducted by Santos (2020) reported that among the metabolites found in urine samples of the subjects enrolled in the clinical trial, six are directly correlated to the metabolism of flavan-3-ols, of which five are PVLs and PVAs and one is a glucuronidated metabolite of methyl-(epi)-catechin. Moreover, the flavone luteolin, present in *P. tenuifila*, and some of its metabolites were also identified in the urine samples (SANTOS, 2020a). What can be inferred from this analysis is that the phenolic compounds related to the intake of passion fruit were bioavailable in these subjects and could be related to the lipid modulation effects found in our studies.

Dietary fibers (DF) are also present in significant amounts in *P. tenuifila*, particularly insoluble ones (IF). DF has long been associated with improved gastrointestinal health, despite being a very heterogenous class of compounds, with different solubility, viscosity, and fermentation properties. Protection against obesity and CVD is also reported for specific DF, with a significant variation in proposed mechanisms (ANDERSON *et al.*, 2009; DAYIB; LARSON; SLAVIN, 2020). An *in vivo* study evaluated the effects of extracted IF from defatted rice bran on the lipidic profile of high-fat-fed rats and observed ameliorated hyperlipidemia, with a decrease in plasmatic TC and LDL (LIU et al., 2021)(LIU et al., 2021)(LIU et al., 2021)(LIU et al., 2021).

Of the general properties attributed to DF as a class, reduction in appetite, improved gastrointestinal transit, and being substrates for the gut microbiota are generally accepted by the scientific community (DAYIB; LARSON; SLAVIN, 2020). SCFA are the main products of fiber fermentation by the gut microbiome, including IF (WIDANINGRUM et al., 2020), and are being investigated for their post-biotic action, including on the energetic metabolism, mainly lipid metabolism. The major SCFA are acetate, butyrate, and propionate, produced in a proportion of 60:20:20 respectively (HE *et al.*, 2020; PORTINCASA *et al.*, 2022).

An increase in fecal acetate was observed for volunteers after the ingestion of *P. tenuifila* for 30 consecutive days, with no statistically significant changes in fecal butyrate and propionate. While the production of propionate and butyrate seems to be more specific to certain bacterial genera, acetate synthesis pathways are commonly shared by the gut microbiome, which can be modulated by certain diets and food components. Butyrate is more readily used by enterocytes and can be less available in feces, which could partially explain the results of our analysis (MORRISON; PRESTON, 2016).

Acetate is believed to activate *free fatty acid receptor 2* (FFAR2), also known as GPR43, a G protein-coupled receptor involved in many signaling pathways associated with metabolic diseases, significantly expressed in white adipose tissue, pancreas, and intestinal enteroendocrine cells (HE *et al.*, 2020; NAKAJIMA *et al.*, 2017). In enteroendocrine cells, activation of FFAR 2 by acetate seems to suppress lipid accumulation in the white adipose tissue and stimulate the secretion of PPY and GLP-1, reducing appetite (DELEU *et al.*, 2021b; KASUBUCHI *et al.*, 2015).

Other animal studies aimed to assess the effects of acetate on lipid metabolism and adipose tissue remodeling, such as the one conducted by Sahuri-Arisoylu *et al*. where nanoparticle-

delivered acetate was administered three times a week, for 6 weeks, in mice fed with either a high-fat diet or normal fat diet. The findings indicate reduced adipose tissue lipolysis, reduced hepatic *de novo* lipogenesis, reduced whole body fat percentage, and circulating plasma FA compared to control (SAHURI-ARISOYLU *et al.*, 2016).

Still, despite the relationship between acetate and lipidic metabolism, no statistically significant correlation was found between the variation of TC and acetate in subjects with basal TC > 190 mg/dL, who presented lipid-lowering effects after the intervention. The small number of subjects in this analysis is a limitation.

A few points can be noted as limitations of the study, such as i) the wide range of age considered for the clinical trial, an important factor in interindividual variability, ii) most subjects are female both at reproductive age and menopausal and no information on oral contraception is available, iii) no placebo control branch was assessed in the clinical trial, so the placebo effect cannot be ruled out completely. The use of oral contraception can affect the plasmatic levels of TC and lipoproteins (FIGUEIREDO *et al.*, 2021), still, hormonal therapy for menopausal women was an exclusion criteria, and subjects were advised to continue their regular habits during the month-long intervention. Despite the study design not being able to rule out a placebo effect, the clinical trial was the first study ever to prospect metabolic health effects for *P. tenuifila*, with no expected outcomes. The importance of diet has been highlighted in this dissertation, but no data on dietary patterns were assessed for the study. But then, again, subjects were oriented not to change their dietary habits, except on the three days before sample collection, when PAC ingestion should be restricted. Despite some noteworthy limitations of the clinical trial design, this project aims to assess the lipidome of the subjects in an exploratory manner.

Importantly, the intervention must consider *P. tenuifila* as a complex food matrix, with a series of components performing a synergic effect over lipid metabolism. One of the objectives of molecular nutrition research is to understand the regulatory mechanisms of lipids in health and disease, making personalized nutrition more accurate when considering the interindividual differences in dietary intervention responsiveness. This work contributed to the available evidence on food bioactives and health, being the first investigation of the effects of *P. tenuifila* over lipid metabolism and suggesting a populational group that can benefit from the health-promoting effects of this passion fruit for upcoming studies.

7. CONCLUSION

The lipidomic analysis showed a change in the plasma lipidome of subjects after the intervention once significant features were nominated. The subjects' lipidome showed that eutrophic and obese subjects respond differently to *P. tenuifila*, despite the limitation of unidentified lipids. The clearest difference in responsiveness is not dependent on BMI. The lipidomic profile for subjects with altered basal TC presented a visible pattern of negative variation for PCs, and negative and positive variation for LPCs, lipids belonging to a class studied as potential biomarkers in CVD. The intervention resulted in an increase of fecal acetate, SCFA with important activity over the lipid metabolism regulation; a reduction in body fat percentage; and a reduction in plasmatic total cholesterol for subjects who started the intervention with this parameter above the reference values.

The results of this study bring to discussion the potential cardioprotective effects of the bioactive compounds present in *P.tenuifila*, especially proanthocyanidins and their bioactive metabolites, and who is the population benefiting the most from the intervention, casting light on possible paths for future research.

8. REFERENCES

AL-ISHAQ, R. K. *et al.* Flavonoids and Their Anti-Diabetic Effects: Cellular Mechanisms and Effects to Improve Blood Sugar Levels. **Biomolecules**, [*s. l.*], v. 9, n. 9, 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31480505/. Acesso em: 19 set. 2022.

AMERICAN HEART ASSOCIATION. **Waist size predicts heart attacks better than BMI, especially in women | American Heart Association**. [*S. l.*], 2018. Disponível em: https://www.heart.org/en/news/2019/03/19/waist-size-predicts-heart-attacks-better-than-bmi-especially-in-women. Acesso em: 8 jun. 2022.

ANDERSON, J. W. *et al.* Health benefits of dietary fiber. **Nutrition reviews**, [*s. l.*], v. 67, n. 4, p. 188–205, 2009. Disponível em: https://pubmed.ncbi.nlm.nih.gov/19335713/. Acesso em: 6 jul. 2022.

ASSOCIAÇÃO BRASILEIRA PARA O ESTUDO DA OBESIDADE E SÍNDROME METABÓLICA. **Mapa da Obesidade**. [*S. l.*], 2022. Disponível em: https://abeso.org.br/obesidade-e-sindrome-metabolica/mapa-da-obesidade/. Acesso em: 30 maio 2022.

BARBALHO, S. M. *et al.* Effects of Passiflora edulis on the metabolic profile of diabetic Wistar rat offspring. **Journal of medicinal food**, [*s. l.*], v. 14, n. 12, p. 1490–1495, 2011. Disponível em: https://pubmed.ncbi.nlm.nih.gov/21663518/. Acesso em: 19 out. 2022.

BASELGA-ESCUDERO, L. *et al.* Grape seed proanthocyanidins repress the hepatic lipid regulators miR-33 and miR-122 in rats. **Molecular Nutrition & Food Research**, [*s. l.*], v. 56, n. 11, p. 1636–1646, 2012. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1002/mnfr.201200237. Acesso em: 30 jun. 2022.

BLÜHER, M. Metabolically Healthy Obesity. **Endocrine reviews**, [*s. l.*], v. 41, n. 3, p. 405–420, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/32128581/. Acesso em: 8 jun. 2022.

BOILEAU, R. A. Advances in body composition assessment. **Cadernos de Saúde Pública**, [*s. l.*], v. 9, n. suppl 1, p. S116–S117, 1993. Disponível em: http://www.scielo.br/j/csp/a/kBCHfpKgThqLgGvRVKph4rg/?lang=en. Acesso em: 18 jul. 2022.

CAMPOS, E. M.; STEHLE, P.; SIMON, M. C. Microbial Metabolites of Flavan-3-Ols and Their Biological Activity. **Nutrients**, [*s. l.*], v. 11, n. 10, 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31546992/. Acesso em: 8 set. 2022.

CAO, H. Adipocytokines in Obesity and Metabolic Disease. **The Journal of endocrinology**, [*s. l.*], v. 220, n. 2, p. T47, 2014. Disponível em: /pmc/articles/PMC3887367/. Acesso em: 12 jun. 2022.

CARVALHO, L. S. F.; CHAVES-FILHO, A. B.; YOSHINAGA, M. Y. Orchestrating a ceramide-phosphatidylcholine cardiovascular risk score: it ain't over 'til the fat layer sings.

European Journal of Preventive Cardiology, [*s. l.*], v. 29, n. 6, p. 892–894, 2022. Disponível em: https://academic.oup.com/eurjpc/article/29/6/892/6120415. Acesso em: 13 out. 2022.

CASTRO-ALVES, V.; OREŠIČ, M.; HYÖTYLÄINEN, T. Lipidomics in nutrition research. **Current opinion in clinical nutrition and metabolic care**, [*s. l.*], v. 25, n. 5, p. 311–318, 2022. Disponível em: https://pubmed.ncbi.nlm.nih.gov/35788540/. Acesso em: 25 out. 2022.

CHEN, L. *et al.* Diverse Mechanisms of Antidiabetic Effects of the Different Procyanidin Oligomer Types of Two Different Cinnamon Species on db/db Mice. **J. Agric. Food Chem.**, [*s. l.*], v. 60, n. 36, p. 9144–9150, 2012. Disponível em: https://pubs.acs.org/sharingguidelines. Acesso em: 25 set. 2022.

CHOI, S. H.; GINSBERG, H. N. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. **Trends in Endocrinology & Metabolism**, [*s. l.*], v. 22, n. 9, p. 353–363, 2011. Disponível em: http://www.cell.com/article/S1043276011000725/fulltext. Acesso em: 1 jan. 2023.

CHOOI, Y. C.; DING, C.; MAGKOS, F. The epidemiology of obesity. **Metabolism**, [*s. l.*], v. 92, p. 6–10, 2019. Disponível em: Acesso em: 30 maio 2022.

CHRISTIE, W. W. **Lipid Essentials: Structures, Occurrence, Biochemistry, and Functions**. [*S. l.*], 2022a. Disponível em: https://www.lipidmaps.org/resources/lipid_web?page=lipids/lipids.html. Acesso em: 16 jul. 2022.

CHRISTIE, W. W. **Lipids: Definitions, Classification and Nomenclature**. [*S. l.*], 2022b. Disponível em: https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/basics/Nomen/index.htm. Acesso em: 16 jul. 2022.

COLE, L. K.; VANCE, J. E.; VANCE, D. E. Phosphatidylcholine biosynthesis and lipoprotein metabolism. **Biochimica et biophysica acta**, [*s. l.*], v. 1821, n. 5, p. 754–761, 2012. Disponível em: https://pubmed.ncbi.nlm.nih.gov/21979151/. Acesso em: 28 dez. 2022.

CORTÉS-MARTÍN, A. *et al.* The Human Metabolism of Nuts Proanthocyanidins does not Reveal Urinary Metabolites Consistent with Distinctive Gut Microbiota Metabotypes. **Molecular nutrition & food research**, [*s. l.*], v. 63, n. 2, 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/30444059/. Acesso em: 25 set. 2022.

DAYIB, M.; LARSON, J.; SLAVIN, J. Dietary fibers reduce obesity-related disorders: mechanisms of action. **Current opinion in clinical nutrition and metabolic care**, [*s. l.*], v. 23, n. 6, p. 445–450, 2020. Disponível em: https://journals.lww.com/co-clinicalnutrition/Fulltext/2020/11000/Dietary_fibers_reduce_obesity_related_disorders_.12.aspx. Acesso em: 19 set. 2022.

DE OLIVEIRA, B. B. R. *et al.* Body fat distribution and its risk for cardiovascular events in 10 years: Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). **Cadernos de saude publica**, [*s. l.*], v. 38, n. 2, 2022. Disponível em: https://pubmed.ncbi.nlm.nih.gov/35170706/. Acesso em: 19 set. 2022.

DELEU, S. *et al.* Short chain fatty acids and its producing organisms: An overlooked therapy for IBD?. **EBioMedicine**, [*s. l.*], v. 66, 2021a. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33813134/. Acesso em: 11 abr. 2022.

DELEU, S. *et al.* Short chain fatty acids and its producing organisms: An overlooked therapy for IBD?. **EBioMedicine**, [*s. l.*], v. 66, p. 103293, 2021b. Disponível em: /pmc/articles/PMC8047503/. Acesso em: 25 set. 2022.

DIBAY MOGHADAM, S. *et al.* Plasma lipidomic profiles after a low and high glycemic load dietary pattern in a randomized controlled crossover feeding study. **Metabolomics : Official journal of the Metabolomic Society**, [*s. l.*], v. 16, n. 12, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33219392/. Acesso em: 8 set. 2022.

DONNELLY, K. L. *et al.* Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. **The Journal of clinical investigation**, [*s. l.*], v. 115, n. 5, p. 1343–1351, 2005. Disponível em: https://pubmed.ncbi.nlm.nih.gov/15864352/. Acesso em: 3 abr. 2022.

DUARTE, I. de A. E. *et al.* Acute Effects of the Consumption of Passiflora setacea Juice on Metabolic Risk Factors and Gene Expression Profile in Humans. **Nutrients**, [*s. l.*], v. 12, n. 4, 2020. Disponível em: /pmc/articles/PMC7231153/. Acesso em: 29 jun. 2022.

DUARTE, I. *et al.* Effect of Passiflora setacea juice and its phenolic metabolites on insulin resistance markers in overweight individuals and on microglial cell activity. **Food & Function**, [*s. l.*], v. 13, n. 12, p. 6498–6509, 2022. Disponível em: https://pubs.rsc.org/en/content/articlehtml/2022/fo/d1fo04334j. Acesso em: 29 jun. 2022.

EICHELMANN, F. *et al.* Deep Lipidomics in Human Plasma: Cardiometabolic Disease Risk and Effect of Dietary Fat Modulation. **Circulation**, [*s. l.*], v. 146, n. 1, p. 21–35, 2022. Disponível em: https://www.ahajournals.org/doi/abs/10.1161/CIRCULATIONAHA.121.056805. Acesso em: 25 out. 2022.

EMBRAPA CERRADOS. **Porque estudar Passifloras?**. [*S. l.*], 2016. Disponível em: https://www.cpac.embrapa.br/passitec/estudarpassifloras/. Acesso em: 26 jun. 2022.

EMBRAPA CERRADOS. **Rede Passitec: Dsenvolvimento tecnológico para uso funcional e medicinal das Passifloras silvestres**. [*S. l.*], 2003. Disponível em: https://www.cpac.embrapa.br/passitec/. Acesso em: 2 jun. 2022.

FABBRINI, E.; SULLIVAN, S.; KLEIN, S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. **Hepatology (Baltimore, Md.)**, [*s. l.*], v. 51, n. 2, p. 679–689, 2010. Disponível em: https://pubmed.ncbi.nlm.nih.gov/20041406/. Acesso em: 3 abr. 2022.

FAHY, E. *et al.* A comprehensive classification system for lipids 1. **Journal Lipid Research**, [*s. l.*], v. 46, p. 839–861, 2005. Disponível em: http://www.jlr.org. Acesso em: 16 jul. 2022.

FALEIRO, F. G. *et al.* **O Gênero Passiflora: diversidade, conservação e uso**. [*S. l.*], 2020. Disponível em: https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1124044/o-genero-passiflora-diversidade-conservacao-e-uso. Acesso em: 29 jun. 2022.

FALUDI, A. *et al.* Atualização da diretriz brasileira de dislipidemias e prevenção da aterosclerose. **Arquivos Brasileiros de Cardiologia**, [*s. l.*], v. 109, n. 2, p. 4–13, 2017. Disponível em: http://publicacoes.cardiol.br/2014/diretrizes/2017/02_DIRETRIZ_DE_DISLIPIDEMIAS.pdf. Acesso em: 8 set. 2022.

FIGUEIREDO, B. Q. de *et al.* Metabolic alterations resulting from the use of hormonal contraceptives: an integrative literature review. **Research, Society and Development**, [*s. l.*], v. 10, n. 13, p. e481101321628–e481101321628, 2021. Disponível em: https://rsdjournal.org/index.php/rsd/article/view/21628. Acesso em: 28 dez. 2022.

FURUKAWA, S. *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome Find the latest version : Increased oxidative stress in obesity and its impact on metabolic syndrome. **The Journal of Clinical investigation**, [*s. l.*], v. 114, n. 12, p. 1752–1761, 2017.

GARCÍA-VILLALBA, R. *et al.* Alternative method for gas chromatography-mass spectrometry analysis of short-chain fatty acids in faecal samples. **Journal of Separation Science**, [*s. l.*], v. 35, n. 15, p. 1906–1913, 2012. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1002/jssc.201101121. Acesso em: 10 abr. 2022.

GUERRERO, L. *et al.* Serum metabolites of proanthocyanidin-administered rats decrease lipid synthesis in HepG2 cells. **The Journal of Nutritional Biochemistry**, [*s. l.*], v. 24, n. 12, p. 2092–2099, 2013. Disponível em: Acesso em: 25 set. 2022.

HE, J. *et al.* Short-Chain Fatty Acids and Their Association with Signalling Pathways in Inflammation, Glucose and Lipid Metabolism. **International journal of molecular sciences**, [*s. l.*], v. 21, n. 17, p. 1–16, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/32887215/. Acesso em: 9 ago. 2022.

HOLSCHER, H. D. Dietary fiber and prebiotics and the gastrointestinal microbiota. **Gut Microbes**, [*s. l.*], v. 8, n. 2, p. 172, 2017. Disponível em: /pmc/articles/PMC5390821/. Acesso em: 4 jul. 2022.

KAHN, B. B.; FLIER, J. S. Obesity and insulin resistance. **The Journal of Clinical Investigation**, [*s. l.*], v. 106, n. 4, p. 473–481, 2000. Disponível em: Acesso em: 2 jun. 2022.

KASUBUCHI, M. *et al.* Dietary Gut Microbial Metabolites, Short-chain Fatty Acids, and Host Metabolic Regulation. **Nutrients**, [*s. l.*], v. 7, n. 4, p. 2839, 2015. Disponível em: /pmc/articles/PMC4425176/. Acesso em: 25 set. 2022.

KHAN, N.; MUKHTAR, H. Tea polyphenols for health promotion. **Life sciences**, [*s. l.*], v. 81, n. 7, p. 519–533, 2007. Disponível em: https://pubmed.ncbi.nlm.nih.gov/17655876/. Acesso em: 25 set. 2022.

KIM, E. J.; RAMACHANDRAN, R.; WIERZBICKI, A. S. Lipidomics in diabetes. **Current opinion in endocrinology, diabetes, and obesity**, [*s. l.*], v. 29, n. 2, p. 124–130, 2022. Disponível em: https://pubmed.ncbi.nlm.nih.gov/34954721/. Acesso em: 20 jul. 2022.

KOLCZYNSKA, K. *et al.* Diacylglycerol-evoked activation of PKC and PKD isoforms in regulation of glucose and lipid metabolism: a review. **Lipids in Health and Disease 2020 19:1**, [*s. l.*], v. 19, n. 1, p. 1–15, 2020. Disponível em: https://lipidworld.biomedcentral.com/articles/10.1186/s12944-020-01286-8. Acesso em: 20 jul. 2022.

KOUDOUFIO, M. *et al.* Intestinal protection by proanthocyanidins involves anti-oxidative and anti-inflammatory actions in association with an improvement of insulin sensitivity, lipid and glucose homeostasis. **Scientific reports**, [*s. l.*], v. 11, n. 1, 2021. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33594093/. Acesso em: 30 jun. 2022.

LAW, S. H. *et al.* An Updated Review of Lysophosphatidylcholine Metabolism in Human Diseases. **International journal of molecular sciences**, [*s. l.*], v. 20, n. 5, 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/30845751/. Acesso em: 25 out. 2022.

LI, Y. O.; KOMAREK, A. R. Dietary fibre basics: Health, nutrition, analysis, and applications. **Food Quality and Safety**, [*s. l.*], v. 1, n. 1, p. 47–59, 2017. Disponível em: https://academic.oup.com/fqs/article/1/1/47/4791730. Acesso em: 4 jul. 2022.

LIPIDMAPS. **Recommendations and tools: classification, nomenclature and structure drawing**. [*S. l.*], 2003. Disponível em: https://www.lipidmaps.org/data/classification/lipid_cns.html. Acesso em: 25 out. 2022.

LIU, Q. *et al.* Positive intervention of insoluble dietary fiber from defatted rice bran on hyperlipidemia in high fat diet fed rats. **Journal of Food Science**, [*s. l.*], v. 86, n. 9, p. 3964–3974, 2021. Disponível em: Acesso em: 4 jul. 2022.

LUCAS-GONZÁLEZ, R. *et al.* Current trends in Passiflora genus research: Obesity and fermented foods systematic review. **Trends in Food Science and Technology**, [*s. l.*], v. 127, p. 143–155, 2022. Disponível em: Acesso em: 19 set. 2022.

MARIATH, A. B. *et al.* Obesidade e fatores de risco para o desenvolvimento de doenças crônicas não transmissíveis entre usuários de unidade de alimentação e nutrição. **Cadernos de Saúde Pública**, [*s. l.*], v. 23, n. 4, p. 897–905, 2007. Disponível em: http://www.scielo.br/j/csp/a/665D7h76C8Zpz9RkNYXcGks/abstract/?lang=pt. Acesso em: 3 abr. 2022.

MARTÍNEZ-LÓPEZ, S. *et al.* Realistic intake of a flavanol-rich soluble cocoa product increases HDL-cholesterol without inducing anthropometric changes in healthy and moderately hypercholesterolemic subjects. **Food & Function**, [*s. l.*], v. 5, n. 2, p. 364–374, 2014. Disponível em: https://pubs.rsc.org/en/content/articlehtml/2014/fo/c3fo60352k. Acesso em: 4 jul. 2022.

MASOOD, A.; STARK, K. D.; SALEM, N. A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. **Journal of lipid research**, [*s. l.*],

v. 46, n. 10, p. 2299–2305, 2005. Disponível em: https://pubmed.ncbi.nlm.nih.gov/16061957/. Acesso em: 11 abr. 2022.

MATTHEWS, D. R. *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. **Diabetologia**, [*s. l.*], v. 28, n. 7, p. 412–419, 1985. Disponível em: https://pubmed.ncbi.nlm.nih.gov/3899825/. Acesso em: 14 ago. 2022.

MAXIMUS, P. S. *et al.* Adipocytokines: Are they the Theory of Everything?. **Cytokine**, [*s. l.*], v. 133, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/32559663/. Acesso em: 26 jun. 2022.

MCGLINCHEY, A. J. *et al.* Metabolic signatures across the full spectrum of non-alcoholic fatty liver disease. **JHEP Reports**, [*s. l.*], v. 4, n. 5, 2022. Disponível em: http://www.jhep-reports.eu/article/S2589555922000490/fulltext. Acesso em: 8 set. 2022.

MENA, P. *et al.* Metabotypes of flavan-3-ol colonic metabolites after cranberry intake: elucidation and statistical approaches. **European journal of nutrition**, [*s. l.*], v. 61, n. 3, p. 1299–1317, 2022. Disponível em: https://pubmed.ncbi.nlm.nih.gov/34750642/. Acesso em: 25 set. 2022.

MILLER, W. M. *et al.* Obesity and lipids. **Current Cardiology Reports 2005 7:6**, [*s. l.*], v. 7, n. 6, p. 465–470, 2005. Disponível em: https://link.springer.com/article/10.1007/s11886-005-0065-8. Acesso em: 1 jun. 2022.

MOREIRA, V. *et al.* Orange juice affects acylcarnitine metabolism in healthy volunteers as revealed by a mass-spectrometry based metabolomics approach. **Food Research International**, [*s. l.*], v. 107, p. 346–352, 2018. Disponível em: Acesso em: 21 out. 2022.

MORRISON, D. J.; PRESTON, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. **https://doi.org/10.1080/19490976.2015.1134082**, [*s. l.*], v. 7, n. 3, p. 189–200, 2016. Disponível em: https://www.tandfonline.com/doi/abs/10.1080/19490976.2015.1134082. Acesso em: 24 abr. 2022.

MYHRSTAD, M. C. W. *et al.* Dietary Fiber, Gut Microbiota, and Metabolic Regulation-Current Status in Human Randomized Trials. **Nutrients**, [*s. l.*], v. 12, n. 3, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/32210176/. Acesso em: 6 jul. 2022.

NAKAJIMA, A. *et al.* The short chain fatty acid receptor GPR43 regulates inflammatory signals in adipose tissue M2-type macrophages. **PloS one**, [*s. l.*], v. 12, n. 7, 2017. Disponível em: https://pubmed.ncbi.nlm.nih.gov/28692672/. Acesso em: 9 ago. 2022.

NELSON, D. L.; COX, M. M. **Princípios de Bioquímica de Lehninger**. 6ªed. [*S. l.*]: Artmed, 2014.

NIE, Y.; LUO, F. Dietary Fiber: An Opportunity for a Global Control of Hyperlipidemia. **Oxidative medicine and cellular longevity**, [*s. l.*], v. 2021, 2021. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33897940/. Acesso em: 14 jul. 2022.

NILSON, E. A. F. *et al.* Custos atribuíveis a obesidade, hipertensão e diabetes no Sistema Único de Saúde, Brasil, 2018. **Revista Panamericana de Salud Pública**, [*s. l.*], v. 44, p. e32, 2020. Disponível em: https://doi.org/10.26633/RPSP.2020.32. Acesso em: 30 maio 2022.

NYGREN, H. *et al.* Liquid chromatography-mass spectrometry (LC-MS)-based lipidomics for studies of body fluids and tissues. **Methods in molecular biology (Clifton, N.J.)**, [*s. l.*], v. 708, p. 247–257, 2011. Disponível em: https://pubmed.ncbi.nlm.nih.gov/21207295/. Acesso em: 3 abr. 2022.

OBRADOVIC, M. *et al.* Leptin and Obesity: Role and Clinical Implication. **Frontiers in Endocrinology**, [*s. l.*], v. 12, 2021. Disponível em: /pmc/articles/PMC8167040/. Acesso em: 3 jan. 2023.

PANG, Z. *et al.* MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. **Nucleic Acids Research**, [*s. l.*], v. 49, n. W1, p. W388, 2021. Disponível em: /pmc/articles/PMC8265181/. Acesso em: 8 jun. 2022.

PAPANDREOU, C. *et al.* Circulating Metabolites Associated with Body Fat and Lean Mass in Adults with Overweight/Obesity. **Metabolites**, [*s. l.*], v. 11, n. 5, 2021. Disponível em: https://pubmed.ncbi.nlm.nih.gov/34068443/. Acesso em: 8 set. 2022.

PETERS, S. A. E.; BOTS, S. H.; WOODWARD, M. Sex differences in the association between measures of general and central adiposity and the risk of myocardial infarction: Results from the UK Biobank. **Journal of the American Heart Association**, [*s. l.*], v. 7, n. 5, 2018. Disponível em: https://www.ahajournals.org/doi/abs/10.1161/JAHA.117.008507. Acesso em: 8 jun. 2022.

PIKÓ, P. *et al.* Obesity-Related Changes in Human Plasma Lipidome Determined by the Lipidyzer Platform. **Biomolecules**, [*s. l.*], v. 11, n. 2, p. 1–20, 2021. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33669967/. Acesso em: 13 out. 2022.

PORTINCASA, P. *et al.* Gut Microbiota and Short Chain Fatty Acids: Implications in Glucose Homeostasis. **International Journal of Molecular Sciences 2022, Vol. 23, Page 1105**, [*s. l.*], v. 23, n. 3, p. 1105, 2022. Disponível em: https://www.mdpi.com/1422-0067/23/3/1105/htm. Acesso em: 17 ago. 2022.

QUESADA, H. *et al.* The lipid-lowering effect of dietary proanthocyanidins in rats involves both chylomicron-rich and VLDL-rich fractions. **British Journal of Nutrition**, [*s. l.*], v. 108, n. 2, p. 208–217, 2012. Disponível em: https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/lipidlowering-effect-of-dietary-proanthocyanidins-in-rats-involves-both-chylomicronrich-and-vldlrich-fractions/688C79B840D9CE18CB26E53618064AC4. Acesso em: 25 set. 2022.

RIVAS SERNA, I. M. *et al.* Lipidomic Profiling Identifies Signatures of Poor Cardiovascular Health. **Metabolites**, [*s. l.*], v. 11, n. 11, 2021. Disponível em: https://pubmed.ncbi.nlm.nih.gov/34822405/. Acesso em: 25 out. 2022.

RUIZ-PEREZ, D. *et al.* So you think you can PLS-DA?. **BMC Bioinformatics**, [*s. l.*], v. 21, n. 1, p. 1–10, 2020. Disponível em:

https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-019-3310-7. Acesso em: 3 set. 2022.

SAHURI-ARISOYLU, M. *et al.* Reprogramming of hepatic fat accumulation and "browning" of adipose tissue by the short-chain fatty acid acetate. **International journal of obesity (2005)**, [*s. l.*], v. 40, n. 6, p. 955–963, 2016. Disponível em: https://pubmed.ncbi.nlm.nih.gov/26975441/. Acesso em: 24 jul. 2022.

SALVADÓ, M. J. *et al.* Roles of proanthocyanidin rich extracts in obesity. **Food & function**, [*s. l.*], v. 6, n. 4, p. 1053–1071, 2015. Disponível em: https://pubmed.ncbi.nlm.nih.gov/25669490/. Acesso em: 3 abr. 2022.

SÁNCHEZ, D.; MIGUEL, M.; ALEIXANDRE, A. Dietary fiber, gut peptides, and adipocytokines. **Journal of medicinal food**, [*s. l.*], v. 15, n. 3, p. 223–230, 2012. Disponível em: https://pubmed.ncbi.nlm.nih.gov/22181071/. Acesso em: 14 jul. 2022.

SANTOS, J. T. do C. *et al.* Brazilian native passion fruit (Passiflora tenuifila Killip) is a rich source of proanthocyanidins, carotenoids, and dietary fiber. **Food Research International**, [*s. l.*], v. 147, p. 110521, 2021. Disponível em: Acesso em: 29 jun. 2022.

SANTOS, J. T. do C. **Caracterização de compostos bioativos de maracujá (Passiflora tenuifila) e efeito do seu consumo sobre marcadores bioquímicos, microbiota intestinal em indivíduos eutróficos e obesos**. 2020a. Tese de Doutorado - Universidade de São Paulo, São Paulo, 2020. Disponível em: https://www.teses.usp.br/teses/disponiveis/9/9131/tde-04122020-153741/. Acesso em: 2 jun. 2022.

SANTOS, J. T. do C. **Caracterização de compostos bioativos de maracujá (Passiflora tenuifila) e efeito do seu consumo sobre marcadores bioquímicos, microbiota intestinal em indivíduos eutróficos e obesos.** 2020b. Doctoral Thesis - University of São Paulo, São Paulo, 2020. Disponível em: https://www.teses.usp.br/teses/disponiveis/9/9131/tde-04122020-153741/pt-br.php. Acesso em: 10 abr. 2022.

SANTOS, K. G. dos *et al.* Orange juice intake by obese and insulin-resistant subjects lowers specific plasma triglycerides: a randomized clinical trial. **Clinical Nutrition ESPEN**, [*s. l.*], v. 0, n. 0, 2022. Disponível em: http://clinicalnutritionespen.com/article/S240545772200434X/fulltext. Acesso em: 8 set. 2022.

SARRIÁ, B. *et al.* Cocoa colonic phenolic metabolites are related to HDL-cholesterol raising effects and methylxanthine metabolites and insoluble dietary fibre to anti-inflammatory and hypoglycemic effects in humans. **PeerJ**, [*s. l.*], v. 8, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/32995094/. Acesso em: 4 jul. 2022.

SHANG, T. *et al.* Protective effects of various ratios of DHA/EPA supplementation on high-fat diet-induced liver damage in mice. **Lipids in health and disease**, [*s. l.*], v. 16, n. 1, 2017. Disponível em: https://pubmed.ncbi.nlm.nih.gov/28356106/. Acesso em: 3 abr. 2022.

SINGLA, P.; BARDOLOI, A.; PARKASH, A. A. Metabolic effects of obesity: A review. **World Journal of Diabetes**, [*s. l.*], v. 1, n. 3, p. 76, 2010. Disponível em: /pmc/articles/PMC3083889/. Acesso em: 3 abr. 2022.

SIQUEIRA, A. F. A.; ABDALLA, D. S. P.; FERREIRA, S. R. G. LDL: da síndrome metabólica à instabilização da placa aterosclerótica. **Arquivos Brasileiros de Endocrinologia & Metabologia**, [*s. l.*], v. 50, n. 2, p. 334–343, 2006. Disponível em: http://www.scielo.br/j/abem/a/KQLfLzRv7x7xXGLCZLCTHPP/?lang=pt. Acesso em: 19 set. 2022.

SMERIGLIO, A. *et al.* Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. **British Journal of Pharmacology**, [*s. l.*], v. 174, n. 11, p. 1244, 2017. Disponível em: /pmc/articles/PMC5429339/. Acesso em: 3 abr. 2022.

SOCIEDADE BRASILEIRA DE DIABETES. **Diretrizes da Sociedade Brasileira de Diabetes 2019-2020**. [*S. l.*], 2020. Disponível em: https://portaldeboaspraticas.iff.fiocruz.br/biblioteca/diretrizes-da-sociedade-brasileira-de-diabetes-2019-2020/. Acesso em: 23 out. 2022.

SOUZA, M. de S. S. de *et al.* Effects of Passiflora edulis (Yellow Passion) on Serum Lipids and Oxidative Stress Status of Wistar Rats. **Journal of Medicinal Food**, [*s. l.*], v. 15, n. 1, p. 78–82, 2012. Disponível em: https://www.liebertpub.com/doi/10.1089/jmf.2011.0056. Acesso em: 29 jun. 2022.

STANLEY, E. G. *et al.* Lipidomics Profiling of Human Adipose Tissue Identifies a Pattern of Lipids Associated with Fish Oil Supplementation. **Journal of proteome research**, [*s. l.*], v. 16, n. 9, p. 3168–3179, 2017. Disponível em: https://pubmed.ncbi.nlm.nih.gov/28587463/. Acesso em: 3 abr. 2022.

TONG, Y. *et al.* Obesity and insulin resistance: Pathophysiology and treatment. **Drug Discovery Today**, [*s. l.*], v. 27, n. 3, p. 822–830, 2022. Disponível em: Acesso em: 26 jun. 2022.

WANG, Y. *et al.* Lipidomic Profile Revealed the Association of Plasma Lysophosphatidylcholines with Adolescent Obesity. **BioMed Research International**, [*s. l.*], v. 2019, 2019. Disponível em: Acesso em: 8 set. 2022.

WANG, T. K. *et al.* Proanthocyanidins Should Be a Candidate in the Treatment of Cancer, Cardiovascular Diseases and Lipid Metabolic Disorder. **Molecules (Basel, Switzerland)**, [*s. l.*], v. 25, n. 24, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33339407/. Acesso em: 30 jun. 2022.

WIDANINGRUM *et al.* Fruit and vegetable insoluble dietary fibre in vitro fermentation characteristics depend on cell wall type. **Bioactive Carbohydrates and Dietary Fibre**, [*s. l.*], v. 23, p. 100223, 2020. Disponível em: Acesso em: 17 ago. 2022.

WORLD HEALTH ORGANIZATION. **Cardiovascular diseases**. [*S. l.*], 2022a. Disponível em: https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1. Acesso em: 2 jun. 2022.

WORLD HEALTH ORGANIZATION. **Controlling the global obesity epidemic**. [*S. l.*], 2022b. Disponível em: https://www.who.int/activities/controlling-the-global-obesity-epidemic. Acesso em: 10 abr. 2022.

WORLD HEALTH ORGANIZATION. **Noncommunicable diseases**. [*S. l.*], 2022c. Disponível em: https://www.who.int/health-topics/noncommunicable-diseases#tab=tab_1. Acesso em: 30 maio 2022.

WORLD HEALTH ORGANIZATION. **Obesity and overweight**. [*S. l.*], 2021. Disponível em: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. Acesso em: 10 abr. 2022.

WORLD HEALTH ORGANIZATION. Waist Circumference and Waist–Hip Ratio. **WHO Expert**, [*s. l.*], v. 64, n. 1, p. 2–5, 2011a. Disponível em: http://www.nature.com/doifinder/10.1038/ejcn.2009.139. Acesso em: 20 out. 2022.

WORLD HEALTH ORGANIZATION. Waist Circumference and Waist–Hip Ratio. **WHO Expert**, [*s. l.*], v. 64, n. 1, p. 2–5, 2011b. Disponível em: http://www.nature.com/doifinder/10.1038/ejcn.2009.139. Acesso em: 8 jun. 2022.

WOZNIAK, S. E. *et al.* Adipose tissue: the new endocrine organ? A review article. **Digestive diseases and sciences**, [*s. l.*], v. 54, n. 9, p. 1847–1856, 2009. Disponível em: https://pubmed.ncbi.nlm.nih.gov/19052866/. Acesso em: 3 abr. 2022.

WU, H.; BALLANTYNE, C. M. Metabolic Inflammation and Insulin Resistance in Obesity. **Circulation Research**, [*s. l.*], p. 1549–1564, 2020. Disponível em: https://www.ahajournals.org/doi/abs/10.1161/CIRCRESAHA.119.315896. Acesso em: 2 jun. 2022.

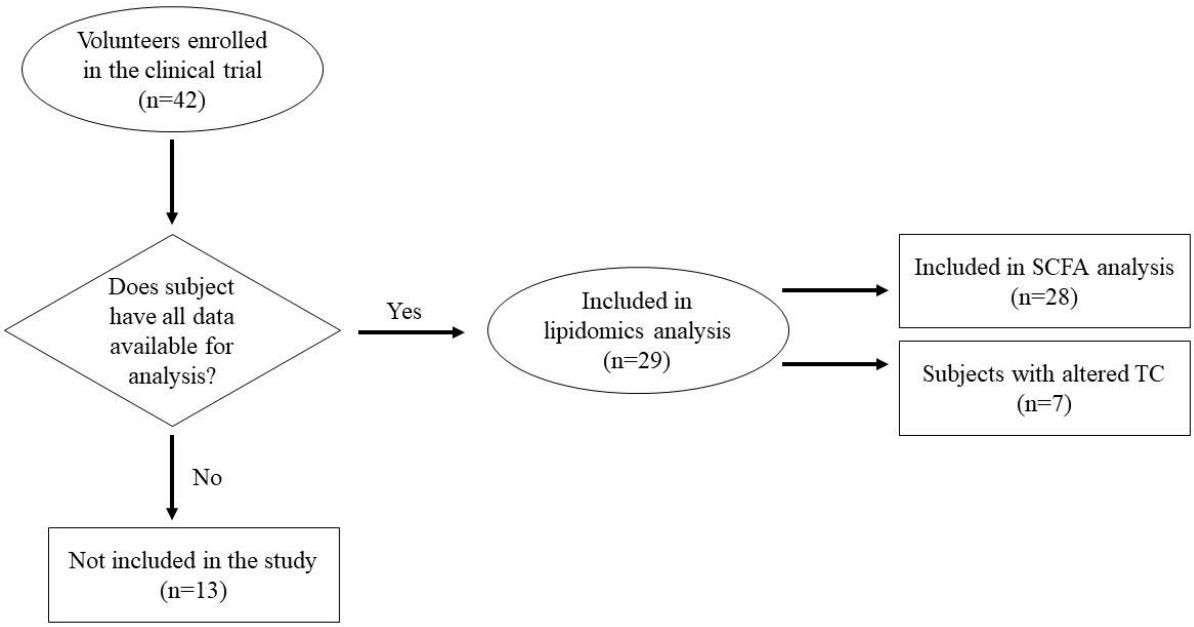
ZANOTTI, I. *et al.* Atheroprotective effects of (poly)phenols: a focus on cell cholesterol metabolism. **Food & Function**, [*s. l.*], v. 6, n. 1, p. 13–31, 2015. Disponível em: https://pubs.rsc.org/en/content/articlehtml/2015/fo/c4fo00670d. Acesso em: 8 set. 2022.

ZERAIK, M. L. *et al.* Maracujá: um alimento funcional?. **Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy**, [*s. l.*], v. 20, n. 3, p. 459–471, 2010. Disponível em: Acesso em: 29 jun. 2022.

ZHENG, X. X. *et al.* Green tea intake lowers fasting serum total and LDL cholesterol in adults: a meta-analysis of 14 randomized controlled trials. **American Journal of Clinical Nutrition**, [*s. l.*], v. 94, n. 2, p. 601–610, 2011. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK82842/. Acesso em: 25 set. 2022.

9. SUPPLEMENTARY MATERIAL

Figure S1: Rationale of study population segmentation.



Subjects were selected for the lipidomic analysis based on sample intersection and available data for: biochemical parameters, anthropometry and short-chain fatty acids (for SCFA, one subject did not present fecal samples, and was excluded of this analysis).

Table S1: Internal standards used in the lipidomic analysis

Internal Standards	Lipid Class	Concentration
PC (17:0)	Glycerophospholipid	2.5 µg/mL
PE (17:0)	Glycerophospholipid	2.5 µg/mL
LPC (17:0)	Glycerophospholipid	2.5 µg/mL
SM (17:0)	Sphingolipid	2.5 µg/mL
Ceramide (d18:1/17:0)	Sphingolipid	2.5 µg/mL
TG (17:0/17:0/17:0)	Glycerolipid	2.5 µg/mL
PC (16:0 D31-18:1)	Glycerophospholipid	2.5 µg/mL
CE (17:0)	Sterolipid	2.5 µg/mL

Abbreviations: CE, cholesterol ester. LPC, lysophosphatidylcholines. PC, phosphatidylcholine. PE, phosphatidylethanolamine. SM, sphingomyelin. TG, triacylglycerol. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains.

Table S2: Lipids used for the calibration curve in the lipidomic analysis

Lipid Species	Lipid Class
CE (16:0)	Sterol lipid
CE (18:0)	Sterol lipid
CE (18:1)	Sterol lipid
CE (18:2) (9 Z)	Sterol lipid
CE (18:2) (12 Z)	Sterol lipid
Cer (d18:1/18:1)	Sphingolipid
DG (18:1)	Glycerolipid
Lyso PC (18:0)	Glycerophospholipid
Lyso PC (18:1)	Glycerophospholipid
Lyso PE (18:1)	Glycerophospholipid
PC (16:0/16:0)	Glycerophospholipid
PC (16:0-18:1)	Glycerophospholipid
PC (18:0/18:0)	Glycerophospholipid
PE (16:0-18:1)	Glycerophospholipid
SM (d18:1/16:0)	Sphingolipid
TG (16:0/16:0/16:0)	Glycerolipid
TG (18:0/18:0/18:0)	Glycerolipid
Cer (d18:0/18:1) 9 Z	Sphingolipid

Abbreviations: CE, cholesterol ester. Cer, ceramide. DG, diacylglycerol. LPC, lysophosphatidylcholines. PC, phosphatidylcholine. PE, phosphatidylethanolamine. SM, sphingomyelin. TG, triacylglycerol. Numbers in parentheses indicate the numbers of carbons and saturation of FA chains.

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Investigação metabolômica da ingestão de maracujá (Passiflora tenuifila)
Pesquisador: Neuza Mariko Aymoto Hassimotto
Área Temática:
Versão: 6
CAAE: 57619616.4.0000.0067
Instituição Proponente: Faculdade de Ciências Farmacêuticas da Universidade de São Paulo
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.816.330

Apresentação do Projeto:
Este é um projeto em andamento que visa um estudo comparativo da biodisponibilidade de compostos fenólicos presentes em maracujá (P. tenuifila), em voluntários obesos e eutróficos, avaliando a médio prazo os efeitos de sua ingestão através de uma abordagem metabolômica. Não houve modificação no desenho nem na amostragem do estudo.

Objetivo da Pesquisa:
Comparar a biodisponibilidade de fenólicos de maracujá (P. tenuifila) entre voluntários obesos e eutróficos e avaliar o efeito a médio prazo de sua ingestão, através de uma abordagem metabolômica.

Avaliação dos Riscos e Benefícios:
A pesquisa é considerada de risco mínimo, com coleta de amostras de urina e fezes (procedimento não invasivo) e de sangue por profissionais treinados. Não houve mudança no protocolo, ou amostragem, portante mantém-se os mesmos riscos e benefícios do projeto em andamento.

Comentários e Considerações sobre a Pesquisa:
O projeto está em andamento e esta emenda pede a inserção de um novo membro na equipe de trabalho. Parte das análises previstas não foram realizadas em um projeto de mestrado anterior e desta maneira a proponente pede que o conste o nome do projeto de mestrado que efetivamente fará estas análises. Não há inserção de análises novas, mas somente a separação de análises já

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

previstas em projetos de mestrado distintos.

Considerações sobre os Termos de apresentação obrigatória:

Não houve mudança nos termos já apresentados e aprovados, de maneira que atendem às recomendações e exigências deste CEP.

Recomendações:

O projeto não foi modificado. Há somente um pedido de inserção de um novo membro na equipe, assim como a inserção do titulo de um novo projeto de mestrado sendo conduzido com análises já previstas. Recomenda-se a aprovação da emenda.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências ou inadequações.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1479318_E4.pdf	05/12/2019 11:42:16		Aceito
Outros	Adendo.pdf	05/12/2019 11:38:41	Neuza Mariko Aymoto Hassimotto	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_infraestrutura_dante.pdf	05/05/2017 11:48:10	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Instrucoes_voluntarios_etapa_2_dante.docx	05/05/2017 11:01:00	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Instrucoes_voluntarios_etapa_1_dante.docx	05/05/2017 11:00:42	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	motivo_adendo_3.pdf	05/05/2017 10:59:35	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Fluxogramas_dante.pdf	05/05/2017 10:58:58	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	cfr_dante.pdf	05/05/2017 10:58:25	Neuza Mariko Aymoto Hassimotto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_etapa2_dante.docx	05/05/2017 10:57:43	Neuza Mariko Aymoto Hassimotto	Aceito
TCLE / Termos de Assentimento / Justificativa de	TCLE_etapa1_dante.docx	05/05/2017 10:57:01	Neuza Mariko Aymoto Hassimotto	Aceito

Continuação do Parecer: 3.816.330

Ausência	TCLE_etapa1_dante.docx	05/05/2017 10:57:01	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Declaracoes_cep_dante.pdf	04/05/2017 13:27:05	Neuza Mariko Aymoto Hassimotto	Aceito
Declaração de Pesquisadores	Declaracao_pesquisadores_dante.pdf	04/05/2017 13:25:40	Neuza Mariko Aymoto Hassimotto	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_coparticipante_dante.pdf	04/05/2017 13:24:32	Neuza Mariko Aymoto Hassimotto	Aceito
Brochura Pesquisa	Projeto_dante.doc	04/05/2017 13:23:32	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Carta_adendo_2.pdf	13/03/2017 12:33:17	Neuza Mariko Aymoto Hassimotto	Aceito
Brochura Pesquisa	Projeto_p_ten_19_59a.docx	13/03/2017 12:31:49	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Adendo_motivo_alt_p_tenuifila.pdf	27/09/2016 17:38:40	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Instrucoes_voluntarios_etapa_2_p_tenuifila.docx	27/09/2016 17:30:17	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Instrucoes_voluntarios_etapa_1_p_tenuifila.docx	27/09/2016 17:17:49	Neuza Mariko Aymoto Hassimotto	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_p_tenuifila.docx	27/09/2016 17:16:30	Neuza Mariko Aymoto Hassimotto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_etapa2_p_tenuifila.docx	27/09/2016 17:16:04	Neuza Mariko Aymoto Hassimotto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_etapa1_p_tenuifila.docx	27/09/2016 17:15:52	Neuza Mariko Aymoto Hassimotto	Aceito
Outros	Carta_correcao_pos_parecer.pdf	17/08/2016 15:45:39	Neuza Mariko Aymoto Hassimotto	Aceito
Cronograma	Cronograma_HU_atualizado.pdf	17/08/2016 13:15:35	Neuza Mariko Aymoto Hassimotto	Aceito
Orçamento	Orcamento_HU_atualizado.pdf	17/08/2016 13:03:49	Neuza Mariko Aymoto Hassimotto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_etapa_1_atualizado.docx	17/08/2016 12:58:59	Neuza Mariko Aymoto Hassimotto	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_instituicao_infraestrutura.pdf	06/07/2016 15:38:52	Neuza Mariko Aymoto Hassimotto	Aceito
Declaração de Pesquisadores	Descricao_declaracao_pesquisadores.pdf	06/07/2016 15:34:48	Neuza Mariko Aymoto Hassimotto	Aceito

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

Continuação do Parecer: 3.816.330

Cronograma	Cronograma_de_execucao_HU.pdf	30/06/2016 16:36:38	Neuza Mariko Aymoto Hassimotto	Aceito
Folha de Rosto	Folha_de_rosto_assinada_PLATAFORMA_BRASIL.pdf	27/06/2016 17:11:02	Neuza Mariko Aymoto Hassimotto	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 30 de Janeiro de 2020

Assinado por:
Mauricio Yonamine
(Coordenador(a))

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

ADENDO

CAAE: 57619616.4.0000.0067

Título da pesquisa: "Investigação metabolômica da ingestão de maracujá (*Passiflora tenuifila*)"

Pesquisador Responsável: Neuza Mariko Aymoto Hassimotto

Venho por meio deste solicitar a inclusão da pesquisadora Laila Guimarães Zeraik Cardoso na equipe de pesquisa.

Solicitamos também que seja incluído o nome de 1 projeto dentro do projeto principal, pois as análises de lipidômica do plasma que não foram realizadas no projeto anterior, serão realizadas nesse novo projeto de mestrado.

- "EFEITO DA INGESTÃO DE MARACUJÁ (*Passiflora tenuifilia*) NO PERFIL LIPIDÔMICO DE PLASMA DE INDIVÍDUOS EUTRÓFICOS E OBESOS".

_____

Pesquisadora responsável: Drª Neuza M. A. Hassimotto

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas
FICHA DO ALUNO

9131 - 7609811/1 - Laila Guimarães Zeraik Cardoso

Email:	laila.cardoso@usp.br
Data de Nascimento:	14/10/1991
Cédula de Identidade:	RG - 48.708.487-1 - SP
Local de Nascimento:	Estado de São Paulo
Nacionalidade:	Brasileira
Graduação:	Farmacêutica-Bioquímica - Faculdade de Ciências Farmacêuticas - Universidade de São Paulo - São Paulo - Brasil - 2017

Curso:	Mestrado
Programa:	Ciência dos Alimentos
Área:	Bromatologia
Data de Matrícula:	22/01/2020
Início da Contagem de Prazo:	22/01/2020
Data Limite para o Depósito:	17/07/2023
Orientador:	Prof(a). Dr(a). Neuza Mariko Aymoto Hassimotto - 22/01/2020 até o presente. Email: aymoto@usp.br
Proficiência em Línguas:	Inglês, 22/01/2020
Data de Aprovação no Exame de Qualificação:	(não exigido)
Data do Depósito do Trabalho:	
Título do Trabalho:	
Data Máxima para Aprovação da Banca:	
Data de Aprovação da Banca:	
Data Máxima para Defesa:	
Data da Defesa:	
Resultado da Defesa:	
Histórico de Ocorrências:	Primeira Matrícula em 22/01/2020

Aluno matriculado no Regimento da Pós-Graduação USP (Resolução nº 7493 em vigor a partir de 29/03/2018).
Última ocorrência: Matrícula de Acompanhamento em 18/07/2022
Impresso em: 18/10/2022 20:59:19

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas
FICHA DO ALUNO

9131 - 7609811/1 - Laila Guimarães Zeraik Cardoso

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
FBA5899-3/2	Biodisponibilidade de Nutrientes e de Substâncias Bioativas em Alimentos e Dietas (1)	04/02/2019	22/03/2019	90	6	75	A	N	Concluída
FBA5905-2/3	Planejamento Experimental e Análise Multivariada	18/02/2020	14/06/2020	60	0	-	-	N	Pré-matrícula indeferida
HNT5709-7/1	Dietas e Doenças Crônicas não Transmissíveis (Faculdade de Saúde Pública - Universidade de São Paulo)	27/04/2020	01/06/2020	60	4	100	A	N	Concluída
ICB5747-2/5	Ciências Ômicas em Doenças Infecciosas (CODI) (Instituto de Ciências Biomédicas - Universidade de São Paulo)	22/06/2020	12/07/2020	90	6	100	A	N	Concluída
FBC5719-4/3	Trato Gastrointestinal: Imunomodulação da Colonização e Infecção Bacteriana	14/08/2020	04/12/2020	90	6	100	A	N	Concluída
FBA5741-4/2	Química e Bioquímica de Alimentos I	01/09/2020	06/10/2020	60	4	100	A	N	Concluída
FBA5908-1/1	Nutrição Humana	05/04/2021	10/05/2021	45	3	100	A	N	Concluída
EFP5779-1/1	Introdução ao software R para análise exploratória de dados em Aprendizagem Motora (Escola de Educação Física e Esporte - Universidade de São Paulo)	04/05/2021	14/06/2021	90	6	100	A	N	Concluída
FBA5909-1/1	Microbiologia de Alimentos	10/05/2021	14/06/2021	45	3	95	A	N	Concluída

	Créditos mínimos exigidos Para depósito da dissertação	Créditos obtidos
Disciplinas:	25	38
Estágios:		
Total:	25	38

Créditos Atribuídos à Dissertação: 71

Observações:
1) Disciplina(s) cursada(s) isoladamente e aceita(s) pelo(a) orientador(a) do(a) candidato(a)

Conceito a partir de 02/01/1997:
A - Excelente, com direito a crédito; B - Bom, com direito a crédito; C - Regular, com direito a crédito; R - Reprovado; T - Transferência.

Um(1) crédito equivale a 15 horas de atividade programada.

Última ocorrência: Matrícula de Acompanhamento em 18/07/2022
Impresso em: 18/10/2022 20:59:20

Currículo Lattes

Imprimir currículo

Laila Guimarães Zeraik Cardoso

Endereço para acessar este CV: https://lattes.cnpq.br/7289104814018443

Última atualização do currículo em 26/10/2022

Resumo informado pelo autor

Possui graduação em Farmácia-Bioquímica pela Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, FCF USP (2017). Tem experiência profissional na área de farmacovigilância, assistência em saúde, organização de eventos científicos e gerenciamento de projetos. É aluna de mestrado do programa Ciência dos Alimentos da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, FCF USP (2020-2022), na área de Bromatologia, onde desenvolve pesquisa na área de biodisponibilidade e investigação da atividade biológica de compostos fenólicos utilizando ferramentas "omicas".

(Texto informado pelo autor)

Nome civil

Nome Laila Guimarães Zeraik Cardoso

Dados pessoais

Formação acadêmica/titulação

2020 Mestrado em Ciência dos Alimentos.
Universidade de São Paulo, USP, Sao Paulo, Brasil
Título: Effects of passion fruit (Passiflora tenuifila Killip) intake on eutrophic and obese subjects: Lipidomic Approach.
Orientador: Neuza M. A. Hasimotto
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2011 - 2016 Graduação em Farmácia e Bioquímica.
Universidade de São Paulo, USP, Sao Paulo, Brasil
Título: PARECER TÉCNICO CIENTÍFICO: USO DE OXCARBAZEPINA SUSPENSÃO ORAL (60MG/ML) PARA TRATAMENTO (MONOTERAPIA) DE EPILEPSIA FOCAL PEDIÁTRICA, Ano de obtenção: 2016
Orientador: Eliane Ribeiro

Atuação profissional

1. International Life Sciences Institute - Brasil - ILSI BRASIL

Vínculo institucional

2019 - 2022 Vínculo: Formal labor contract , Enquadramento funcional: Coordenadora de Projetos, Regime: Parcial
Outras informações:
Trabalho conjunto com especialistas da Academia, Governo e Indústrias para promover as Ciências da Vida por meio de atividades que tenham um propósito e benefício público. Responsável pela condução dos projetos de todas as forças-tarefa do Instituto, desde o planejamento das reuniões às atividades de execução e pós-execução (seminários, workshops, publicação de folhetos e outros projetos).
Responsável pela comunicação do Instituto: mídias sociais, website, newsletter e comunicação interna.
Parte do Subcomitê de Comunicação do ILSI Global, atuando no desenvolvimento de um plano de comunicação para todas as Entidades da ILSI (2021). Realizou a adequação de toda a presença digital do ILSI às novas leis de proteção de dados digitais (2021). Durante todo o tempo de trabalho para o ILSI, (co)organizou 126 eventos institucionais (reuniões, seminários, workshops e simpósios) e 96 publicações (folhetos, artigos científicos e material de comunicação).newsletter, site e comunicação interna.

2017 - 2018 Vínculo: Formal labor contract , Enquadramento funcional: Analsita de Projetos, Regime: Parcial
Outras informações:
Responsável pelas forças-tarefa Biotecnologia, Avaliação do Risco de Agroquímicos e Food Safety.
Manutenção da comunicação do instituto.

2015 - 2016 Vínculo: Formal labor contract , Enquadramento funcional: Estagiária, Regime: Parcial
Outras informações:
Responsável pelas forças-tarefas Biotecnologia, Avaliação do Risco de Agroquímicos e Food Safety.

2. Farmácia Universitária da Universidade de São Paulo - FARMUSP

Vínculo institucional

2015 - 2015 Vínculo: Civil servant , Enquadramento funcional: Estagiária, Regime: Parcial
Outras informações:
Acompanhamento e assistência às consultas farmacêuticas para pacientes com câncer de próstata em tratamento feito exclusivamente no Hospital Universitário (HU-USP). Desenvolvimento de material básico para educação primária em saúde nas principais DCNT. Assistência na implementação de procedimentos para notificações de reações adversas a medicamentos.

3. Centro de Vigilância Sanitária (SP) - CVS SP

Vínculo institucional

2013 - 2014 Vínculo: Scholarship , Enquadramento funcional: Estagiária, Regime: Parcial
Outras informações:
Estágio no Núcleo de Farmacovigilância do Centro de Vigilância Sanitária através do programa "PET-Vigilância", financiado pelo Ministério da Saúde. Analista de causalidade das notificações de suspeita de Reação Adversa a Medicamento encaminhadas ao CVS; Gerenciamento de informações através extração de relatórios específicos do Banco de Dados PERIweb; Análise farmacoterapêutica de classes específicas de medicamentos, com a finalidade de avaliação do perfil risco x benefício e possível identificação de sinais de segurança. Desenvolvimento de estudo farmacoterapêutico: PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY ASSOCIATED TO NATALIZUMAB: THE IMPORTANCE OF MRI BASED DIAGNOSIS IN ASYMPTOMATIC CASES.

Áreas de atuação

1. Farmácia
2. Ciência de Alimentos
3. Metabolismo e Bioenergética

Idiomas

Inglês Compreende Bem , Fala Bem , Escreve Razoavelmente , Lê Bem

Espanhol Compreende Razoavelmente , Fala Razoavelmente , Escreve Pouco , Lê Razoavelmente

Francês Compreende Pouco , Fala Pouco , Escreve Pouco , Lê Razoavelmente

Português Compreende Bem , Fala Bem , Escreve Bem , Lê Bem

Producão

Produção bibliográfica

Apresentação de trabalho e palestra

1. **CARDOSO, L. G. Z.**; RIBEIRO, A. G.; KANO, E. K.
Progressive Multifocal Leukoencephalopathy Associated to Natalizumab: The Importance of MRI Based Diagnosis in Asymptomatic Cases, 2014. (Congresso,Apresentação de Trabalho)

Produção técnica

Demais produções técnicas

1. **CARDOSO, L. G. Z.**; RIBEIRO, A. G.; KANO, E. K.
A IMPORTÂNCIA DA RESSONÂNCIA NUCLEAR MAGNÉTICA NO DIAGNÓSTICO DE LEUCOENCEFALOPATIA MULTIFOCAL PROGRESSIVA ASSINTOMÁTICA ASSOCIADO À TERAPIA COM NATALIZUMABE., 2014. (Outra produção técnica)

Eventos

Eventos

Participação em eventos

1. **3rd International Conference on Food Bioactives & Health**, 2022. (Congresso)
.
2. **1st International Symposium on Citrus Bioactive compounds and health benefits**, 2018. (Simpósio)
.
3. Apresentação Oral no(a) **XIX Semana Farmacêutica de Ciência e Tecnologia**, 2014. (Feira)
A IMPORTÂNCIA DA RESSONÂNCIA NUCLEAR MAGNÉTICA NO DIAGNÓSTICO DE LEUCOENCEFALOPATIA MULTIFOCAL PROGRESSIVA ASSINTOMÁTICA ASSOCIADO À TERAPIA COM NATALIZUMABE..
4. Apresentação de Poster / Painel no(a) **XLIX Semana Universitária Paulista de Farmácia e Bioquímica (SUO+PFAB)**, 2014. (Feira)
A IMPORTÂNCIA DA RESSONÂNCIA NUCLEAR MAGNÉTICA NO DIAGNÓSTICO DE LEUCOENCEFALOPATIA MULTIFOCAL PROGRESSIVA ASSINTOMÁTICA ASSOCIADO À TERAPIA COM NATALIZUMABE.

Página gerada pelo sistema Currículo Lattes em 27/10/2022 às 14:42:16.