UNIVERSITAT ROVIRA i VIRGILI

OBESITY AND BARIATRIC SURGERY MODIFY PLASMA BIOACTIVE LIPID SPECIES

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Bachelor's Degree Final Project

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ABBREVIATIONS

ALA: α-linolenic acid

ARA: Arachidonic acid

ASP: Acylation stimulating protein

BMI: Body mass index

BS: Bariatric surgery

CKD: Chronic kidney disease

COX: Cyclooxygenases

CVD: Cardiovascular diseases

CYP450: Cytochrome P450

CysLT: Cysteinyl-leukotriene

DAP: Diastolic arterial blood pressure

DHA: Docosahexaenoic acid

DLP: Dyslipidemia

EET: Epoxyeicosatrienoic acid

EPA: Eicosapentaenoic acid

FC: Fold Change analysis

FFA: Free fatty acid

GERD: Gastroesophageal reflux disease

GI: Gastrointestinal

GPCR: G protein-coupled receptors

HDL: High-density lipoprotein

HDL-C: High-density lipoprotein cholesterol

HETE: Hydroxyeicosatetraenoic acid

HOMA IR: Homeostatic model assessment for insulin resistance

HPETE: Hydroperoxy-eicosatetraene

HPLC: High performance liquid chromatography

HTA: Arterial hypertension

IL-6: Interleukin 6

IR: Insulin resistance

LA: Linoleic acid

LDL: Low-density lipoprotein

LDL-C: Low-density lipoprotein cholesterol

LOX: Lipoxygenases

LX: Lipoxin

MS: Metabolic syndrome

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

PCA: Principal Component Analysis

PGE2: Prostaglandin E2

PLA2: Phospholipase A2

PLS-DA: Partial Least Squares – Discriminant Analysis

PPAR: Peroxisome proliferator-activated receptors

PUFA: Polyunsaturated fatty acid

RYGB: Roux-en-Y gastric bypass

SAP: Systolic arterial blood pressure

SCFA: Short-chain fatty acid

SG: Sleeve gastrectomy

sHE: Soluble epoxide hydrolase

SPM: Protectins, resolvins and maresins

SREBP: Sterol regulatory element-binding protein

T2DM: Type 2 diabetes mellitus

TG: Triglyceride

TNF-α: Tumor necrosis factor alpha

VIP: Variable Importance in the Projection

VLDL: Very low-density lipoprotein

WHO: World Health Organization

ABSTRACT

Background: Obesity is a condition whose prevalence is increasing all around the world and it has become one of the main causes of mortality in first world countries. It comes with the appearance of comorbidities such as non-alcoholic fatty live disease (NAFLD), type 2 diabetes mellitus (T2DM), hypertension (HTA), dyslipidemia (DLP) and metabolic syndrome (MS), reducing patients' life expectancy. NAFLD is a disease that implies hepatic steatosis or fatty liver, and it can progress to non-alcoholic steatohepatitis (NASH), in which there are also cellular damage and inflammation. Obesity's gold standard treatment is bariatric surgery (BS). It has shown to be effective in the disappearance of other comorbidities. Oxylipins, also known as bioactive lipid species, are lipidic mediators derived from fatty acids that are involved in inflammatory processes, such as adipose tissue inflammation in obesity and liver inflammation in NASH.

Objectives: To quantify plasma oxylipin profile in obese patients to determine if there are modifications due to obesity, obesity-related comorbidities and BS, and if BS can restore normal oxylipin concentrations.

Methods: Plasma samples from non-obese (n=50), obese pre-BS (n=118) and obese post-BS (n=44) patients were analyzed to obtain biochemical parameters and were subjected to LC-MS/MS assay to identify and quantify its oxylipin profile. Histological samples were obtained during and one year after BS procedure and analyzed to determine their liver severity status.

Results: Oxylipins were increased in obese patients in comparison with the non-obese group. Oxylipins levels in obese patients with comorbidities gave no significant variations respect to obese volunteers without comorbidities. Oxylipin levels in NASH patients where not modified in relation with non-NASH patients. Some obese patients were subjected to a very low calory diet (VLCD) before the BS procedure, which also did not produce changes in plasma oxylipins. BS modified plasma oxylipins by increasing and decreasing certain species. Finally, post BS patients did not restore non-obese oxylipin levels, as there were significant differences in concentration between the two groups.

Conclusion: Obesity and bariatric surgery modify plasma oxylipin profile, whereas obesity-related comorbidities and VLCD not. Oxylipin profile modifications persist after weight loss.

Key words: morbid obesity, NASH, bariatric surgery, oxylipins, obesity-related comorbidities.

INTRODUCTION

1. OBESITY

The World Health Organization (WHO) defines obesity as abnormal and/or excessive fat accumulation that may damage our health. Obesity is a condition that changes how the body functions, harms the organism and it is a response to different environmental factors and genetic predisposition. Suffering from obesity involves a high risk of developing cardiovascular diseases, diabetes, musculoskeletal disorders, some cancers (1) and mental health problems (2).

Obesity is diagnosed by the body mass index (BMI), which is the result of dividing a person's weight in kilograms by the square of his height in meters. Specifically, an adult has overweight if their BMI is 25 kg/m^2 or over, and obesity if their BMI is 30 kg/m^2 or over (1). A BMI greater than 40 kg/m^2 is considered a sign of morbid obesity.

Obesity is caused by multiple factors. The simplest explanation is due to a chronic positive energy intake, meaning that the energy consumption is bigger than its expense. This energy accumulation in the patients' bodies is transformed into deposits of triglycerides in their adipose tissue (2). A person's tendency to develop obesity is also influenced by genetic predisposition and the environmental and social aspects of their development (1).

There is a wide variety of phenotypes among patients with obesity, as it is a multifactorial disease. That means, for example, that some people show central obesity (visceral obesity) and have a waist-to-hip ratio bigger than 1, whereas others show peripheric obesity and have a waist-to-hip ratio lower than 1 and lower metabolic risk (3).

1.1. OBESITY: AN EPIDEMIC

Obesity has been recognized as an epidemic by the WHO. Not only it is a condition that affects millions of people, but also it is one of the biggest causes of mortality in high- and medium-income countries. Nowadays, nearly two thirds of the world population have overweight or obesity (2), and there are more overweight than underweight people in every region of the world except some parts of Africa and Asia (1).

In 2016, it was determined that about 40% of adults suffered from overweight and obesity globally (4). Its prevalence is slightly lower in young women but eventually it is bigger in women between 50-65 years old, age that corresponds with the appearance of menopause (Figure 1) (2).

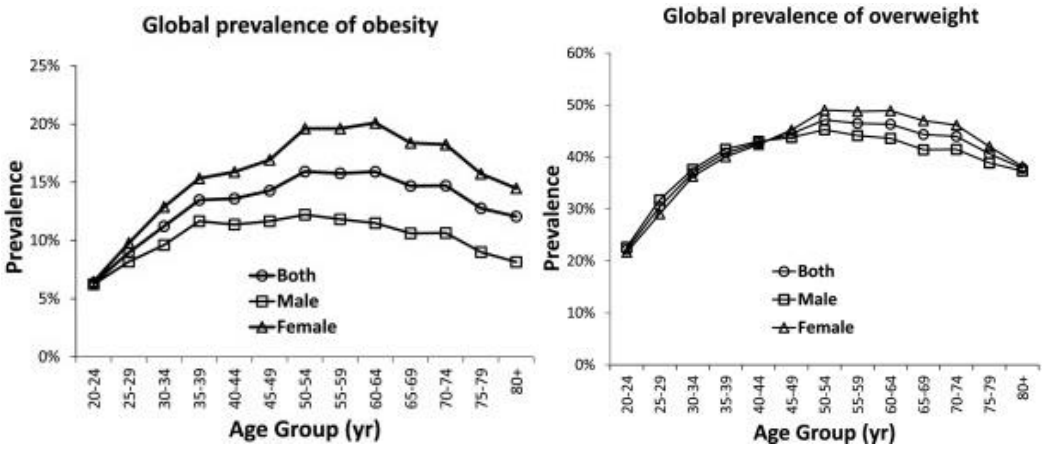

Figure 1. Prevalence of overweight and obesity in adults (> 20 years old) by sex and age. Extracted from (2).

The reasons for the raise in obesity in menopausal women are yet to be defined. It is known that estrogens are important regulators in glucose and lipid metabolism. The low production of estrogens during menopause it's a signal for the appearance of metabolic dysfunctions that lead eventually to obesity related diseases as type 2 diabetes mellitus (T2DM), metabolic syndrome (MS) and cardiovascular risk (5). The change in body fat distribution related to age and the arrival of menopause along with the decrease in sexual hormones seems to be the reason for the higher prevalence of obesity between menopausal women (5).

The prevalence of obesity in children is a growing problem both in first and third world countries. In the USA, obesity has doubled in children aged 2-5 years and tripled in children aged 6-11 years (2). Children in the UK, France, China, South Africa and Saudi Arabia are increasingly suffering from obesity (6). At first, obesity was a condition that used to be commoner in upper class people, but now the epidemic affects children from every socioeconomic status (2).

The biggest rise in overweight and obesity in the last 35 years has been registered in the USA and Europe (2). However, it is undeniable that the prevalence rates of this disease are increasing in every part of the globe. In general, the prevalence rates have doubled since 1980 in almost every region of the world (Figure 2) (2).

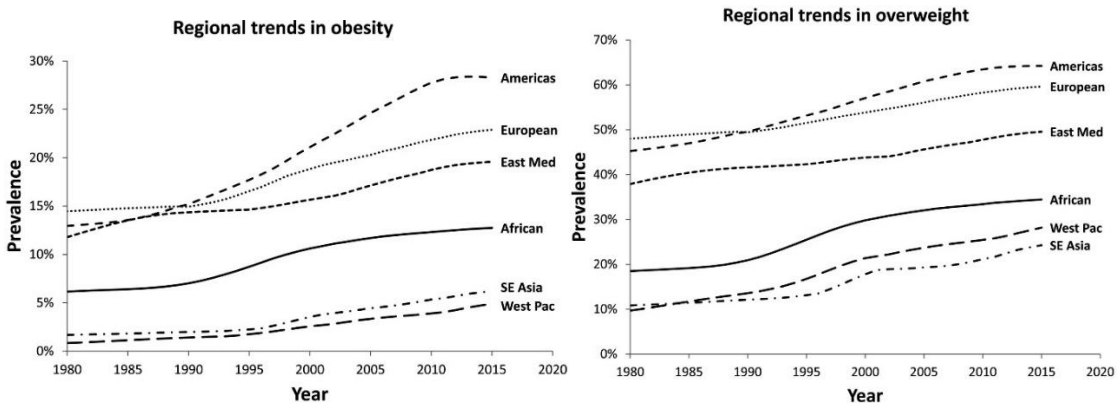

Figure 2. Prevalence of overweight and obesity in adults (> 20 years old) by region. Extracted from (2).

1.2. OBESITY ASSOCIATED COMORBIDITIES

Insulin resistance and type 2 diabetes mellitus

One of the main side effects of obesity is insulin resistance (IR). IR corresponds to the inability of target cells to answer to insulin secretion by the pancreas, which leads to higher production of insulin provoking hyperinsulinemia (7). Patients developing IR manifest inflammation of the adipose tissue. This tissue participates in body homeostasis and metabolism by being an endocrine organ that produces adipokines, such as the acylation stimulating protein (ASP), tumor necrosis factor alpha (TNF-α), leptin, adiponectin and interleukin 6 (IL-6). Inflammation of the adipose tissue leads to macrophage infiltration into the adipocytes that causes the disruption of the tissue homeostasis and affects the whole body increasing IR (7). Macrophage accumulation in the adipose tissue and pancreatic isles leads to the development of crown-like structures in the adipose tissue and the decrease of insulin sensitivity in adipocytes. Increased macrophages increases β-cell number, which leads to insulin secretion to overcome insulin resistance. Systemic inflammation eventually leads to the failure of β-cells and the decrease in insulin secretion, which added to increased insulin resistance produces hyperglycemia (8).

Early identification of IR is a way to prevent type 2 diabetes mellitus (T2DM) and reduce the mortality rates of obesity (9).

Cardiovascular diseases

Cardiovascular diseases (CVD) related to obesity are due to a condition called dyslipidemia, which is a disorder of lipoprotein metabolism and causes an accumulation of lipids in the blood (9). Around 65-75% of hypertension cases are obesity-associated. Obesity leads also to a higher chance of showing chronic kidney disease (CKD), that derives in higher cardiovascular risk and chance of mortality (9).

Furthermore, abdominal fat accumulation leads to fat deposits around the heart. This provokes a higher synthesis of leptin and lower of adiponectin, which promotes inflammation and can cause leptin resistance, that increases obesity as it reduces energy expenditure (10) and is common in patients with metabolic syndrome. Additionally, IR causes a dysfunction in the heart muscle that affects negatively to its functioning (9).

Dyslipidemia

Dyslipidemia consists in higher levels of triglycerides (TG) and free fatty acids (FFA), while there is a decrease in high-density lipoprotein-cholesterol (HDL-cholesterol) and normal or increased levels of low-density lipoprotein-cholesterol (LDL-cholesterol). This is due to an increase in FFA in the liver, which leads to TG accumulation and very low-density lipoproteins (VLDL) synthesis. This affects reduces chylomicron lipolysis due to impaired lipoprotein lipase (LPL) activity, and leads to a remain of TG to be transported into the liver (11).

Owing to the accumulation of certain FFA, this disorder is one of the causes of a higher chance of developing IR (11).

Gastroesophageal reflux disease

Gastroesophageal reflux disease (GERD) is defined as complications that derive from the reflux of stomach contents into the esophagus (12). In nonobese people the most common way of developing GERD is the increased periods of transient lower esophageal sphincter relaxations. This phenomenon has been observed to be more usual in obese patients, added to the higher quantity of visceral fat, that is also related to heartburn and regurgitation and can result in GERD (9).

Pulmonary diseases

Overweight and obese people lose lung capacity consistently, especially patients with visceral obesity. This is due to the reduction of lung volume. It is common in obesity patients that they experience breathlessness regularly. Obesity can also worsen asthma (9).

Sleep disorders

Weight gain and metabolic dysfunction are common causes of sleep disorders, disrupted circadian rhythms and stress. Additionally, having obesity can increase the chance of showing sleep-disordered breathing, a condition that involves sleep apnea, hypopneas, and respiratory effort-related arousals during sleep (9).

Major Depressive disorder

Patients with obesity have a 55% chance of suffering from major depressive disorder. This is consistent in both genders but specially women. Other factors like age, education, conjugal and financial status and other chronic diseases can aggravate this disorder. General anxiety disorder is also more frequent in obesity patients (13).

Furthermore, depressive people have a 58% chance of getting obese (13).

Cancer

Hyperadiposity and inflammation produced by obesity alter the function of the adipose tissue leading to adipocyte death. Eventually, the harmed adipose tissue is infiltrated by immune cells, with changes the microenvironment of the tissue and can be followed by the promotion of some cancers (14). Metabolic syndrome (MS), DLP and IR can worsen the damage of the tissue causing the growth of the tumor (14).

2. NAFLD

Non-alcoholic fatty liver disease (NAFLD) is the most usual liver disease worldwide (15). It is a disease that also affects certain extrahepatic organs and regulatory pathways. Like obesity, the presence of NAFLD increases the risk of suffering from T2DM, CVD and CKD (16).

The prevalence rates of this disease are increasing along with the rates of obesity, T2DM, and MS, and it is projected to keep rising from 20% to 27% of adults by 2030 in the United States (17). NAFLD is more common in men than women, and more present in patients above 50 years old (16). As NAFLD increases, the number of patients with cirrhosis and in need of liver transplantation is also on the rise (16).

NAFLD is a progressive disease that implies hepatic steatosis or fatty liver. When it progresses to non-alcoholic steatohepatitis (NASH) there are also cellular damage and inflammation. NASH tends to appear with pericellular fibrosis, and this can eventually lead to cirrhosis and hepatocellular carcinoma (17). Cirrhosis is a response of the hepatic tissue to injuries, characterized by tissue disruption. Fibrous tissue deposits between the cells and changes the extracellular matrix (18). 40% of NAFLD patients show fibrosis, and 20% of the total advance to cirrhosis (18).

It has been determined that there are some genetic factors that make people more propense to develop fibrosis and NASH. Studies have shown that close relatives of people diagnosed with NASH presented fibrous tissue at some level (17). Patients with NASH remain undiagnosed, as the development of this disease is asymptomatic until it reaches irreversible stages (17).

2.1. FROM NAFLD TO NASH

NAFLD starts with insulin resistance. Insulin is a key element in lipid synthesis and storage. The inability to respond to insulin by the target cells leads to hyperinsulinemia in the blood, which activates lipogenesis by induction of sterol regulatory element-binding protein (SREBP) and fatty acid synthase (19). The increase of FFA delivered to the liver by the big intake of them by the diet overwhelms the production of VLDL, so fatty acids accumulate in the liver (19).

FFA accumulation leads to hepatocyte apoptosis that increase inflammation and fibrogenesis, and macrophage activation, promoting the recruitment of monocytes and leucocytes. Eventually this activates hepatic stellate cells and the accumulation of excessive extracellular matrix that would form pericellular fibrosis (20).

Inflammation is carried out by Kupffer cells, which respond to the damaged tissue by liberating cytokines and signaling molecules (Figure 3) (17).

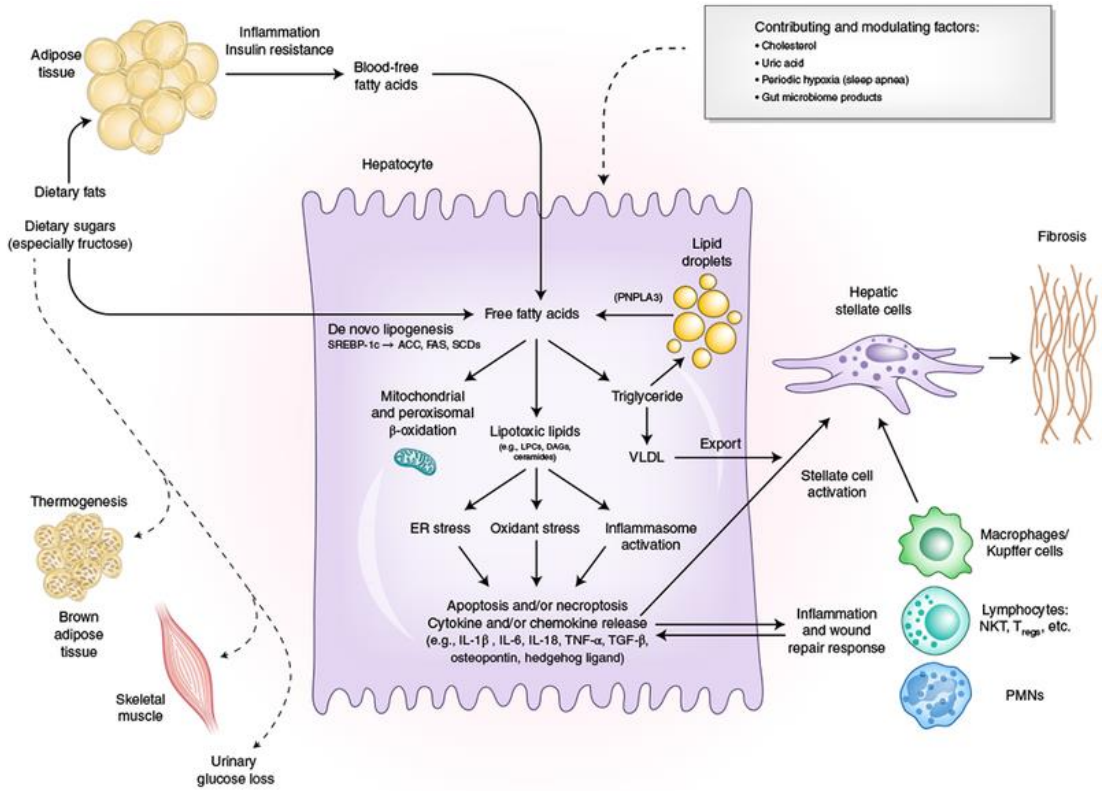

Figure 3. Overview of NASH pathogenesis. Extracted from (17).
Free fatty acids from lipolysis of TG in adipose tissue are delivered to the liver by blood circulation. The major fates of FFA in hepatocytes are mitochondrial beta-oxidation and re-esterification to form TG.TG are exported as VLDL. When there is an accumulation of FFA, exceeding FFA are led to the formation of lipotoxic species that lead to ER stress, oxidant stress and inflammasome activation. These processes are responsible for the development of NASH. SCD: steroyl CoA-desaturase; FAS: fatty acid synthase; NKT: natural killer T cell; Tregs: regulatory T cells; PMNs: polymorphonuclear leukocytes.

2.2. NAFLD AND NASH DIAGNOSIS

As discussed before, most NAFLD patients remain asymptomatic. It is crucial to know how NAFLD manifests to diagnose it before there is too much hepatic damage. Nowadays, a screening is carried out to people with obesity or metabolic syndrome to look for NAFLD (21).

To determine the presence of NAFLD the best scan is ultrasound, offering 60-94% sensitivity and 66-97% specificity. Once it has been stablished that the patient has steatosis, it is important to exclude people that regularly consume alcohol, people who take certain medications and other conditions such as celiac disease (21).

However, ultrasound cannot be used in morbidly obese patients, and does not inform about the inflammation state of the liver. For these reasons, liver biopsy, a highly invasive and costly procedure, is the gold standard technique for the diagnosis of NASH (21).

Kleiner et al., in 2005 described a method to determine the presence of NASH by evaluating the degree of steatosis, necroinflammatory lesions, ballooning and fibrosis from formalin-fixed paraffin-embedded hepatic tissue (22).This system analyzes these four characteristics and gives a score from 0 to 8 that allows us to determine in which state of the disease the patient is. Samples rated 5 or over are diagnosed as NASH, while

the ones rated less than 3 are labeled as non-NASH. Samples that score 3 or 4 are in an intermediate state, considered uncertain or borderline (22).

3. BARIATRIC SURGERY

The gold-standard technique to treat obesity and obesity-associated comorbidities is bariatric surgery (BS). It is widely extended as it has proved its effectiveness in the vast majority of patients (23).

BS is recommended for all people whose BMI is over 40, and people whose BMI is 30-40 if they have a serious comorbidity that can threaten their health, and some patients are required to take a very low-calorie diet (VLCD), as it has proven to improve the prognosis after surgery (24).

There are four different surgical procedures to perform bariatric surgery, but the most common interventions for weight loss surgery are sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB) (Figure 4) (23).

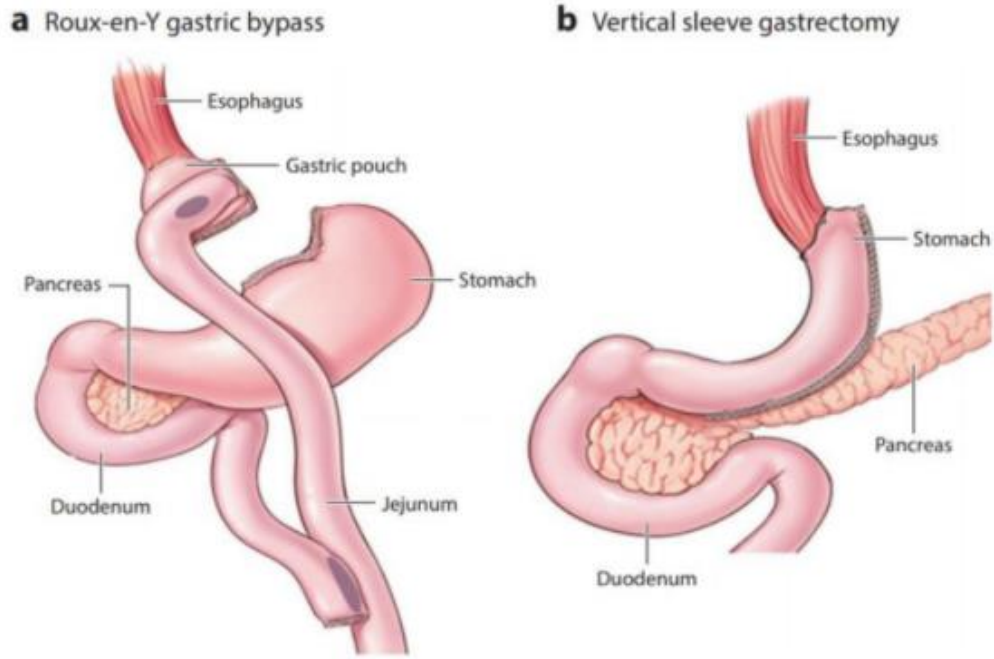

Figure 4. Main types of bariatric surgery. Extracted from (25).
(a) In a Roux-en-Y gastric bypass, a small pouch is created under the esophagus that is connected to the jejunum. (b) In a vertical sleeve gastrectomy, roughly 80% of the stomach along the greater curvature is removed.

3.1. SLEEVE GASTRECTOMY

Procedure

Consists of the resection of the bigger curve of the stomach to give it a tubular structure with reduced capacity (23). It is one of the most extended treatments nowadays, but as it is fairly recent, its long-term consequences are yet to be studied (26).

Outcome

It is an efficient procedure in weight loss, reducing approximately 25% of total body weight and 60% of excess weight and the excess BMI is also reduced by 65%. The first couple of years after the surgery a big weight loss can be observed, and then the weight stabilizes. Its long-term effects are yet to be determined and its durability and effectiveness have not been determined after 10 years or more (23).

Complications

Short-term complications include bleeding and gastrointestinal (GI) leak, however the incidence of these is lower in SG than RYGB (23). On the other hand, it has been observed that SG can produce oxygen dependence, hypertension, hypoalbuminemia, and diabetes. Also, 32% of GERD patients that go through SG have experienced that their symptoms have worsened. (23)

3.2. ROUX-EN-Y GASTRIC BYPASS

Procedure

This procedure was the most popular until 2013 when it was taken over by SG. It is characterized by the separation of the stomach into 2 different sections. The new part is called "gastric pouch" and it is connected with the small intestine by the "roux limb", located in the distal part of the jejunum. After the procedure, the food will go straight to the intestine from the gastric pouch without having to go down most part of the stomach and the beginning of the small intestine (23).

Outcome

Weight loss rate in RYGB is bigger than in SG, and its durability and effectiveness have been proven after 10 years or more (23).

Complications

Approximately 8% of people that go through this procedure experience some type of complication, and 3% have serious problems. The most common short-term ones are bleeding, gastrointestinal leaks, and bowel obstruction (23).

The main complications are long-term vitamin deficiencies after the procedure. Other complications include abdominal pain, ulceration of the gastrojejunal anastomosis (marginal ulcer), herniation of the small intestine and Dumping Syndrome (23).

3.3. IMPACT ON OBESITY ASSOCIATED COMORBIDITIES

BS improves insulin resistance and reduces chronic inflammation, which leads to an advance in recovering normal lipid metabolism (27). This eventually reduces the incidence of NASH in most patients after the procedure. About 85% of NASH patients are cured a year after the surgery, which leads to think that BS is an appropriate treatment not only for obesity but also NAFLD (27).

It has been observed that BS reduces hypertension to 84% of operational patients. As a result, heart function is restored, and cardiovascular risk and mortality is reduced notably (27).

BS has been proved to be a good treatment in T2DM, as almost half (49%) of the patients that subjected to the procedure experimented an improvement in sugar blood levels, blood pressure and cholesterol levels. Moreover, 36% of surgical patients experimented total or partial diabetes remission (27).

BS also has an effect in diabetes prevention, as non-diabetic surgical patients have a very low risk of developing the disease (3%), compared to obese people that go through different treatments and end up suffering from T2DM (26%) (23).

4. OXYLIPINS

Oxylipins, also known as bioactive lipid species, are lipidic mediators molecules derived from fatty acids that are involved in inflammatory processes (28). Specifically, oxylipins are formed from eicosanoids that are polyunsaturated fatty acids (PUFA) that regulate many homeostasis and inflammatory processes. Although the name 'eicosanoid' indicates 20 carbon atoms, there are eicosanoids between 18 and 22 carbon atoms (28). Some eicosanoid precursors are arachidonic acid (ARA 20:4), linoleic acid (LA 18:2), α-linolenic acid (ALA 18:3), eicosapentaenoic acid (EPA 20:5), and docosahexaenoic acid (DHA 22:6) (28).

Oxylipins are obtained from the diet or from mono or dioxygen-dependent reactions. The first step for the formation of oxylipins is the release of PUFAs from phospholipids, carried out by Phospholipase A2 (PLA2) in tissues such as liver, adipose, intestine and kidneys. These oxylipins can have a short life and act locally, but they have also able to travel and interact to produce biological effects. When they are no longer needed, oxylipins can be re-esterified to lipids. The formation of PUFAs to oxylipins can occur via 3 main pathways: cyclooxygenases (COX), lipoxygenases (LOX) and cytochrome P450 enzymes (CYP450) (28). The type of PUFA that generates the oxylipins is what gives them their function in the inflammatory process. Generally, ω-3 PUFA result in anti-inflammatory oxylipins, whereas ω-6 PUFA produce a pro-inflammatory response. Oxylipins act by connecting with peroxisome proliferator-activated receptors (PPAR), and G protein-coupled receptors (GPCR). The plasmatic oxylipin profile is altered in response to COX and LOX modified metabolism, obesity, cardiovascular diseases, and metabolic syndrome (28).

Oxylipins are a very heterogenous group that tend to circulate at nanomolar concentrations, which makes it difficult to quantify them. The most used technique for oxylipin measure is high performance liquid chromatography (HPLC) coupled to mass spectrometry (28).

There are oxylipins in all tissues, some of them are free and therefore biologically active, and others are esterified to phospholipids. The latter's functions are yet to be defined (29).

Biosynthesis

Eicosanoid is an umbrella term that includes lipid mediators that come from C20 PUFAs. In this category there are prostaglandins, thromboxanes, leukotrienes, hydroxyeicosatetraenoic acids, and related other derivatives. The main precursors for these oxylipins are ARA and EPA (30).

ARA is an essential fatty acid and must be provided by the diet. The oxylipins derived from ARA are synthetized by a process called eicosanoid cascade. They are found in cell membranes conjugated with phospholipids and can function as a signaling molecule in the central nervous system or act as an apoptosis regulator in some animal tissues (30).

Their synthesis begins with the activation of PLA2. This activation can be produced by a number of factors, some of them include variation in hormone and cytokine levels (30). It's been studied that ceramide-1-phosphate and phosphatidylinositol 4,5-bisphosphate bind to the enzyme and stimulate it. Activated PLA2 produces ARA from the membrane phospholipids, which can generate different oxylipins. The product will variate whether the enzyme that carries out the process is COX (prostaglandins, prostacyclin and thromboxanes), CYP450 (hydroxyeicosatetraenoic acids (HETEs) and epoxides (EETs)), or LOX (leukotrienes, cysteinyl-leukotrienes (CysLTs) and lipoxins (LXs)) (Figure 6) (30).

Oxylipins derived from COX

Oxylipins produced by the COX enzyme cause the main signs of inflammation (heat, swelling, redness, pain and loss of function) (31). Some examples of these oxylipins include prostanoids (prostaglandins, prostacyclin and thromboxanes). To express their activity, they interact with certain GPCRs (30).

Oxylipins derived from LOX

The 5-LOX pathway plays a more specific role in the inflammatory process to promote bronchoconstriction and leukocyte recruitment to the site of tissue damage. Some products may be ligands for PPARα and PPARγ, which produce anti-inflammatory effects and modulate the liver X receptor (LXR) which regulates cholesterol homeostasis (31).

Some of these oxylipins are hydroperoxy-eicosatetraenes (HPETEs), which have a short life as they are quickly modified into their stable form hydroxyeicosatetraenoic acids (HETE), leukotrienes and lipoxins, and protectins, resolvins and maresins (SPM) (32).

Oxylipins derived from CYP450

Many CYPs are expressed in the liver, but also in other tissues where they inactivate toxins and metabolites. CYPs higher up in the synthesis pathway convert ARA to epoxyeicosatrienoic acids (EETs) or ω-HETEs. Some CYP and soluble epoxide hydrolase (sEH) may be responsible for the synthesis of SPM (31).

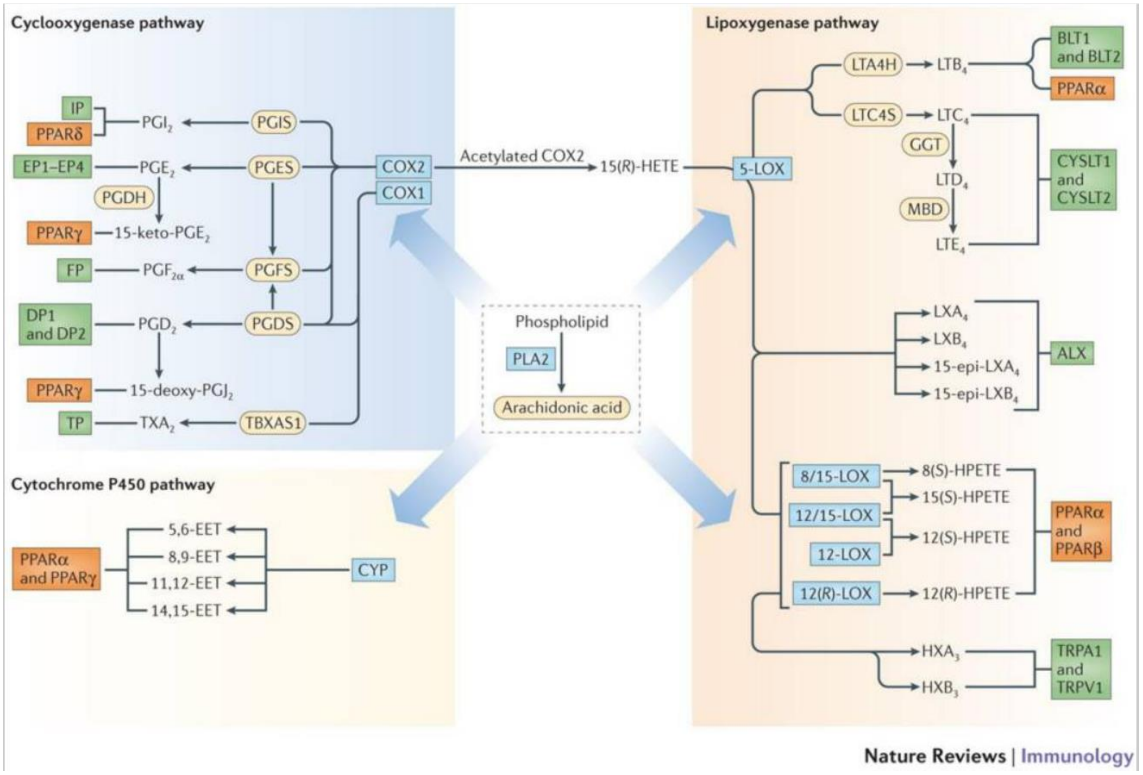

Figure 5. Main pathways for the synthesis of oxylipins. Extracted from (31).
Lipidomic view of phospholipase A2 (PLA2), cyclooxygenase-1 and -2 (COX1/2), 5-lipoxygenase (5-LOX), 8-, 12-, and 15-lipoxygenases (8/12/15-LOX) and cytochrome P450 epoxyhydrolase (CYP) pathways of eicosanoid biosynthesis derived from arachidonic acid.
COX: cyclooxygenase; CYP: cytochrome P450; EET: epoxyeicosatrienoic acid; GGT: gamma-glutamyl transferase; HETE: hydroxyeicosatetraenoic acid; HX: hepoxilin; LOX: lipoxygenase; LT: leukotriene; LTAH: LTA4 hydrolase; LTCS: LTC4 synthase; LX: lipoxin; MBD: membrane bound dipeptidase; PLA2: phospholipase A2; PPAR: peroxisome proliferator-activated receptor; TRPA1: transient receptor potential ankyrin 1; TRPV1: transient receptor potential vanilloid 1; TX: thromboxane.

Efficient degradation and deactivation mechanisms of eicosanoids are essential for the control of their functions. There is a main catabolic pathway that is common to most of them. The first step in this process is the oxidation of a hydroxyl group carried out by a dehydrogenase. Then the intermediary is reduced by a ketoprostaglandin reductase, giving an inactive product that is then catabolized by beta-oxidation, giving prostaglandin E2 as a final product (30).

4.1. FUNCTIONS OF OXYLIPINS

Since they induce the symptoms of inflammation, which include heat, swelling, redness, discomfort, and loss of function, studies of eicosanoids in inflammation have mainly focused on signaling pathways mediated by lipids formed by COX enzymes. The great variety of functions these lipids play in different tissues makes it difficult to understand their physiological effects; for example, binding of prostaglandin E2 (PGE2) to its receptors, the EP receptor family, on neurons causes pain associated with inflammation, while autocrine EP signalling by PGE2 on macrophages can reduce TNF output while increasing IL-10 production, resulting in decreased inflammatory signals (31).

More details and other oxylipin functions are explained in Table 1.

Table 1. Functions of some oxylipins analyzed in this study. Extracted from (31).

PATHWAY	MEDIATOR	RECEPTOR	BIOCHEMICAL EFFECT
COX	PGE2	EP1, EP2, EP3 and EP4	Vasodilation and vascular leakage; hyperalgesia; fever; IL-10 ↑ TNF-α ↓; PMN eicosanoid class switching
	PGD2	DP1	Mast cell maturation, vasodilation, neuroprotection
		DP2	Eosinophil recruitment and allergic response ↑
	PGF2α	FP	Uterine, vascular and respiratory smooth muscle contraction
			Intraocular pressure ↓
5-LOX	LTE4, LCD4	CysLT1, CysLT2	Bronchoconstriction; vascular leakage; neutrophil extravasation
8-, 12-, 15-LOX	HpETEs, HETEs and diHETEs	TRPV1	Hyperalgesia
		PPAR-α, γ	Fatty acid translocase/CD36 ↑
CYP	EETs	PPAR-α, γ	Vasodilation; antihyperalgesia; COX-2 expression ↓

COX: cyclooxygenase; PGE2: prostaglandin E2; EP: prostaglandin E receptor; IL-10: interleukin 10; TNF-α: tumor necrosis factor α; PMN: polymorphonuclear neutrophils; PGD2: prostaglandin D2; DP: prostaglandin D receptor; PGF2α: prostaglandin F2α; FP: prostaglandin F receptor; LOX: lipoxygenase; LTE4: leukotriene E4; LCD4: lipoxygenase catalytic domain 4; CysLT: cysteinyl leukotriene receptor; HpETE: hydroperoxyeicosatetraenoic acid; HETE: hydroxyeicosatetraenoic acid; TRPV1: transient receptor potential V1 channel; PPAR-α: peroxisome proliferator-activated receptor α; CYP: cytochrome P450; EET: epoxyeicosatrienoic acids.

The change in the oxylipin profile in obesity, related comorbidities, and bariatric surgery has not been studied yet. Only 64 results show up in PubMed if the words “oxylipins + obesity” are searched, and none show up if the search is “oxylipins + bariatric surgery”. It is necessary a bigger investigation in this area of knowledge as it can answer some questions still remaining about these diseases and their treatment, and it can provide with valuable markers to diagnose asymptomatic conditions like NAFLD which are usually found too late to treat in most patients.

HYPOTHESIS AND OBJECTIVES

Obesity is a major problem nowadays. Its prevalence is increasing all around the world and it has become one of the main causes of mortality in first world countries. Along with it comes the appearance of obesity-related comorbidities such as non-alcoholic fatty live disease (NAFLD) and non-alcoholic steatohepatitis (NASH), that start with insulin resistance derived from type 2 diabetes mellitus (T2DM) and dyslipidemia (DLP) due to obesity.

NAFLD is a condition that is very hard to diagnose, as it is asymptomatic until it becomes irreversible. The only way to diagnose it nowadays is by liver biopsy, a very invasive procedure. Also, NAFLD and IR patients that go through bariatric surgery have experienced the disappearance of these diseases in a year. Oxylipins are mediators involved in inflammatory processes such as adipose tissue inflammation in obesity and liver inflammation in NASH. Consequently, it was hypothesized that the plasma oxylipin profile is modified by obesity and NASH. Patients that undergo bariatric surgery, as their comorbidities disappear, are expected to have their plasmatic oxylipins restored to normal levels.

The aim of this study was to determine changes in plasma oxylipin profile related to obesity, obesity-associated NASH and bariatric surgery.

Specific objectives:

- To quantify oxylipin profile in plasma samples from healthy controls, obese patients without NASH, obese patients with NASH and patients with obesity that had been operated on of BS 12 months after the procedure.
- To compare the different oxylipin profiles to find:
    - Changes related to obesity: healthy controls compared to obesity patients.
    - Changes related to NASH: non-NASH obese patients compared to NASH obese patients.
    - Changes related to the administration of a very low calory diet before BS, and to other comorbidities such as T2DM, DLP, HTA and MS.
    - Changes related to BS: obese pre-surgery patients compared to obese post-surgery patients.
- To determine if oxylipin levels are restored after BS: healthy controls compared to post-surgery patients.

MATERIALS AND METHODS

1. STUDY PARTICIPANTS

The participants of this study were morbid obesity patients that underwent bariatric surgery in Sant Joan de Reus hospital. The main exclusion criteria were a BMI lower than 35 and an age under 18. Other exclusion criteria included having acute illness, chronic or acute inflammation, infectious diseases, or terminal illness.

The total number of participants was 118. Among those patients, 50 were distinguished by having NASH (NAS $\geq$ 5), 48 by not having NASH (NAS $\leq$ 2), and 20 by having uncertain NASH (NAS = 3 and 4). In addition, 44 patients out of the 118 were followed-up 12 months after the procedure.

For the control group, 50 age- and sex-matched blood samples from the Bank of biological samples from *Institut d'Investigació Sanitària Pere Virgili (Biobanc-IISPV)* were used.

The complete design of the study is shown in Figure 6.

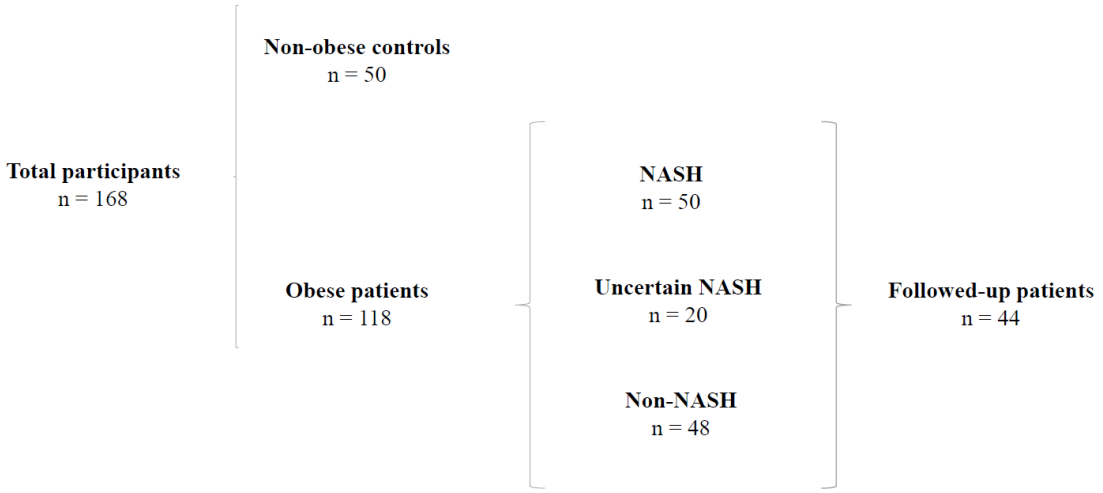

Figure 6. Design of the study.

2. SAMPLING

Blood samples from patients were collected right before the surgery was performed. The patients that agreed to be followed-up 12 months after the intervention had blood extracted at the hospital.

These samples were collected in tubes and centrifuged at 2500 rpm for 15 minutes at 4ºC to obtain plasma and serum. Finally, samples were stored at -80ºC for further analyses.

During bariatric surgery, a percutaneous hepatic biopsy was performed, and 12 months after the surgery, 44 of the patients who needed to be followed-up for their liver severity status at the time of the surgery, had a follow-up biopsy. Hepatic tissue samples were maintained in formaldehyde for 24 hours and then were paraffin-embedded to be able to use them for histological analyses.

3. BIOCHEMICAL ANALYSIS

Biochemical parameters like glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) were determined by an automatic analyzer (COBAS 8000, Roche Farma).

Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR) by the formula: [fasting insulin (μU/L) x fasting glucose (nmol/L)]/22.5 (33)

4. HISTOLOGICAL ANALYSES

Hepatic tissue samples that were being kept in formaldehyde were subjected to a dehydration progress that consists in their immersion in several solutions with different concentrations of xylenes and ethanol. Once they are dehydrated, they are included in paraffin and cut in 2 μm pieces using the microtome. They are then dyed with three different stains to determine their Non-Alcoholic Fatty Liver Activity Score (NAS): Hematoxylin and Eosin (to dye acidic and basic structures, respectively), Sirius Red (to dye collagen fibers) and Masson's Trichrome (to dye the different types of collagen fibers in different colors).

Once they are stained, NAS is determined by their observation with the microscope. By the evaluation of the components that conform NASH (steatosis, lobular inflammation, fibrosis and ballooning effect), a number from 0 to 7 was assigned. Samples with NAS 0-2 werere labelled as non-NASH, NAS 3-4 werelabelled as uncertain NASH and NAS 5-7 werelabelled as NASH (22).

5. LIPIDOMICS

Materials, standards and reagents:

- Oasis PRIME HLB SPE cartridges (30 mg, 1 cc) (Waters)
- Vacuum Manifold for SPE (Teknokroma)
- Chromatographic column: Agilent Eclipse XDB-C18 1.8 μm, 2.1 x 150 mm (Agilent Technologies)
- Acetic acid LC-MS (Sigma Aldrich)
- Water LC-MS (Scharlab)
- Acetonitrile LC-MS (Merck)
- Methanol LC-MS (Merck)

- Formic acid (Sigma Aldrich)
- Butylated hydroxytoluene (BHT) (Sigma Aldrich)
- Standards and internal standards were acquired from Cayman Chemical (Ann Arbor, USA) (Supplementary table S1).

Sample preparation:

A volume of 250 µL of plasma was mixed with 100 µL of internal standard prepared in MeOH (BHT 0.001 M) in a 2 mL tube. A volume of 500µL of MeOH (BHT 0.001 M) was added to the plasma, and the sample was incubated 30 minutes at -20 ºC. Then, the sample was centrifuged, and supernatant was recovered and diluted with 4 mL of Milli-Q water (Formic acid 0.1%). Afterwards, a cleanup was applied using OASIS HLB PRIME eluting with 600 µL of ACN:MeOH (9:1, v/v) twice. The elute was evaporated to dryness in nitrogen and reconstituted in 100 µL of Milli-Q water:MeOH (1:1, v/v).

LC-MS/MS assay:

An UHPLC 1290 Series coupled to a QqQ/MS 6490 series instrument were used to analyze the extracts. Separation of oxylipins was carried out onto an analytical column Eclipse XDB C18 1.8 µm, 2.1 x 150 mm from Agilent Technologies. The chromatographic separation was performed with the gradient elution detailed in Table 2 using water (0.01% acetic acid) and acetonitrile:methanol (85:15, v/v) as mobile phases at a flow rate of 0.400 mL/min and 45 ºC. The injection volume was 10 µL (4 ºC).

6. DATA ANALYSIS AND STATISTICS

Identification of many oxylipins with the corresponding standard was limited because these standards are not commercially available. Therefore various tools to identify the maximum number of oxylipins were used: MS confirmation with the molecular ion [M-H]- and fragmentation patterns using ESI in negative ionization. In addition, published data in relation to the main oxylipins in the studied matrices was used for identification purposes.

To quantify oxylipins the linear calibration curves used were constructed from available commercial standards using internal standard correction. Internal standard was selected based on chromatographic behavior criteria. For the compounds with non-available commercial standard the calibration curve of a similar compound was used and, therefore, are semi-quantified. Outliers were detected by the Iglewicz and Hoaglin's robust test for multiple outliers, using the outlier criterion that the modified Z score had to be equal to or higher than 3.5. Outliers and missing values were replaced by the median of their group.

Statistical assessments were carried out using SPSS 25 (IBM Corp., Chicago, IL, USA), considering differences between groups significant when their p-value was <0.05. Non-parametric variables were compared with the Mann-Whitney U test, matched comparisons with the Wilcoxon signed-rank test, several-group comparison with

Kruskall-Wallis test, and categorical variables with chi-squares test and contingency tables. Quantitative variables were expressed as median and interquartile range, and qualitative variables as % of participants.

Metaboanalyst 5.0 (https://www.metaboanalyst.ca/) was used to generate different scores and loading plots. Data was normalized by log transformation. Univariate analysis consisted in the generation of several plots that were based on ratios of concentrations and p-values. The analyses that were carried out were: Fold change analysis, Volcano plots, Heatmaps, Principal Component Analysis (PCA) and Partial Least Square – Discriminant Analysis (PLS-DA). Fold Change analysis was used to determine concentration differences between each condition in a group. Volcano plots were generated to visualize significant oxylipins along with their Fold Change. Heatmaps were useful to determine the relative concentration of each compound from each patient and were applied to visualize global relative concentrations and correlations between variables. PCA and PLS-DA were used to reduce the dimensionality of the database. From PLS-DA it was generated the Variable Importance in the Projection (VIP) scores to visualize the variables with more separation capacity. Bubble Plots and Correlation Plots were generated to determine the concentration changes between oxylipin species, and to visualize the relationship between variables and BMI, respectively, with RStudio (R version 4.0.2). Package used were ggplot2, ggpubr, deplyr and ggrepel, and PerformanceAnalytics, respectively. All packages were used at most recent version available at CRAN (https://cran.r-project.org/) on May 17th of 2021.

RESULTS

In this study a lipidomic analysis was performed to quantify plasma concentrations of oxylipins in non-obese voluntaries and morbid obese patients, to determine variations due to obesity, associated comorbidities and bariatric surgery. The main objectives in this study were:

1. Modification of plasma oxylipin profile by obesity. Comparison of non-obese and obese individuals.
2. Modification of plasma oxylipin profile by obesity-related comorbidities. Comparison of obese patients with and without T2DM, HTA, DLP and MS, and comparison of non-NASH, uncertain NASH and NASH individuals.
3. Modification of plasma oxylipin profile by a VLCD. Comparison of obese people that followed this diet before BS, obese people who followed a mixed diet, and obese people who did not follow any diet.
4. Modification of plasma oxylipin profile by BS. Comparison of individuals before and 12 months after surgical intervention.
5. Restoration of plasma oxylipin profile to normal levels by BS. Comparison of non-obese individuals and patients followed-up 12 months after their procedure.

CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE POPULATION

Comparing the clinical characteristics of the three main groups (healthy controls, obese patients and post-BS patients), most results were as expected. Similar results in sex and age were observed. The most significant difference was found in BMI, as obese patients had the greatest BMI of the three groups, and the followed-up patients 12 months after BS had greater BMI than the healthy controls, but lower than the obese group. It was also observed a slighter increase in systolic arterial pressure (SAP) in obese and post BS patients compared to non-obese controls, as well as a slight decrease in diastolic arterial pressure (DAP) in obese and post-BS patients compared to the healthy individuals. Significant differences in comorbidities' prevalence were also found, as it was very high in obese patients and low in non-obese and the followed-up group.

The biochemical variables were also different between the three groups, for example, glucose, insulin and HOMA IR were significantly greater in obese patients compared to the non-obese and post-BS patients.

Transaminases levels were also slightly higher in the obese group. In most cases, transaminases decreased 12 months after the surgical procedure but did not reach the level of the control patients.

Finally, the percentages of drug treatments between groups were also very different, and notably higher in the obese group in comparison with the non-obese and post-BS groups.

The complete results of the clinical and biochemical analysis of the patients are shown in Table 8.

Table 2. Clinical and biochemical characteristics of the patients.

	Healthy controls	Obese patients	12 months after BS	p-value
Clinical characteristics				
Sex (woman, %)	62	75	63	
Age (years)	44 (38-59)	50 (41-56)	47 (41-62)	
BMI (kg/m2)	27 (25-31)	44 (40-50)	34 (31-38)	a, b, c
SAP (mmHg)	125 (110-146)	130 (122-144)	134 (116-147)	b, c
DAP (mmHg)	80 (70-94.5)	77 (69-84)	76 (67-88)	a, b, c
T2DM (%)	2	39	15	a, b, c
HTA (%)	14	59	20	a, b, c
Dyslipidaemia (%)	6	42	6	a, b, c
MS (%)	18	59	14	a, b
Steatosis (%)	-	66	2	b
NAS	-	4 (2-5)	0	b
Biochemical variables				
Total cholesterol (mM)	4.95 (4.60-5.83)	3.87 (3.45-4.47)	4.77 (4.44-5.64)	a, b, c
HDL-cholesterol (mM)	1.46 (1.31-1.78)	0.93 (0.80-1.09)	1.55 (1.32-1.82)	a, b, c
LDL-cholesterol (mM)	3.09 (2.44-3.74)	2.20 (1.74-2.86)	2.95 (2.51-3.32)	a, b, c
Triglycerides (mM)	1.00 (0.70-1.40)	1.38 (1.12-1.87)	0.99 (0.80-1.21)	a, b, c
Glucose (mM)	4.50 (4.30-4.90)	6.75 (5.62-8.54)	4.77 (4.39-6.22)	a, b, c
Insulin (mM)	49.42 (32.37-74.27)	82.30 (42.19-124.67)	46.53 (15.97-86.12)	a, b, c
HOMA IR	1.44 (0.92-2.25)	3.52 (1.60-5.93)	1.85 (1.23-2.54)	a, b, c
Transaminases				
ALT (µKat/L)	0.25 (0.22-0.36)	0.56 (0.42-0.95)	0.23 (0.19-0.29)	a, b, c
AST (µKat/L)	0.33 (0.27-0.40)	0.57 (0.36-0.78)	0.39 (0.23-0.32)	a, b, c
GGT (µKat/L)	0.20 (0.14-0.29)	0.36 (0.25-0.56)	0.26 (0.16-0.38)	a, b, c
Medication				
Metformin (%)	4	60	4	b, c
Sulfonylureas (%)	2	9	2	a
Insulin (%)	0	38	2	a, b, c
ACE-ARBS (%)	6	48	10	
Diuretic (%)	14	13	4	
Statins (%)	2	27	6	a, b, c

BMI: body mass index; SAP: systolic arterial blood pressure; DAP: diastolic arterial blood pressure; T2DM: type 2 diabetes mellitus; MS: metabolic syndrome; NAS: NAFLD activity score; HDL: high-density lipoproteins; LDL: low-density lipoproteins; HOMA IR: homeostatic model assessment for insulin resistance; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase; ACE-ARBS: angiotensin converting enzyme and angiotensin receptor blockers. Non-parametric variables are expressed as median and interquartile range (25-75%). Qualitative variables are expressed as percentage (%) of participants. Groups were compared using Mann-Whitney U test or Wilcoxon test. p-values <0.05: significant differences between (a) non-obese controls and obese patients, (b) obese patients and followed-up patients 12 months after BS, (c) non-obese controls and followed-up patients 12 months after BS.

LIPIDOMICS

1. OBESITY MODIFIES PLASMA OXYLIPIN PROFILE

Oxylipin concentrations were compared between obese patients and non-obese controls. The main difference observed was that some oxylipin species were increased in obese patients compared to non-obese group.

Univariate analysis was first performed. Fold Change between obese and non-obese individuals revealed which oxylipins variate between this two groups. Some species like 12-HETE, 12-HEPE and 14-HDHA were significantly increased in obese patients (Figure 7A). The hierarchical clustered heatmap in Figure 7B showed that total oxylipin levelswere lower in non-obese individuals, in comparison with obese patients..

It was also questioned if oxylipin variation was somehow correlated to BMI. The performance of this correlation revealed the 5 species (9,12,13-TriHOME, 12-HEPE, 12-HETE, 11-HDHA and 14-HDHA) shown in Figure 7C that most positively and significantly correlated to BMI.

Multivariate analysis, performed with PCA and PLS-DA analyses shown in Figure 7D and E, exposed separation between obese and non-obese individuals. This indicated that concentration changed in most oxylipin species. In PLS-DA VIP scores, 12-HETE, 14-HDHA and 12-HEPE were highlighted for their separation capacity, meaning they were given more importance in the generation of the PLS-DA so that the groups were visibly separated.

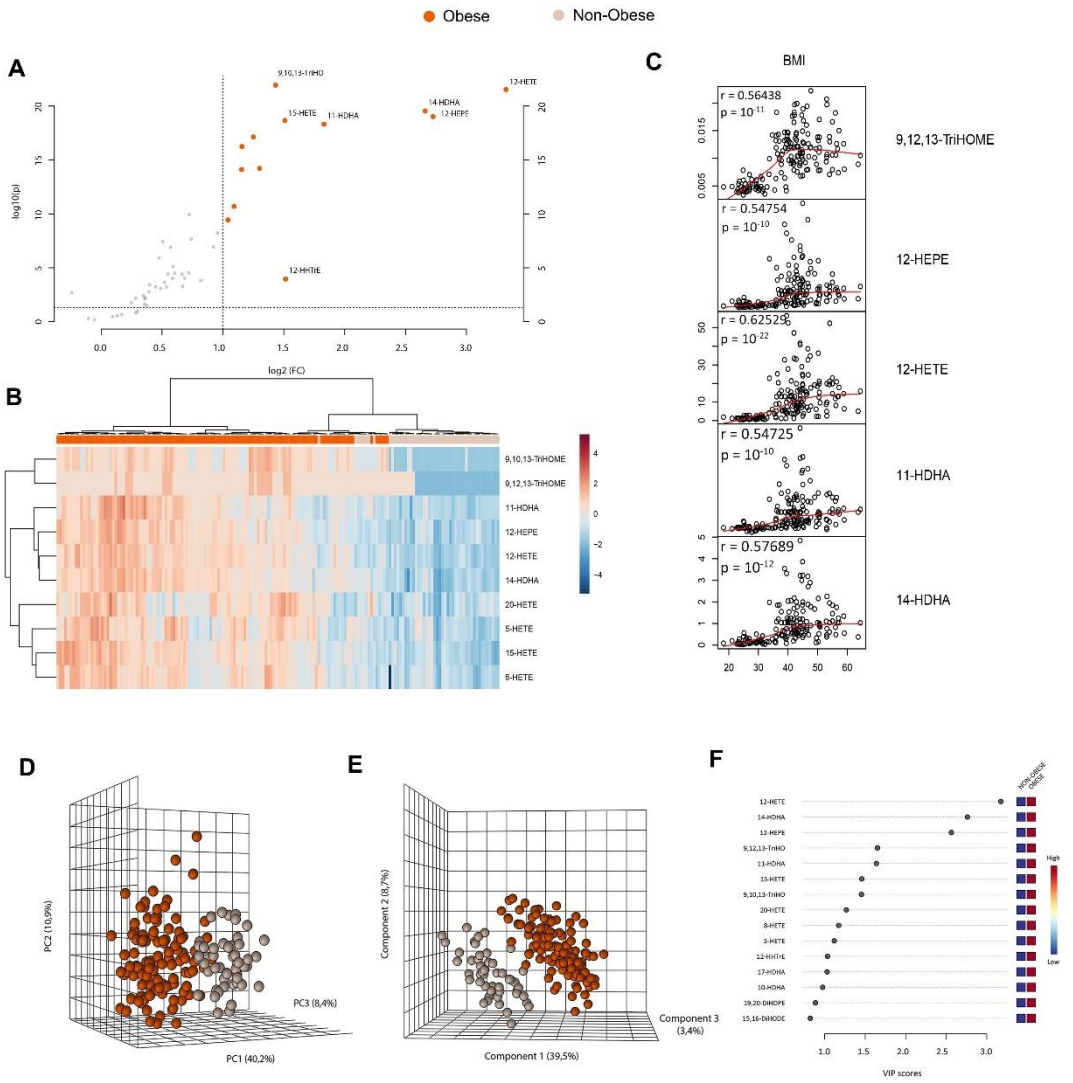

Figure 7. Effect of obesity in oxylipin profile. A: Volcano plot; B: hierarchical clustered heatmap with top 10 species ranked by t-test; C: top 5 lipid species correlated with body mass index; D: 3D plot of PCA; E: 3D plot of PLS-DA; F: variable importance in projection (VIP) scores from PLS-DA.
PCA: Principal Component Analysis; PLS-DA: Parcial Least Squares – Discriminant Analysis; HETE: hydroxyeicosatetranoid acid; HEPE: hydroxyeicosapentanoid acid; HDHA: hydroxydocosahexaenoic acid; TriHOME: trihydroxyoctadecenoid acid; HHTrE: hydroxyheptadecatrienoid acid; DiHDPE: dihydroxydocosapentaenoid acid; DiHODE: dihydroxyoctadecadienoid acid.

To see if there were changes associated with an oxylipin' subclass, a Bubble Plot was generated (Figure 8). This plot, which presents the fold change of each species, and the associated significance according to the size of the point (the larger the point, the higher the significance), revealed that octanoids were the oxylipin species that less variate in case of obesity, as their Fold Change is the closest to 0. In general, the class with most significant changes was eicosanoids.

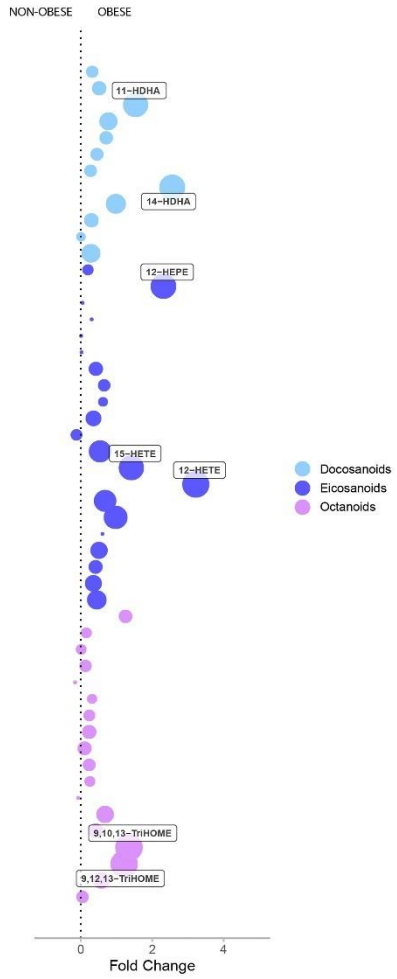

Figure 8. Bubble Plot between obese and non-obese patients. Bigger bubbles indicate bigger significance. HETE: hydroxyeicosatetranoid acid; HEPE: hydroxyeicosapentanoid acid; HDHA: hydroxydocosahexaenoic acid.

Oxylipin concentrations and p-value of the comparison are shown in Supplementary table S4.

2. OBESITY-RELATED COMORBIDITIES DO NOT MODIFY PLASMA OXYLIPIN PROFILE

Uni and multivariate analysis of oxylipin concentrations between patients with T2DM, HTA, DLP and MS and obese patients with no comorbidities revealed that oxylipins' concentrations were not affected by these obesity-related comorbidities. Fold Change analysis did not show any significant oxylipins modified between patients with and without comorbidities, and PLS-DA analysis could not separate the groups (Figure 9).

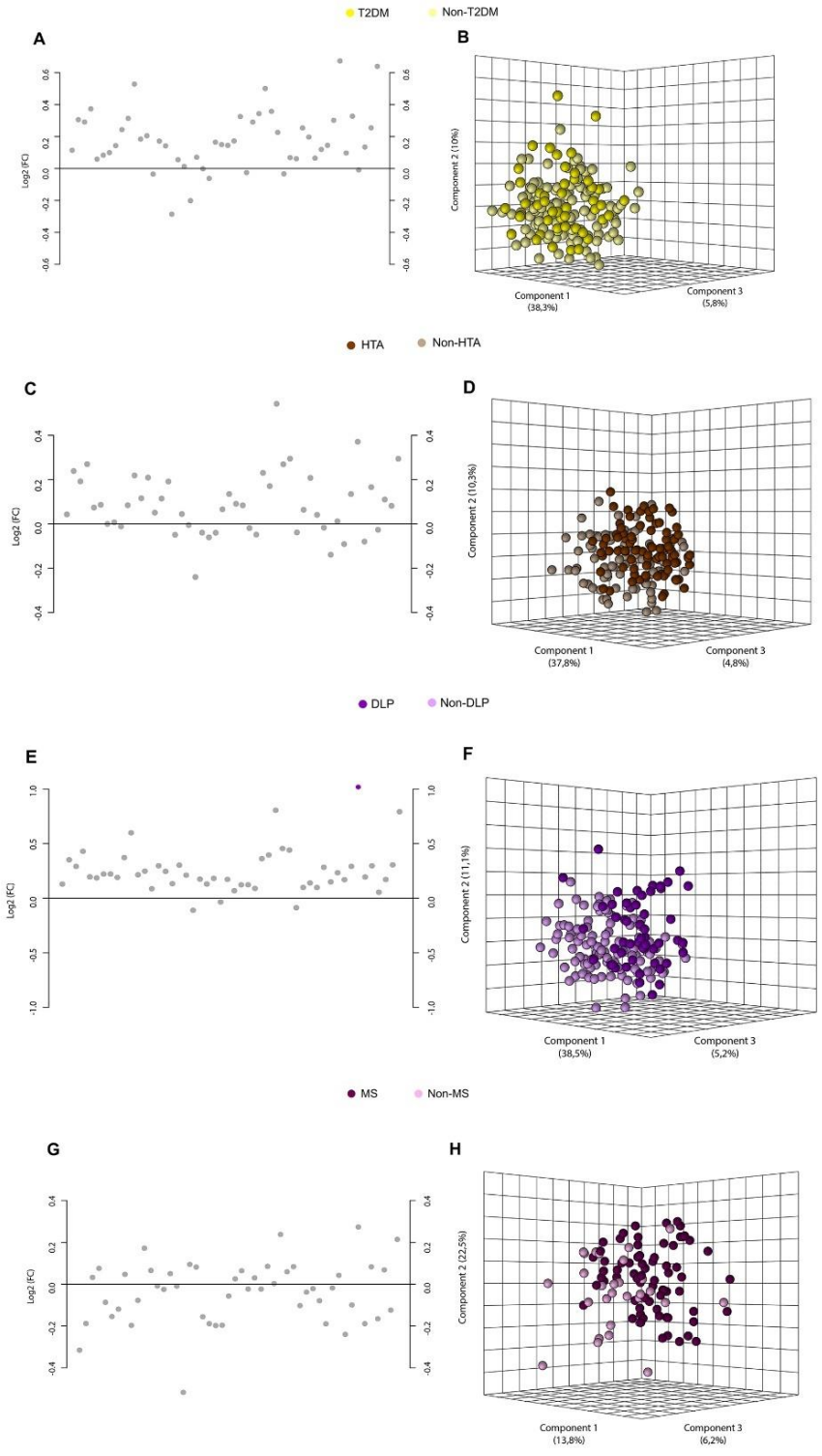

Figure 9. FC and PLS-DA analysis between obese patients with comorbidities and obese patients without comorbidities. A, C, E, G: Fold Change analysis; B, D, F, H: 3D plot of PLS-DA.
FC: Fold Change; PLS-DA: Parcial Least Squares – Discriminant Analysis; T2DM: type 2 diabetes mellitus; HTA: arterial hypertension; DLP: dyslipidemia; MS: metabolic syndrome.

When Kruskall-Wallis test was performed between NASH, uncertain and non-NASH individuals, 6 lipid species (11-HDHA, 12-HEPE, 12-HETE, 14-HDHA, 19,20-DiHDPE and TXB2) appeared as significant (Figure 10A). Each of these were individually plotted to show how the concentration varied between NASH, uncertain NASH and non-NASH (Figure 10B), and no tendency was noted with the progression of NAFLD. 11-HDHA, 12-HEPE, 12-HETE and 14-HDHA were increased in uncertain NASH, 19,20-DiHDPE was increased in non-NASH, and TXB2 was increased in NASH. Furthermore, with PCA and PLS-DA multivariate analysis, no separation was observed between NASH, uncertain and non-NASH patients (Figures 10C and D). Finally, univariate and multivariate analyses were performed between the three groups, compared two by two, to see if there were any changes in oxylipins as shown in Figures 10E-J, and no significant oxylipins appeared in Fold Change, nor the groups were separated in PLS-DA.

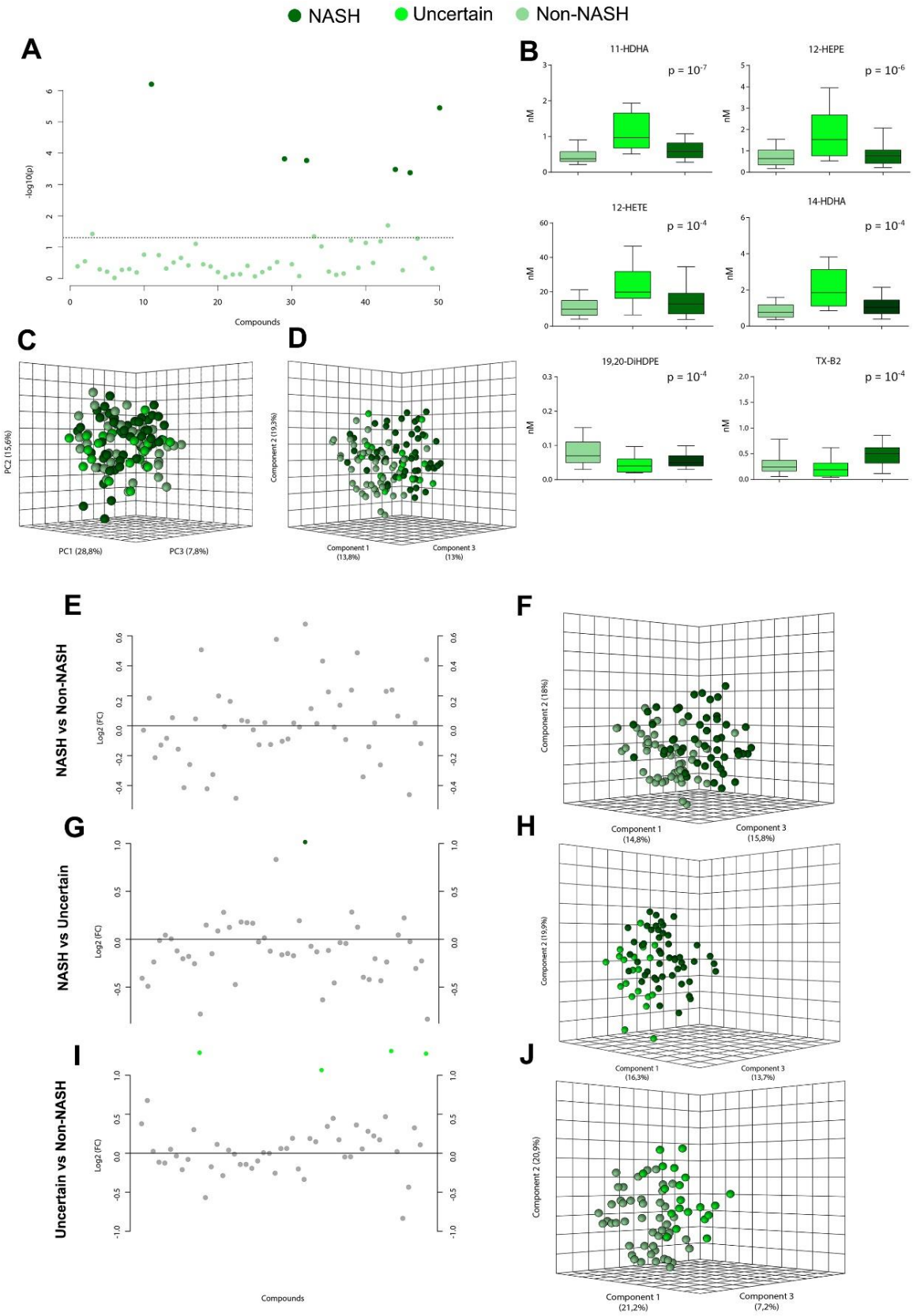

Figure 10. Uni and multivariate analysis between NASH, uncertain and non-NASH individuals. A: Kruskall Wallis test; B: Concentration plots of the six significant species; C: 3D plot of PCA; D: 3D plot of PLS-DA; E, G, I: Fold Change analysis; F, H, J: 3D plot of PLS-DA.
PCA: Principal Component Analysis; PLS-DA: Parcial Least Squares – Discriminant Analysis; HETE: hydroxyeicosatetranoid acid; HEPE: hydroxyeicosapentanoid acid; HDHA: hydroxydocosahexaenoic acid; DiHDPE: dihydroxydocosapentaenoid acid; TX: thromboxane.

3. FOLLOWING A VERY LOW CALORY DIET BEFORE BS DOES NOT MODIFY PLASMA OXYLIPIN PROFILE.

Multivariate analysis of oxylipin concentrations between patients that underwent BS and followed a VLCD, patients who followed a mixed diet, and patients that did not follow any diet revealed that oxylipin levels were not affected. Kruskall-Wallis test did not show any soxylipin that was significantly modified between these three groups, and PLS-DA analysis did not separate the groups (Figure 11).

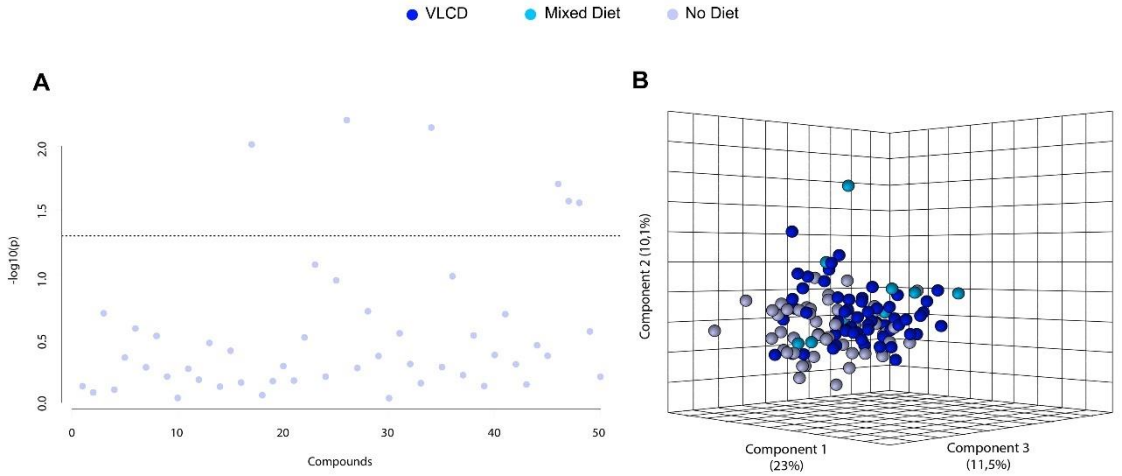

Figure 11. Uni and multivariate analysis between patients that followed a VLCD and patients that did not. A: Kruskall Wallis test; B: 3D plot of PLS-DA.
PLS-DA: Parcial Least Squares – Discriminant Analysis; VLDL: very low calory diet.

4. BARIATRIC SURGERY MODIFIES PLASMA OXYLIPIN PROFILE

Univariate analysis between pre- and post-operated obese patients revealed that several oxylipin species were significantly increased (TXB2, PGE2, 12-HHTrE, 14-HDHA and 11-HDHA) and decreased (20-HETE, 12,13-DiHOME, 19,20-DiHDPE and 15, 16-DiHODE) in post-operated patients (Figure 12A). This could also be observed in hierarchical clustered heatmaps (Figures 12B and C, top 10 species ranked by t-test, and all species, respectively) that showed at a glance that some species were decreased in post-operated patients, whereas others were significantly increased. The Bubble Plot (Figure 12D) showed important variations in all three types of oxylipins analyzed being docosanoids the less variated as their significances were smaller.

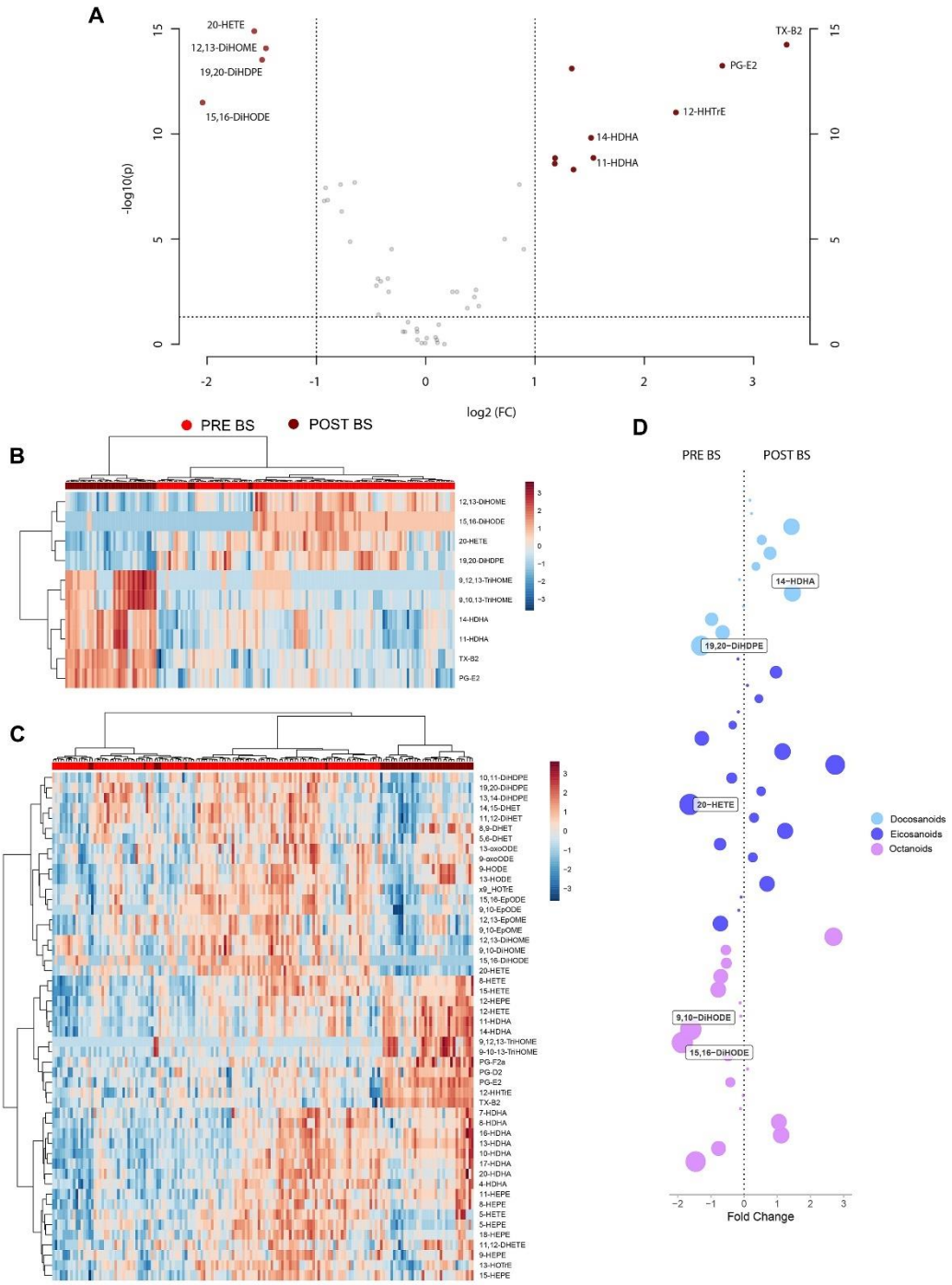

Figure 12. Univariate analysis between pre- and post-operated patients. A: Volcano plot; B: hierarchical clustered heatmap with top 10 species ranked by t-test, C: hierarchical clustered heatmap with all species; D: Bubble Plot (bigger bubbles indicate bigger significance).
HETE: hydroxyeicosatetranoid acid; HEPE: hydroxyeicosapentanoid acid; HDHA: hydroxydocosahexaenoic acid; HOME: hydroxyoctadecenoid acid; HHTrE: hydroxyheptadecatrienoid acid; HDPE: hydroxydocosapentaenoid acid; HODE: hydroxyoctadecadienoid acid.; TX: thromboxane; PG: prostaglandin; EpODE: epoxyoctadecenoic acid; oxoODE: oxooctadecadienoic acid; HOTrE: hydroxyoctadecatrienoic acid; DHET: dihidroxyeicosatrienoic acid.

Multivariate analysis carried out with PCA showed separation between groups (Figure 13A). Separation between groups was higher in PLS-DA analysis by giving more importance in the projection of TXB2, 12-HHTrE and PGE2 (Figures 13B and C).

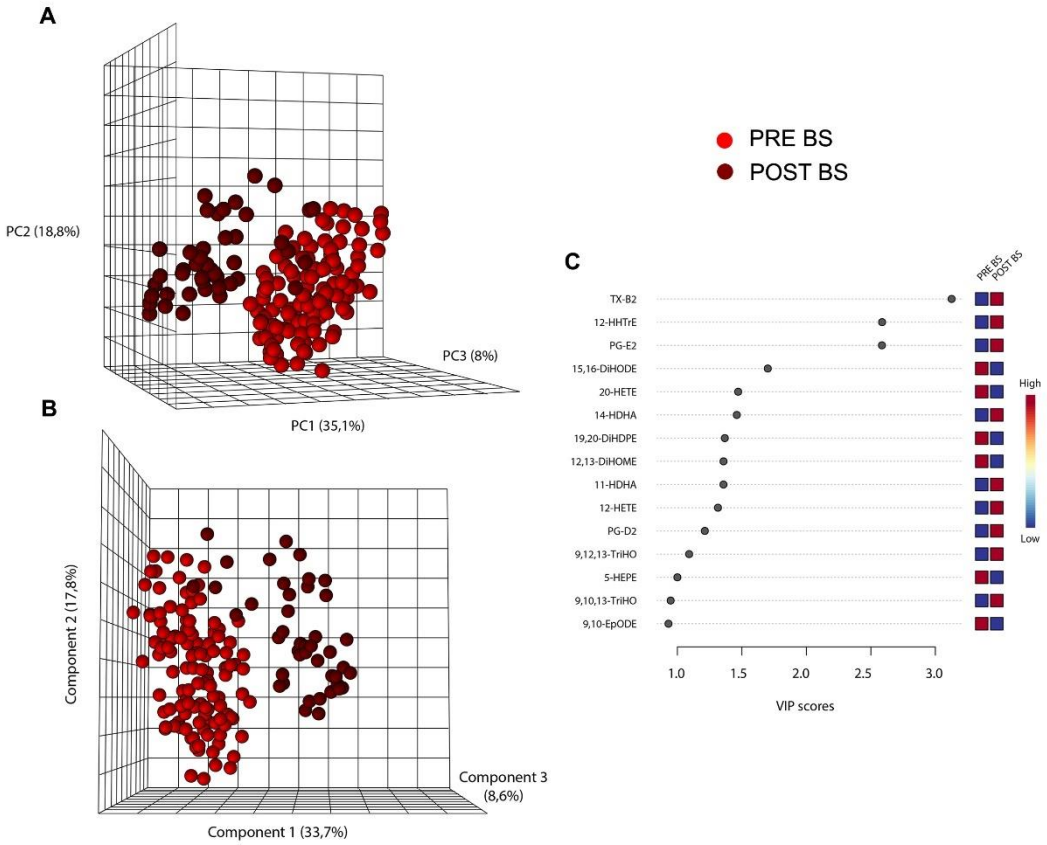

Figure 13. Multivariate analysis between pre- and post-operated patients. A: 3D plot of PCA; B: 3D plot of PLS-DA; C: variable importance in projection (VIP) scores from PLS-DA.
PCA: Principal Component Analysis; PLS-DA: Parcial Least Squares – Discriminant Analysis; HETE: hydroxyeicosatetranoid acid; HEPE: hydroxyeicosapentanoid acid; HDHA: hydroxydocosahexaenoic acid; HOME: hydroxyoctadecenoid acid; HHTrE: hydroxyheptadecatrienoid acid; HODE: hydroxyoctadecadienoid acid.; TX: thromboxane; PG: prostaglandin.

Oxylipin concentrations and p-value of the comparison is shown in Supplementary table S5.

5. PATIENTS THAT UNDERWENT BARIATRIC SURGERY DID NOT RESTORE PLASMA OXYLIPIN PROFILE TO NORMAL LEVELS

Univariate analysis between non-obese controls and post-operated obese patients revealed several oxylipin species were significantly increased (12-HETE, 14-HDHA, 12-HEPE, 12-HHTrE, 11-HDHA, TXB2, 9,10,13-TriHOME and 15-HETE) and one species was decreased (15,16-DiHODE) in post-operated patients (Figure 14A), stating that these values were very different between non-obese and post-BS patients. This could also be observed in hierarchical clustered heatmaps (Figures 14B and C, top 10 species ranked

by t-test, and all species, respectively) that showed at a glance that most species (14-HDHA, 12-HETE, 12-HEPE, 11-HDHA, 15-HETE, 8-HETE, 9,10,13-TriHOME, 9,12,13-TriHOME, 12-HHTrE and PGE2) were decreased in non-obese controls with respect of post-operated obese patients. The Bubble Plot showed important variations in all three types of oxylipins analyzed being docosanoids the less varied.

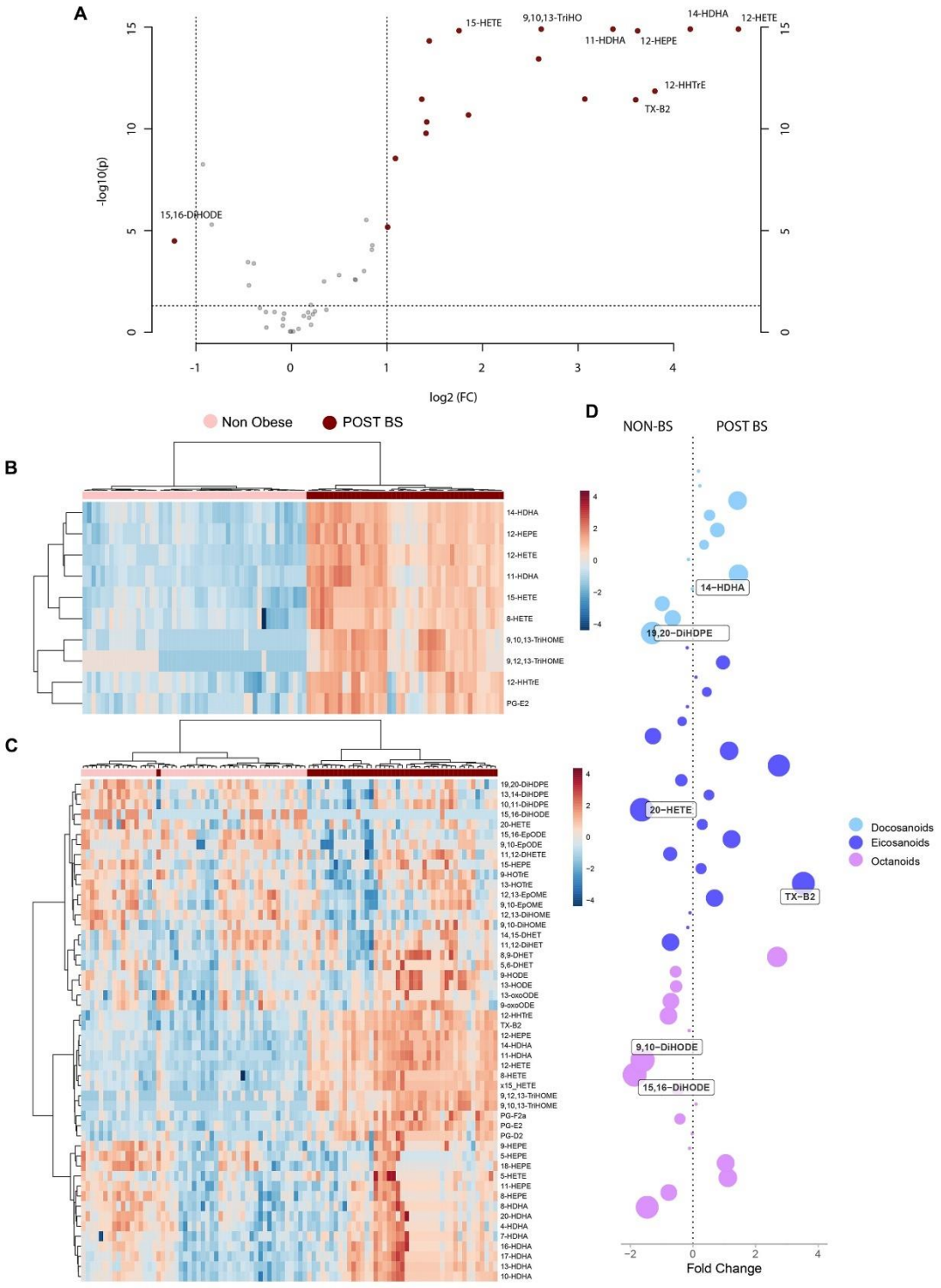

Figure 14. Univariate analysis between healthy controls and post-operated patients. A: Volcano plot; B: hierarchical clustered heatmap with top 10 species ranked by t-test, C: hierarchical clustered heatmap with all species; D: Bubble Plot (bigger bubbles indicate bigger significance).
HETE: hydroxyeicosatetranoid acid; HEPE: hydroxyeicosapentanoid acid; HDHA: hydroxydocosahexaenoic acid; HOME: hydroxyoctadecenoid acid; HHTrE: hydroxyheptadecatrienoid acid; HDPE: hydroxydocosapentaenoid acid; HODE: hydroxyoctadecadienoid acid.; TX: thromboxane; PG: prostaglandin; EpOME: epoxyoctadecenoic acid; oxoODE: oxooctadecadienoic acid; HOTrE: hydroxyoctadecatrienoic acid; DHET: dihidroxyeicosatrienoic acid.

Multivariate analysis carried out with PCA analysis showed separation between groups (Figure 15A). The separation was increased in PLS-DA analysis (Figure 15B). TXB2, 12-HHTrE and PGE2 were the oxylipin species with greater VIP scores, used to generate PLS-DA (Figure 15C).

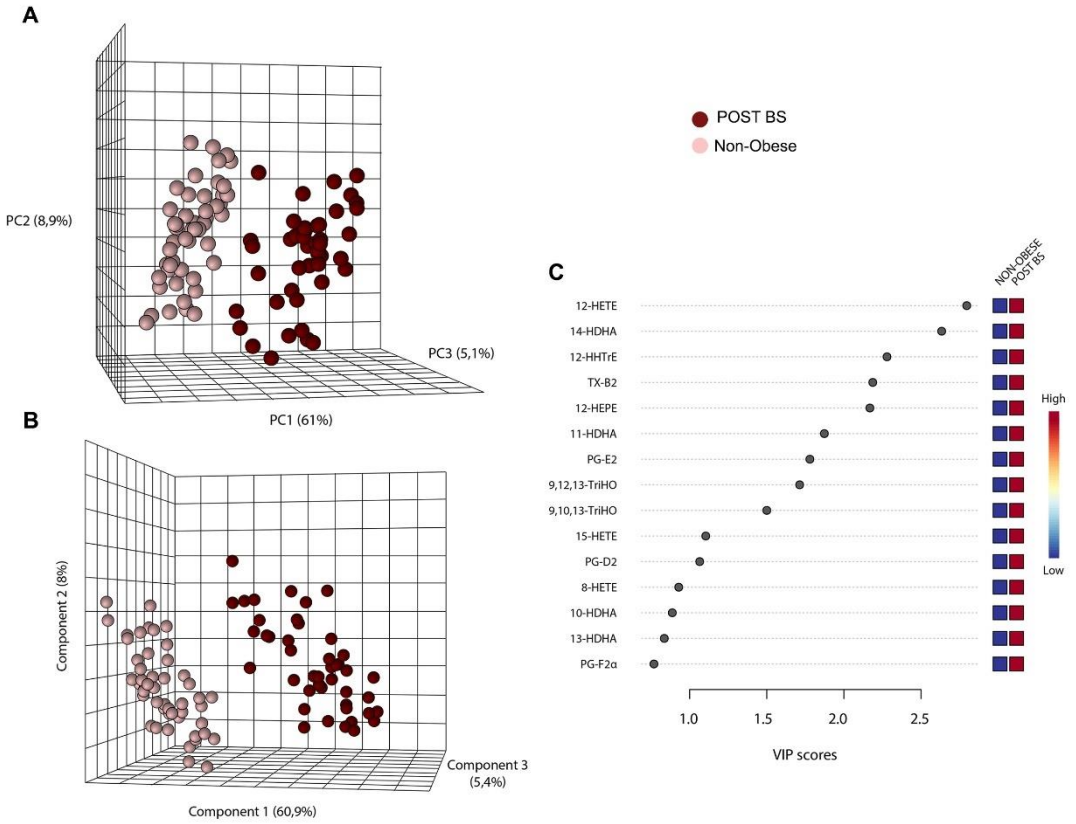

Figure 15. Multivariate analysis between healthy controls and post-operated patients. A: 3D plot of PCA; B: 3D plot of PLS-DA; C: variable importance in projection (VIP) scores from PLS-DA.
PCA: Principal Component Analysis; PLS-DA: Parcial Least Squares – Discriminant Analysis; HETE: hydroxyeicosatetranoid acid; HEPE: hydroxyeicosapentanoid acid; HDHA: hydroxydocosahexaenoic acid; HOME: hydroxyoctadecenoid acid; HHTrE: hydroxyheptadecatrienoid acid; HODE: hydroxyoctadecadienoid acid.; TX: thromboxane; PG: prostaglandin.

Oxylipin concentrations and p-value of the comparison is shown in Supplementary table S6.

DISCUSSION

Obesity and overweight are a major health issue nowadays, with over 1.5 billion people suffering from overweight all around the globe, being over 200 million men and 300 million women of those obese (34). Along with obesity comes the rise of obesity-related comorbidities that involve a high risk of mortality, the main ones are T2DM, NAFLD, CVD (such as HTA), dyslipidemia, and cancer (34). Cancer was not considered as a parameter in this study about oxylipins as only 5% of the population that took part in it had cancer.

The prevalence of NAFLD has increased to the point that nowadays 1 in 4 people in the world suffer from this condition (35). This disease may evolve in some cases to NASH, and then to cirrhosis, a condition in which liver damage accumulation leads to fibrogenesis of the liver (36).

Analyzing the clinical and biochemical characteristics of the non-obese and obese patients, it was observed that the parameters associated with obesity like BMI, blood pressure and obesity-related comorbidities were higher in the obese group, as expected. As dyslipidemia increased, there was also a decrease in HDL-cholesterol levels and an increase in TG (37). A reduction in LDL-cholesterol levels was observed, this contradicts most studies, which agree that the results expected were normal or slightly higher levels of LDL (37,38). It could be related to the use of statins by some patients, a medication that reduces LDL-C levels to treat DLP (39).

HOMA IR (a marker of IR) was also greater in the obese group, as IR leads to hyperinsulinemia (7,40), which was also one of the phenomena observed from this analysis. Additionally, hyperinsulinemia and IR are one of the main causes from the higher blood pressure usually observed in obese patients (40).

High glucose levels are related to obesity and hepatic damage as they are derived from the deregulation of transaminases (41). Obese people showed greater levels of ALT, AST and GGT, as well as higher levels of glucose. Some of these obese patients had also NASH, which is one of the main reasons for the greater quantity of plasma glucose.

The obese group was characterized by greater prevalence of obesity-related comorbidities. Along with it, comorbidity treatments were administrated to the members of this group. Metformin, sulfonylureas, and insulin were prescribed to treat T2DM, ACE-ARBS and diuretics were prescribed to treat HTA, and statins was prescribed to treat DLP.

Bariatric surgery is the best treatment nowadays to deal with obesity (36). It also has shown to be effective in the cure of NASH, over 59% of patients studied experienced the disappearance of NASH after the surgical procedure (42).

Comparing the clinical and biochemical characteristics of the obese group pre-BS and the followed-up group 12 months after BS, it was observed that the parameters associated with obesity as BMI and obesity-related comorbidities were lower in the post BS group, as expected. As dyslipidemia remitted, there was also an increase in HDL-cholesterol levels and a decrease in TG (37). As in the previous comparison, LDL levels were not the expected, as they rose instead of staying at the same level or slightly decreasing from the

values of the obese group, in comparison with what was observed in previous studies (37,43).

BS has been proved effective in reducing hepatic damage, as ALT, AST and GGT levels significantly lowered 12 months after the surgery. Glucose levels also lowered significantly due to the decrease in IR. This was a sign of NASH remission, consistent with the NAS value (0) obtained on histologic assessment of post-surgery patients.

HOMA IR was lower 12 months after BS, a sign of the reduction of IR like the other comorbidities. Hyperinsulinemia was also reverted with the procedure (7,40). Hyperinsulinemia and IR reduction should have led to blood pressure levels dropping from obese values, but this was not the case (40). This could be explained by findings in recent studies that showed that blood pressure can worsen in the first year after BS. (44)

Lower prevalence of obesity-related comorbidities in post-BS patients compared to obese patients led to a reduction in medication treatments in the followed-up group, as fewer patients were prescribed metformin, sulfonylureas, insulin, ACE-ARBS, statins and diuretics.

The third comparison done to evaluate biochemical characteristics was to observe if post-BS patients recovered the non-obese group levels. Comparing obesity-related comorbidities similar values between the two groups were observed, obese levels dropped to non-obese values in DLP and NASH, but not in HTA and T2DM which lowered from the obese group but was still high in post BS patients. Nevertheless, BMI remained in obese-diagnosed spectrum (45).

Regarding transaminases values, ALT values were observed to be slightly lower in post BS patients than non-obese patients, whereas AST and GGT levels were slightly higher in post-BS patients than non-obese controls. This can be a sign of hepatic damage accumulation: even if NASH is cured, transaminases values are still a mark of past damage. Glucose levels were also similar than those of non-obese patients as BS has been proved effective in recovering glucose control (46).

Whereas T2DM levels did not drop to non-obese levels, there was a significant reduction of HOMA IR and insulin levels, reaching lower values than the non-obese group. This effect has been proved in several studies: hyperinsulinemia reduction is bigger in bariatric surgery than other obesity treatments like caloric restriction, but it has been observed a remission in diabetes in operated patients after a few years (46). Hyperinsulinemia and IR reduction should have led to blood pressure levels reaching non-obese values, but this was not the case (40).

Analyzing plasma oxylipin profile in obese and non-obese patients showed some oxylipin species increasing in obesity. This agrees with several studies that observed increased HETEs in obese patients (47). 12-HETE has vasoconstrictor (48) and pro-inflammatory (49,50) effects which could be a reason for its increase in obesity, a condition characterized by higher blood pressure and adipose tissue inflammation. 14-HDHA is also elevated in obesity, this oxylipin has the function of reducing inflammation, which could seem like a contradiction in this case. This phenomenon was also experienced in other studies (51), which could indicate that a higher concentration of 14-HDHA could lead to a better capacity of resolution of inflammation in case of obesity remission.

Referring to the role of obesity-related comorbidities in oxylipin profile change, no effect on plasma oxylipins' concentration by T2DM was observed. This agrees with a study that stated that diabetes does not modify oxylipin profile (52).

Studies like (53) did not reveal a change in plasma oxylipin profile in obese patients, whereas demonstrated a change in T2DM patients. However, the cohort in their study was much smaller than ours.

Individuals with HTA did not show modifications in plasma oxylipins in comparison with non-HTA patients, either. Some oxylipins participate in vasoconstriction process (31). In this sense, it has been demonstrated that HETEs are related to the development of HTA (31). However, considering that, as some of the obese population received antihypertensive treatments, and some of them can control their HTA, this could be the reason why plasma oxylipins did not change in our analysis. DLP and MS did not affect plasma oxylipin profile either. However, it has been studied that HETEs are increased in DLP patients. The reason for the negative result that was observed could be related with the species being inhibited in this case, as HETEs have pro-inflammatory effects, and the statin treatment received by many of the population in this study is anti-inflammatory (54). Furthermore, MS is characterized by early inflammation (47), so this effect could be decreased in the presence of lipid modifying drugs.

When NASH, uncertain and non-NASH patients were compared, 6 significantly different lipids were obtained. This has also been experienced in other studies, but the reason for the increase in 12-HETE is not clear as HETEs had been proved not to have an effect in NAFLD (55). Nevertheless, other oxylipins like 11-HDHA and 14-HDHA could be related to NASH as they are platelets inhibitors (56), and it has been studied that this inhibition is common in NAFLD patients (57).

There were no significant differences between NASH and uncertain NASH patients, or between uncertain NASH and non-NASH patients. This confirmed the hypothesis that NAFLD is not affected by oxylipins (55).

When patients that had been following a VLCD, patients that followed a mixed diet and patients that did not follow any diet were compared, it was observed that this diet did not have an effect on plasma oxylipin profile. This did not match other studies that stated that a VLCD does reduce plasma oxylipin concentrations (58). Although the diet is effective in prognostic of bariatric surgery, it did not change oxylipin concentrations. It was suggested that a reason for this could be that the diet was not correctly followed by some of the patients. It has been studied that a very low calory ketogenic diet did have an effect on oxylipin concentrations (59).

Analyzing the oxylipin differences in pre- and post-operated patients, some species significantly reduced in post-BS individuals, like 20-HETE, which makes sense given that their effects are vasoconstriction and inflammation (31), both of which were reduced with weight loss, which is a reason for the reduction in HETEs and DiHODEs (60). Other oxylipin species were increased in post-BS patients, some of which (HDHAs and TXB2) agree with previous studies (51). This is consistent with their functionality, as 14-HDHA has an anti-inflammatory effect (61) and PGE2 reduces blood pressure (31), both effects amplified in weight loss post bariatric surgery.

Finally, the last comparison between non-obese controls and post-operated patients showed that the post BS group did not recover normal levels of oxylipins. If they had been restored to control levels, there should not be a significant variation between groups. This agrees with a recent study that has proved that obesity induced changes in oxylipin profile persist even after weight loss (62).

As the results of this study did not gave a correlation between oxylipins and obesity-related comorbidities in obese patients, it is not considered that oxylipins can be useful biomarkers for obesity, as the methods for obesity diagnosis are anthropometrics and less invasive than blood extraction. However, this could be an interesting point to start further investigations in the future, as it would be of interest to do a retrospective study with overweight patients, followed-up for a certain amount of time, to observe which ones end up developing obesity, and if their oxylipin profile modifications are able to predict the evolution and prognostic of these patients. Other future lines of study could be to investigate if oxylipins could indicate the metabolic status of lean individuals. It would be interesting to know if plasma oxylipin profile is modified by T2DM, HTA, DLP, NAFLD and MS in lean individuals, as they are not developing adipose tissue inflammation like obese patients, so oxylipins in their bodies would not be functioning as inflammation promoters in this tissue.

CONCLUSIONS

After the analyses performed in this study, several conclusions have been reached:

1. Circulating oxylipins are increased in patients with obesity, which could mean that they are involved in obesity common symptoms like adipose tissue inflammation and higher blood pressure. Also, the concentration of these species is sufficiently different between obese patients and non-obese individuals that obesity has its own plasma oxylipin signature.

2. Obesity-related comorbidities like T2DM, HTA, DLP, MS and NAFLD do not have an effect in plasma oxylipin profile, as no modifications in oxylipin concentrations were found.

3. Patients that took a very low calory diet before BS did not experiment a change in their oxylipin profile. Although the diet is effective in prognostic of bariatric surgery, it does not change circulating bioactive lipid concentrations.

4. BS could modify oxylipin profile of obese patients. The changes observed in concentrations were both increased and decreased in most analyzed oxylipins.

5. Plasma oxylipin modifications due to obesity persist 12 months after BS and weight loss, and post BS patients could not recover a non-obese profile.

This study had limitations that will be overcome in future studies. These include that inaccuracy in the number of patients selected for the study, and it would have been interesting to have followed-up all pre-BS patients. Aside from this, some controls were not completely healthy and had overweight and other diseases. Finally, to know exactly the effect of the medication administrated to the obese population, it would have needed to have them untreated, which is not ethical.

To summarize, most objectives of this study have been achieved. However, it is needed further investigation in this field to find the answer to more questions that could not be found in this project.

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SUPPLEMENTARY MATERIAL

TABLE OF CONTENTS

Supplementary table S1. Oxylipin standards and internal standards.

Product name	Oxylipin	Formula
SPM D-series LC-MS Mixture (100ng/ml ethanol)	Resolvin D3	C22H32O5
	Resolvin D2	C22H32O5
	17 (R) - Resolvin D1	C22H32O5
	Resolvin D1	C22H32O5
	Resolvin D5	C22H32O4
	Docosahexaenoic acid	C22H32O2
Lipoxin LC-MS Mixture (100ng/ml ethanol)	Lipoxin B4	C20H32O5
	Lipoxin A4	C20H32O5
	15(R)-Lipoxin A4	C20H32O5
	Arachidonic acid	C20H32O2
EPA Oxylipin LC-MS Mixture (1ug/ml ethanol)	Prostaglandin E3	C20H30O5
	Prostaglandin F3a	C20H32O5
	5(s)-HEPE	C20H30O3
	8-HEPE	C20H30O3
	9-HEPE	C20H30O3
	11-HEPE	C20H30O3
	12(S)-HEPE	C20H30O3
	15(S)-HEPE	C20H30O3
	18-HEPE	C20H30O3
Primary COX and LOX LC-MS Mixture (1ug/ml ethanol)	6-keto Prostaglandin F1a	C20H34O6
	Thromboxane B2	C20H34O6
	Prostaglandin F2a	C20H34O5
	Prostaglandin E2	C20H32O5
	Prostaglandin D2	C20H32O5
	12(s)-HHTrE	C17H28O3
	15(s)-HETE	C20H32O3
	12(s)-HETE	C20H32O3
	5(s)-HETE	C20H32O3
Leukotriene B4 Pathway LC-MS Mixture (1ug/ml ethanol)	18-carboxy dinor Leukotriene B4	C18H26O6
	20-carboxy Leukotriene B4	C20H30O6
	20-hydroxy Leukotriene B4	C20H32O5
	Leukotriene B4	C20H32O4
	5(s)-HETE	C20H32O3
	12(13)-DiHOME	C18H34O4

Linoleic Acid Oxylipins LC-MS Mixture (10ug/ml ethanol)	9(10)-DiHOME	C18H34O4
	9(s)-HODE	C18H32O3
	13(s)-HODE	C18H32O3
	13-oxoODE	C18H30O3
	9-oxoODE	C18H30O3
	12(13)-EpOME	C18H32O3
	9(10)-EpOME	C18H32O3
10(s),17(s)-DiHDHA (NPD1)	10(s),17(s)-DiHDHA (NPD1)	C22H32O4
20-HETE	20-HETE	C20H32O3
(±)11(12)-DiHET	(±)11(12)-DiHET	C20H34O4
8-iso Prostaglandin F2α	8-iso Prostaglandin F2α	C20H34O5
SPM E-series LC-MS Mixture (1ug/ml ethanol)	Resolvin E1	C20H30O5
	18-HEPE	C20H30O3
	EPA	C20H30O2
ALA and GLA Oxylipin LC-MS Mixture (1ug/ml ethanol)	9(s)-HOTrE	C18H30O3
	13(s)-HOTrE	C18H30O3
	13(s)-HOTrE (g)	C18H30O3
Deuterated Primary COX and LOX LC-MS Mixture (1ug/ml ethanol)	Prostaglandin D2-d4	C20H28D4O5
	Prostaglandin E2-d4	C20H28D4O5
	6-keto Prostaglandin F1a-d4	C20H30D4O6
	Prostaglandin F2a-d4	C20H30D4O5
	Thromboxane B2-d4	C20H30D4O6
	5(s)-HETE-d8	C20H24D8O3
	12(s)-HETE-d8	C20H24D8O3
	15(s)-HETE-d8	C20H24D8O3
Leukotriene B4-d4	Leukotriene B4-d4	C20H28D4O4
Deuterated Linoleic Acid Oxylipins LC-MS Mixture (1ug/ml ethanol)	(±)12(13)-DiHOME-d4	C18H30D4O4
	13(s)-HODE-d4	C18H28D4O3
	13-OxoODE-d3	C18H27D3O3
	(±)12(13)-EpOME-d4	C18H28D4O3
Lipoxin A4-d5	Lipoxin A4-d5	C20H27D5O5
Arachidonic Acid-d8	Arachidonic Acid-d8	C20H24D8O2
Resolvin D1-d5	Resolvin D1-d5	C22H27D5O5
8-iso Prostaglandin F2α-d4	8-iso Prostaglandin F2α-d4	C20H30D4O5

Supplementary table S2. Gradient elution conditions.

Time	Mobile Phase Composition	
	Eluent A Solution (%)	Eluent B Solution (%)
0.00 min	90.00 %	10.00 %
0.01 min	90.00 %	10.00 %
3.93 min	65.00 %	35.00 %
6.18 min	60.00 %	40.00 %
7.87 min	58.00 %	42.00 %
10.12 min	50.00 %	50.00 %
16.87 min	35.00 %	65.00 %
19.12 min	25.00 %	75.00 %
20.80 min	15.00 %	85.00 %
21.93 min	5.00 %	95.00 %
24.18 min	5.00 %	95.00 %
24.75 min	90.00 %	10.00 %
29.25 min	90.00 %	10.00 %

Supplementary table S3. Lipid spieces analyzed in the study.

Lipid spieces	Main Class	Sub Class	Systematic name
12(13)-DiHOME	Octadecanoids	Other Octadecanoids	12,13-dihydroxy-9Z-octadecenoic acid
9(10)-DiHOME	Octadecanoids	Other Octadecanoids	9,10-dihydroxy-12Z-octadecenoic acid
9,12,13-TriHOME	Octadecanoids	Other Octadecanoids	9S,12S,13S-trihydroxy-10E-octadecenoic acid
9,10,13-TriHOME	Octadecanoids	Other Octadecanoids	9,10,13-trihydroxy-11-octadecenoic acid
9-HODE	Octadecanoids	Other Octadecanoids	9-hydroxy-10E,12Z-octadecadienoic acid
13-HODE	Octadecanoids	Other Octadecanoids	13S-hydroxy-9Z,11E-octadecadienoic acid
20-HDHA	Octadecanoids	Other Octadecanoid	
16-HDHA	Octadecanoids	Other Octadecanoid	
13-HDHA	Octadecanoids	Other Octadecanoid	
10-HDHA	Octadecanoids	Other Octadecanoid	
11-HDHA	Octadecanoids	Other Octadecanoid	
7-HDHA	Octadecanoids	Other Octadecanoid	
8-HDHA	Octadecanoids	Other Octadecanoid	
12(13)-EpOME	Octadecanoids	Other Octadecanoid	(+/-)-12(13)-epoxy-9Z-octadecenoic acid
4-HDHA	Octadecanoids	Other Octadecanoid	
9(10)-EpOME	Octadecanoids	Other Octadecanoid	9,10-epoxy-12Z-octadecenoic acid
15,16-DiHODE	Octadecanoids	Other Octadecanoids	(+/-)-15,16-dihydroxy-9Z,12Z-octadecadienoic acid
9,10-DiHODE	Octadecanoids	Other Octadecanoids	(+/-)-9,10-dihydroxy-12Z,15Z-octadecadienoic acid
13-oxoODE	Octadecanoids	Other Octadecanoids	13-keto-9Z,11E-octadecadienoic acid
9-oxoODE	Octadecanoids	Other Octadecanoids	9-oxo-10E,12Z-octadecadienoic acid
15(16)-EpODE	Octadecanoids	Other Octadecanoids	15(16)-epoxy-9Z,12Z-octadecadienoic acid
9(10)-EpODE	Octadecanoids	Other Octadecanoids	9(10)-epoxy-12Z,15Z-octadecadienoic acid
9-HOTrE	Octadecanoids	Other Octadecanoids	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid
13-HOTrE	Octadecanoids	Other Octadecanoids	13S-hydroxy-9Z,11E,15Z-octadecatrienoic acid
12-HHTrE	Eicosanoids	Hydroxy/ hydropreoxyeicosatetraenoic acids	12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid

14,15-DHET	Eicosanoids	Hydroxy/ hydropreoxyeicosatetraenoic acids	14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid
8,9-DHET	Eicosanoids	Hydroxy/ Hydroperoxyeicosatetraenoic acids	8,9-dihydroxy-5Z,11Z,14Z-eicosatrienoic acid
5,6-DHET	Eicosanoids	Hydroxy/ hydroperoxyeicosapentaenoic acids	5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid
PG F2α	Eicosanoids	Prostaglandins	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid
TX B2	Eicosanoids	Thromboxanes	9S,11,15S-trihydroxy-thromboxa-5Z,13E-dien-1-oic acid
8-HETE	Eicosanoids	Hydroxy/ hydroperoxyeicosatetraenoic acids	8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid
5-HETE	Eicosanoids	Hydroxy/ hydroperoxyeicosatetraenoic acids	5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid
12-HETE	Eicosanoids	Hydroxy/ hydroperoxyeicosatetraenoic acids	12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid
15-HETE	Eicosanoids	Hydroxy/ hydroperoxyeicosatetraenoic acids	15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid
20-HETE	Eicosanoids	Hydroxy/ hydroperoxyeicosapentaenoic acids	20-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid
11,12-DiHETE	Eicosanoids	Hydroxy/ hydropreoxyeicosatetraenoic acids	(+/-)-11,12-dihydroxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid
11(12)-DiHETE	Eicosanoids	Hydroxy/ hydroperoxyeicosatetraenoic acids	(+/-)-11,12-dihydroxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid
PG E2	Eicosanoids	Prostaglandins	9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid
PG D2	Eicosanoids	Prostaglandins	9S,15S-dihydroxy-11-oxo-5Z,13E-prostadienoic acid
5-HEPE	Eicosanoids	Hydroxy/hydroperoxyeicosapentaenoic acids	(+/-)-5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid
18-HEPE	Eicosanoids	Hydroxy/hydroperoxyeicosapentaenoic acids	(+/-)-18-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid
15-HEPE	Eicosanoids	Hydroxy/hydroperoxyeicosapentaenoic acids	(+/-)-15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid

11-HEPE	Eicosanoids	Hydroxy/hydroperoxyeicosapentaenoic acids	(+/-)-11-hydroxy-5Z,8Z,12E,14Z,17Z-eicosapentaenoic acid
8-HEPE	Eicosanoids	Hydroxy/hydroperoxyeicosapentaenoic acids	(+/-)-8-hydroxy-5Z,9E,11Z,14Z,17Z-eicosapentaenoic acid
12-HEPE	Eicosanoids	Hydroxy/hydroperoxyeicosapentaenoic acids	(+/-)-12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid
9-HEPE	Eicosanoids	Hydroxy/hydroperoxyeicosapentaenoic acids	(+/-)-9-hydroxy-5Z,7E,11Z,14Z,17Z-eicosapentaenoic acid
19,20-DiHDPE	Docosanoids	Other Docosanoids	(+/-)-19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid
13,14-DiHDPE	Docosanoids	Other Docosanoids	(+/-)-13,14-dihydroxy-4Z,7Z,10Z,16Z,19Z-docosapentaenoic acid
10,11-DiHDPE	Docosanoids	Other Docosanoids	(+/-)-10,11-dihydroxy-4Z,7Z,13Z,16Z,19Z-docosapentaenoic acid
17-HDHA	Docosanoids	Other Docosanoids	17R-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid
14-HDHA	Docosanoids	Other Docosanoids	14S-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid

Supplementary table S4. Concentration comparison carried out with Mann Whitney U test between controls and obese patients. Significant p-values are in bold.

	Control	Obese	p-value
12(13)-DiHOME	4.44 (3.2-5.52)	5.89 (3.77-7.8)	**3.97E-04**
9(10)-DiHOME	4.33 (3.63-6.29)	7.91 (5.62-11.79)	**4.06E-08**
9,12,13-TriHOME	0.005 (0.004-0.006)	0.012 (0.01-0.014)	**3.79E-24**
9,10,13-TriHOME	0.012 (0.011-0.015)	0.032 (0.028-0.037)	**2.07E-24**
9-HODE	26.39 (19.94-33.64)	40.15 (26.73-53.72)	**3.02E-06**
13-HODE	17.23 (12.45-25.25)	29.50 (21.80-37.90)	**6.41E-09**
20-HDHA	0.23 (0.16-0.32)	0.31 (0.23-0.43)	**3.17E-04**
16-HDHA	0.27 (0.18-0.37)	0.37 (0.27-0.54)	**1.45E-04**
13-HDHA	0.34 (0.26-0.48)	0.56 (0.35-0.84)	**5.97E-05**
10-HDHA	0.35 (0.23-0.55)	0.64 (0.47-1.06)	**1.48E-09**
11-HDHA	0.18 (0.16-0.19)	0.58 (0.36-0.87)	**5.56E-20**
7-HDHA	0.14 (0.1-0.19)	0.2 (0.14-0.27)	**1.01E-05**
8-HDHA	0.11 (0.078-0.14)	0.15 (0.11-0.2)	**4.58E-04**
12(13)-EpOME	12.68 (10.1-18.58)	12.46 (9.57-16.46)	4.90E-01
4-HDHA	0.22 (0.13-0.3)	0.26 (0.21-0.37)	**5.03E-03**
9(10)-EpOME	3.17 (2.11-4.23)	4.18 (3.21-5.65)	**2.14E-04**
15,16-DiHODE	0.005 (0.003-0.007)	0.007 (0.005-0.013)	**2.11E-05**
9,10-DiHODE	0.0006 (0.0005-0.001)	0.001 (0.0007-0.002)	**1.45E-05**
13-oxoODE	2.65 (1.91-3.47)	3.35 (2.43-4.23)	**1.19E-03**
9-oxoODE	2.95 (2.29-4.16)	3.85 (2.87-4.58)	**1.34E-02**
15(16)-EpODE	0.12 (0.08-0.2)	0.12 (0.09-0.17)	6.89E-01
9(10)-EpODE	0.014 (0.011-0.018)	0.018 (0.013-0.028)	**5.50E-04**
9-HOTrE	0.5 (0.39-0.58)	0.55 (0.41-0.78)	**6.00E-03**
13-HOTrE	0.93 (0.69-1.26)	1.18 (0.80-1.64)	**4.30E-03**
12-HHTrE	0.29 (0.13-0.46)	0.67 (0.21-1.48)	**5.79E-05**
14,15-DHET	0.68 (0.49-0.91)	1.09 (0.87-1.38)	**2.54E-11**
8,9-DHET	0.06 (0.05-0.07)	0.085 (0.066-0.11)	**2.90E-08**
5,6-DHET	0.13 (0.1-0.18)	0.19 (0.13-0.26)	**2.50E-05**
PG F2α	0.14 (0.12-0.17)	0.20 (0.17-0.24)	**1.22E-08**
TX B2	0.24 (0.095-0.42)	0.29 (0.18-0.52)	5.71E-02
8-HETE	0.36 (0.30-0.46)	0.8 (0.56-1.05)	**8.99E-18**
5-HETE	0.48 (0.38-0.66)	1.1 (0.75-1.42)	**1.69E-15**
12-HETE	1.24 (0.97-1.90)	12.97 (7.58-19.81)	**1.12E-23**

15-HETE	1.44 (1.17-1.9)	4.19 (2.85-5.44)	**2.13E-20**
20-HETE	1.40 (0.94-1.81)	3.25 (2.16-4.61)	**1.02E-15**
11,12-DHETE	0.054 (0.043-0.064)	0.043 (0.031-0.060)	**1.30E-03**
11(12)-DiHET	0.54 (0.41-0.76)	0.77 (0.60-0.97)	**3.87E-07**
PG E2	0.11 (0.07-0.14)	0.15 (0.08-0.23)	**1.64E-02**
PG D2	0.0724 (0.072-0.073)	0.11 (0.07-0.16)	**3.76E-04**
5-HEPE	0.19 (0.12-0.30)	0.32 (0.22-0.48)	**1.26E-05**
18-HEPE	0.083 (0.057-0.14)	0.097 (0.066-0.16)	1.86E-01
15-HEPE	0.57 (0.40-0.84)	0.61 (0.42-0.87)	3.31E-01
11-HEPE	0.068 (0.045-0.11)	0.083 (0.050-0.120)	1.09E-01
8-HEPE	0.08 (0.05-0.12)	0.083 (0.062-0.12)	3.31E-01
12-HEPE	0.13 (0.09-0.2)	0.77 (0.42-1.27)	**6.30E-21**
9-HEPE	0.12 (0.07-0.16)	0.15 (0.10-0.20)	**2.69E-03**
19,20-DiHDPE	0.031 (0.024-0.038)	0.055 (0.037-0.082)	**1.20E-10**
13,14-DiHDPE	0.086 (0.068-0.1)	0.098 (0.07-0.144)	**2.39E-02**
10,11-DiHDPE	0.046 (0.037-0.058)	0.072 (0.048-0.098)	**8.01E-06**
17-HDHA	0.19 (0.15-0.26)	0.41 (0.27-0.62)	**4.35E-12**
14-HDHA	0.15 (0.078-0.28)	1.04 (0.61-1.60)	**1.91E-21**

Supplementary table S5. Concentration comparison carried out with Wilcoxon test between obese and post BS patients. Significant p-values are in bold.

	Obese	Post BS	p-value
12(13)-DiHOME	5.89 (3.77-7.8)	2.14 (1.71-2.81)	**4.86E-16**
9(10)-DiHOME	7.91 (5.62-11.79)	4.65 (3.12-6.39)	**5.75E-08**
9,12,13-TriHOME	0.012 (0.01-0.014)	0.026 (0.014-0.043)	**3.45E-10**
9,10,13-TriHOME	0.032 (0.028-0.037)	0.066 (0.039-0.111)	**2.75E-09**
9-HODE	40.15 (26.73-53.72)	37.31 (25.05-61.15)	9.86E-01
13-HODE	29.50 (21.80-37.90)	29.11 (20.75-40.27)	7.98E-01
20-HDHA	0.31 (0.23-0.43)	0.28 (0.21-0.35)	1.51E-01
16-HDHA	0.37 (0.27-0.54)	0.47 (0.34-0.73)	**1.09E-02**
13-HDHA	0.56 (0.35-0.84)	0.97 (0.63-1.31)	**4.26E-06**
10-HDHA	0.64 (0.47-1.06)	0.93 (0.76-1.17)	**1.62E-03**
11-HDHA	0.58 (0.36-0.87)	1.55 (0.80-2.43)	**2.75E-10**
7-HDHA	0.2 (0.14-0.27)	0.23 (0.18-0.27)	5.96E-02
8-HDHA	0.15 (0.11-0.2)	0.17 (0.11-0.19)	5.23E-01
12(13)-EpOME	12.46 (9.57-16.46)	9.34 (6.75-14.27)	**1.92E-03**
4-HDHA	0.26 (0.21-0.37)	0.28 (0.17-0.35)	8.60E-01
9(10)-EpOME	4.18 (3.21-5.65)	2.99 (2.05-4.57)	**5.39E-04**
15,16-DiHODE	0.007 (0.005-0.013)	0.002 (0.001-0.003)	**9.04E-18**
9,10-DiHODE	0.001 (0.0007-0.002)	0.0003 (0.0002-0.0004)	**4.21E-18**
13-oxoODE	3.35 (2.43-4.23)	3.13 (2.61-3.44)	2.21E-01
9-oxoODE	3.85 (2.87-4.58)	3.55 (2.64-4.76)	5.59E-01
15(16)-EpODE	0.12 (0.09-0.17)	0.070 (0.055-0.092)	**3.96E-09**
9(10)-EpODE	0.018 (0.013-0.028)	0.011 (0.007-0.015)	**5.18E-08**
9-HOTrE	0.55 (0.41-0.78)	0.38 (0.23-0.70)	**3.57E-04**
13-HOTrE	1.18 (0.80-1.64)	0.81 (0.52-1.34)	**8.61E-04**
12-HHTrE	0.67 (0.21-1.48)	4.32 (1.76-7.07)	**1.61E-12**
14,15-DHET	1.09 (0.87-1.38)	0.67 (0.58-0.93)	**5.83E-09**
8,9-DHET	0.085 (0.066-0.11)	0.076 (0.057-0.124)	5.10E-01
5,6-DHET	0.19 (0.13-0.26)	0.18 (0.14-0.21)	1.85E-01
PG F2α	0.20 (0.17-0.24)	0.33 (0.25-0.45)	**6.81E-09**
TX B2	0.29 (0.18-0.52)	3.40 (1.78-5.56)	**2.18E-16**
8-HETE	0.8 (0.56-1.05)	0.96 (0.85-1.15)	**2.16E-03**

5-HETE	1.1 (0.75-1.42)	0.67 (0.56-0.85)	**1.43E-05**
12-HETE	12.97 (7.58-19.81)	30.56 (14.40-49.13)	**1.37E-09**
15-HETE	4.19 (2.85-5.44)	5.16 (4.30-5.66)	**2.06E-03**
20-HETE	3.25 (2.16-4.61)	1.05 (0.88-1.54)	**2.56E-17**
11,12-DHETE	0.043 (0.031-0.060)	0.061 (0.034-0.093)	**3.69E-03**
11(12)-DiHET	0.77 (0.60-0.97)	0.60 (0.51-0.76)	**3.14E-04**
PG E2	0.15 (0.08-0.23)	1.00 (0.60-1.71)	**5.50E-15**
PG D2	0.11 (0.07-0.16)	0.25 (0.21-0.33)	**2.92E-10**
5-HEPE	0.32 (0.22-0.48)	0.13 (0.12-0.20)	**6.77E-08**
18-HEPE	0.097 (0.066-0.16)	0.077 (0.060-0.095)	**1.69E-02**
15-HEPE	0.61 (0.42-0.87)	0.54 (0.43-0.76)	2.02E-01
11-HEPE	0.083 (0.050-0.120)	0.11 (0.064-0.13)	**1.15E-02**
8-HEPE	0.083 (0.062-0.12)	0.089 (0.071-0.110)	2.63E-01
12-HEPE	0.77 (0.42-1.27)	1.50 (0.90-2.35)	**1.51E-05**
9-HEPE	0.15 (0.10-0.20)	0.13 (0.094-0.150)	6.22E-02
19,20-DiHDPE	0.055 (0.037-0.082)	0.02 (0.017-0.029)	**1.77E-15**
13,14-DiHDPE	0.098 (0.07-0.144)	0.062 (0.050-0.080)	**8.40E-08**
10,11-DiHDPE	0.072 (0.048-0.098)	0.036 (0.025-0.063)	**1.94E-06**
17-HDHA	0.41 (0.27-0.62)	0.41 (0.32-0.54)	9.61E-01
14-HDHA	1.04 (0.61-1.60)	2.84 (1.70-4.37)	**2.70E-11**

Supplementary table S6. Concentration comparison carried out with Wilcoxon test between controls and post BS patients. Significant p-values are in bold.

	Control	Post BS	p-value
12(13)-DiHOME	4.44 (3.2-5.52)	2.14 (1.71-2.81)	**1.87E-09**
9(10)-DiHOME	4.33 (3.63-6.29)	4.65 (3.12-6.39)	8.80E-01
9,12,13-TriHOME	0.005 (0.004-0.006)	0.026 (0.014-0.043)	**2.92E-16**
9,10,13-TriHOME	0.012 (0.011-0.015)	0.066 (0.039-0.111)	**1.99E-16**
9-HODE	26.39 (19.94-33.64)	37.31 (25.05-61.15)	**5.05E-04**
13-HODE	17.23 (12.45-25.25)	29.11 (20.75-40.27)	**2.35E-05**
20-HDHA	0.23 (0.16-0.32)	0.28 (0.21-0.35)	**3.92E-02**
16-HDHA	0.27 (0.18-0.37)	0.47 (0.34-0.73)	**2.69E-06**
13-HDHA	0.34 (0.26-0.48)	0.97 (0.63-1.31)	**5.72E-11**
10-HDHA	0.35 (0.23-0.55)	0.93 (0.76-1.17)	**1.95E-11**
11-HDHA	0.18 (0.16-0.19)	1.55 (0.80-2.43)	**8.15E-17**
7-HDHA	0.14 (0.1-0.19)	0.23 (0.18-0.27)	**6.73E-07**
8-HDHA	0.11 (0.078-0.14)	0.17 (0.11-0.19)	**1.07E-03**
12(13)-EpOME	12.68 (10.1-18.58)	9.34 (6.75-14.27)	**3.05E-03**
4-HDHA	0.22 (0.13-0.3)	0.28 (0.17-0.35)	7.00E-02
9(10)-EpOME	3.17 (2.11-4.23)	2.99 (2.05-4.57)	8.98E-01
15,16-DiHODE	0.005 (0.003-0.007)	0.002 (0.001-0.003)	**3.74E-09**
9,10-DiHODE	0.0006 (0.0005-0.001)	0.0003 (0.0002-0.0004)	**1.22E-09**
13-oxoODE	2.65 (1.91-3.47)	3.13 (2.61-3.44)	**2.94E-02**
9-oxoODE	2.95 (2.29-4.16)	3.55 (2.64-4.76)	7.75E-02
15(16)-EpODE	0.12 (0.08-0.2)	0.070 (0.055-0.092)	**1.49E-06**
9(10)-EpODE	0.014 (0.011-0.018)	0.011 (0.007-0.015)	**1.81E-02**
9-HOTrE	0.5 (0.39-0.58)	0.38 (0.23-0.70)	9.70E-02
13-HOTrE	0.93 (0.69-1.26)	0.81 (0.52-1.34)	4.13E-01
12-HHTrE	0.29 (0.13-0.46)	4.32 (1.76-7.07)	**2.44E-13**
14,15-DHET	0.68 (0.49-0.91)	0.67 (0.58-0.93)	6.38E-01
8,9-DHET	0.06 (0.05-0.07)	0.076 (0.057-0.124)	**2.26E-03**
5,6-DHET	0.13 (0.1-0.18)	0.18 (0.14-0.21)	**3.95E-03**
PG F2α	0.14 (0.12-0.17)	0.33 (0.25-0.45)	**9.93E-13**
TX B2	0.24 (0.095-0.42)	3.40 (1.78-5.56)	**9.31E-13**
8-HETE	0.36 (0.30-0.46)	0.96 (0.85-1.15)	**6.57E-16**
5-HETE	0.48 (0.38-0.66)	0.67 (0.56-0.85)	**3.89E-05**
12-HETE	1.24 (0.97-1.90)	30.56 (14.40-49.13)	**7.56E-17**

15-HETE	1.44 (1.17-1.9)	5.16 (4.30-5.66)	**1.45E-16**
20-HETE	1.40 (0.94-1.81)	1.05 (0.88-1.54)	7.25E-02
11,12-DHETE	0.054 (0.043-0.064)	0.061 (0.034-0.093)	5.00E-01
11(12)-DiHET	0.54 (0.41-0.76)	0.60 (0.51-0.76)	1.28E-01
PG E2	0.11 (0.07-0.14)	1.00 (0.60-1.71)	**6.96E-13**
PG D2	0.0724 (0.072-0.073)	0.25 (0.21-0.33)	**6.80E-12**
5-HEPE	0.19 (0.12-0.30)	0.13 (0.12-0.20)	5.89E-02
18-HEPE	0.083 (0.057-0.14)	0.077 (0.060-0.095)	3.91E-01
15-HEPE	0.57 (0.40-0.84)	0.54 (0.43-0.76)	9.15E-01
11-HEPE	0.068 (0.045-0.11)	0.11 (0.064-0.13)	**9.68E-04**
8-HEPE	0.08 (0.05-0.12)	0.089 (0.071-0.110)	1.45E-01
12-HEPE	0.13 (0.09-0.2)	1.50 (0.90-2.35)	**1.95E-16**
9-HEPE	0.12 (0.07-0.16)	0.13 (0.094-0.150)	1.70E-01
19,20-DiHDPE	0.031 (0.024-0.038)	0.02 (0.017-0.029)	**1.71E-04**
13,14-DiHDPE	0.086 (0.068-0.1)	0.062 (0.050-0.080)	**1.51E-04**
10,11-DiHDPE	0.046 (0.037-0.058)	0.036 (0.025-0.063)	1.37E-01
17-HDHA	0.19 (0.15-0.26)	0.41 (0.32-0.54)	**8.80E-10**
14-HDHA	0.15 (0.078-0.28)	2.84 (1.70-4.37)	**9.83E-17**