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The Role of Eating Patterns and Biological Factors in Inducing Metabolic Improvement Following Bariatric Surgery or Dietary Intervention

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Abstract

Obesity and type 2 diabetes are major public health concerns with significant implications for patients and healthcare systems worldwide. Bariatric surgery and dietary interventions have been considered effective strategies for weight loss and metabolic improvement in individuals with obesity and diabetes. However, the effects of eating patterns on the results of these interventions as well as the comparison of the low-calorie diet and the bariatric surgery had not been investigated thoroughly.

This thesis, which consists of 2 projects, investigates the short-term and medium-term metabolic improvements in patients with different eating patterns following bariatric surgery or low-calorie diet. The first project focused on examining the effects of five different eating patterns, such as meal frequency and timing, on post-operative metabolic outcomes. The second project compares the short-term metabolic effects of the low-calorie diet and bariatric surgery. During both projects, the levels of different plasma metabolites such as blood glucose were analyzed before and after the interventions using R programming. A comprehensive literature review was conducted to gather existing knowledge on the subject.

The findings suggest that eating patterns could be associated with diabetes remission after bariatric surgery. Furthermore, the short-term metabolic improvement following the low-calorie diet and bariatric surgery are almost the same and the surgery could be replaceable by the diet in some subjects. The outcomes contribute to a better understanding of these interventions' success and provide important implications for clinical practice and the development of personalized treatment strategies for obesity management.

Keywords: Obesity, Type 2 Diabetes, Eating Behaviours, Bariatric Surgery, Low-Calorie Diet

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1. Introduction

1.1 Obesity

1.1.1 Definition and Classification

Obesity is defined by the World Health Organisation (WHO) as a multi-factorial chronic disease with multiple complications and serious public health challenges, characterized by an abnormal or excessive accumulation of fat. Obesity is identified as a major determinant of disability and death in the WHO European Region. One of the common ways to measure obesity is the body mass index (BMI), which is calculated by the weight in kilograms divided by the square of the height in meters. In clinical practice, body mass is usually calculated by BMI, which is age- and sex-independent. Overweight and obesity are defined as having a BMI of between 25 and 30 and a BMI of 30 or more respectively. To be more precise, as is described in Table 1, we can speak of first-degree obesity if the BMI is between 30 and 34.9, second-degree if it is between 35 and 39.9, and finally, third-degree obesity if the value of 40 is exceeded [1].

Table 1. Classification of overweight and obesity by BMI

Definition	Obesity Class	BMI (kg/m^2)
Underweight		<18.5
Normal		18.5 – 24.9
Overweight		25 – 29.9
Obesity	I	30 – 34.9
	II	35 – 39.9
	III	≥40

These BMI values were chosen based on various research, which focused on the relationship between the body mass index and the risk of all-cause mortality [2].

However, BMI alone is an inaccurate index as a measure of body adiposity and could have some errors in assessing the effects of obesity on health. It can be because of some reasons:

1) BMI does not show an indication of fat distribution, which is a significant factor in developing obesity-related complications. To illustrate, patients with a visceral fat distribution are in more danger than patients with adipose tissue at the peripheral level

with the same BMI [1]. Waist circumference is the most used indicator of the visceral distribution of adipose tissue which should also be considered [3].

2) BMI is not an index of adiposity and it cannot differentiate between different body compositions. For instance, the BMI of a subject with a high lean body mass and a subject with a high fat mass with the same height and weight is the same. Besides, the BMI of a subject with a high lean mass could be high and be considered as obesity or a subject with a normal BMI could have a high percentage of fat mass which is a factor of obesity and overweight [1], [4].
3) The effects of different comorbidities and the changes following getting older are not considered in measuring BMI [1], [4]. Besides, the same BMI might not correspond to the same amount of fat mass in different subjects and differ across populations, especially by sex, age and ethnic groups [5].

Despite these important issues, due to some reasons such as simplicity and low cost, the use of indirect measures such as BMI is recognized as a practical approach and remains the most used parameter for diagnosis of the presence and the degree of obesity, especially in large-scale public health and clinical studies. However, more evaluations might be necessary to decide about the type of therapeutic intervention appropriate for each subject [6], [7]. For instance, based on the 2015 European guidelines for the treatment of obesity, it is not recommended to rely only on BMI to identify the best treatment for each individual, but it is also necessary to consider the waist circumference, as an indicator of adipose tissue distribution [1].

It has been shown that BMI and waist circumference have a positive correlation in the diagnosis of obesity [8] and it is suggested to use both of them for the diagnosis of obesity and overweight. Measurements of waist circumference, including waist-to-hip ratio, could be used for abdominal fat mass [9] and demonstrate a similar association with morbidity and mortality as BMI. Alterations in waist circumference correlate with changes in the risk of obesity-related comorbidities, in particular cardiovascular diseases. The cut-offs for waist circumference are sex-specific and lower for women [5].

1.1.2 Epidemiology of Obesity

Globally, obesity has reached epidemic proportions [1] and every country is affected by obesity and overweight, with some lower-income countries dealing with the highest increases in the last years. No country has reported a decrease in obesity prevalence across their entire population, and none is on track to meet the WHO's target of 'no increase on 2010 levels by 2025' [10]. This serious health issue has to be prevented as it has been considered the largest global chronic health issue in adults, which is increasingly converting into a more serious problem than malnutrition [1]. Moreover, obesity, as one of the leading causes of disability and death, is a gateway to ill health [1] and can increase the risk of a variety of diseases such as 13 types of cancer, type 2 diabetes, hypertension, dyslipidemia, as well as severe COVID-19 outcomes [5].

Furthermore, to compare with the normal BMI, obesity classes II and III are significantly associated with higher all-cause mortality, morbidity, and lower quality of life [1]. Class I obesity overall is not associated with higher mortality and overweight is associated with significantly lower all-cause mortality [11]. Based on WHO statistics, obesity and overweight, as the fifth leading risks for global deaths, result in at least 2.8 million deaths in adults each year.

1.1.2.1 Obesity in the World

Based on WHO reports, the worldwide prevalence of obesity tripled between 1975 and 2016 and doubled from 15% to 33% between the 1980s and 2004. In 2020, more than 2.6 billion people, aged 2 years old and more, (38% of the population) had overweight (BMI≥25 kg/m^2) and over 980 million (14% of the population) were dealing with obesity (BMI≥30 kg/m^2) in the worldwide. It is predicted that these figures will increase to more than 4 billion (51% of people) and more than 1.9 billion subjects (24% of people) living with overweight and obesity respectively in 2035. Besides, the number of adults (aged 20 years and above) with obesity will rise from over 800 million in 2020 (14% of men and 18% of women) to more than 1.5 billion in 2035 (23% of men and 27% of women) [10].

1.1.2.2 Obesity in Europe

As is shown in the WHO Global Health Observatory, Age-standardized estimates for 2016 indicate that the highest obesity prevalence for adults is in the American Region and then the European Region among all the WHO regions. Besides, The highest prevalence of both overweight and obesity is found in the Mediterranean and Eastern European countries [5]. The increasing trend of obesity and overweight among adults of different genders in the WHO European Region between 1975 and 2016 is indicated in Figure 1.

Obesity and overweight in adults have reached epidemic proportions in the WHO European Region, in which 59% and 23% of adults are living with overweight and obesity in turn. Obesity is more prevalent among females than among males, in contrast, overweight levels are higher among males than among females across the WHO European Region and in most countries [5].

In 2020, approximately 192 million adults (26% of men and 28% of women) were living with obesity in the European Region. It is predicted that the prevalence of obesity will rise to around 263 million adults (39% of men and 35% of women) in 2035 [10].

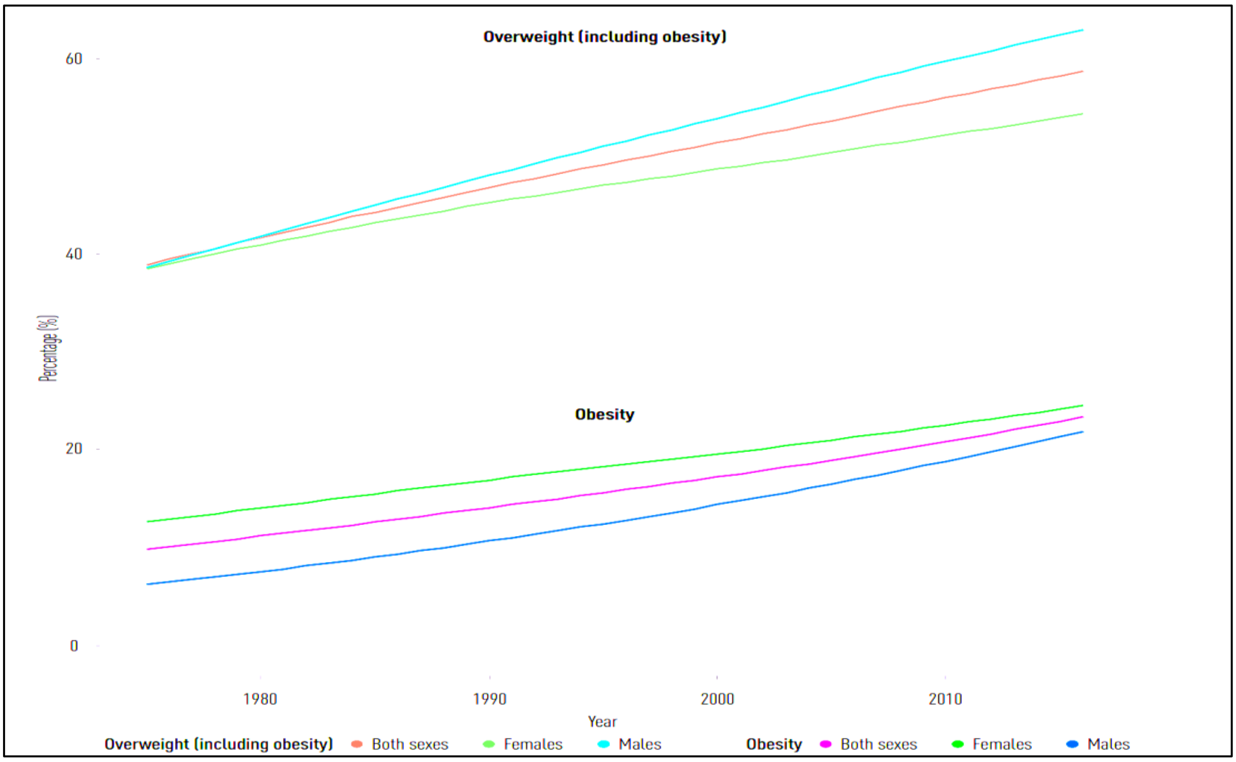

Figure 1. Prevalence of overweight and obesity among adults in WHO European Region by sex, (1975–2016), source: WHO estimates, [5]

1.1.2.3 Obesity in Italy

Based on OECD (The Organisation for Economic Co-operation and Development) data, obesity rates among adults are mild in Italy but are very high among children in comparison with other countries. Around 1 in 10 people are dealing with obesity and more than 1 in 2 men and 1 in 3 women have overweight in Italy [12].

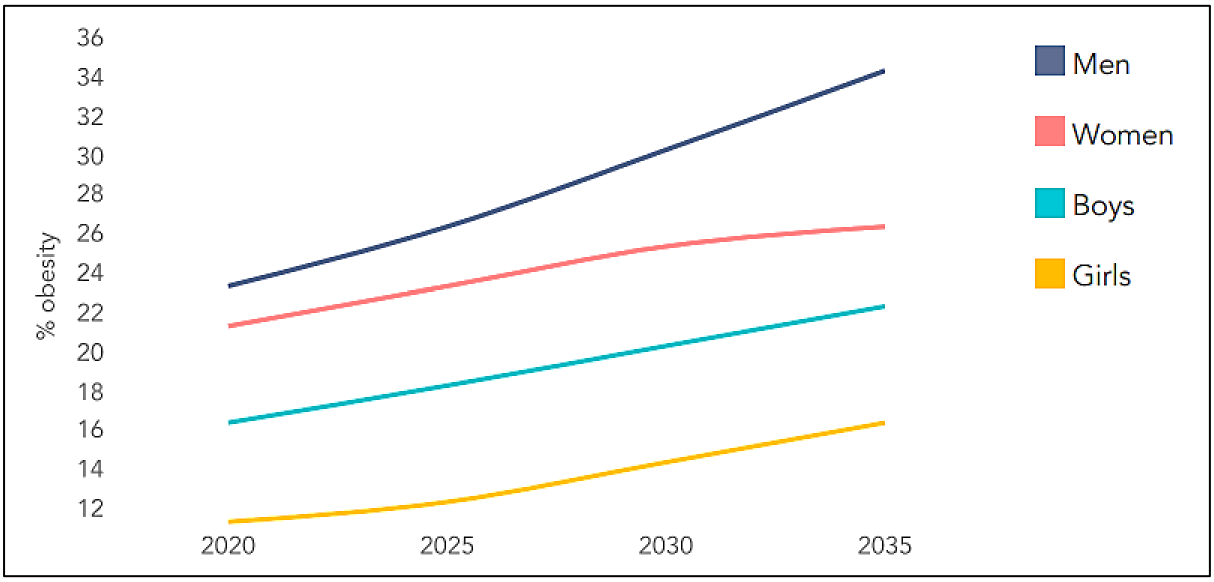

Figure 2. Prevalence of obesity (BMI ≥30 kg/m^2) in Italy between 2020 and 2035, source: World Obesity Atlas 2023

As can be seen in Figure 2, the annual increase in adult obesity in the 15-year period between 2020 and 2035 is expected to be 2.0%, which is considered a medium increase. This rate for childhood obesity is 2.1%, which is a high value in this category. Besides, the average percentage of adults with obesity will be 31% in 2035, which is a very high rate [10].

1.1.2.4 Obesity in Norway

The Norwegian Institute of Public Health reported that around 1 in 4 middle-aged (40-45 years old) men and 1 in 5 women live with obesity in Norway. Approximately 2400 annual deaths in Norway and many cases of diabetes, cardiovascular disease and other chronic diseases are the result of obesity and high BMI.

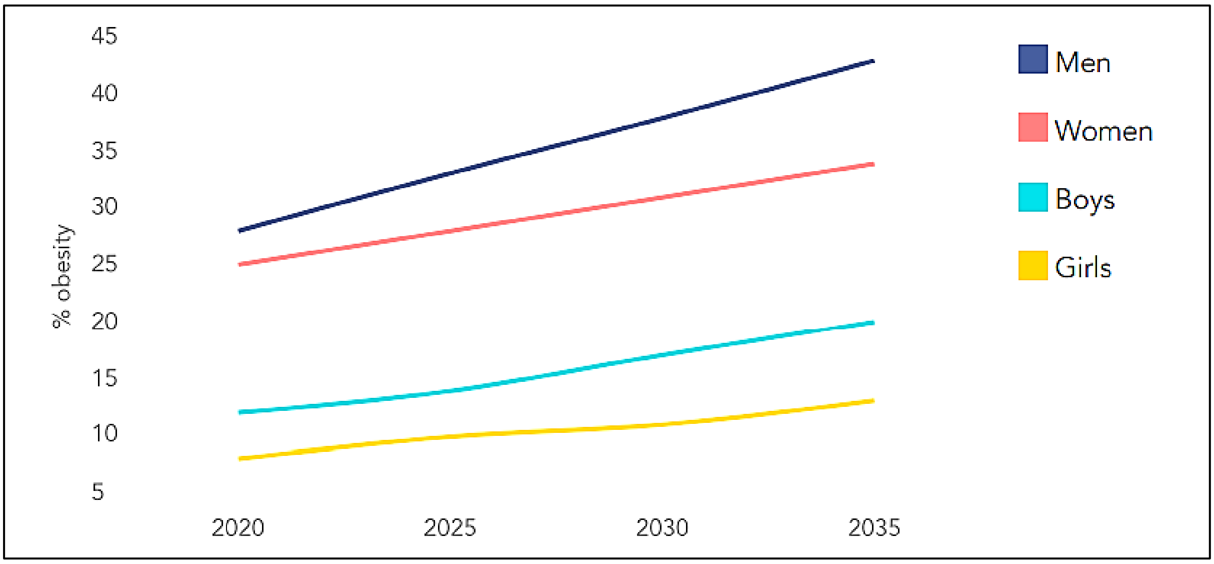

Figure 3. Prevalence of obesity (BMI ≥30 kg/m^2) in Norway between 2020 and 2035, source: World Obesity Atlas 2023

Figure 3 illustrates that the annual increase in adult and childhood obesity is expected to be 2.5% and 3.0% between 2020 and 2035, which are high and very high values. The average percentage of adults with obesity will be 40% in 2035, which is a very high rate [10]. Therefore, obesity is more prevalent in Norway than in Italy, which could be the cause of other health issues.

1.1.3 Complications of Obesity

Obesity is a serious chronic disease associated with comorbidities and complications that involve most systems of the body, which can reduce the quality of life and also shorten the life expectancy. Even in the absence of complications, obesity increases the risk of early mortality [13]. The most important consequences of obesity are as follows: metabolic syndrome (including hypertension and diabetes mellitus), dyslipidemia, ischaemic heart disease, certain types of cancers, obstructive sleep apnea syndrome (OSAS), liver and gallbladder diseases, osteoarthritis, reproductive and urologic problems as well as social, economic, and psychological issues [14].

Besides, individuals with obesity often face stigma and bias in society, including from healthcare professionals, which can affect the support and treatment they receive. Moreover, people with obesity have a substantially higher risk of developing severe forms of COVID-19 compared to those without obesity. There is also a positive correlation between COVID-19 mortality rates and the proportion of overweight individuals in a country's adult population [5].

In this dissertation, we will focus mainly on the relationship between obesity and type 2 diabetes mellitus as well as the improvement of these diseases after different treatments.

1.2 Type 2 Diabetes Mellitus

1.2.1 Definition

Diabetes is a group of metabolic disorders characterized by hyperglycemia secondary to defects in insulin secretion, insulin action, or both [15]. The most common diabetes classifications include Type 1 diabetes mellitus, Type 2 diabetes mellitus, gestational diabetes and some specific types of diabetes. Type 2 diabetes, known in the past as "non-insulin-dependent diabetes" or "adult-onset diabetes", is the most prevalent and makes up approximately 90-95% of all diabetes cases. It involves individuals who experience a relative insulin deficiency and display peripheral insulin resistance. In the beginning, and frequently throughout their lives, these patients might not require insulin therapy to maintain their health and survive [16].

The chronic hyperglycemia of diabetes mellitus can cause long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Some of the symptoms of diabetes include polyuria, polydipsia, polyphagia, unintentional weight loss, dehydration, and blurred vision. Furthermore, type 2 diabetes might be asymptomatic and present for a long period of time before diabetes is diagnosed due to the gradual development of hyperglycemia at earlier stages, which is often not severe enough for the patient to notice the typical diabetes symptoms caused by hyperglycemia, namely weight loss [16]. A degree of hyperglycemia is sufficient to result in pathologic and functional alterations in different tissues but without clinical symptoms [15].

1.2.2 Diagnosis and Screening of Type 2 Diabetes and Prediabetes

The diagnostic criteria of prediabetes and type 2 diabetes are available in Table 2. Usually, plasma glucose levels, either the fasting plasma glucose (FPG) value or the 2-h plasma glucose (2-h PG) value during a 75-g oral glucose tolerance test (OGTT), or HbA1C level is used for the diagnosis of diabetes [16].

Table 2. Diagnostic criteria of prediabetes and type 2 diabetes, source: ADA guidelines

	FPG	2h-OGTT	HbA1C
Normal	< 100 mg/dl	< 140 mg/dl	< 5.7 %
Prediabetes	100 – 126 mg/dl	140 – 200 mg/dl	5.7 - 6.5 %
Type 2 diabetes	≥ 126 mg/dl	≥ 200 mg/dl	≥ 6.5 %

Screening for the diagnosis of diabetes and pre-diabetes should be performed in all adults, even if they are asymptomatic if they have obesity or overweight and bear one or more of the risk factors for diabetes. To illustrate, a history of cardiovascular disease (CVD), hypertension, previous gestational diabetes mellitus (GDM), and dyslipidemia are some of the risk factors for diabetes [16].

1.2.3 Epidemiology of Type 2 Diabetes

It is estimated that diabetes affects around 537 million adults globally, with a prevalence of 10.5 percent among adults aged 20 to 79 years. Among all diabetes diagnoses worldwide, approximately 98 percent are dealing with type 2 diabetes [17]. Type 2 diabetes is considered a significant public health issue and the ninth leading cause of mortality, having a considerable impact on both human life and healthcare expenditure [18]. Besides, there is a growing concern that the prevalence of diabetes will continue to rise significantly in the future [17].

1.2.3.1 Type 2 Diabetes in the World

The worldwide prevalence of diabetes mellitus is increasing. In 2017, 6.28% of the world's population, approximately 462 million subjects, were affected by type 2 diabetes (6059 cases per 100,000). By the year 2030, it is estimated that the prevalence of type 2 diabetes worldwide will rise to 7079 individuals per 100,000 population [18].

1.2.3.2 Type 2 Diabetes in Europe

As mentioned in the IDF (International Diabetes Federation) Diabetes Atlas, the number of adults with diabetes is approximately 61 million people and it is predicted to rise to 69 million by 2045, an increase of 13%. It is calculated that the prevalence of type 2 diabetes was 8529 cases per 100,000 individuals in Europe in 2017 [18]. Furthermore, as is seen in Figure 4, the number of adults with diabetes increased noticeably from 16.8 million to 32.3 million in Europe in 2000 and 2019.

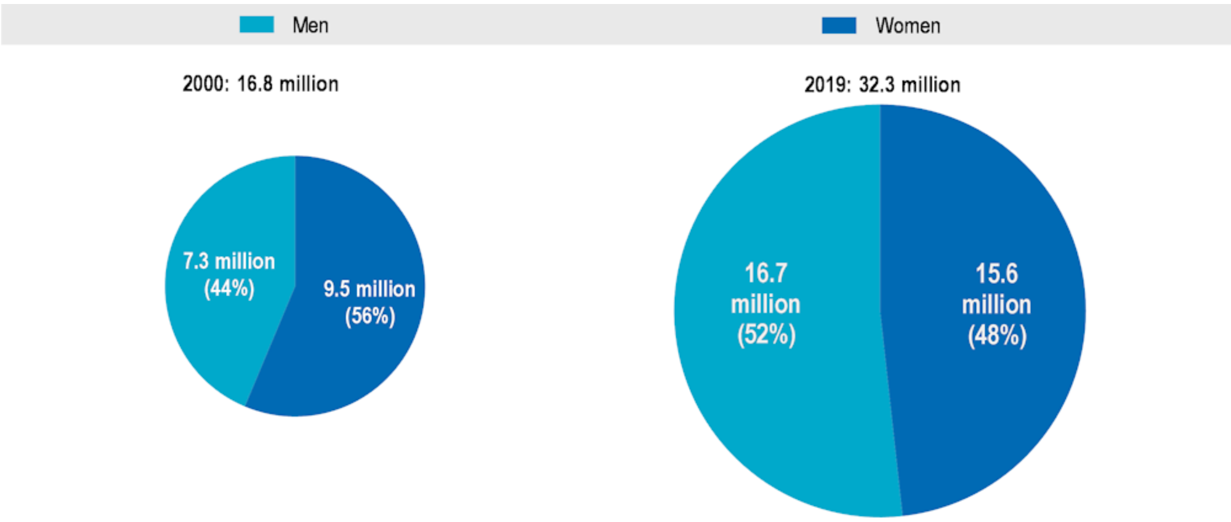

Figure 4. Number of people aged 20-79 with Type 1 or Type 2 diabetes in Europe in 2000 and 2017, source: OECD library

1.2.3.3 Type 2 Diabetes in Italy and Norway

Based on International Diabetes Federation (IDF) information, In 2021, 9.9% and 4.8% of adults were living with diabetes in Italy and Norway respectively. In 2017, the prevalence of type 2 diabetes in Italy was 9938 cases per 100,000 individuals [18]. The prevalence of diabetes has significantly risen over the past two decades in Norway and around 270,000 people live with diagnosed diabetes in Norway in 2020 [19].

1.3 Diabesity

There is a strong relationship between obesity and type 2 diabetes mellitus, in all ethnic groups and genders [20]. The most important metabolic consequence of obesity is type 2 diabetes mellitus, which noticeably increases cardiovascular disease-related morbidity and mortality and presents significant difficulties in managing the disease [21]. Most, but not all, patients with type 2 diabetes live with obesity, and obesity itself causes some degree of insulin resistance [15]. Each kilogram rise in body weight increases the risk of diabetes by 4.5% [22]. The term "diabesity", which demonstrates the strong connection between obesity and diabetes, with or without associated risk factors such as dyslipidemia and hypertension, was introduced in the 1970s [21]. Diabesity forms a subset of metabolic syndrome [22].

Various types of medications used to treat diabetes, including insulin, have been linked to the possibility of weight gain and could worsen "diabesity." As a result, it is a necessity to carefully choose the right antidiabetic pharmacotherapy when managing diabesity. In addition, non-drug approaches such as dietary treatments, exercise programs, and bariatric procedures should be highlighted as potential treatments for diabesity [21].

Weight management and glycaemic control should be the goal of diabesity management [22]. Consistent and strong evidence indicates that effective management of obesity can delay the progression of prediabetes to type 2 diabetes. Additionally, managing obesity proves to be highly advantageous in the treatment of type 2 diabetes, based on the amount of weight loss achieved. By losing excess weight, glycemic control is improved, resulting in a decreased reliance on glucose-lowering medications. Moreover, substantial reductions in HbA1C levels can be achieved, and in some cases, sustained remission of diabetes can be attained through weight loss [23].

1.4 Obesity Management

As mentioned before, obesity is linked to numerous additional non-communicable diseases (NCDs), including cardiovascular diseases (CVD), type 2 diabetes (T2DM), specific forms of cancer, and various mental health concerns [5]. Therefore, obesity, as a chronic disease, should be treated as soon as possible and over a period. Appropriate objectives of weight management are to achieve a reduction in health risks, promotion and maintenance of weight loss, and prevention of weight regain [1].

There are various approaches used for obesity management. To illustrate, there should be a multidisciplinary team, consisting of an obesity medical specialist, a dietitian, a psychologist or psychiatrist, a specialist in physical activity, a general practitioner, and a nurse. This team should try to reach obesity management goals such as achieving weight loss and treating comorbidities through avoiding stigmatization in a health care setting, improving motivation and communication with the patients, considering lifestyle behaviour change, increasing physical activity, and avoiding weight cycling [24].

Besides, Weight reduction is an integral part of the therapy for type 2 diabetes in many patients. Numerous studies have confirmed the efficacy of weight reduction in both treating and preventing type 2 diabetes. Based on some research, through lifestyle intervention, the conversion of prediabetes to type 2 diabetes was decreased by more than 50% [25].

There are different interventions for obesity management, which might be done in parallel for the best result. Some of them are briefly explained below.

1.4.1 Lifestyle Modification

Multiple groups of specialists have advised individuals with overweight and obesity to aim for a 10% reduction in their initial body weight in 16-26 weeks of treatment using a combination of diet, physical activity, and behaviour therapy, commonly known as lifestyle modification [26]. For optimal short- and long-term weight loss results, combining weight loss medications with a comprehensive lifestyle modification program is likely to be the most effective approach [27].

Typically, comprehensive lifestyle modification programs offer weekly individual or group sessions aimed at modifying eating and activity patterns as part of the treatment plan. Lifestyle modification promotes weight loss by assisting individuals in managing their external food environment. Patients are advised, for instance, to avoid fast-food restaurants and convenience stores, follow a grocery list while shopping, store food items out of sight at home, and avoid doing other activities, such as watching television, while eating. By minimizing exposure to food-related stimuli, the urge to eat is regulated [27].

Besides, physical activity is a vital factor in enhancing cardiovascular health for individuals with both average weight and obesity. Even without significant weight loss, engaging in regular aerobic activities has proven to be beneficial. It can lead to reduced blood pressure, improved lipid levels, and a decrease in visceral fat. Moreover, it is linked to better glucose tolerance and insulin sensitivity in nondiabetic individuals, and improved glycemic control for those with type 2 diabetes [28]. On average, the expected weight loss achieved through exercise training is less than 3 kg. To preserve lean mass while losing weight, it is recommended to follow an exercise training program that focuses on resistance training at a moderate-to-high intensity level. This approach can help individuals retain their muscle mass while shedding excess body weight [5].

1.4.2 Diet Therapy

Dietary intervention is a key component of overweight and obesity management, and adopting a healthy dietary pattern has positive effects on metabolic health, reduces inflammation, and lowers the risk of noncommunicable diseases (NCDs) [29]. Dietary and nutrition therapy should be individualized for each patient according to their comorbidities, physical activity, food preferences, cultural factors, etc [30].

In general, the dietary recommendations should promote a balanced and healthy diet by encouraging the consumption of nutrient-rich foods such as vegetables, beans, legumes, grains, and fiber. It's advisable to replace high-fat dairy products and meats with their low-fat counterparts. Additionally, incorporating more seafood into the diet is essential. To maintain a healthy lifestyle, it is advised to avoid foods with added sugars and solid fats, as well as sugary drinks and alcoholic beverages. Furthermore, it is recommended to decrease the calorie intake and portion size, not skip meals, and avoid having snacks between meals [1].

For individuals dealing with overweight or obesity and type 2 diabetes, it is advised to pursue nutrition therapy, physical activity, and behavioural therapy as means to attain and maintain a weight loss of at least 5%. More weight loss usually results in further improvements in diabetes and cardiovascular risk management [31].

A variety of dietary interventions are shown to be successful in the treatment of obesity and its comorbidities, namely low-calorie diet, Mediterranean diet, intermittent fasting, ketogenic diet, etc. Dietitians should explore these diverse approaches and provide personalized treatments to their patients [32].

In this dissertation, the low-calorie diet and fasting will be discussed.

1.4.2.1 Low-Calorie Diet And Fasting

A low-calorie diet typically consists of consuming 1,200 to 1,500 calories per day. To promote weight loss, deficits of 500 to 750 calories per day are often utilized, as suggested by numerous obesity societies and guidelines [33]. Besides, a very low-calorie diet (VLCD) provides less than 800 kcal a day, which is not recommended for long-term consumption although it is helpful for a short-term period. VLCD diet could result in 20-30% reductions in body weight. In some cases, these remarkable weight loss results can be achieved within a relatively short period of 12 to 16 weeks. Some research and evidence indicate that adhering to a VLCD in adults with type 2 diabetes (T2D) can lead to substantial improvements in glycemic control. In some cases, it has shown the potential to achieve complete remission of type 2 diabetes [34]. The exact effects of these low-calorie diets require further research.

A 2-week VLCD has proven to be an effective method for pre-operative weight reduction in patients. During this period, considerable amounts of both fat mass and lean body mass are lost, with the loss of lean body mass exceeding that of fat mass. Interestingly, it appears that patients who achieve greater overall weight loss during the VLCD period do not necessarily experience a corresponding increase in fat mass loss. Instead, they tend to lose more lean body mass, which might have implications for their overall health and surgical outcomes [35].

Fasting methods, including intermittent fasting and alternate-day fasting, have been linked to more significant weight changes and reductions in body fat, potentially leading to substantial improvements in lipid profiles, although the evidence for the latter is not as definitive. However, the impact of intermittent fasting on individuals with obesity and type 2 diabetes requires further investigation [32].

Furthermore, intermittent fasting, which involves regular periods with no or very limited calorie intake, has various benefits not only from calorie reduction. Its effects on metabolic switching can help reverse insulin resistance, while also contributing to the strengthening of the immune system and enhancing physical and cognitive functions [36]. Intermittent fasting focuses on the specific time window for eating rather than calorie intake calculation or the composition of macronutrients. This approach enables individuals to limit their food consumption without the need to count calories and also helps them steer clear of late-night snacking [33].

In this thesis, the effects of the low-calorie diet on type 2 diabetes remission in people with obesity are discussed.

1.4.3 Pharmacological Treatment

In Europe, pharmacotherapy is commonly recommended for weight loss and weight-loss maintenance in individuals with a body mass index (BMI) equal to or higher than 30, or those with a BMI greater than or equal to 27 kg/m2 along with adiposity-related complications. The use of pharmacotherapy is meant to complement behavioural and psychological interventions in these cases. By incorporating pharmacotherapy, the potential for achieving greater weight loss beyond what can be accomplished through health behaviour changes alone is enhanced. Additionally, it plays a crucial role in preventing weight regain, making it an important consideration in weight management strategies [5].

Weight loss medications, such as Sibutramine or Rimonabant, modify internal signals that control feelings of hunger and fullness. On the other hand, medications like Orlistat induce nutrient malabsorption. Medications in the first category seem to diminish the urge to start or continue eating, potentially making obese individuals less susceptible to the constant presence of food in their environment [37]. New research indicates that the potential for achieving significant initial weight loss and sustaining it over the long term can be optimized by combining lifestyle adjustments and consistent pharmacotherapy [26].

1.4.4 Surgical Treatment

The rising prevalence of morbid obesity has led to a significant increase in the use of surgery as a treatment option. Bariatric surgery has become a popular choice due to its significant impact on weight loss compared to conservative methods [35]. Surgery could be considered for patients between the ages of 18 and 60 years, who have a BMI of 40.0 kg/m2 or higher, or a BMI between 35.0 and 39.9 kg/m2 with co-morbidities, in whom surgically induced weight loss is expected to improve obesity complications (such as type 2 diabetes and other metabolic disorders) [1].

Additionally, it offers the advantage of treating related issues such as diabetes and metabolic syndrome, making it a favourable intervention for individuals dealing with morbid obesity [35]. Therefore, patients with a BMI of between 30 and 35 kg/m2 with type 2 diabetes may also be considered for bariatric surgery on an individual basis [1]. Long-term data indicates that bariatric surgery offers the most sustained and long-lasting weight loss compared to all other weight loss strategies. This means that individuals who undergo bariatric surgery are more likely to maintain their reduced weight over an extended period compared to those who opt for different weight loss methods [38].

There are different types of bariatric surgery. The most common types of bariatric surgery are sleeve gastrectomy (LSG), Roux-en-Y gastric bypass (RYGB), and adjustable gastric banding. In the research of this thesis, LSG and RYGB are investigated.

1.5 "Metabolic Surgery"

The term "metabolic surgery" has gained popularity as a more suitable and accurate descriptor for weight-loss surgery. This shift in terminology is primarily due to the fact that these surgical procedures result in altered gastrointestinal anatomy, which subsequently leads to beneficial effects on metabolism. Rather than solely focusing on weight loss, as mentioned before, the term "metabolic surgery" highlights the significant impact these procedures have on metabolic processes, especially in relation to conditions like type 2 diabetes and other metabolic disorders [39].

1.5.1 Bariatric Surgery and Type II Diabetes Mellitus

Bariatric surgery has demonstrated remarkable effects on reducing the incidence of type 2 diabetes mellitus (T2DM) by achieving improved glycemic control and enhancing cardiovascular health, ultimately leading to decreased morbidity and mortality rates. Although the exact mechanisms behind these positive outcomes require further research, some potential factors include weight loss resulting from reduced caloric intake, decreased insulin resistance leading to improved glucose tolerance, and increased insulin sensitivity. These combined effects contribute to the significant benefits observed in patients undergoing bariatric surgery in relation to T2DM management and overall health outcomes [40].

1.6 Predictors of Diabetes Remission After Bariatric Surgery

Numerous prediction models have been developed to evaluate the potential for diabetes remission after bariatric surgery. These models include various scoring systems and logistic regression models such as ABCD, Individualized metabolic surgery (IMS), DiaRem, Advanced-DiaRem (Ad-DiaRem), and DiaBetter. Each of these models incorporates a range of preoperative variables, allowing for personalized predictions regarding the success of bariatric surgery in achieving remission of Type 2 diabetes. These preoperative variables typically include age, BMI, C-peptide, diabetes duration, number of diabetes medications, insulin use, sex, fasting glucose, and HbA1c, with each prediction model utilizing two to six of these variables [41], [42].

Furthermore, there is evidence suggesting that the Amino acid metabolites (AAM) profile, which is linked to glucose and energy homeostasis, obesity, and Type 2 diabetes (T2DM), might offer a more precise prediction of T2DM remission following bariatric surgery compared to the currently available prediction models [43], [44].

These predictors are not complete and accurate as they do not include other important factors such as preoperative eating behaviours and diet, which are discussed in this thesis.

1.7 Eating Behaviours

There is some literature that shows eating habits proved to be significant predictors of weight loss six months after surgery and it is crucial to continuously evaluate eating behaviour both before and after the surgical procedure to attain favourable weight loss results [45].

There are several types of unhealthy eating behaviours among patients with obesity. Binge eating disorder stands out as the most common eating disorder among candidates for bariatric surgery. The majority of those with binge eating disorder typically seek treatment primarily for their weight issues rather than addressing an eating disorder or unhealthy eating habits [46].

In recent years, researchers have been exploring whether pre-surgical binge eating disorders have any connection to less successful weight loss results following bariatric surgery. Although numerous studies have been carried out, most of them do not provide evidence of a significant relationship, with only a few suggesting that binge eating disorder might be associated with less favourable weight loss outcomes. As a result, binge eating disorders are not considered as factors that would rule out or contraindicate bariatric surgery [47]. Nonetheless, individuals with pre-surgical binge eating or BED should receive diligent post-surgery monitoring, as there is substantial evidence indicating that disordered eating habits can resurface following the surgical procedure [48].

Besides, Eating behaviour therapy could be an option to prevent metabolic disorders. There are some randomized controlled trials (RCTs) that provide strong evidence that employing behavioural approaches to support alterations in the eating habits and physical activity of adults with overweight results in weight reduction ranging from 10 percent over a span of 4 months to 1 year. Moreover, there is no clear advantage of one behaviour therapy over another when it comes to its impact on weight loss. Instead, it seems that employing multimodal strategies has the best results, and interventions with the highest level of intensity are linked to the most substantial weight loss [49].

1.8 Blood Metabolites Before and After The Interventions

In addition to both short- and long-term effects on weight loss, a rapid post-operative improvement in metabolic parameters has been demonstrated. It is uncertain what the reasons for this are, whether it is solely due to metabolic improvement as a result of the surgical procedure, or whether the reduction in energy intake in the postoperative phase is also important [50], [51]. A better understanding of the reason for the initially favourable effect of bariatric surgery on metabolic parameters before weight reduction is essential and will be able to provide increased knowledge about mechanisms for the development and maintenance of obesity, which can later be used in preventive and therapeutic treatment.

1.8.1 Blood Glucose and Insulin Levels

Numerous studies have underscored bariatric surgery's remarkable impact on glycemic control and metabolic function. One of the foremost advantages of Bariatric surgery in the context of type 2 diabetes is its capacity to induce a substantial decrease in blood glucose levels [52]. This effect is often rapid and pronounced, leading to improved short-term outcomes for patients. By altering the anatomical structure of the gastrointestinal tract and affecting the hormonal balance involved in glucose regulation, these procedures contribute to a profound normalization of blood sugar levels [53].

Furthermore, Bariatric surgery has been shown to enhance insulin sensitivity, a key factor in the effective utilization of glucose by the body's cells. This improved sensitivity to insulin enables cells to take up and utilize glucose more efficiently, thereby reducing the burden on the pancreas to produce excessive amounts of insulin. The result is not only better glycemic control but also a reduced risk of diabetes-related complications [54].

Besides, the regulatory effect of calorie restriction on the metabolism of obese patients with type 2 diabetes is profound and independent of any changes in body weight. By enhancing insulin sensitivity, optimizing energy utilization, reducing circulating fatty acids, and mitigating inflammation, calorie restriction offers a multifaceted approach to managing and improving the metabolic health of individuals with type 2 diabetes. This underscores the importance of dietary interventions as a powerful tool in the management of type 2 diabetes, offering hope for better control of blood sugar levels and a reduced risk of related complications [55], [56].

1.8.2 Ketones

An increase in the levels of ketone bodies, specifically 3-hydroxybutyrate and acetoacetate, was notably observed following bariatric surgery and low-calorie diets or fasting in a short time after the interventions. One of the primary mechanisms that causes the increase in ketone bodies is the reduction in food intake and caloric restriction as after bariatric surgery and during fasting, patients typically consume significantly fewer meal portions and calories, which leads to a decrease in the availability of glucose, the body's primary energy source [57].

As a consequence of this decreased glucose supply, the body initiates a change in energy metabolism. It begins to rely more heavily on stored fat as the energy source. This shift will activate the pathways that promote the breakdown of fats into fatty acids, which will convert to ketone bodies in the liver through some pathways. These ketone bodies are released into the blood and serve as an alternative fuel source for various tissues, including the brain [58].

This rise in ketone bodies is typically temporary and over time, as patients continue to lose weight and their dietary habits stabilize, the production of ketone bodies may decrease. Nonetheless, monitoring ketone levels can be important for healthcare providers to ensure patients' nutritional needs are met and to control any potential complications associated with ketogenic states [59].

1.8.3 Amino acids

The levels of amino acids alter following bariatric surgery and energy-restricted dietary interventions. The alterations differ between studies. For instance, in one research, three months post-RYGB surgery, there was a notable rise in the levels of the majority of amino acids. However, by the six-month mark, concentrations of glutamic acid, serine, arginine, alanine, methionine, valine, phenylalanine, isoleucine, and tyrosine had decreased [60].

One important group of amino acids is branched-chain amino acids (BCAA), including valine, leucine, and isoleucine. Their levels are often increased in individuals with obesity and insulin resistance, and this elevation is associated with adverse metabolic outcomes. Following the surgical procedure, the concentrations of branched-chain amino acids (BCAAs) showed a significant reduction [61].

The changes in the concentrations of amino acids following a low-calorie diet will be explained in the systematic review of this dissertation.

2. Purpose of the Studies

The purpose of this thesis is to evaluate the effects of bariatric surgery in short- and long-term periods as well as a low-calorie diet in a short time on patients with obesity. Through the evaluations, we analyzed the blood metabolites in the groups of patients to figure out the alterations in their levels during pre- and post-stages of interventions.

Besides, the eating behaviours of patients prior to bariatric surgery were analyzed to show the potential influences of eating patterns on the remission of obesity complications, especially type 2 diabetes.

This thesis consists of 2 projects at the University of Padua, Italy, and the University of Bergen, Norway. The aims of each study are explained below.

2.1 Aim of the Study at the University of Padua

(The Effects of Different Eating Behaviours on the Type 2 Diabetes Remission Following Bariatric Surgery in Patients with Obesity)

There are several pieces of evidence in previous literature indicating the positive impacts of bariatric surgery on the remission of the complications of obesity. This literature focuses on different obesity complications such as type 2 diabetes, hypertension, dyslipidemia, and OSAS following different procedures such as gastric bypass and sleeve gastrectomy. For instance, in most cases, there will be a partial or complete remission of type 2 diabetes after the surgery.

Furthermore, some studies show the effects of some factors on the level of remission in these diseases, especially diabetes. There are some prediction models to calculate the probability of remission in each patient based on their individualized characteristics. However, there is a lack in these models, which is the eating behaviours and patterns of patients prior to the interventions.

In this study, we analyzed the impacts of different eating behaviours and habits on the result of the surgery through follow-ups until a year after bariatric surgery. Therefore, patients were divided into five different clusters based on their meal frequency and eating behaviours to analyze the difference between their remission postoperatively. Their blood samples were collected before and during the follow-up sessions and analyzed.

The main purpose of this study is to understand the influences of health behaviours, especially eating patterns on the result of bariatric surgery in patients with obesity with the main focus on type 2 diabetes.

2.2 Aim of the Study at the University of Bergen

(Short-Term Effects of the Low-Calorie Diet and Bariatric Surgery on Blood Metabolites in Patients with Obesity)

Bariatric surgery is an effective method of weight reduction. Studies show a rapid improvement in metabolic parameters postoperatively, which occurs before the primary weight loss. It is uncertain whether this effect can primarily be attributed to low caloric intake in the postoperative phase or whether it is due to factors triggered by the operative intervention. There is a need for increased knowledge about the cause and mechanisms for the initial metabolic improvement of bariatric surgery.

In this clinical trial, we carried out a study where we collected blood samples from obesity surgery patients before and six days post-operatively after two different surgical procedures, gastric bypass and gastric sleeve. We supplemented this material with a group of 14 patients who carried out a six-day low-calorie diet in order to compare these with bariatric surgery patients with a correspondingly low-calorie intake after surgery. Fasting blood samples were taken initially and after six days of low-calorie intake. The results of the study will contribute to a better understanding of mechanisms in adipose tissue that underlie the improvement in metabolic parameters due to the surgical intervention, and which are not primarily due to the reduced energy intake.

Besides, a systematic review of previous literature was done to understand the impacts of a low-calorie diet on blood metabolites, especially amino acids in a short-term period. Most of the studies in the review focused on only fasting and energy-restricted diets and there were only a few studies to compare the short-term impacts of this diet and bariatric surgery.

The primary endpoints for the study are changes in metabolic parameters measured by biochemical analyses and adipose tissue biopsies. We want to compare changes in blood and adipose tissue after six days of low-calorie intake found from this study with postoperative samples taken six days after either gastric sleeve or gastric bypass operation.

3. Materials and Methods

This thesis consists of two relevant projects on different subjects through their specific methodologies at two different universities.

The first project was done at the University of Padua, Italy, and the other one at the University of Bergen, Norway.

Ethics: During both projects, the research participation had been done voluntary and all subjects were informed about the aims and the protocols of the studies and their rights, such as the right to withdraw from the research at any point. Subjects' privacy and anonymity while analyzing and reporting the data were valued.

3.1 The Methodology of the Project at the University of Padua

3.1.1 Participants, Study Setting and Ethics

The subjects included in this study are 173 patients who were referred to the Center for the Study and Integrated Management of Obesity in Padua (Clinica Medica 3, the University Hospital of Padua) between the years 2019 and 2021. These patients were invited to participate in this study, including the assessments in different follow-ups in the process of the research. Among them, 126 patients underwent bariatric surgery and follow-ups until the time of the data collection in this investigation (July 2022), while the remaining are going to undergo surgery in the near future. The target groups of this analysis are 31 and 70 patients who were already dealing with type 2 diabetes and prediabetes, respectively, before the operation.

The selected patients with obesity had been affected by different obesity complications, especially, hypertension, type 2 diabetes, OSAS, and dyslipidemia, which were the focus of this clinical trial. Prior to the decision about the candidacy for surgery, all other treatments such as weight-managing dietary interventions and exercises had been prescribed for these subjects several times and none of them had helpful, long-lasting, and practical results. Therefore, following various multidisciplinary meetings with dietitians, surgeons, internal medicine specialists, and psychologists, with or without the presence of the patients, the last and best treatment for them was decided to be bariatric surgery.

The study protocol and patients' rights were clarified to them before the start of the study and their identity was protected during the data analysis.

The baseline characteristics of these patients are available in Table 3.

Table 3. Baseline Characteristics of the Patients

Characteristics	**Measurements**
Age	
Mean	45
Range	19-71
Gender	
Male (n, %)	37, 30%
Female (n, %)	89, 70%
BMI, kg/m^2	
Mean (SD)	44.05 (6.32)

3.1.2 Interventions and Study Visits

This research project was a quantitative non-randomized clinical trial on patients with obesity and other complications, who were approved for bariatric surgery. As discussed in the mentioned multidisciplinary meetings the selected procedure of bariatric surgery for all of the subjects was Roux-en-Y Gastric Bypass (RYGB).

The data collection was carried out by exploiting, for each patient, the data available on the electronic records on the Padua University Hospital’s system (E-Health). These data consist of the data from various evaluations and experiments in different stages of the intervention and the follow-ups in the project.

During the sessions prior to the surgery, the pre-intervention evaluations including the collection of clinical history and comorbidities, anthropometric parameters such as BMI, the metabolic profile, complete blood chemistry tests, and their medications with dosages have been done for all 173 cases.

The total study period was over 2 years since the day of the surgery with assessments and examinations in the meanwhile. Before patients had undergone bariatric surgery, mostly in the morning on the day of the surgery, the complete blood chemistry and anthropometric measurements, namely weight, height and BMI had been collected and measured.

During the pre-intervention evaluations, the patients were divided into five different clusters based on their eating habits and meal frequencies. The subjects in each cluster had different eating behaviours and patterns and the number of meals during the day varied in the clusters, which could be influenced by their mental and psychological status. For instance, the individuals in the last two groups were dealing with emotional and psychological eating during the day in the fourth group and during both day and night in the sleep-time in the fifth group. These clusters and their meal frequency are shown in Table 4.

Table 4. Five different eating behaviours clusters in the patients

Eating bahaviour clusters	1. Three meals/day (breakfast, lunch, dinner)
	2. Variable (sometimes skip lunch)
	3. Five regular meals (breakfast, snack, lunch, snack, dinner)
	4. Eat all the time during the day
	5. Having contact with food even at night due to the psychological effects

3.1.3 Analysis of Serum and Plasma Samples

The blood chemistry tests were the measurement of HbA1c, blood glycemia, HDL, LDL, TG, total cholesterol, ALT, AST, etc. For diagnostic and screening purposes, blood chemistry measurements have been performed on venous plasma before and after the operation in the laboratory. The OGTT test was conducted in some patients.

In the post-surgical period, the patients were evaluated with follow-ups, one, three, six, and 12 months after the surgery. During the follow-up visits, the same assessments as the pre-intervention phase were repeated, in addition to the search for possible micronutrient deficiencies (vitamin B12, folate, iron, ferritin).

3.1.4 Outcome Measurements

The primary outcome of this study was to examine the relationship between eating habits and the effectiveness of bariatric surgery on the remission of type 2 diabetes based on the alterations in serum glucose and HbA1c concentrations before and following surgery.

3.1.5 Statistical Analyses

After evaluating the data, we would be able to figure out any probable relationship between various eating bahaviours and the remission of patients' comorbidities especially T2DM in patients with obesity following bariatric surgery. Therefore, it would be possible to realize if having a healthier diet and eating patterns could have positive effects on faster and long-lasting remission of T2DM.

The diagnosis criteria of four important diseases in this project, T2DM, hypertension, dyslipidemia, and OSAS are as described below:

During the post-intervention period, all patients who had normal fasting glycaemia and HbA1C levels or below the limits for the diagnosis of type 2 diabetes or prediabetes in the absence of pharmacotherapy, namely Metformin and Insulin therapy, as proposed by the ADA criteria, were considered in the remission of the disease.

Patients already in hypertensive therapy or who had blood pressure values compatible with the criteria proposed by the ESH / ESC (European Society of Hypertension and European Society of Cardiology) guidelines for the diagnosis of hypertension were considered hypertensive. The classes of drugs considered were: ACE inhibitors, sartans, β-blockers, α-lytics, calcium channel blockers, and diuretics.

The lipid profile was assessed according to the ESC / EAS (European Society of Cardiology and European Society of Atherosclerosis), in relation to the individual cardiovascular risk class for the diagnosis of dyslipidemia; patients already on lipid-lowering therapy at first were also considered with dyslipidemia. The drug classes considered were statins, ezetimibe, and fenofibrates.

All patients who were already on CPAP therapy or who had measured AHI values during polysomnography comparable to those proposed with the International Classification of Sleep Disorders (ICSD-3) criteria were considered to be affected by OSAS.

The data collection in this project was faced with a few limitations and difficulties. As an illustration, the medical records of the patients were not complete and easy to access. Some information regarding the blood chemistry of the subjects and their medical history was not found such as the level of HbA1c in some cases.

All data analyses were conducted using statistical programs such as Excel Microsoft and R programming.

3.2 The Methodology of the Project at the University of Bergen

3.2.1 Participants, Study Setting and Ethics

Three groups of patients were included in this study. One group received the dietary intervention and the other two groups underwent bariatric surgery.

The main focus of this study is on a group of candidates for bariatric surgery (RYGB), for whom a low-calorie diet was prescribed during the last week before the surgery. In total, 15 patients were included in this group during the years 2016 and 2017 at the Haugesund Hospital, Norway. The table of the baseline characteristics can be found below.

Table 5. Baseline Characteristics of the patients in the diet study

Characteristics	Measurements
Age	
Mean (SD)	44.26 (6.6)
Range	30-53
Gender	
Male (n, %)	6, 40%
Female (n, %)	9, 60%
BMI, kg/m^2	
Mean (SD)	42.04 (1.88)
Weight, kg	
Mean (SD)	122.98 (15.39)
Height, m	
Mean (SD)	1.70 (0.08)

The other two groups are the patients approved for Laparoscopic Sleeve Gastrectomy (LSG) at the Voss Hospital, Norway, or Roux-en-Y Gastric Bypass (RYGB) at the Haugesund Hospital, Norway in the period from September 2011 to November 2014 were invited to participate in the study. In total, 39 patients were included in the LSG group and 27 patients were included in the RYGB group. Of these, 21 patients and 24 patients in the RYGB group and LSG group respectively completed the assessments and tests. As an illustration, they completed the oral glucose tolerance test (OGTT) at both visits before and 7 days after the surgery. Participants were not required to specify why they withdrew from the study.

In Table 6, the characteristics of patients before the surgery are shown.

Table 6. Baseline Characteristics of the patients in surgery groups

Characteristics	Intervention Groups	
	LSG	RYGB
Age Mean (Range)	37.4 (35.0–49.9)	40.5 (36.0–53.3)
Gender Male (n, %) Female (n, %)	6, 40% 9, 60%	5, 31,25% 11, 68,75%
BMI, kg/m^2 Mean (Range)	43.1 (41.5–44.6)	42.1 (40.3–44.6)

3.2.2 Inclusion and Exclusion Criteria

Inclusion criteria for all the subjects were 18–60 years of age, body mass index (BMI) 40–45 kg/m2, fasting blood glucose < 7 mmol/L at the medical examination 2 to 3 months prior to the surgery, and living in close proximity to either Haugesund Hospital or Voss Hospital in Norway. Patients included in the study received the same pre- and post-surgery treatment with regard to medications and diet. The participating subjects were exempted from the weight loss program with a low-calorie diet before surgery otherwise recommended at these hospitals, and had no restrictions on their dietary intake before surgery and before the second study visit.

Exclusion criteria for all the subjects were smoking, known T2DM, treatment with anti-diabetic medication (for example Metformin and Glucophage), and complications during surgery leading to blood transfusion. Patients who become pregnant during the follow-up period will exit the study, but data collected in the follow-up until the pregnancy will still be included. In other words, they will be treated as "lost to follow up".

The study was designed as an intervention study with a quasi-experimental pre-test post-test design, as the criteria for the randomization of participants were not met. The research was conducted based on the guidelines and principles laid down in the Declaration of Helsinki and all procedures involving participants were approved by the Norwegian Regional Committee for Medical and Health Research Ethics (approval number: 2010/504). Prior to their participation, written informed consent was secured from all individuals. Furthermore, all data were subjected to anonymous analysis.

3.2.3 Interventions and Study Visits

The patients in the diet group were hospitalized one week prior to bariatric surgery and a specific diet was prescribed for them during this six-day period. The planned dietary intervention was an in-house low-calorie diet under the direct supervision of the dietitians and researchers at the hospital.

The bioimpedance data was collected every day in the diet group as they were hospitalized in the hospital. As an illustration, their weight, BMI and fat-free mass (FFM) were measured every day.

This energy-restricted diet consisted of a daily intake of approximately 400 to 600 kcal/day (550 kcal/day on average), with 4 meals per day which are mainly based on crispbread with different toppings. The approximate amount of protein was 44 g/day and the patients could drink plenty of water between meals. The focus will be on the patients getting an adequate intake of vitamins/minerals and supplementation with 1 Multivitamins or 1 Kostpluss and daily omega 3 intake is recommended. Plenty of fluid intake will be emphasized. Their meals were prepared based on the calculated amounts of the macro-nutrients (protein, carbohydrate, and fat) in the hospital's kitchen and provided to the hospitalized subjects [62]. The details of this low-calorie diet are indicated in Table 7.

Table 7. The foods in the meals of the low-calorie diet

Meal	
Breakfast	160g Q Skyr yogurt (pineapple & mango, blueberry & vanilla or wild berries & vanilla) (79 kcal, 16g protein)
Lunch and Dinner	2dL Soup of the day with Tine Styrk milk and Meritene nutritional powder (approx. 90kcal, 7g protein) Day 1. Toro original tomato soup Day 2. Toro creamy chicken soup with corn and spring onions Day 3. Toro Bergen fish soup Day 4. Toro original tomato soup with half a hard-boiled egg Day 5. Toro creamy chicken soup with corn and spring onions Day 6. Toro Bergen fish soup with prawns
Snacks After Lunch and After Dinner	(probably one of the alternatives – 4 yogurts and 8 x ½ YT distributed over the days) a. 1.25dL (1pk) Tine Biola Forest Berry Yogurt (110kcal, 4.3g protein) b. 1.5dL YT Smoothie (99kcal, 5.4g protein) c. 1.5dL YT Protein drink Drinking yogurt (99kcal, 6.1g protein)

As previously indicated, two distinct forms of bariatric surgeries were carried out: LSG and RYGB. In the case of LSG, approximately 80% of the stomach was removed, resulting in the formation of a slim gastric tube along the lesser curvature. Conversely, RYGB involved the creation of a small stomach pouch just below the cardia, which was then connected to the mid-jejunum. This surgical procedure effectively bypassed the remaining portion of the stomach and the proximal intestine in terms of nutrient flow.

The total study periods in all the patient groups were seven days, with assessments and examinations the day before the interventions and six days after surgery and diet. Assessments were conducted in the morning after fasting during the night before. The patients were instructed not to eat or drink anything except water after 22.00 the previous day. The body impedance assessments were done every day during the 7-day hospitalization in the patients of the diet study.

Body height and body weight were measured on the day and 6 days after surgery and every day in the diet therapy. Blood samples were collected the day before and six days after the interventions, and the staff complied with a strict protocol for pre-analytical sample handling to eliminate differences in sample handling at the two hospitals and over time.

During both assessments, a standardized Oral Glucose Tolerance Test (OGTT) was administered, consisting of 75 grams of glucose dissolved in 100 mL of lukewarm water. Patients were directed to consume this solution within 10 minutes on the day preceding the surgical procedure, as it was anticipated that they would need a minimum of 10 minutes to drink this quantity six days after surgery. Blood samples were obtained before (while fasting) and 30 and 120 minutes after consuming the glucose solution. [62].

3.2.4 Analysis of Serum and Plasma Samples

The amount of amino acids, ketones and AGEs (Advanced Glycated End products) in the blood tests in all three groups of patients were measured to be used for the data analysis to realize the impacts of these biological factors on the result of bariatric surgery and diet and the difference between this result in these groups of patients.

Blood samples were collected using specific tubes: VACUETTE® TUBE 4 ml Z Serum Separator Clot Activator tubes from Greiner Bio-one, Austria, were used to isolate serum, while BD Vacutainer P800 8.5 ml tubes from Becton, Dickinson and Company, containing K2EDTA and a proprietary cocktail of protease, esterase, and dipeptidyl peptidase-IV inhibitors, were used to isolate plasma for the analysis of GLP-1, glucagon, and peptide YY (PYY) [62]. These tests were performed using standard procedures at the Laboratory of Clinical Biochemistry and the Hormone Laboratory at Haukeland University Hospital in Bergen, Norway. The reference values used were based on these laboratory standards [62].

The following analyses were conducted on various serum components: glucose, insulin, amino acids, ketones and Advanced Glycated End Products (AGEs). The full list of the measured metabolites is shown below:

1. Amino acids: Alanine, Asparagine, Aspartic acid, Carboxyethyllysine, Carboxymethyllysine, Cystathionine, Fumarate, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Ornithine, Phenylalanine, Proline,

Sarcosine, Serine, Threonine, Tryptophan, Tyrosine, Valine, β-Alanine, β-Aminoisobutyrate

2. Ketones: Acetoacetate, β-Hydroxybutyrate
3. AGEs: α- Hydroxybutyrate, Carboxymethyllysine (CML), Carboxyethyllysine (CEL)

Among these, the most important metabolites with the most significant changes following the interventions were analyzed.

For the analysis of serum non-esterified fatty acids (NEFA) and albumin, the Cobas c111 system from Roche Diagnostics GmbH in Mannheim, Germany, was employed. The NEFA FS kit from DiaSys Diagnostic Systems GmbH, Germany, and the ALB2 kit for Cobas c111 were used for these measurements. Plasma concentrations of active GLP-1 were determined using the GLP-1 (active) ELISA kit (EIA-3056, DRG Instruments GmbH, Marburg, Germany), while plasma concentrations of PYY were determined using the Human PYY (total) ELISA kit. The between-day coefficient of variations (CVs) for these methods ranged from 2.1% to 11%. All samples were kept anonymous to the laboratory personnel [62].

In all the groups of patients, the following blood tests were taken after a minimum of 8 hours of fasting: Glucose, HbA1c, CRP, LPK, ALT, ALP, Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides.

3.2.5 Outcome Measurements

The primary outcome of this study revolved around examining alterations in fasting and postprandial serum glucose concentrations following RYGB or LSG surgery. Secondary outcomes were variations in circulating concentrations of insulin, glucagon, GLP-1, PYY, NEFA and lipids after surgery.

3.2.6 Statistical Analyses

Statistical analyses were conducted using RStudio version 2023.03.0+386 and Excel. Participants who did not finish the study were excluded from statistical analyses. The outliers in the data were recognized using the Rosner test. All comparisons were two-sided, and the P-value < 0.05 was considered statistically significant.

3.3 The Systematic Review at the University of Bergen

Besides the clinical trial at the University of Bergen, a comprehensive systematic review on “the short-term effects of a low-calorie diet or fasting on blood metabolites, especially amino acids in patients with obesity” was conducted by searching electronic databases, including "PubMed," with no restriction on the publication timeframe to cover all accessible evidence and literature, with a particular emphasis on the most up-to-date articles. The searches were carried out between February and April 2023.

3.3.1 Search Strategy

The Medical Subject Headings (MeSH) were used when necessary. We looked through the reference lists of relevant papers. The search was conducted in English using the following search terms:

((("Fasting"[MeSH Terms] OR "diet, reducing"[MeSH Terms] OR "Caloric Restriction"[MeSH Terms] OR "low-calorie diet"[Title/Abstract] OR "fasting-mimicking"[Title/Abstract] OR "fast*"[Title/Abstract]) AND ("obesity"[MeSH Terms] OR "obes*"[Title/Abstract])) OR "obesity/diet therapy"[MeSH Terms]) AND ("Clinical Trials as Topic"[MeSH Terms] OR "Clinical Trial"[Publication Type]) AND ("week*"[Title/Abstract] OR "wk"[Title/Abstract] OR "day"[Title/Abstract] OR "hour*"[Title/Abstract] OR "hr"[Title/Abstract] OR "short-term"[Title/Abstract] OR "acute"[Title/Abstract]) AND "Humans"[MeSH Terms] AND ("Metabolomics"[MeSH Terms] OR "Amino Acids"[MeSH Terms] OR "metabolite*"[Title/Abstract] OR "metabolomic*"[Title/Abstract] OR "amino acid*"[Title/Abstract])

3.3.2 Study Selection

We considered human short-term or acute clinical trials that involved patients with obesity undergoing low-calorie diets or fasting and the effects of these interventions on blood metabolites, especially amino acids were examined. Studies involving animals and those not categorized as original research, such as reviews, observational studies, comments, surveys, letters, editorials, expert opinions, abstracts from conference meetings, case reports, case series, systematic reviews, and meta-analyses were excluded from our analysis.

3.3.3 Inclusion Criteria

The studies were chosen based on these criteria:

1. Population: The subjects in the studies would be patients with obesity, who could be the candidates for bariatric surgery.

2. Intervention: Studies investigate the effects of a low-calorie diet or fasting on blood metabolites with the main focus on amino acids during a short period until a month of dietary intervention.

3. Study Design: The study designs of the papers could be a type of clinical trial such as randomized or non-randomized controlled trials.

4. Publication Language, Date and Geographic Location: The language of the articles should be English. The date of publication and the location of the studies are not limited.

5. Publication Type: In order for papers to qualify for inclusion, they need to have received acceptance for publication.

6. Exclusion Criteria: Studies with the main focus on supplementations, microbiota, genes, hormones and micronutrients as well as animal studies and clinical trials on children and women with pregnancy will be excluded from the review.

3.3.4 Data Extraction

The search results were downloaded and subjected to analysis, with duplicate articles being excluded using the Rayyan software. The process of article selection and evaluation involved an initial screening based on title and abstract. Subsequently, the full text of selected articles was thoroughly reviewed, and data extraction was performed once the inclusion criteria were confirmed.

The following information was taken from each study: authors, published year, study region, study design, sample size, participant characteristics, study length and measurement points, intervention and diet details, measured metabolites primary outcomes and limitations.

As information was collected and synthesized from previous research, the systematic review and meta-analysis were considered exempt from requiring ethical approval, and there was no involvement of patients or the general public in this review.

We included 10 articles for this systematic review. The flowchart of the identification and screening of the studies in the review is shown in Figure 5, which was made using the PRISMA flowchart template for systematic reviews.

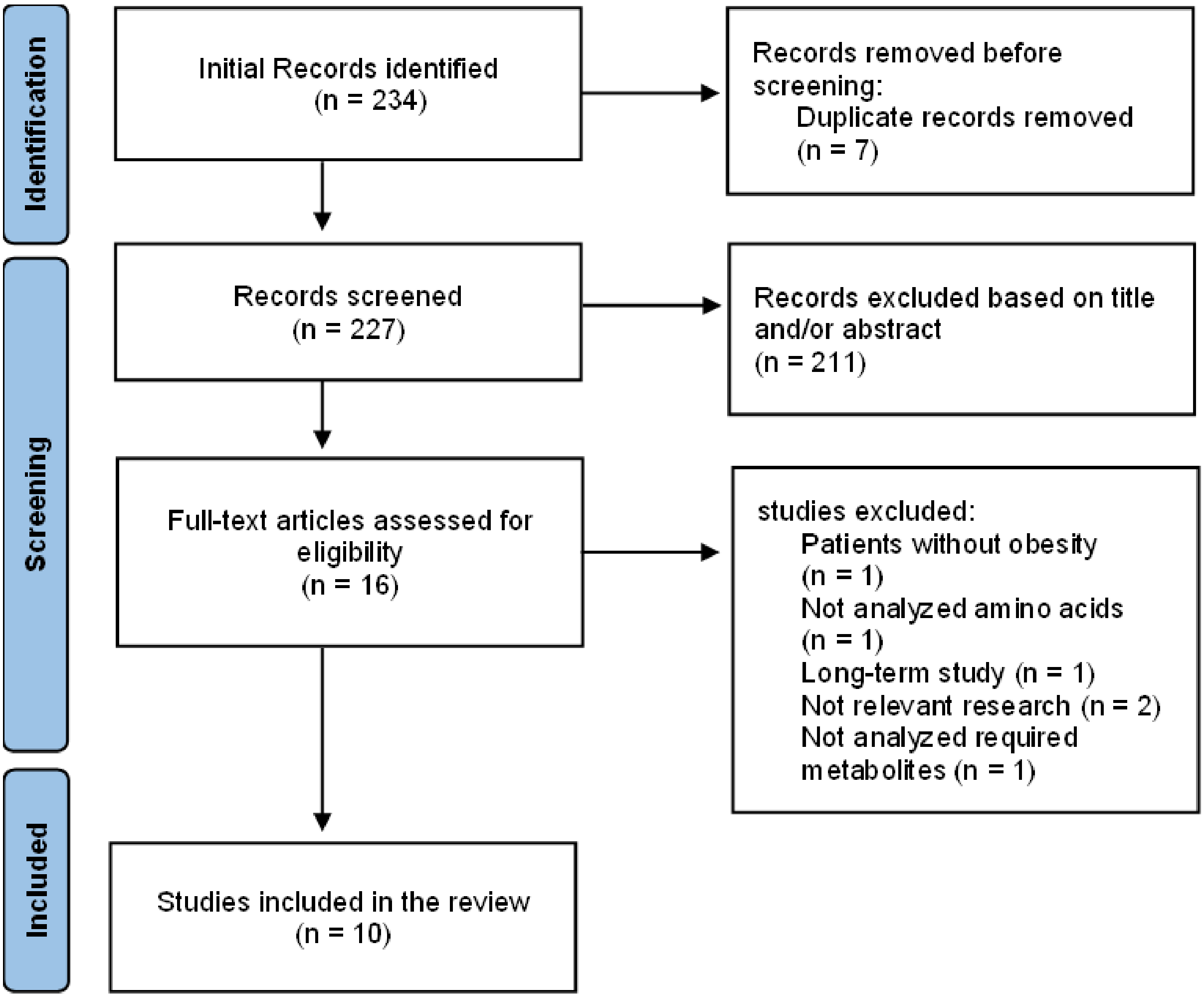

Figure 5. The flow chart of study selection

Besides these 10 studies, one other study was recommended to be reviewed as it was relevant research on people without obesity.

4. Results

4.1 Results of the Project at the University of Padua

(The Effects of Different Eating Behaviours on the Type 2 Diabetes Remission Following Bariatric Surgery in Patients with Obesity)

4.1.1 Patient Characteristics

The age and gender distribution of patients before and after the surgery were the same, as is shown in Table 3.

In addition to the baseline characteristics, alcohol consumption and smoking were analyzed. Of the total of 173 patients, 124 subjects did not drink alcohol or smoke, 24 subjects only smoked, 16 subjects only drank alcohol and 9 subjects both drank alcohol and smoked. An analysis of this data is visible in Figure 6.

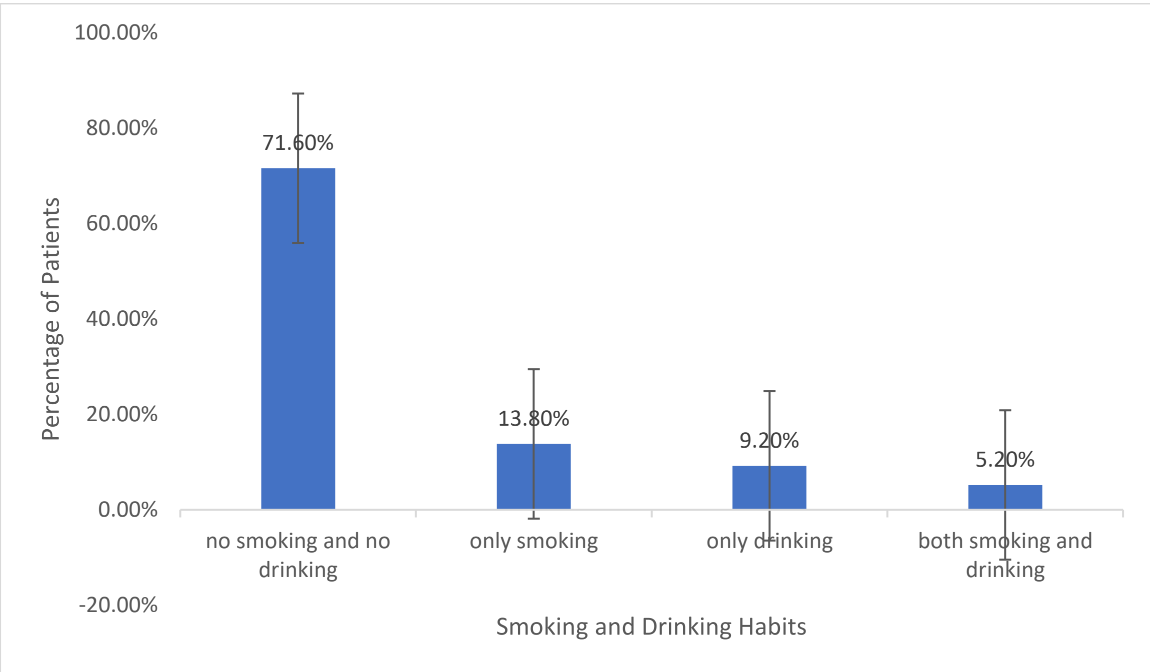

Figure 6. Analysis of patients drinking and smoking prior to surgery

Prior to bariatric surgery, the number of cases with and without physical activity was collected. Among 173 patients with obesity, 144 patients did not have any physical activity and the rest, 29 subjects, were physically active. This data was analyzed and is available in Figure 7.

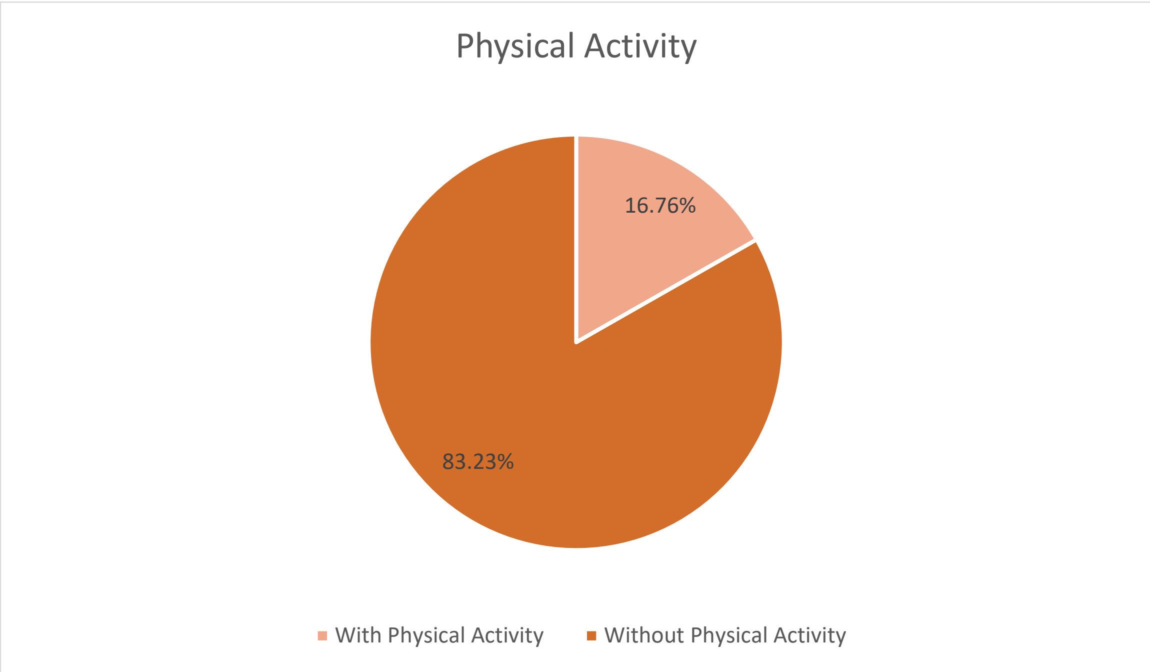

Figure 7. Analysis of the physical activity of the patients

4.1.2 Comorbidities of Patients Before Bariatric Surgery

The candidates for bariatric surgery are patients with obesity, who mostly are affected by obesity complications such as type 2 diabetes, dyslipidemia, hypertension and Obstructive Sleep Apnea Syndrome (OSAS). Our subjects were dealing with these comorbidities as well. Among our 173 candidates, 70 and 31 subjects had prediabetes or type 2 diabetes in turn. 76 patients had hypertension, 86 patients were living with dyslipidemia and 35 subjects were dealing with OSAS. 12 patients had all of these comorbidities.

Figure 8 shows the distribution of these diseases in our subjects.

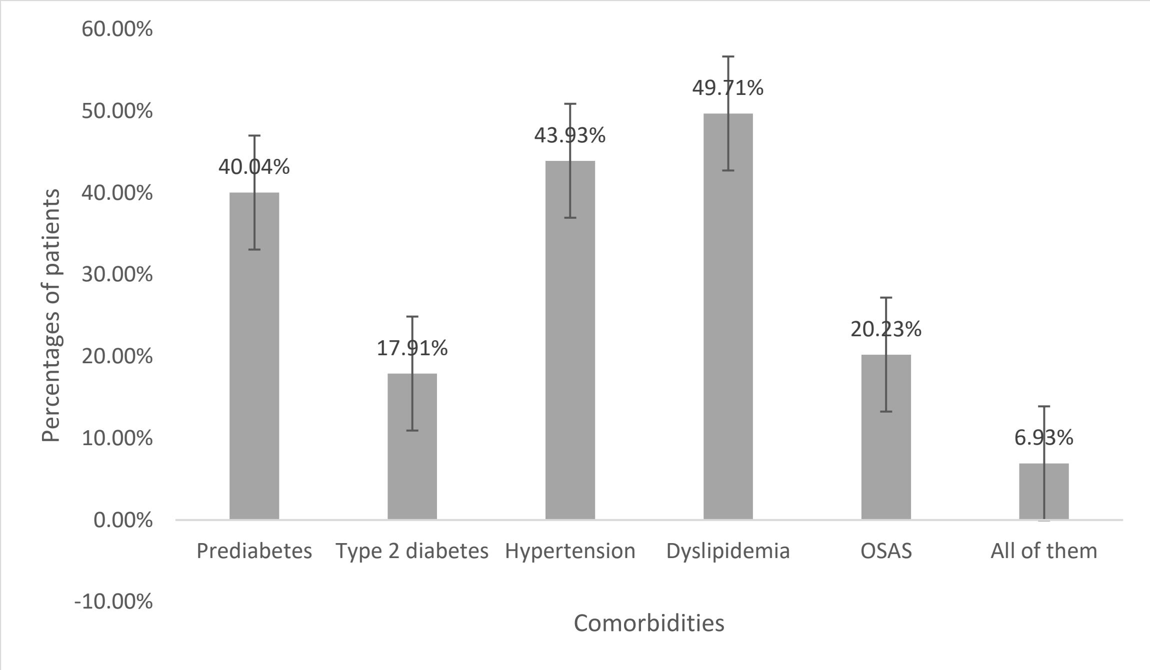

Figure 8. Complications of obesity in patients prior to the surgery

4.1.3 Eating Behaviours Clusters

As mentioned in the methods, subjects were divided into five clusters of eating patterns based on their eating habits prior to the surgery in order to analyze any possible relationships between eating behaviours and the remission of the diseases of the patients following bariatric surgery, especially type 2 diabetes and prediabetes.

The number of patients in each cluster and the population of the cluster's subjects with type 2 diabetes and prediabetes before and one year after the surgery are indicated in Table 8.

As the main focus of this research is on diabetes remission after the intervention, we analyzed the data of patients' blood glucose and HbA1c concentration before and in four follow-up sessions, one month, three months, six months and one year after the surgery. Besides, the data on the consumed medications were collected. Based on this collected data we analyzed the population of patients with or without type 2 diabetes and prediabetes one year following the surgery. This collected data is shown in Table 8 and Figure 9.

Table 8. The number of patients in different eating bahaviours clusters and the number of patients with type 2 diabetes (T2DM) and prediabetes (Pre-DM) in each cluster before and one year after bariatric surgery

		Before Bariatric Surgery		After Bariatric Surgery	
Cluster of Eating Habits	Total Number of Patients	Patients with T2DM	Patients with Pre-DM	Patients with T2DM	Patients with Pre-DM
1	21	2	3	0	2
2	27	4	12	0	3
3	19	2	5	1	1
4	38	6	16	2	3
5	20	4	11	2	3

The data in Table 8 was analyzed and is shown in Figure 9.

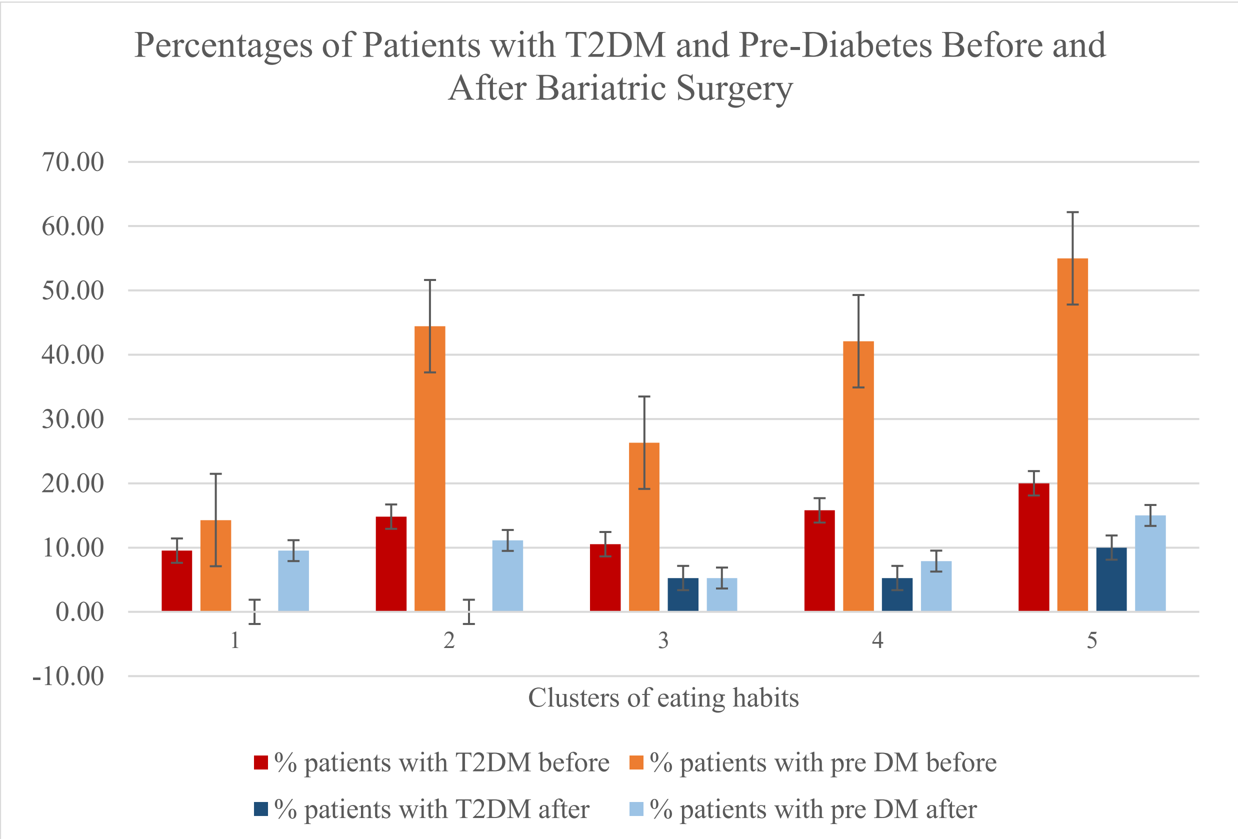

Figure 9. The percentages of patients with type 2 diabetes (T2DM) and prediabetes (pre-DM) before and after bariatric surgery in each eating behaviour cluster

As is shown in Figure 9 and Table 8, a large number of patients in all of the eating clusters were dealing with type 2 diabetes or prediabetes in the pre-intervention period. 10% to 20% of patients in each cluster had T2DM and 1% to 55% of patients had prediabetes before the surgery. As was already known and expected, most of the patients had partial or complete remission of their diseases.

Table 9 shows the percentages of success in the remission of type 2 diabetes or prediabetes in subjects of the eating patterns clusters. As is indicated, the patients with type 2 diabetes in clusters 1 and 2, who ate three times a day and had variable meals, had complete remission after the surgery. The patients in other clusters had success of 50% to 66% in the treatment of type 2 diabetes in the post-intervention period. The success in the remission of prediabetes in the first cluster was less than the other clusters, which had a high success of 70% to 80%.

Table 9. The percentages of success in the remission of type 2 diabetes or prediabetes in the eating behaviours clusters

Clusters of eating habits	**% of the success of the remission of T2DM**	**% of the success of the remission of pre-DM**
1	100	33.33
2	100	75
3	50	80
4	66.66	81.25
5	50	72.72

All in all, we could not find an obvious relationship between eating behaviours and the remission of diabetes after bariatric surgery, but this relationship is possible and more follow-ups are needed.

4.2 Results of the Clinical Trial at the University of Bergen

4.2.1 Patient Characteristics

There were no differences in gender distribution, age and BMI between the diet group and LSG and RYGB groups of patients (Tables 5 and 6). Also, there were no differences between the 2 groups of surgery with respect to fasting concentrations of the measured parameters before the surgery, including glucose and insulin. These values were different from the numbers in the diet group (Table 11).

The bioimpedance of the subjects in the diet group was measured every day during the hospitalization. The average percentage change in the data collected from the first to the last day is shown in Table 10.

Table 10. The average percentage changes of the bioimpedance data of the diet group on the last day of dietary intervention in comparison with the first day

Subject	Weight (%)	BMI (%)	FFM (%)
1	-3.81471	-3.81471	
2	-3.29761	-3.29761	-5.70033
3	-3.88098	-3.88098	
4	-3.22581	-3.22581	-5.98592
5	-3.48292	-3.48292	-3.31361
6	-5.0536	-5.0536	-6.27353
7	-3.68932	-3.68932	-6.50995
8	-3.45111	-3.45111	-3.04636
9	-3.57925	-3.57925	-3.56704
10	-1.91606	-1.91606	0.740741
11	-2.71528	-2.71528	-4.92063
12	-4.25844	-4.25844	-6.84524
13	-3.4632	-3.4632	-4.87395
14	-3.62117	-3.62117	-4.51895
15	-2.69266	-2.69266	-4.65116
Mean	-3.47614	-3.47614	-4.5743
SD	0.69176	0.69176	1.930698

4.2.2 Fasting Glucose and Insulin

There were decreasing patterns in both fasting glucose and insulin concentrations in all patient groups. The mean values and the range of the metabolites are shown in Table 11.

Table 11. The concentrations of Fasting Plasma Glucose (FPG) and insulin during fasting and 2 hours after the OGTT test in samples collected the day before and six days after the interventions

	Diet		RYGB		LSG	
	day 1	day 7	day 1	day 7	day 1	day 7
FPG (mg/dl)	6.25 (4.9-7.4)	5.31 (4.4-6.3)	5.5 (4.8-6.6)	5.03 (4.4-6)	5.21 (4.3-7.5)	5 (3.8-7)
2h-OGTT Glucose (mg/dl)	8.57 (4.4-13.5)	8.42 (4.6-11.6)	7.2 (4.8-11.3)	5.6 (3.5-8.2)	6.55 (3.2-12.7)	5.59 (4.2-7.5)
Insulin, fasting (mg/dl)	26.16 (9.7-45.6)	15.81 (3-23.7)	20.96 (5.9-47.5)	13.02 (2.2-30.7)	17.88 (8.3-40.8)	11.42 (1.8-21.3)
Insulin, 2h-OGTT (mg/dl)	149.59 (32.8->300)	128.86 (25.9->300)	132.86 (48-310)	73.19 (19.3-223)	76.43 (12-239)	69.62 (13-275)

4.2.3 Oral Glucose Tolerance Test (OGTT)

An OGTT (75 g glucose) was carried out the day before and six days after the interventions. In addition to the clinically relevant 120-minute blood sample, we included a blood sample at 30, 60 and 90 minutes. The 120-minute glucose and 120-minute insulin decreased in both surgery groups. there was also a decrease in these numbers in the diet group after the 2h-OGTT test (Table 11).

Table 12. The percentage changes in Glucose and Insulin before and after the interventions in Fasting and after 2 hours OGTT in the 3 groups of patients

	Diet	**RYGB**	**LSG**
FPG (%)	-0.1504	-0.08545	-0.04031
2h-OGTT Glucose (%)	-0.0175	-0.22222	-0.14656
Insulin, Fasting (%)	-0.39564	-0.37882	-0.3613
Insulin, 2h-OGTT (%)	-0.13858	-0.44912	-0.0891

As can be seen in Table 12, the percentage changes of glucose and insulin before and after the interventions in fasting situation and 120-minute OGTT tests were calculated and collected in a table. Glucose and insulin experienced reductions in all three intervention groups. The highest reduction in these values was in the diet group and the RYGB group.

4.2.4 Amino acids

Among all of the measured metabolites, there were some of them that had the most significant changes after the interventions to compare with pre-intervention concentrations, especially in the group of patients with the low-calorie diet.

Considering the measured amino acids, there were seven amino acids with significant changes, which was more than 20% change after the dietary intervention: Cystathionine, Glycine, Isoleucine, Leucine, Proline, Sarcosine, Serine.

There was little difference between the patterns of the metabolites in some of the patients and the mean patterns.

As is indicated in Figure 10, The concentrations of three amino acids, Isoleucine, Serine and Leucine experienced rises after six days of the interventions, on day 7. These increases were more significant in the diet group in comparison with the two surgery groups. The growth in the level of Isoleucine was more than the other amino acids in the diet group.

The amount of these metabolites went back to their initial levels or even lower than the initial levels one year after bariatric surgery through the RYGB procedure.

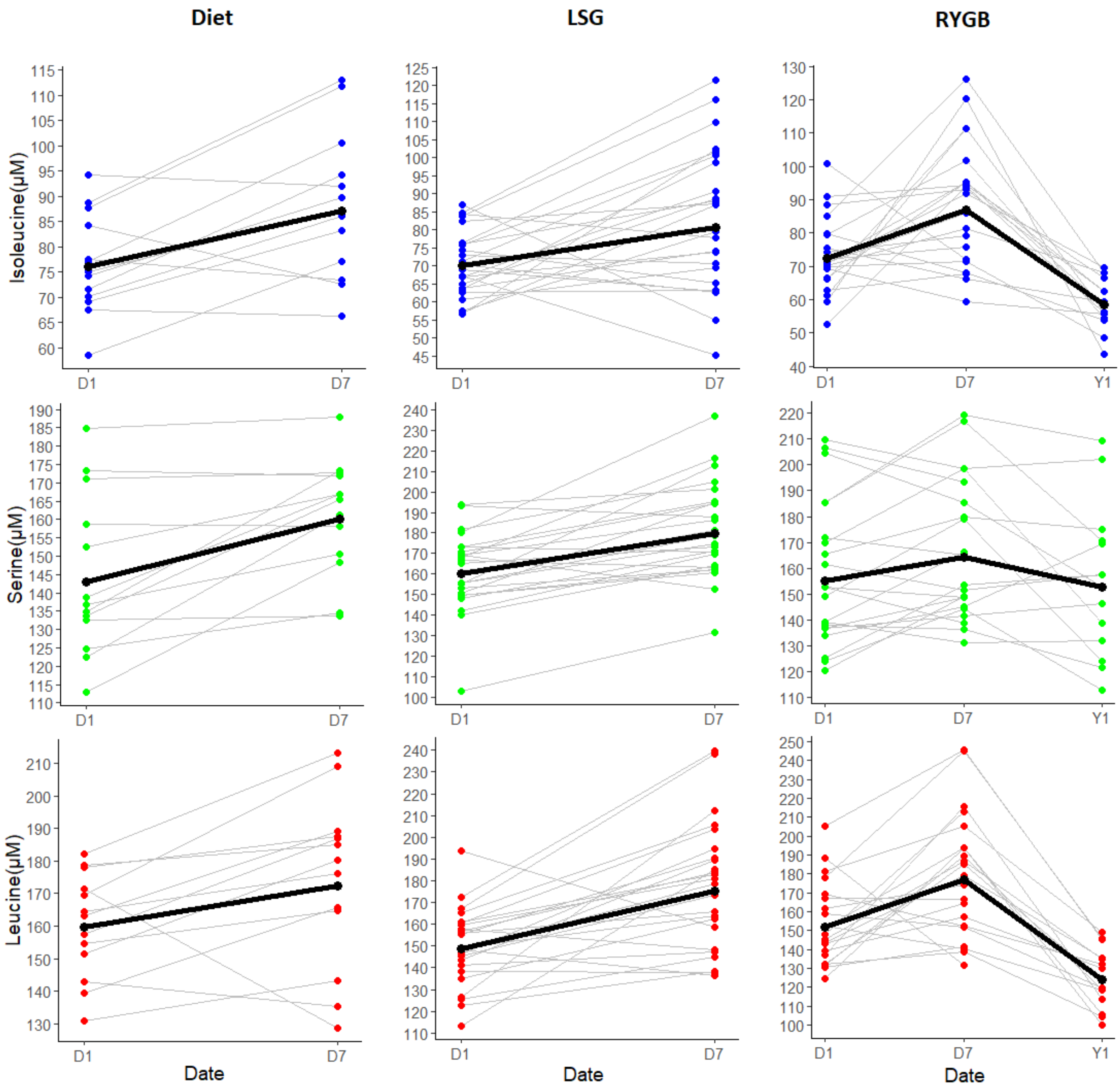

Figure 10. The alterations of Isoleucine, Serine and Leucine, before and after the interventions

The levels of the other four analyzed amino acids, Glycine, Cystathionine, Sarcosine and Proline declined in the post-intervention stage. These changes are available in Figure 11.

As is seen in Figure 11, the level of Glycine stayed almost stable, with a slight increase in the diet group following the interventions. There was also a modest growth in the level of this metabolite one year after RYGB.

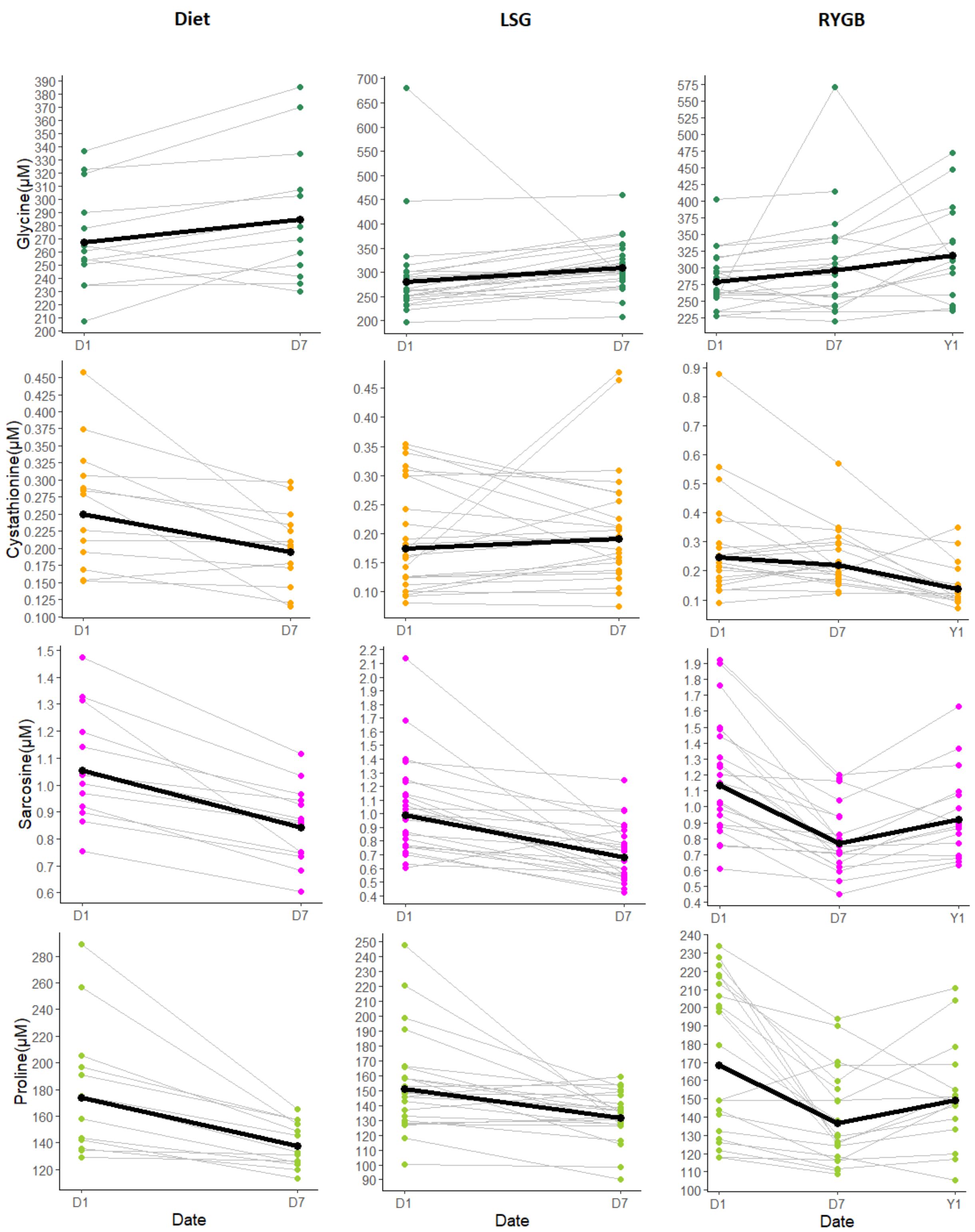

Figure 11. The alterations of Glycine, Cystathionine, Sarcosine and Proline, before and after the interventions

The concentrations of the other amino acids dropped six days after the interventions, especially since this decrease was more noticeable in the levels of Proline in the diet group.

The amounts of Sarcosine and Proline elevated to approximately their initial concentrations following the significant decreases in a week after RYGB. These changes in the levels of Glycine and Cystathionine were with modest increase and decrease to higher and lower than their amounts before the surgery respectively.

4.2.5 Ketones

As ketones have important roles in metabolic disorders, especially obesity, we measured and calculated the concentrations of two of the most important ketone bodies, Acetoacetate (AcAc) and β-Hydroxybutyrate (bHB), which is the most abundant ketone body. We also measured the total amounts of ketones in our three groups of patients.

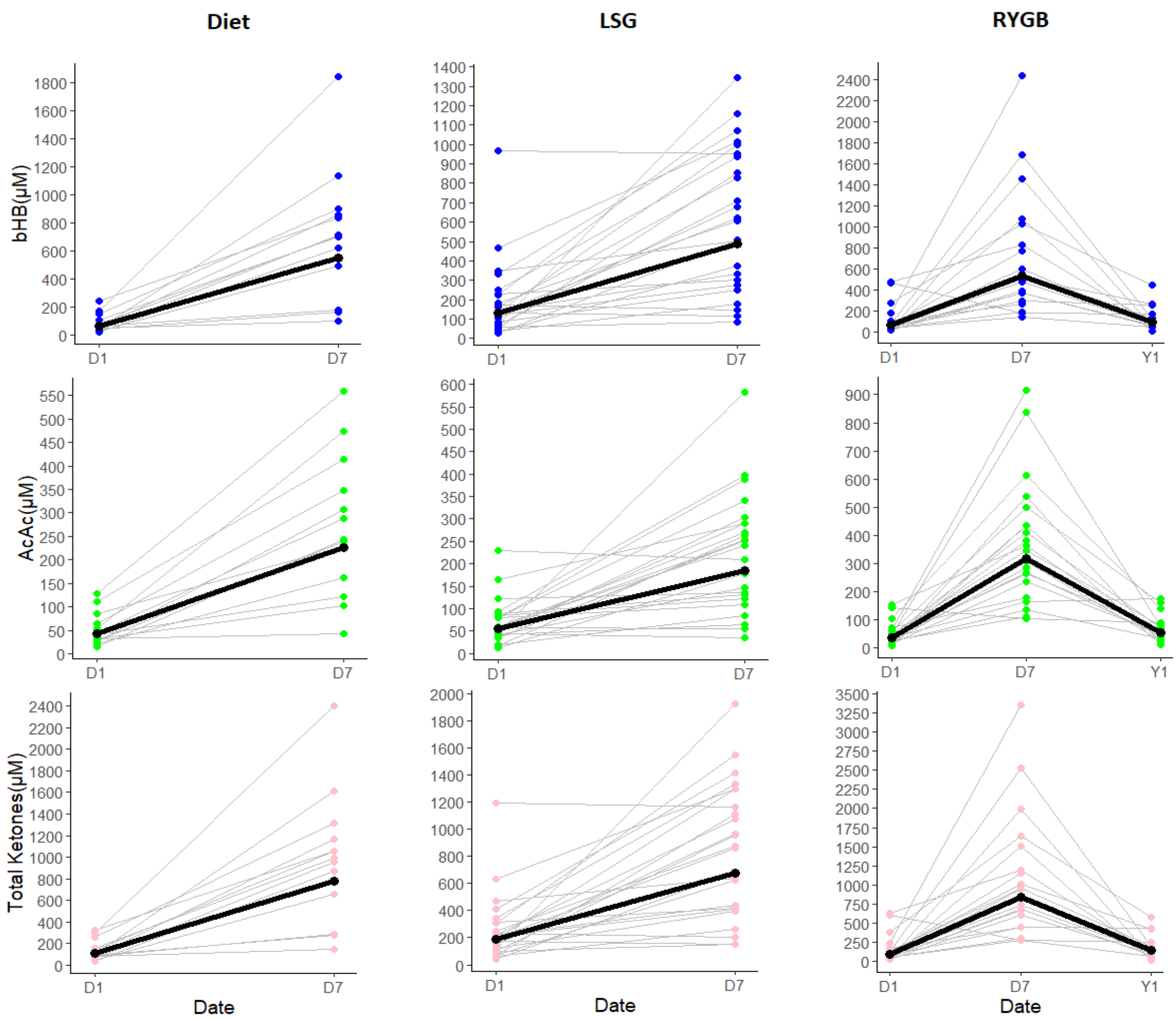

Figure 12. The alterations of Acetoacetate and β-Hydroxybutyrate, before and after the interventions

Because of the low energy intake in both the low-calorie diet and after bariatric surgery, the levels of ketone bodies in the blood increase, which will go back to the starting point after a while and following receiving the normal diet and energy intake.

To illustrate, as is shown in Figure 12, the concentrations of Acetoacetate and β-Hydroxybutyrate noticeably boosted on day 7 of the diet study and six days after bariatric surgery. These levels went back to the onset levels, which were almost zero in a year after RYGB surgery.

There were also some outliers in the numbers of β-Hydroxybutyrate and total ketones.

4.2.6 Advanced Glycated End Products (AGEs)

The concentrations of Advanced Glycated End Products (AGEs), including α-Hydroxybutyrate (aHB), Carboxymethyllysine (CML) and Carboxyethyllysine (CEL) were measured and analyzed as well.

Carboxyethyllysine (CEL), among the advanced glycation end products (AGEs), has received less research attention compared to its counterpart, CML. Both CML and CEL concentrations tend to increase as individuals age and have important roles in the development of age-related chronic conditions such as diabetes, cardiovascular disease, Alzheimer's disease, and kidney dysfunction.

α- Hydroxybutyrate (aHB) is an early biomarker of insulin resistance and impaired glucose regulation that seems to arise due to increased lipid oxidation and oxidative stress.

As indicated in Figure 13, the concentrations of CML and CEL dropped significantly after six days of dietary therapy and had marginal decreases six days after the surgeries. These levels rose to their starting amounts one year after the RYGB surgery.

The alterations in the levels of α- Hydroxybutyrate (aHB) were different and increased noticeably until one week after the interventions and dropped back to their initial level a year following RYGB.

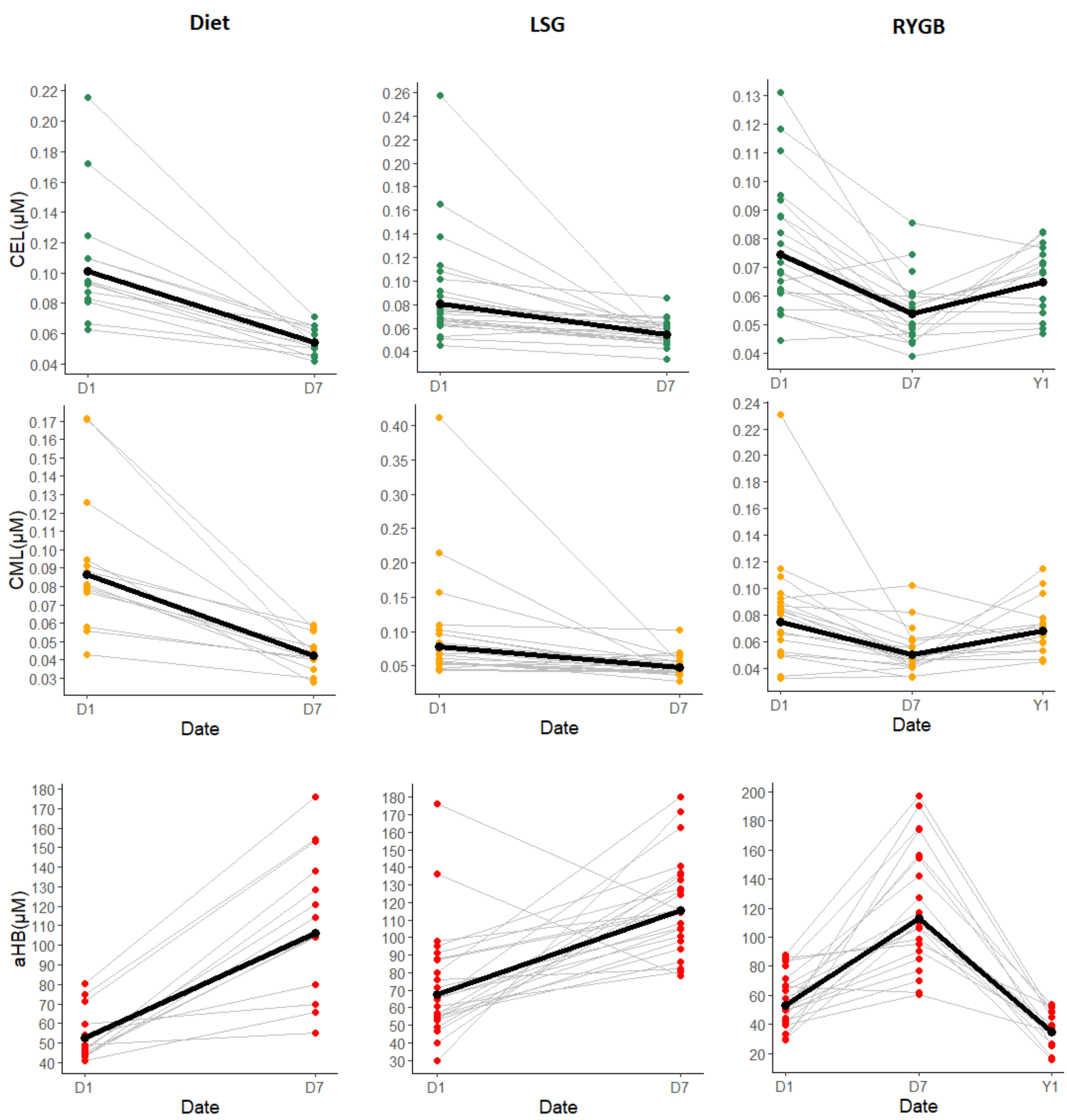

Figure 13. The alterations of Advanced Glycated End Products (AGEs), including α-Hydroxybutyrate, CML and CEL, before and after the interventions

4.2.7 α-ketoglutarate (aKG)

α-ketoglutarate (aKG) is a keto acid of the Krebs cycle formed from isocitrate. Circulating α-ketoglutarate has been associated with heart failure, obesity, diabetes and ageing.

The changes in the levels of the metabolite were analyzed and are indicated in Figure 14.

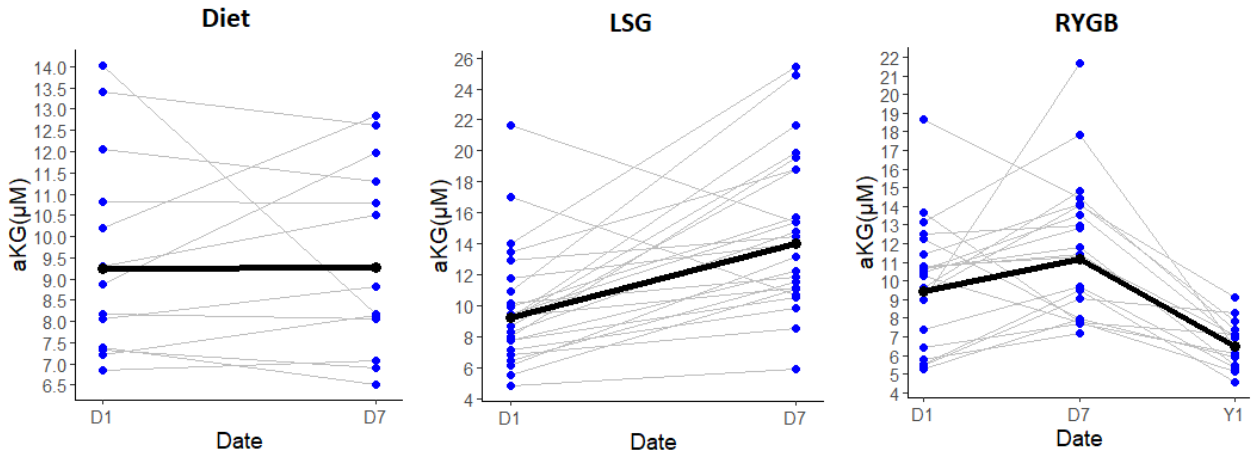

Figure 14. The alterations of α-ketoglutarate (aKG) before and after the interventions

The concentrations of aKG remained the same in the diet group although had an increase in the surgery groups. Its level dropped significantly to lower than the amount on the day of the surgery one year after the RYGB procedure.

4.3 Result of the Systematic Review at the University of Bergen

Summaries of selected studies in the systematic review are shown in the following tables. Among the chosen 11 studies, five articles analyzed the amino acid levels in the blood samples of people with obesity after a low-calorie diet (<1000 kcal). The summary of these studies is shown in Table 13.

As can be seen in Table 13, in some studies the concentrations of amino acids, especially Branched Chained Amino Acids (BCAA) were higher in individuals with obesity and type 2 diabetes than in the lean group [63]. The results following dietary and surgical interventions varied. The concentration of the examined amino acids in the study carried out by Lips MA et al. did not change three weeks after a very low-calorie diet (VLCD) and Gastric Bypass surgery (GB), while decreased three weeks after the RYGB surgery, which proposed to be caused by the surgery procedure.

The alterations of amino acids were different in other studies. For instance, in the clinical trial conducted by Vazquez JA et al., the levels of amino acids after low-calorie diets decreased except that of Glycine and Threonine in patients with morbid obesity. Besides, the enhancement in the amounts of ketones and the decrease in the amount of glucose were measured [64].

The level of fasting and postprandial amino acids following RYGB surgery and calorie-restricted diet in subjects with obesity dropped although ketones had an increasing trend two weeks after the interventions in the Non-Randomized Clinical Trial conducted by Khoo CM et al. [65].

In another Randomized Clinical Trial carried out by Hoffer LJ et al. on patients with moderate obesity, Circulating alanine decreased, leucine increased, β-hydroxybutyrate increased and glucose and insulin decreased three weeks following very low-calorie diets [66].

Different types of amino acids were measured in the clinical trial by Gougeon-Reyburn R et al., which analyzed the effects of two weeks of hypoenergetic diets in individuals with obesity. The BCAAs decreased significantly after one of the diets and remained the same and the blood glucose levels decreased after the other diet [67].

Table 13. Summary of the studies analyzing blood amino acids, glucose and ketones levels in subjects with obesity following a low-calorie diet

Author, year, Study region Study design	Sample size and participant characteristics	Number of patients in Interventions	Study length and measurement points	Types of diets	Measured metabolites	Primary outcomes	Limitations
Lips MA et al.[63], 2014, Netherlands Controlled Clinical Trial	32 females with obesity and T2DM (oral medication only, Average duration of diabetes: 3.8±0.7 years) (DM-VLCD: Age: 50.8±2.1, BMI: 40.2±1.9 kg/m2) (DM-RYGB: Age: 51.3±1.9, BMI: 43.5±1.1) 30 Normal Glucose-Tolerant (NGT) obese females (NGT-GB: Age: 46.3±1.9, BMI: 43.1±0.9) (NGT-RYGB: Age: 48.6±1.6, BMI: 44.2±0.8) Control: 12 lean	DM-VLCD (n = 12) DM-RYGB (n = 15) NGT-GB (n = 11) NGT-RYGB (n = 16)	after ≥10 h overnight fasting a month before Baseline 3 weeks after 3 months after the intervention	VLCD: (commercial high-protein, low-calorie meal) (600 kcal/day in total) the first 3 weeks Up to 2 months: expand their intake with vegetable and light dairy products (800 kcal/day in total).	29 detected metabolites (Glucose, leucine, valine, isoleucine, 2-aminoadipic acid, glutamic acid, kynurenine, asparagine, histidine, tryptophan, lysine, methionine, threonine, glutamine, ornithine, glycine, serine, sarcosine, Phenylalanine, tyrosine, alanine, proline)	BCAA levels tend to be higher in NGT subjects with obesity and are significantly higher in T2DM subjects compared with lean subjects. Weight loss by VLCD and GB did not result in lower plasma BCAA concentrations (in comparison with RYGB)., The decrease in BCAA levels after RYGB is predominantly caused by the bypass procedure and is not due to weight loss.	low number of subjects in the comparison, short-term follow-up to dissociate the effect of the intervention from the effect of weight loss

	healthy females (Age= 49.2±1.8, BMI = 21.7±0.5) dropouts: 8						
Vazquez JA et al.[64], 1992, USA Clinical Trial	16 healthy women with morbid obesity 2 groups: (similar in age and BMI): Subjects on the ketogenic diet: Mean age: 45±4, Mean BMI: 47±2 Subjects on non-ketogenic diet: Mean age: 43±5, Mean BMI: 49±4	Ketogenic Non-ketogenic diet	31 days: Run-in period: 3 days Diet: 28 days (measurements: baseline, 14 and 28 days)	Ketogenic diet: 590 kcal/day, Isonitrogenous nonketogenic diet: 590 kcal/day	13 AAs (Alanine, Glutamic acid, Glutamine, Glycine, Isoleucine, Leucine, Methionine, Phenylalanine, Serine, Threonine, Tyrosine, Valine) Ketones (β-hydroxybutyrate and AcAc) Glucose	The plasma concentrations of all AAs decreased except that of Glycine and Threonine increased. β-hydroxybutyrate and AcAc increased, more significantly in the ketogenic diet Glucose decreased in both groups, but more significantly in the ketogenic diet	(1) short (10 days) duration of treatment, (2) dissimilarity in amounts of protein, calories, or minerals
Khoo CM et al.[65], 2014, USA Non-Randomized Clinical Trial	RYGB surgery: 10 subjects with obesity (Mean age: 46.3 ± 6.6, Mean BMI: 44.0 ± 2.8): 7 females Calorie Restriction (CR): 10 control subjects matched for	10 Bariatric Group 10 CR group	Baseline 10-14 days post-intervention + measurement during fasting and 2hr	Four 8-oz cans of Ensure High Protein/day in CR group: 920kcal/day	AAs (proline, histidine, valine, BCAA, aromatic AA, serine, aspartate, ornithine, citrulline, glycine, glutamine,	Significant reductions in fasting proline, histidine, valine, BCAA, aromatic AA and total AA were observed after RYGB, but not with CR. Fasting alanine was reduced significantly in both groups. the mean postprandial responses for valine, proline, histidine,	Static metabolite profiles can identify perturbations in fuel metabolism, but they do not provide definitive information about metabolic flux.

	age, sex and BMI (Mean age: 49.6 ± 3.5, Mean BMI: 45.6 ± 2.4): 7 females		postprandial		methionine, alanine), Ketones (β-hydroxybutyrate) Glucose and Insulin	serine, aspartate, ornithine, citrulline, and BCAA were highly significant after both interventions. However, the mean postprandial responses for phenylalanine, glycine, glutamate/glutamine and aromatic AA were significant with RYGB, but not with CR. The postprandial trajectories for valine, leucine/ isoleucine, phenylalanine, tyrosine, proline, serine, methionine, and arginine were significantly altered after RYGB, but not after CR. Almost all amino acid levels were significantly lower after RYGB than CR at the end of meal challenge. Both interventions resulted in significant increases in fasting total ketones and β-hydroxybutyrate, with a trend towards larger responses with RYGB. The postprandial trajectories for glucose and insulin showed a steeper increase after RYGB, but not after CR.	

Hoffer LJ et al.[66], 1984, USA Randomized Clinical Trial	17 young women with moderate obesity in good health and receiving no medication Mean Age: (25±4 in 1.5 diet group, 26±4 in 0.8 diet group)	VLCD (1.5 g/kg IBW/day protein): 9 subjects VLCD (0.8 g/kg IBW/day protein): 8 subjects	Control: (run-in period with maintenance energy) 4 days Baseline diet: 3 weeks 8 weeks (in some cases)	2 VLCD (559±79 kcal/day in 1.5 diet and 501±39 kcal/day in 0.8 diet) the difference in the amount of protein (1.5 g and 0.8 g) and carbohydrate	plasma leucine plasma alanine, β-hydroxybutyrate whole blood glucose and insulin	Circulating alanine decreased with both diets. leucine increased, at 3 weeks of dieting. The decrease in alanine and increase in leucine plasma concentrations were significantly greater with the 1.5 diet. β-hydroxybutyrate increased Circulating glucose and insulin decreased with both diets	
Gougeon-Reyburn R et al.[67], 1989, Canada Clinical Trial	11 healthy adults with obesity and without diabetes (6 women and 5 men) Age: 20-51 Mean BMI: 41.3 ± 2.0 kg/m2	Protein diet: 6 subjects (4 men, 2 women) Carbohydrate diet: 5 subjects (1 man, 4 women)	2-5 days: baseline (weight-maintaining diet) 2 weeks: hypoenergetic diets 2-4 weeks: total fast 2 weeks: refeeding	baseline (weight-maintaining diet: ≥2500 kcal) hypoenergetic diets: 400 kcal/d (protein alone or glucose polymer) refeeding period: first 9 days: 800 kcal/d then: 1000 kcal/d	AAs: Histidine, Lysine, Ornithine, Alanine, Isoleucine, Leucine, Phenylalanine, Tyrosine, Valine, Glycine, Serine, Threonine Blood glucose	Alanine: decreased significantly after glucose and remained low after protein. Glycine: increased and reached elevated values typical of prolonged starvation after both diets. Serine: increased after protein and remained high after glucose. The BCAAs decreased significantly after protein to levels not different from those at baseline; no change after glucose. Threonine: did not change with the 400-kcal diets, showed a significant increase with fasting. Plasma glucose decreased	Values are not presented for tryptophan, glutamine, glutamate, asparagine, and aspartate because they are not reliably determined by the sampling and analysis procedures employed.

Besides, the blood glucose levels were investigated in three other studies on subjects with obesity following a low-calorie diet (<1000 kcal). The summary of these studies is available in Table 14.

A Randomized Clinical Trial on patients with obesity was done by Arciero PJ et al. by analyzing a low-calorie diet. The concentration of plasma glucose was significantly decreased ten days after the intervention [68].

The same result, lowered plasma glucose, happened in the other two studies. One of which was a Cross-over Randomized Double-Blind Placebo controlled trial done on patients with overweight and obesity undergoing three weeks of a very low-calorie diet[69]. The other one was a Randomized Clinical Trial analyzing four weeks of a very low-calorie diet in patients with moderate obesity [70].

Two articles conducted clinical trials on dietary interventions with a total daily calorie intake of more than 1000 kcal and analyzed the amino acid levels. These studies were three-week caloric-restricted dietary interventions on people with healthy weight and obesity. In both research studies, the concentrations of most of the amino acids rose followed by dietary therapy and weight loss [71], [72]. The summary of these articles is shown in Table 15.

The last study, which was recommended by colleagues, is research on individuals with normal weight undergoing fasting and the concentrations of amino acids were analyzed. The summary of this study is indicated in Table 16. As can be seen, this study is a Highly Controlled Human Trial carried out by Krug S et al. on healthy individuals with normal weight undergoing fasting for a short period of time, 36 hours. The levels of 275 metabolites, including amino acids were analyzed. Although the concentrations of BCAAs increased, the levels of most of the amino acids dropped or did not change following the intervention [73].

Table 14. Summary of the studies analyzing blood glucose and glycemia levels in subjects with obesity following a low-calorie diet

Author, year, country Type of Study	Number and type of patients (sample size)	Number of patients in Interventions	Duration of the study and measurement points	Types of diets	Measured metabolites	Primary findings	limitations
Arciero PJ et al.[68], 1999, USA Randomized Clinical Trial	9 men with obesity 7 women with obesity With: IGT (n=9) or mild Type 2 diabetes mellitus(n=7) Mean age: LCD:53±1, ET: 51±2	8 patients on a low-calorie diet (LCD): 3 women and 5 men 8 patients on the exercise training (ET) group: 4 women and 4 men	3 days: weight-maintaining diet + 10 days: interventions Measurements: Before and after the interventions	low-calorie diet (LCD): 50% of the calories required to maintain energy balance Mean energy: 1154±53 kcal/day	Fasting plasma glucose, plasma insulin	Fasting plasma glucose levels were significantly reduced by both the LCD and ET programs	Not measuring AAs levels
Merra G et al.[69], 2017, Italy Cross-over Randomized Double-Blind Placebo Controlled-Trial	age: 18 – 65 BMI ≥25kg/m2 percentage of body fat (PBF) ≥ 25% for males, and ≥ 30% for females	three groups: intervention group (IG): VLCKD1: 20 subjects two control groups (CG1 and CG2): VLCKD2 and VLCKD3:each group 20 subjects	baseline 3 weeks	3 types of VLCKD: (difference in type of protein) Females: 450-500 kcal/day Males: 650-700 kcal per/day	Glycemia	favorable acute effects on some risk factors, such as glycemia, inflammatory markers	small number of participants, the follow-up was short, lacked in some evaluation of biomarkers, such as ketogenic bodies and AAs
Hoffer LJ et al.[70], 1984, USA	11 healthy young women with moderate obesity	6 subjects: Diet A 5 subjects: Diet B	4 weeks the first four days subject received a	2 very low-calorie weight-reduction diets:	Serum glucose	Serum glucose decreased	No data about plasma AAs

Randomized Clinical Trial	receiving no medication (Mean age: 26 + 5 Mean weight: 87.0 ± 11.2)		formula diet providing maintenance energy (130% basal energy expenditure measurements: during the control period and after 3 weeks of dieting	Diet A (360 kcal) + 1.5 g egg protein/ kg IBW + no carbohydrate Diet B (340 kcal) + 0.8 g egg protein per kg IBW + 0.7 g carbohydrate / kg IBW		There was no significant difference between the results of the diets.	

Table 15. . Summary of the studies analyzing blood amino acids and glucose levels in subjects with obesity following a low-calorie diet (>1000 kcal)

Author, year, country Type of Study	Number and type of patients (sample size)	Number of patients in Interventions	Duration of the study and measurement points	Types of diets	Measured metabolites	Primary findings	limitations
Zou H et al.[71], 2020, China Uncontrolled Longitudinal Study, Clinical Trial	41 healthy patients (24 females and 17 males) Age: 30 ± 6 BMI < 28 kg/m2	All patients: calorie-restricted (CR) diet	Baseline: a one-week, run-in period a three-week CR dietary intervention	CR diet: ~60% calories of the recommended daily calorie intake Men: 1414.9 kcal/day Women: 1210.6 kcal/day	31 AAs and their derivations (2-Aminoadipic acid, Alanine, Asparagine, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Ornithine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine, β-Alanine)	levels of 13 blood AAs and their derivatives (such as α-aminoisobutyric acid, β -alanine, serine, glycine, lysine, and 2-aminoadipic acid (an intermediate in lysine metabolism)) significantly increased except tyrosine significantly decreased.	the measures of fat and lean mass before and after CR were lacking no control group there is no result for all 31 AAs
Moszak M et al.[72], 2018, Poland Controlled Clinical Trial	24 patients (10 men and 14 women) BMI: 34 - 49 kg/m2 (mean BMI: 40±4 kg/m2) Age: 24 – 66 Mean age: 46±12 years	All patients: hypocaloric diet	3 weeks	hypocaloric diet based on a 25–30% reduction of caloric dietary intake (2,016.0±281.7 kcal) (1,880.0±234.0 kcal for women; 2,220.0±219.1 for men)	Glucose 42 free AAs (33 AAs measured: (Alanine, Asparagine, Aspartic acid, β aminoisobutyric acid, β-alanine, Glutamine, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Ornithine, Phenylalanine, Proline, Sarcosine, Serine, Threonine, Tryptophan, Tyrosine, Valine)	the levels of 10 (α-amino-n-butyric acid, alanine, citrulline, glutamine, glycine, hydroxyproline, isoleucine, proline, sarcosine, and threonine) out of the 42 AAs measured were significantly higher after weight loss. The highest differences in serum levels: glycine, α-amino-n-butyric acid, proline, and glutamine. Only aspartic acid was lower after the program.	Not long-term Lack of measurements of some AAs

Table 16. Summary of the study analyzing blood amino acids levels in subjects without obesity following fasting

Author, year, country Type of Study	Number and type of patients (sample size)	Number of patients in Interventions	Duration of the study and measurement points	Types of diets	Measured metabolites	Primary findings	limitations
Krug S et al.[73], 2012, Germany Highly Controlled Human Trial	15 healthy, young, and normal weight Men Mean Age: 27.8±2.9 Mean BMI: 23.1±1.8	All the patients on the same challenge	4d challenge protocol, including 36 h fasting (6 challenges within 2 test periods, each lasting 2 days and 2 nights) Measurements during the 36 hr fasting	6 challenges: fasting, SLD, OGTT, PAT, OLTT, and stress (cold stress test)	275 metabolites quantified in plasma (14 AAs) (Isoleucine, Leucine, Valine, β - aminoisobutyrate) Glutamine, Glycine, Histidine, Isoleucine, Leucine, Methionine, Ornithine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine	Fasting for 36 h increased branched-chain amino acids in plasma Most of the 14 AAs had declined or stayed on the same levels	

The 11 studies in this systematic review showed some different alterations in the levels of amino acids. In most of the studies, the concentrations of Branched Chain Amino Acids (BCAA), which were higher in people with obesity decreased following both bariatric surgery and low-calorie diet.

The amounts of ketones, including Acetoacetate and β-Hydroxybutyrate, experienced significant increases after fasting and bariatric surgery as a result of the low-calorie intake in both interventions.

Furthermore, the levels of plasma glucose and insulin decreased after the interventions carried out in these articles.

The results of this systematic review and the clinical trials in this thesis produce almost similar outcomes and there is a consensus among the research studies, which is discussed and concluded in the next section of the thesis.

5. Discussion

5.1 Main Findings

This thesis consisted of relevant studies at the University of Padua and the University of Bergen. As already mentioned, the clinical trial at the University of Padua included candidates for bariatric surgery with obesity and different complications and eating behaviours prior to the surgery. The clinical trial at the University of Bergen included patients with obesity who underwent bariatric surgery or a low-calorie diet.

The results of the first study demonstrate that bariatric surgery is one of the best treatments for obesity, type 2 diabetes and other complications that are prevalent in individuals with obesity. The effects of different eating habits and patterns were analyzed by dividing the patients into five different clusters based on the patients' meal frequencies and patterns prior to bariatric surgery. The diabetes remission in these clusters was investigated and the results provide a new insight into the relationship between eating habits and post-operative diabetes remission, which could be possible although our data of follow-ups did not show obvious connections.

The results of the clinical trial and the systematic review at the University of Bergen indicate that although bariatric surgery is one of the best and fastest treatments for type 2 diabetes and obesity, less invasive treatments and interventions such as low-calorie diet could have the same results. In these studies, different blood metabolites such as blood amino acids, plasma glucose and ketones were analyzed a short time after the interventions. The assessments of the alterations of blood metabolites confirmed our hypothesis that both low-calorie diet and bariatric surgery have positive impacts on the remission of obesity and diabetes and the concentrations of these metabolites were on approximately the same level as a person in a healthy condition. Therefore, the result of bariatric surgery is possible following a low-calorie diet as well.

Both findings confirm the effectiveness of bariatric surgery and calorie-restricted dietary interventions in the remission of type 2 diabetes and obesity, which might differ from person to person based on their habits and lifestyle. These results are in line with our hypothesis.

5.2 Eating Behaviours and Diabetes Remission

These findings build on existing evidence of the effects of lifestyle and eating behaviours on the remission of type 2 diabetes. As is also concluded in the literature, eating behaviours are not just an aspect of patients' lives. they are influential and important components, which can significantly influence the success of weight loss and complications remission after bariatric surgery [74]. Continuous detection of these behaviours, both in pre- and post-operational assessments is a crucial tool for both healthcare providers and patients. It makes early detection and management of potential problems, and the development of sustainable, personalized strategies for achieving and maintaining weight loss and diabetes remission goals achievable [75].

The available research highlights that bariatric surgery candidates mostly indicate unhealthy eating patterns such as emotional eating, continuous snacking (grazing) such as our two last clusters of patients, night eating syndrome (NES) the same as the last cluster in our study, and intense food cravings and addiction. Moreover, these patterns appear to be more prevalent among bariatric surgery candidates when compared to the general population or those without overweight or obesity. Furthermore, literature has suggested that there are a few identifiable groups or 'clusters' of problematic eating-related behaviours experienced by these patients. It is necessary for further investigations into the prevalence, characteristics, experiences, and consequences of these disordered eating behaviours among individuals preparing for bariatric surgery [76].

5.3 Bariatric Surgery and Low-Calorie Diet

The results of our study should be taken into account when considering how bariatric surgery and low-calorie diets can have the same metabolic effects in a short while after the interventions. Restricted calorie intake not only leads to weight reduction but also improves insulin sensitivity, enhances glucose metabolism, and lowers circulating levels of ketones and inflammatory markers. These alterations collectively contribute to better glycemic control and overall metabolic health in patients with obesity and T2DM.

The existing literature also supports the fact that calorie restriction is the important factor behind the immediate metabolic improvements seen in patients with obesity and T2DM who undergo RYGB surgery [77].

5.4 Limitations of the Study

This study had some limitations regarding gathering data from the medical records of the patients. In some cases, the data in the records were not complete and some information was lost in the blood test data. This issue was mostly because some patients (also for logistical reasons) had their blood tests carried out at different hospitals using alternative diagnostic kits and the patients did not carry out the indicated blood tests to our center.

Besides, the lack of a control group in the clinical trials is important, as both research were non-randomized clinical trials and the patients were chosen between the candidates for bariatric surgery.

5.5 Future Research

Further research is needed to establish the effects of different eating behaviours such as meal patterns and frequencies and emotional eating on the results of low-calorie diets to compare it with the outcomes from the surgery study. This research might be the comparison on a long-term basis following the interventions.

There could also be a control group of patients with normal and healthy body weight, BMI and blood glucose levels undergoing the same dietary interventions as the patients with obesity and diabetes.

5.6 Conclusion

In conclusion, in our study, the effectiveness of bariatric surgery procedures, including RYGD and LSG in the treatment of obesity and the management of related comorbidities was confirmed, in particular with regard to type 2 diabetes mellitus. The short-term improvements induced by the surgical interventions on both anthropometric and metabolic parameters such as circulating amino acids, ketones and glucose appeared following the low-calorie dietary intervention as well.

Furthermore, it is worth exploring the potential significance of various preoperative eating behaviors in relation to the outcomes of bariatric surgery although further research on the long-term influences of eating habits is necessary to confirm this hypothesis.

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