Universitat Autònoma de Barcelona

Biomarkers of response to bariatric surgery

Enzamaria Fidilio

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Biomarkers of response to bariatric surgery

Doctoral Thesis

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Biomarkers of response to bariatric surgery

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Dr. Andreea Ciudin and Dr. Rafael Simó Canonge

CERTIFY:

That the thesis titled "Biomarkers of response to bariatric surgery" was realized under my direction, by Enzamaria Fidilio, to opt for the title of Doctor of Medicine.

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And for the record, we sign this document, at the request of the interested party,

Dr. Rafael Simó Canonge Dra. Andreea Ciudin

Barcelona, 2nd of December of 2021

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Abbreviations

ABCD: adiposity-based chronic disease

ACE: Angiotensin I converting enzyme

ADA: American Diabetes Association

ADIPOQ: Adiponectin, C1Q and collagen domain containing

ADRB3: Adrenoceptor beta 3

AEE: activity energy expenditure

AGRP: Agouti related neuropeptide

AGTR1: Angiotensin II receptor type 1

AIC: Akaike Information Criterion

AOO: age of onset of obesity

APOA2: Apolipoprotein A2

APOA5: Apolipoprotein A5

APOC3: Apolipoprotein C3

ASMBS: American Society for Metabolic and Bariatric Surgery

AT: adipose tissue

AUROC: area under the receiver operating characteristic

BDNF: Brain derived neurotrophic factor

BIA: bioimpedanciometry

BMI: body mass index

BS: bariatric surgery

CCDC93: Coiled-coil domain containing 93

CDKAL1: CDK5 regulatory subunit associated protein 1 like 1

CDKN2B: Cyclin dependent kinase inhibitor 2B

CGRS: clinical genetic risk score

CLOCK: Clock circadian regulator

CNR1: Cannabinoid receptor 1

CRS: clinical risk score

CVD: cardiovascular disease

DEXA: dual-energy x ray absorptiometry

DIT: diet-induced thermogenesis

DLW: doubly labelled water

EASO: European Association for the Study of Obesity

EBW: excess body weight

ELOVL6: Fatty acid elongase 6

EOSS: Edmonton obesity staging system

eREE: estimated resting energy expenditure

ESR1: Estrogen receptor 1

EWL: excess of weight loss

FFA: free fatty acids

FTO: fat mass and obesity-associated gene

GHRL: Ghrelin and obestatin prepropeptide

GLUT-4: Glucose transporter type 4

GNAS1: G protein alpha subunit 1

GNB3: G Protein Subunit Beta 3

GPS: genetic predisposition score

GRS: genetic risk score

GWAS: genome-wide association studies

HBE: Harris-Benedict Equation

HEC: hyperinsulinemic- euglycemic clamp

IBW: ideal body weight

IC: indirect calorimetry

ICD: international classification of diseases

IGF2: Insulin like growth factor 2

IKKβ/NF-κB: IκB kinase /Nuclear Factor-kappa B

IL-1B: Interleukin 1 beta

IL-6: interleukin-6

INSIG2: Insulin induced gene 2

IQR: interquartile range

IR: insulin resistance

IRS-1: insulin receptor substrate-1

JNK: c-Jun N-terminal kinases

LEP: Leptin

LEPR: Leptin receptor

LPL: lipoprotein lipase

MC4R: Melanocortin 4 receptor gene

MCP-1: monocyte chemotactic protein-1

MO: morbid obesity

mREE: measured resting energy expenditure

MSJ: Mifflin-St Jeor

MTCH2: Mitochondrial carrier 2

NAFLD: Non-Alcoholic Fatty Liver Disease

NEGR1: Neuronal growth regulator 1

NMR: nuclear magnetic resonance

OBEGENCGRS: OBEGEN clinical-genetic risk score

OSA: Obstructive Sleep Apnea

PAI-1: Plasminogen activator inhibitor-1

PCSK1: proprotein convertase subtilisin / kexin type 1

PI3K: phosphatidylinositol 3-kinase

PLIN1: Perilipin 1

POMC: Pro-opiomelanocortin

PON1: Paraoxonase 1

PPARA: Peroxisome proliferator activated receptor alpha

PPARG: Peroxisome proliferator activated receptor gamma

REE: resting energy expenditure

RYGB: Roux-Y gastric bypass

SAA3: serum amyloid A-3

SD: standard deviation

SG: sleeve gastrectomy

SIRT1: Sirtuin 1

SNPs: single nucleotide polymorphisms

SO: severe obesity

SOCS: suppressor of cytokine signaling

T2D: type 2 diabetes

TCF7L2: Transcription factor 7 like 2

TEE: total energy expenditure

TG: triglycerides

TMEM18: Transmembrane protein 18

TNF-α: tumor necrosis factor-α

TWL: total weight loss

UCP1: Uncoupling protein 1

UCP2: Uncoupling protein 2

UCP3: Uncoupling protein 3

VEGF: Vascular Endothelial Growth Factor

WC: waist circumference

WFS1: Wolframin ER transmembrane glycoprotein

WHO: World Health Organization

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SUMMARY

Obesity represents a major public health problem and its associated comorbidities have increased the economic burden for the health systems. Bariatric surgery (BS) is consider the most effect treatment for obesity, due to high rates of weight loss and remission of associated comorbidities, especially type 2 diabetes (T2D). However, there is a significant proportion of non-responders to BS; around 20–25% of patients that undergo BS do not achieve successful excess of weight loss (EWL), about 30–35% fail to maintain weight loss or experience significant weight regain. Additionally, about a 20–35% relapses of T2D after Y-de-Roux gastric by-pass (RYGB). At present, predictors of response to BS, based on anthropometric and psychosocial factors still lack of precision to fully differentiate responders from non-responders. A more comprehensive insight of obesity complexity has challenged investigators to discover new biomarkers able to identify beforehand those patients non-responder in order to design personalized medicine programs and optimize resources. On one hand, inheritance is responsible for 40–75% of all the causes of obesity, a percentage modulated by the epigenetic influence. On the other hand, energy balance is one the major determinants of weight loss. The following study aimed to investigate the utility of clinical-genetic predisposition score and the resting energy expenditure in the response to BS, in terms of weight loss and remission of T2D. For this purpose, we conducted 3 studies. A) A pilot study, as proof of concept, DNA sample was collected, for a genetic score development, from women with severe obesity that had have a RYGB, and they were stratified in 4 groups match by age and BMI; responders (EWL >70%), non-responders (EWL <40%), diabetes remission, diabetes non- remission, B) A validation study, multicenter, including men and women that had underwent sleeve gastrectomy (SG) or RYGB. DNA sample and clinical features was collected from subjects to develop a clinical-genetic score. And C) A prospective study, including patients with BMI >50kg/m^2, were followed- up 5 years after surgery, an indirect calorimetry (IC) was performed before and after surgery to evaluated the resting energy expenditure (REE). With the pilot study, we developed a genetic-based algorithm for the prediction of %EWL after BS and for T2D remission with high sensitivity and specificity. In the second study, the clinical-genetic score developed was a reliable method to predict the weight response after BS. Finally, the study of the REE showed that is drastically decreased very early after BS, and was able to predict insufficient weight loss and weight regain after 5 years of BS. In conclusion, this new biomarker approaches through genetic predisposition assessment, united to clinical data and the resting energy metabolism evaluation can help clinicians to personalize the therapeutic approach in severe obesity thus optimizing the limited health resources.

RESUMEN

La obesidad representa un importante problema de salud pública y sus comorbilidades asociadas han aumentado la carga económica para los sistemas de salud. La cirugía bariátrica (CB) se considera el tratamiento más eficaz para la obesidad, debido a las altas tasas de pérdida de peso y remisión de las comorbilidades asociadas, especialmente la diabetes tipo 2 (DM2). Sin embargo, existe una proporción significativa de pacientes que no responden a la CB; alrededor del 20-25% de los pacientes que se someten a CB no logran un exceso de pérdida de peso exitoso (EPP), alrededor del 30-35% experimentan una reganancia de peso significativa. Además, en alrededor de un 20 a 35% reaparece la DM2 después del bypass gástrico Y-de-Roux (BGYR). En la actualidad, los predictores de respuesta a CB, basados en factores antropométricos y psicosociales, aún carecen de precisión para diferenciar a los respondedores de los no respondedores. Una visión más completa de la complejidad de la obesidad ha desafiado a los investigadores a descubrir nuevos biomarcadores capaces de identificar de antemano a los pacientes que no responden. Por un lado, la herencia es responsable del 40-75% de todas las causas de obesidad, porcentaje modulado por la influencia epigenética. Por otro lado, el equilibrio energético es uno de los principales determinantes de la pérdida de peso. El siguiente estudio tuvo como objetivo investigar la utilidad del puntaje clínico-genético de predisposición y el gasto energético en reposo en la respuesta al CB, en términos de pérdida de peso y remisión de la DM2. Para ello, realizamos 3 estudios. A) Un estudio piloto, como prueba de concepto, se tomó una muestra de ADN, para el desarrollo de un puntaje genético, de mujeres con obesidad severa que habían tenido BGYR, y se estratificaron en 4 grupos emparejados por edad e IMC; respondedoras (EPP> 70%), no respondedoras (EPP <40%), remisión de diabetes, no remisión de diabetes. B) Un estudio de validación, multicéntrico, que incluyó a hombres y mujeres que se habían sometido a gastrectomía vertical (GV) o BGYR. Se recogieron muestras de ADN y características clínicas de los sujetos para desarrollar una puntuación clínico-genética. Y C) Un estudio prospectivo, que incluyó a pacientes con IMC> 50 kg/m^2, fueron seguidos 5 años después de la cirugía, se realizó una calorimetría indirecta (IC) antes y después de la cirugía para evaluar el gasto energético en reposo (GER). Con el estudio piloto, desarrollamos un algoritmo de base genética para la predicción del % EPP después de la CB y para la remisión de la DM2 con alta sensibilidad y especificidad. En el segundo estudio, la puntuación clínico-genética desarrollada fue un método fiable para predecir la respuesta ponderal tras la CB. Finalmente, el estudio del GER mostró que se reduce drásticamente muy temprano después de la CB, y fue capaz de predecir la pérdida de peso insuficiente y la reganancia de peso después de 5 años de la CB. En conclusión, estos nuevos enfoques de biomarcadores a través de la evaluación de la predisposición genética, unidos a los datos clínicos y la evaluación del metabolismo energético en reposo, pueden ayudar a los médicos a personalizar el enfoque terapéutico en la obesidad severa optimizando así los recursos limitados de salud.

1 INTRODUCTION

1.1 Obesity as a disease

Obesity represents a major public health problem and its associated comorbidities have increased the economic burden for the health systems (1,2). Obesity related comorbidities, such as, type 2 diabetes (T2D) and cardiovascular disease (CVD) challenge investigators to discover new biomarkers able to identify patients at risk for these complications along with a comprehensive insight of obesity complexity, in order to design personalized medicine programs.

In terms of evolution, genetic of the human race adapted to an energy-storage prone metabolism making possible for humans to survive during times of starvation (3). The economic development over the last century has provided better health, wealth and food supply (4). This positive shift on global society wellbeing has, on counterpart, rapidly transformed our dietary pattern. However, evolutionary changes in metabolism take time. A rapid change in food availability over a relatively short period of time lead to a miss-adaptation of a metabolism that is still genetically adapted to store fat, despite of the widely available food supply in developed countries. The shift in food supply is considered a major trigger of the obesity epidemic and the increasing prevalence of associated comorbidities, such as T2D, CVD and cancer (5).

In 2012, over one third of the total world population was overweight or obese (6). By 2030 it is estimated that 38% of the world's adult population will be overweight (BMI >25kg/m^2) and 20% will be obese (BMI >30kg/m^2). Of even more concern, morbid obesity (MO; BMI>40kg/m2) is rapidly increasing, especially in younger ages (7).

1.1.1 Obesity definition

Broadly, obesity is defined as a disproportionate weight-height ratio, mainly due to excessive deposition of adipose tissue (AT). Initially the AT was thought to represent an energy storage tissue. In the recent years AT was proven to be an active tissue, having an increased hormonal activity, leading mainly to early insulin resistance (IR) (8). Moreover, IR itself is an independent and a major risk factor for the development of T2D and CVD (9), making the early detection and management of high clinical relevance.

The most globally accepted definition of obesity is a body mass index (BMI) over 30kg/m^2 (10). The BMI is a formula that is calculated by dividing the weight, always expressed in Kg, by the height, always in meters squared. A BMI above 30kg/m^2 is associated with exponential increase in the mortality and comorbidities such as T2D (11).

The BMI reflects a disproportion between the weight and the height, but lacks of sensibility to discriminate the real body composition and the proportion and distribution of AT. Visceral and abdominal adiposity are shown to be metabolically active and associated to increased mortality and obesity related comorbidities (12). At present there are several methods that can estimate the body composition and the distribution of fat.

Waist circumference (WC) is a widely used parameter in the clinical practice that correlates with abdominal adiposity (13). The World Health Organization (WHO) established that the WC is measured at the midpoint between the highest point of the iliac crest and the lowest rib. A WC ≥94 cm in European men, and ≥80 cm in

European women, was associated with increased cardiovascular risk. Different cut points are recommended in other races and ethnicities (14). Other strategies such as biompedanciometry (BIA), nuclear magnetic resonance (NMR) and Dual-energy x-ray absorptiometry (DEXA) have better sensibility and specificity to analyze the body fat distribution and quantity. Nevertheless they are not widely available, and due to their cost, need for trained personnel and risks (x-ray exposition only for DEXA) are not suitable for use in the daily clinical practice (15).

In light of the deeper understanding of obesity and its pathophysiological mechanisms, scientific societies have recently taken a position in favor of recognizing obesity as a chronic disease (16–19). This fact is linked to a change in the definition of obesity, which reflects the role of adipose tissue in pathophysiology, as well as the underlying complexity of this pathology. Recently, the European Association for the Study of Obesity (EASO) has coined the term adiposity-based chronic disease (ABCD) to allude to obesity redefinition, as it is of particular relevance and in line with EASO's proposal to improve the International Classification of Diseases ICD-11 diagnostic criteria for obesity based on three dimensions, namely etiology, degree of adiposity, and health risks (18).

1.1.2 Physiopathological mechanism: Insulin-resistance and obesity.

Obesity is considered a multifactorial pathology, still not fully understood. Along with increased food intake, many other factors have been associated to the development of obesity, such as genetic, physiologic, environmental, psychological, social, economic, and political (20). Interaction between these factors results in body

fat accumulation, changes at a cellular level in the AT, and finally resulting in systemic alterations related to obesity, mainly IR.

Although the mechanism for IR are several and not fully elucidated, there is robust evidence supporting a tight relationship with obesity (21). In fact, IR is considered the main underlying mechanism leading to most obesity related comorbidities (22). Under normal conditions, the storage and release of triglycerides (TG) and free fatty acids (FFA), respectively, are both coordinated and tightly regulated in the AT. When AT regulation of energy is impaired, an increase in plasma FFA levels and metabolism occurs, including storage of TG in non-adipose tissues (23).

In normal conditions, insulin effect on its receptors in adipocytes induces: a) FFA uptake by stimulating lipoprotein lipase (LPL) activity, b) TG storage by inducing maturation of pre-adipocytes into adipocytes, c) induction of lipogenesis-regulating genes, d) stimulates glucose transport and e) suppression of lipolysis (24). Adipocytes are probably the most insulin-dependent cells in humans. The insulin signaling cascade which initiates these events is largely conserved in evolution from *C. elegans* to humans, thus highlighting the fundamental biological importance of insulin actions in AT (25).

Resistance to insulin refers to an impaired capacity of insulin to induce its physiological effects, mainly the glucose uptake by the tissues. In the hyperinsulinemic- euglycemic clamp (HEC), this impairment results in an increased need of insulin to achieve a normal glucose uptake by the insulin-dependent organs

(mainly muscles, AT and liver). Impaired insulin action is, in part explained by an altered insulin signaling pathway (26).

In adipocytes from obese humans with T2D, IRS-1 expression is reduced (IRS-1 is the first substrate of insulin after binding to its receptor), resulting in decreased IRS-1–associated PI3K activity, thus undocking the signaling (27). In both muscle and adipocytes from obese subjects, insulin binding to its receptor, receptor phosphorylation and tyrosine kinase activity, and phosphorylation of IRS-1 are reduced (27) (**Figure 1**).

Adipocytes were proven to release the inflammatory adipokines that have been related to IR (28). These findings have led recently to consider the AT an endocrine organ (29). Adipokines, along with FFA, have significant effects on total body glucose and lipid metabolism, and insulin sensitivity (23). The main adipokines associated with IR are resistin, adiponectin, IL-6, visfatin, SAA3, and PAI-1, while also leptin and VEGF account for CVD risk(28).

Other pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), C-reactive protein, and interleukins, are upregulated in IR (30,31). Interestingly animals deficient in TNF-α receptor are protected against IR (32). TNF-α impairs insulin signaling via serine phosphorylation of IRS-1 (the first substrate of insulin after binding with its receptor) and reduces GLUT-4 (an insulin-dependent glucose transporter) expression (32).

Obesity-induced chronic inflammation likely plays an important role in IR in obese subjects (31). Alterations in the above mentioned cytokines and metabolic pathways are considered the early triggers of IR. Numerous stimuli can activate

these pathways and increase the expression of inflammatory agents involved in IR. Activation of IKKβ/NF-κB and JNK pathways is a possible link between inflammation and IR (33). The IKKβ signaling pathway is a key element in tissue inflammation, its inhibition is accompanied by improved insulin sensitivity (31,34). Additionally, JNK pathway promotes serine phosphorylation in IRS-1 and impairs insulin signaling (35). IL-6 induces IRS degradation in a dependent manner via suppressor of cytokine signaling 1 (SOCS1) and SOCS3 activation (36), a family of negative-feedback regulating proteins in the intracellular signaling of cytokines (37,38) (**Figure 1**).

Moreover, higher levels of plasma lipids activate serine–threonine kinase and thereby inhibit the insulin signaling pathway (39). Also, FFA can promote inflammation by binding to toll-like receptors 2 and 4 through the adaptor protein fetuin-A, resulting in activation of NF-κB and JNK (40). Thus, two of the main features of obesity: increased AT and FFA release, are related to insulin-signaling impairment via pro-inflammatory adipokines and cytokines secretion.

These initial findings lead to the idea that adipokines secretion and the microenvironment within the AT are responsible for a low-grade of inflammation, that translates into an impaired metabolism phenotype (41). Recent data suggest that changes in adipokines secretion, adipocyte deregulation and FFA release into circulation contribute to maintain immune cells activation as well as their infiltration into regulatory organs (42).

In obesity, AT may become severely dysfunctional and not expand properly to store the energy excess, causing impaired adipogenesis, unbalanced systemic

energy homeostasis and redistribution of the amount and proportion of the immune cells within the AT (43). Main changes in the AT resident immune-cells are: a) increased number and activity of macrophages, mast-cells, neutrophils and lymphocytes, b) decreased number of eosinophils, Th2, T-reg and NKT. Besides the well-established role of macrophages in the AT, recent findings showed the involvement of T- reg and neutrophils in the onset of inflammation and multisystemic alterations (44), suggesting the crucial role of both the innate and the adaptive immune system on the onset low-grade inflammation and development of IR (45).

1.2 Genetic risk factors for obesity

Inheritance is responsible for 40–75% of all the causes of obesity, a percentage modulated by the epigenetic influence (46,47). Through genome-wide association studies (GWAS), a series of gene variants and single nucleotide polymorphisms (SNPs) in more than 120 genes, have been linked with eating behavior, energy expenditure, response to diet, or lifestyle interventions (48,49). These genes include, for example, INSIG2 (insulin induced gene 2), FTO (fat mass and obesity-associated gene) or MC4R (Melanocortin 4 receptor gene). As each variant alone has little effect on body weight, genetic predisposition is conditioned by the simultaneous presence of SNPs in multiple genes (50). The possibility of elucidating the best combination of SNPs responsible for the variability of the response to BS in terms of weight loss and remission of comorbidities, offers the opportunity to design individualized therapy strategies.

In recent years, interest in the genetic influence on the response of different treatments for obesity has increased. Two retrospective studies (51,52) showed that

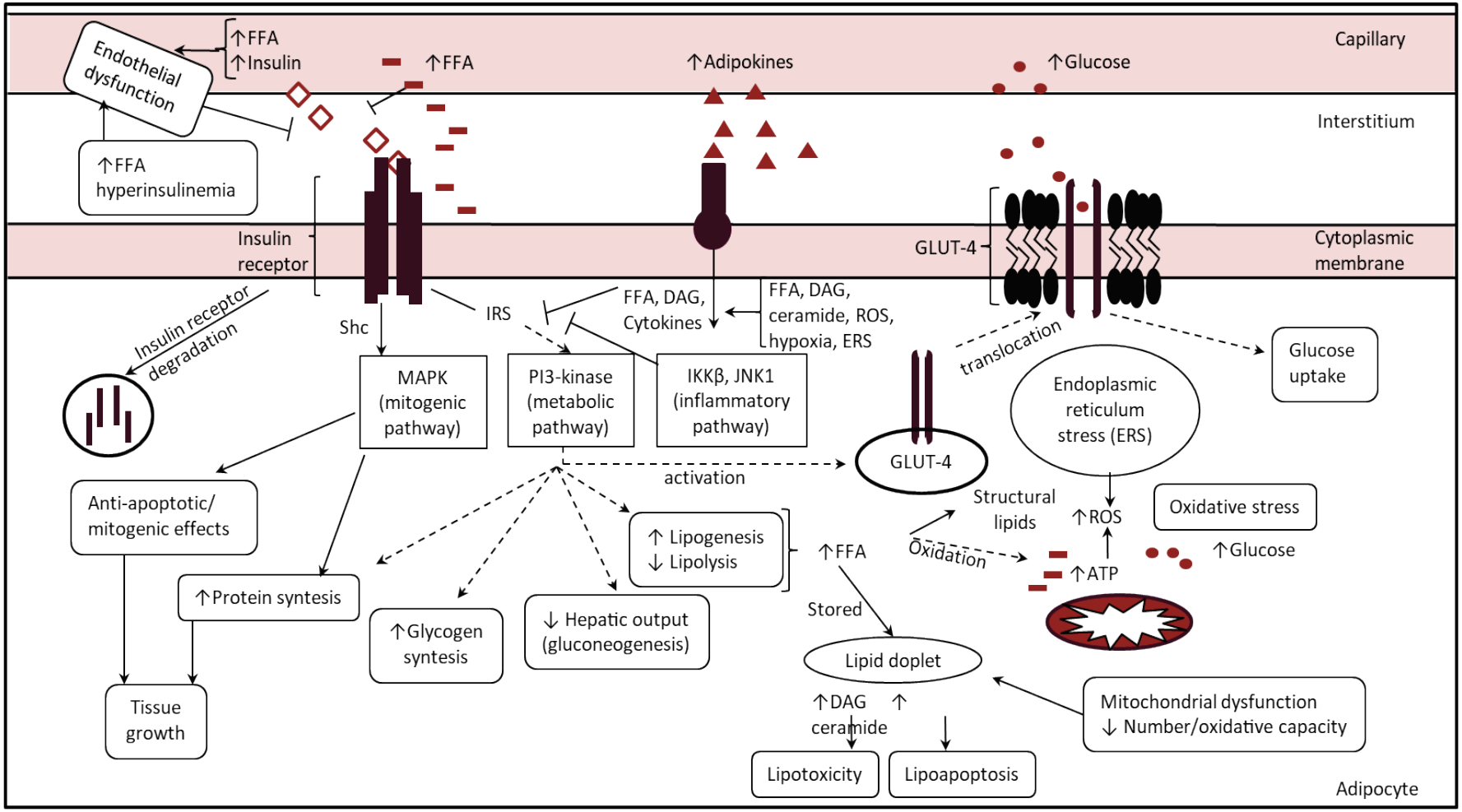


Figure 1. Summary of the main molecular mechanisms involved in the development of insulin resistance associated with obesity.– Insulin resistance associated with obesity, occurs due to pre-receptor, receptor and/or post-receptor impairments, mainly secondary to elevated FFA, hyperinsulinemia and increased cytokines. Insulin access to the interstitial space (pre-receptor impairment) may be induced by the excess of FFA and their metabolites as well by endothelial dysfunction secondary to increased circulating insulin. Hyperinsulinemia, secondary to the decrease of FFA-induced insulin clearance and the increase of insulin secretion, causes downregulation of insulin receptors. In addition, insulin receptor downstream signaling (post-receptor impairment) is inhibited by FFA and cytokines. Increased intracellular FFA also contribute to excessive production of ATP, oxidative stress and mitochondrial dysfunction, production of reactive oxidative species, endoplasmic reticulum stress and lipid storage and accumulation of non-oxidative toxic derivatives (dyacylgycerol and ceramide). These factors also activate inflammation pathways. Independently of the upstream or downstream level of the insulin receptor impairment, insulin resistance occurs by the inhibition of the phosphorylation of the insulin receptor substrates (IRS-1 or 2) and the subsequent inhibition of PI3K pathway, responsible for metabolic effects. Consequently to this inhibition occurs 1) decrease in the activation of GLUT-4 which impairs glucose uptake; 2) increase of glucose production by the liver (either by inhibiting glucogenesis and/or promoting glycogenolysis) and 3) increase of *de novo* lipogenesis, storage of lipid (lipid droplets) and toxic derivatives. On the other hand, the insulin receptor substrate, Shc, is spared from inhibition by FFA or cytokines and is stimulated by hyperinsulinemia. Consequently, MAPK pathway is activated leading to anti-apoptotic and proliferation effects, culminating with tissue growth. ATP; adenosine triphosphate, DAG; diacylglycerol, FFA; free fatty acids, GLUT-4; glucose transporter type 4, ROS; reactive oxidative species, MAPK; mitogen-activated protein kinase, PI3-Kinase; phosphatidylinositol-3 kinase, IKKβ; inhibitor of nuclear factor kappa-B kinase subunit beta, JNK1; Jun N-Terminal Kinase.

several single nucleotide polymorphisms (SNPs) were associated with a poor response to BS. However, in these studies the discrimination capacity of the GPS was not significant, and the role of T2D in the response to BS and the impact of these genetic factors on diabetes remission were not evaluated.

We know of a large number of genes associated with baseline anthropometric measurements, but very few studies have addressed their influence on long-term dynamic changes in body weight. Still et al showed, in 1011 subjects, how SNPs in the FTO, INSIG2, MC4R and PCSK1 genes (proprotein convertase subtilisin / kexin type 1) negatively influenced weight loss after Roux-Y gastric bypass (RYGB) (51). In turn, Mirshahi et al described, in 1433 subjects, how the I251L variant of the MC4R gene predisposed to a better outcome after 2 years of a RYGB (52). In the SOS study (Swedish Obesity Study), after analyzing the impact of various SNPs in 1,443 obese patients for 6 years, only FTO (rs16945088) was associated with maximum weight loss after gastric band, but not in vertical gastroplasty or in the RYGB (53).

More recently, in a group of 146 individuals, carriers of the FTO variant (rs9939609) showed a lower success rate, as well as a greater and faster recovery, after 2 years of follow-up. However, the same SNPs were not associated with different weight loss after 6 months of vertical gastrectomy in 74 severely obese patients (54). Lastly, Velázquez-Fernández D et al have evaluated the presence of 20 SNPs in 249 morbidly obese subjects undergoing RYGB, with POMC (rs1042571) being the only SNP associated with greater weight loss (55). And two genetic risk scores, based on the genotyping of 35 SNPs, have shown that scores in the lower quartile are associated with the greatest weight loss in 238 subjects

undergoing RYGB (56). These data, beyond supporting the potential usefulness of genetic markers in predicting the response to BS, suggests that different types of surgery exert their therapeutic effect in different ways. This feature can be helpful when choosing the most appropriate type of surgery for each patient.

Data on genetic susceptibility, resolution of comorbidities and BS are still scarce. But it is an important issue given the rise of “metabolic surgery”, since the possibility of resolving T2D becomes a reason for opting for surgical treatment (57). In the study by Liou et al, 5 SNPs (the ESR1, FTO, PPARG and UCP genes) were genotyped in 520 patients with severe obesity: the synergistic effect of the ESR1 (estrogen receptor 1 gene) and FTO genes in improving the HbA1c was superior to any of these genes alone (58). More recently, at 12 months after a RYGB, the highest score obtained in the combination of 7 polymorphisms (rs1801282 in PPARG2, rs4994 in ADRB3, rs1800592 in UCP1, rs659366 and rs669339 in UCP2, rs7121 in GNAS1, and rs5443 in GNB3) of 150 patients were associated with a greater reduction in blood glucose, triglycerides and total cholesterol (59).

The molecular mechanisms and the function of the genes involved in the development of obesity, its comorbidities and the response to BS are not well defined. In recent years, the metabolomic approach has been used successfully to identify new biomarkers associated with different diseases and traits, and thus try to bridge the gap between genomics and phenotype (60,61). In this sense, the possibility of identifying metabolites associated with certain SNPs offers a unique opportunity to infer some of the underlying biological mechanisms in the therapeutic response of obesity (62–64). An example is the analysis of the polymorphism rs9939609 in the FTO gene, which identified 7 metabolites related to the

phosphatidylcholine metabolic pathway expressed in a differential way in carriers, and that helped to understand the role that this gene exerts on the metabolism of amino acids such as valine and some phospholipids (65).

Genetic factors can influence the outcome of BS, although the available studies are far from conclusive. This project proposes progress in understanding the variability in the therapeutic effects of BS and delving into the metabolic pathways that justify such an effect. This is to bring us closer to the precision treatment of obesity, by being able to establish a more precise selection method than the current clinical predictors. Discourage BS in a certain group of patients, or opt for more aggressive surgical techniques and / or more intense postoperative follow-up in subjects with a more unfavorable profile, reducing costs and unnecessary adverse effects. In short, a more effective and personalized treatment for patients with morbid obesity.

1.3 Energy expenditure balance in obesity

The physiology of weight gain and weight loss is complex, multifactorial, and by far to be completely elucidated. A recent systematic review identified 124 determinants of weight loss maintenance. Of those, reducing energy intake, increasing energy expenditure, and monitoring behaviors showed the strongest level of evidence (66). One of the major determinants is the balance between energy intake and energy expenditure.

Weight variations are associated with variations in total energy expenditure (TEE) (67). TEE is influenced by factors such as age, gender, weight, body composition, diet, and physical activity (68). TEE is defined as the amount of heat

energy used by the human body for daily physiological functions and is divided into 3 main components: (a) resting energy expenditure (REE)—accounting for around 70% of TEE; (b) diet-induced thermogenesis (DIT); and (c) activity energy expenditure (AEE) (69).

Historically, several methods have been developed for assessing TEE. However, each approach has its advantages and disadvantages. If the purpose is to assess free-living TEE, doubly labelled water (DLW) is recommended. DLW provides information on TEE for a 4–20-day period, likely to reflect the normal energy requirement of individuals. DLW is proven to be safe and useful in all age groups and in several clinical settings. On the other hand, it is highly expensive, and proper equipment and specialized expertise are required to analyze isotope concentration in body fluids by mass spectrometry (70).

Direct calorimetry measures total heat loss from the body while the participant is isolated in a thermally controlled chamber. Although very accurate, it is unpractical for measuring TEE in a free-living population context. On the other hand, indirect calorimetry measures CO2 production and VO2 consumed in a controlled environment (closed-circuit) to calculate the amount of energy expended. It should be noted that if performed in a resting state, IC will allow the measurement of REE, which is not provided by other techniques. For this reason, IC is considered the gold standard for REE measure (69).

Additionally, the technique for REE measure is timesaving and requires minimal training, making it feasible and practical for study populations. Furthermore, in order to assess exercise metabolism, open-circuit portable indirect calorimetry

techniques are more suitable. More recently, heart rate monitoring portable devices may be useful for assessment of physical activity rather than TEE. Finally, questionnaires of activity recall and motion sensors, such as pedometers and accelerometers, may have a role in evaluating interventions aimed at increasing physical activity; instead, its use to quantify REE is very limited (70).

Some data in the literature suggested that REE is increased in patients with morbid obesity (71). Reliable data on REE in these cases is necessary to personalize calorie intake in order to assure a safe and effective weight loss and, more importantly, weight maintenance after successful weight loss. Nevertheless, REE is calculated in the daily clinical practice by means of estimation equations. Although widely used in clinical settings, it should be noted that these equations were validated based on data from healthy normoweighted subjects.

These estimations are not always accurate for REE in subjects with overweight or obesity (72). Actual published evidence is reflecting great disparities between predicted and measured energy expenditure values in patients with obesity (73–75). Additionally, at present, there is no reliable data regarding their accuracy in estimating REE in patients with severe obesity (SO).

Bariatric surgery (BS) has proven to be an effective treatment for obesity resulting in sustainable and substantial weight loss and improvement of related comorbidities (76). Furthermore, BS was proven to be safe and effective in patients with SO at short-medium follow-up, representing the preferred treatment for obesity in these patients (77). Some authors suggested that BS can modify the REE and

have proposed that the greater long-term success of BS as a treatment for obesity could be partially explained by the effects of BS on REE (78).

Nevertheless, others have found no influence of REE on outcomes after BS, rather a compensatory adaptive thermogenesis mechanism that occurs in response to a decreased energy intake (79). Whether the changes in REE after BS act as determinants of weight loss maintenance is still under investigation. One possible mechanism comes from evidence in rodents. In obese mice, bariatric surgery seems to increase brown adipose tissue activity postoperatively resulting in increased energy consumption and decreased respiratory exchange frequency. These effects deteriorated when mice experienced weight regain 8 weeks after surgery (80). In humans, evidence supporting a “browning” of adipose tissue after BS is increasing. However, evidence in the literature is contradictory (81).

1.4 Response to bariatric surgery

Our understanding of the obesity complex physiopathology is increasing rapidly due to basic and clinical research. As obesity prevalence increases, bariatric surgery is becoming more popular. Bariatric surgery is considered, at present, the most effective treatment for obesity (76). Definition of successful is heterogeneous across literature. Recently, the American Society for Metabolic and Bariatric Surgery (ASMBS) outcome reporting standard recommended using percentage of excess of weight loss (%EWL) and percentage of total weight loss (%TWL) for reporting outcomes in BS. A %EWL >50 or a %TWL > 20 after 1 year of BS is broadly accepted as successful (82). Other main factor to consider a BS effective is

remission of comorbidities associated to obesity. In this regard, T2D is probably in the main stream.

Most predictors factors studied are focused on anthropometric, psychological and social characteristics (83). It seems that age, BMI and Edmonton obesity staging system (EOSS) are positive predictors of success after bariatric surgery, while the age of onset of obesity (AOO) and the years of obesity did not influence surgery outcomes (84). Several studies have suggested that the presence of preoperative psychopathology is associated with suboptimal weight losses, postoperative complications, and less positive psychosocial outcomes (85). Other factors that may be positively associated with weight loss after surgery include mandatory preoperative weight loss (50).

However, it should be noted that around 20–25% of patients that undergo BS do not achieve successful weight loss (50), or, more importantly, about 30–35% fail to maintain weight loss (86), experiencing significant weight regain starting from 3 years after the BS (87). Additionally, weight loss is often less significant than would be expected for a given degree of caloric restriction or BS technique (88). While it is clear that individuals differ in the susceptibility to weight loss (and their subsequent ability to sustain this lower body weight), robust predictors of response to a weight loss intervention remain unclear. Data regarding REE is very scarce in patients with SO, and practically there is no reliable data on the impact of BS on REE in this population (89).

Moreover, BS leads to a dramatic improvement in obesity-related comorbidities (90). The remission rate of T2D after BS is around 60–70% after 1

year of follow-up (91). Therefore, there is a significant proportion of non-responders to BS in terms of diabetes remission. Additionally, after 5 years, there is about a 20–35% relapse of T2D after Y-de-Roux gastric by-pass (RYGB) (92–94). A score based on clinical variables for the pre-operative prediction of T2D remission following RYGB surgery (DiaRem) was proposed (95). However, this model has several limiting factors (96) and it has not been generally adopted in clinical practice. At present, there are no reliable predictors of T2D remission and relapse after BS.

2 HYPOTHESIS

The identification of predictive factors may improve patient selection and help develop interventions targeting specific needs of patients.

The hypothesis of this project was that genetic factors and resting energy expenditure are major determinants of effective weight loss and maintenance after bariatric surgery, as well as for the remission of obesity-related complications.

3 OBJECTIVES

3.1 Main Objective

To evaluate, both the genetic predisposition and the resting energy expenditure in patients with severe obesity, by means of a clinical-genetical predisposition score and indirect calorimetry, to predict the response of bariatric surgery in terms of weight loss and remission of type 2 diabetes.

3.2 Secondary Objectives

1. To evaluate whether genetic markers can be used for the prediction of adequate weight loss and diabetes remission after BS.
2. To explore whether a combined score (using clinical and genetic data) can be a reliable marker for predicting the weight loss after BS.
3. To evaluate the REE in patients with Extreme Obesity by means of the gold standard method (IC) and compare to the values of the REE estimated by the equation that are currently used in the daily clinical practice (eREE);
4. To evaluate the impact of BS on the REE, and the relationship with the evolution post-BS.

4 MATERIAL AND METHODS

4.1 Genetic testing to predict weight loss and diabetes remission and long-term sustainability after bariatric surgery: a pilot study (97) (Annex 10.1.1.1).

4.1.1 Study Design and Population

A single-center, retrospective observational pilot study in a third-level university hospital (Vall d´Hebron University Hospital, Barcelona, Spain) was conducted following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (98). The study comprised patients that underwent RYBG surgery between January 2010 and December 2012. The study was approved by the Local Ethics Committee and registered at Clinical.Trials.gov, NCT02405949.

4.1.2 Inclusion and exclusion criteria

The inclusion criteria were women, stable weight in the prior 6 months before BS, and minimum of 5 years of follow-up after BS. In order to avoid heterogeneity and given that the vast majority of the patients under bariatric surgery were women, we decided to rule out the inclusion of men in this pilot study. The patients were informed about the study and they all signed the written informed consent form.

The exclusion criteria were male, marked mobility problems, a different BS technique apart from RYBG, and severe psychiatric or eating disorders. For the genetic study, a sample of saliva was collected. The characteristics of RYBG were food loop length: 150–180 cms and biliopancreatic loop length: 120 cm, gastric pouch 30 cc. The technique was the same in all cases, performed by the same surgical team in our hospital.

4.1.3 Outcome variables definitions

Excess body weight (EBW) was defined as the amount of weight that was in excess of the ideal body weight (IBW). The percentage of excess weight loss (EWL) was calculated according to the formula: %EWL = (weight before BS (kg) - weight after BS (kg)/ EBW (kg)) X 100. The post-BMI weight regain was defined as a 10% regain of the minimal weight after BS. The minimal weight after BMI was achieved at 2 years follow-up for all of the patients.

Diabetes remission was defined according to American Diabetes Association (ADA) criteria (99). Relapse of T2D was defined as one or more of the following conditions: (a) restarting diabetes medication; (b) one or more HbA1c measures ≥6.5%; and/or (c) one or more fasting glucose measures ≥126 mg/dL (100).

4.1.4 Genotyping and Sequencing

The DNA was extracted from saliva samples and processed by Golden Gate® Genotyping Assay for Vera Code. The genetic predisposition was assessed using Nutri inCode (NiC) (Ferrer inCode, SL) and selecting the 57 SNPs associated with susceptibility to diabetes, obesity, appetite regulation, weight loss in response to hypocaloric diet, and the response to BS. The details about the SNPs are reflected in the **Table 1**. The selected SNPs were grouped into three genetic predisposition risk scores (GPS): diabetes remission, weight loss in non-diabetic subjects, and weight loss in subjects with diabetes.

4.1.5 Statistical Analysis

In order to assess the best predictive GPS, patients were distributed into 4 subgroups according to the BS response (%EWL) and the presence of T2D: **1)** %EWL < 40% without diabetes (n = 15); **2)** %EWL < 40% with diabetes (n = 16); **3)** %EWL > 75% without diabetes (n = 35); and **4)** %EWL > 75% with diabetes (n = 31). Univariate and multivariate logistic regressions were used to establish associations. Akaike Information Criterion (AIC)-based backward selection was used to remove insignificant terms from an initial model containing all the candidate predictors. The calibration of the model's adequacy was determined by the Hosmer–Lemeshow test. The area under the ROC curve (AUROC) was used for evaluating the prediction performance of the models. The cut-off for the developed algorithms were selected as the point which maximizes the Youden index.

Table 1. GPSs evaluated in the study and the risk score calculation.

GPS DIABETES

SNP	GENE	Minor Allele	Major allele	Risk allele	Protective allele	Risk homo-zygote	Risk hetero-zygote
rs4343	ACE	G	A	G	n.a	2	1
rs16861209	ADIPOQ	A	C	A	n.a	2	1
rs5186	AGTR1	C	A	C	n.a	2	1
CD010	APOC3	A	G	A	n.a	2	1
rs7754840	CDKAL1	C	G	C	n.a	2	1
rs10811661	CDKN2B	C	T	C	n.a	2	1
rs696217	GHRL	T	G	T	n.a	2	1
rs1800795	IL6	C	G	n.a	C	-2	-1
rs12970134	MC4R	A	G	A	n.a	2	1
rs1800206	PPARA	G	C	G	n.a	2	1
rs1801282	PPARG	G	C	C	n.a	2	1

rs7903146	TCF7L2	T	C	T	n.a	2	1
rs10010131	WFS1	A	G	n.a	A	-2	-1
CD014	PON1	T	A	A	n.a	1	0

GPS APPETITE REGULATION

SNP	GENE	Minor Allele	Major allele	Risk allele	Protective allele	Risk homo-zygote	Risk hetero-zygote
rs6265	BDNF	A	G	G	n.a	2	1
rs925946	BDNFOS	T	G	T	n.a	2	1
rs12535708	LEP	A C	C	n.a	2	1	1
rs52820871	MC4R	G	T	n.a	G	-2	-1
rs17700633	MC4R	A	G	A	n.a	2	1
rs2229616	MC4R	A	G	n.a	A	-2	-1
rs10838738	MTCH2	G	A	G	n.a	2	1
rs4580704	CLOCK	G	C	C	n.a	1	0
rs4864548	CLOCK	A	G	A	n.a	2	1
rs17782313	MC4R	C	T	C	n.a	2	1

GPS- WEIGHT LOSS IN RESPONSE TO EXERCISE

SNP	GENE	Minor Allele	Major allele	Risk allele	Protective allele	Risk homo-zygote	Risk hetero-zygote
rs328	LPL	G	C	n.a	G	2	1
rs696217	GHRL	T	G	n.a	T	2	1
rs4994	ADRB3	C	T	n.a	C	2	1
rs1800795	IL6	C	G	n.a	C	2	1
rs9693898	Chr.8	G	A	G	n.a	2	1

GPS WEIGHT LOSS IN RESPONSE TO DIET

SNP	GENE	Minor Allele	Major allele	Risk allele	Protective allele	Risk homo-zygote	Risk hetero-zygote
rs2419621	ACSL5	T	C	n.a	T	2	2
rs5082	APOA2	T	C	n.a	C	2	0
rs651821	APOA5	C	T	n.a	C	2	1
rs894160	PLIN1	A	G	n.a	A	2	1
rs1137100	LEPR	G	A	n.a	A	2	0

rs1800849	UCP3	T	C	n.a	C	2	0
rs659366	UCP2	T	C	n.a	T	2	1
rs1801282	PPARG	G	C	G	n.a	-2	-2
rs6824447	Chr.4	G	A	G	n.a	2	1
rs1052700	PLIN	A	T	n.a	A	2	0
GPS-LIFE STYLE INTERVENTIONS (MOLERES)							
SNP	**GENE**	**Minor Allele**	**Major allele**	**Risk allele**	**Protective allele**	**Risk homo-zygote**	**Risk hetero-zygote**
rs9939609	FTO	A	T	A	n.a	2	1
rs17782313	MC4R	C	T	C	n.a	2	1
rs1800795	IL-6	C	G	C	n.a	2	1
rs1801282	PPARG	G	C	G	n.a	2	1
rs2241766	ADIPOQ	G	T	T	n.a	2	1
GPS-BARIATRIC SURGERY (STILL)							
SNP	**GENE**	**Minor Allele**	**Major allele**	**Risk allele**	**Protective allele**	**Risk homo-zygote**	**Risk hetero-zygote**
rs7566605	INSIG2	C	G	C	n.a	2	1
rs9939609	FTO	A	T	A	n.a	2	1
rs17782313	MC4R	C	T	C	n.a	2	1
rs6235	PCSK1	C	G	C	n.a	2	1

GPS: genetic prediction risk score. SNP: single nucleotide polymorphism. GPSs are calculated by using an additive model based on the total number of risk alleles in each genotype.

4.2 A clinical-genetic score for predicting weight loss after bariatric surgery: the OBEGEN study (101) (Annex 10.1.1.2).

4.2.1 Study Design and Population

The OBEGEN study was approved by the human ethics committee of the University Arnau de Vilanova Hospital of Lleida (CEIC-1743). All potential participants gave written informed consent to join the study, which was conducted

according to the Helsinki Declaration and the Good Clinical Practice Guidelines (102). The study was registered in Clinical.Trials.gov - NCT02405949**.**

The OBEGEN project was a multicenter, retrospective, longitudinal, and observational study investigating the role of some genetic variants added to clinical variables to predict weight loss after BS. A total of 416 patients who underwent BS between January 2017 and August 2018 at the Obesity Units of four University Hospitals in Catalonia (Spain) were included. Eligible patients were men and women ≥18 years old, which underwent BS at least 18 months prior to the study. Among the 449 patients who met these criteria, 33 were excluded because of the following reasons: current pregnancy (n = 2), development of drug or alcohol abuse or eating disorders after bariatric surgery (n = 5), markedly mobility problems (n = 4), a different bariatric surgery technique apart from RYGB or SG (n = 9), use of weight lowering pharmacotherapy (n = 7) or a second surgical intervention required during follow-up (n = 6). The study flow chart is displayed in **Figure 2**.

All the patients that agreed to participate in the study and signed the informed consent underwent complete medical history, anthropometric measurement, physical examination, and DNA sampling.

4.2.2 Outcome Weight Measures

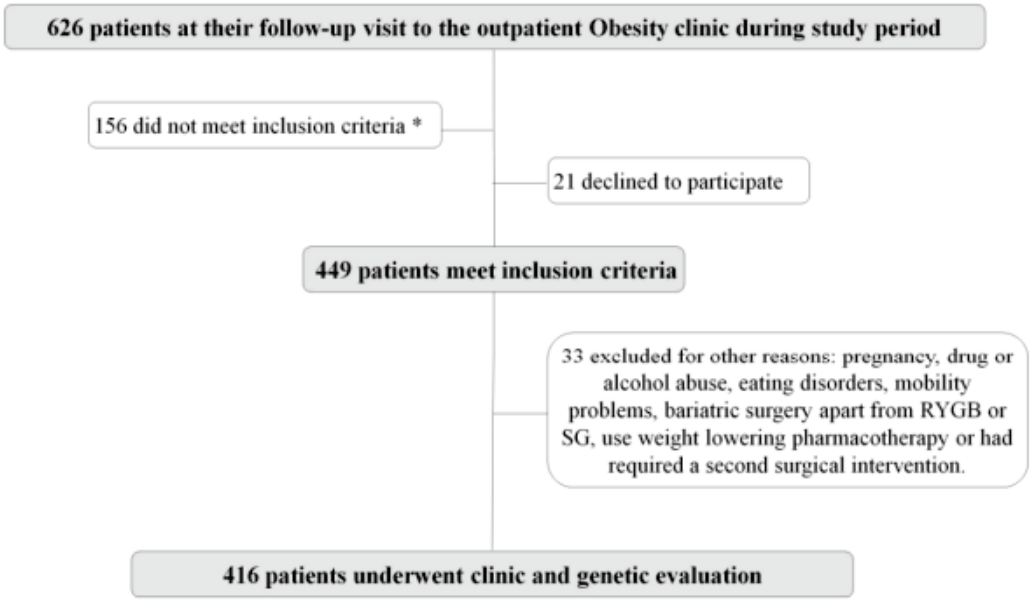
The primary endpoint was the %EWL at nadir. Excess body weight (EBW) was defined as the amount of weight that was in excess from the ideal body weight (IBW). IBW was estimated according to the 1983 Metropolitan Life Insurance Tables (use the midpoint for medium frame) (103). The %EWL was calculated according to the formula: [total preoperative weight (kg) − weight after bariatric surgery (kg)/EBW

(kg)] × 100. Those with a reduction in their %EWL >50% at nadir were considered "good responders" (104).

4.2.3 Genotyping

DNA was extracted from saliva samples and processed by GoldenGate® Genotyping Assay for Vera Code. The genetic predisposition was assessed using the 50 SNPS in 39 genes included in a commercial nutrigenomic product, the Nutri inCode (NiC) (Ferrer inCode, Barcelona, Spain). This product includes SNPs that had previously been associated with susceptibility to weight loss, both in response to lifestyle intervention and BS (52,105,106). In addition, Nutri inCode also includes selected variants of published GWAS studies or replication studies related to genetic susceptibility to regulate appetite and develop type 2 diabetes and obesity

Figure 2. Flow chart of the OBEGEN study population.



* The inclusion criteria were: men and women 18 years of age or older who underwent bariatric surgery at least 18 months prior. RYGB: Roux-en-Y gastric bypass; SG: sleeve gastrectomy.

(107,108). Finally, in the present study, the panel has been enriched with new 11 SNPs compared to our pilot study (**Table 2**).

Table 2. Selected genes and single nucleotide polymorphisms evaluated in the OBEGEN study.

GENE	NAME	CHROMOSOME ALLOCATION OF HUMAN ORTHOLOGUE	SNP
ACE	Angiotensin i converting enzyme	17q23.3	rs4343
ADIPOQ	Adiponectin, c1q and collagen domain containing	3q27.3	rs16861209 rs2241766
ADRB3	Adrenoceptor beta 3	8p11.23	rs4994 rs9693898
AGRP	Agouti related neuropeptide	16q22.1	rs11575892*
AGTR1	Angiotensin ii receptor type 1	3q24	rs5186
APOA2	Apolipoprotein a2	11q23.3	rs5082
APOA5	Apolipoprotein a5	11q23.3	rs651821
APOC3	Apolipoprotein c3	11q23.3	cd010
BDNF	Brain derived neurotrophic factor	11p14.1	rs6265 rs925946
CCDC93	Coiled-coil domain containing 93	2q14.1	rs10490628*
CDKAL1	Cdk5 regulatory subunit associated protein 1 like 1	6p22.3	rs7754840
CDKN2B	Cyclin dependent kinase inhibitor 2b	9p21.3	rs10811661
CLOCK	Clock circadian regulator	4q12	rs4580704 rs4864548
CNR1	Cannabinoid receptor 1	6q15	rs6454674*
ELOVL6 ELOVL	Fatty acid elongase 6	4q25	rs682447
ESR1	Estrogen receptor 1	6q25.1	rs3778099*
FTO	Fat mass and obesity associated	16q12.2	rs9939609
GHRL	Ghrelin and obestatin prepropeptide	3p25.3	rs696217
IGF2	Insulin like growth factor 2	11p15.5	rs680*
INSIG2	Insulin induced gene 2	2q14.1	rs7566605 rs3771942*
IL-1B	Interleukin 1 beta	2q14.1	rs1143643*
IL6	Interleukin 6	7p15.3	rs1800795
LEP	Leptin	7q32.1	rs12535708
LEPR	Leptin receptor	1p31.3	rs1137100
LPL	Lipoprotein lipase	8p.22	rs328
MC4R	Melanocortin 4 receptor	18q21.32	rs12970134 rs52820871 rs17700633 rs2229616 rs17782313
MTCH2	Mitochondrial carrier 2	11p11.2	rs10838738
NEGR1	Neuronal growth regulator 1	1p31.1	rs2568958*
PLIN1	Perilipin 1	15q26.1	rs1052700 rs894160

PPARA	Peroxisome proliferator activated receptor alpha	22q13.31	rs1800206
PPARG	Peroxisome proliferator activated receptor gamma	3p25.2	rs1801282
PCSK1	Proprotein convertase subtilisin/kexin type 1	5q15	rs6235
PON1	Paraoxonase 1	7q21.3	cd014
SIRT1	Sirtuin 1	10q21.3	rs7069102*
TCF7L2	Transcription factor 7 like 2	10q25.2	rs7903146
TMEM18	Transmembrane protein 18	2p25.3	rs2867125*
UCP1	Uncoupling protein 1	4q31.2	rs45539933*
UCP2	Uncoupling protein 2	11q13.4	rs659366
UCP3	Uncoupling protein 3	11q13.4	rs1800849
WFS1	Wolframin transmembrane glycoprotein	4p16.1	rs10010131

SNP: single nucleotide polymorphism. *: genetic variants that have been added from the pilot study (97).

4.2.4 Statistical Analysis

A normal distribution of the variables was established using the Kolmogorov–Smirnov test, and data are expressed as the mean ± standard deviation (SD), median (interquartile range), or as a percentage. Comparisons between groups were made using the Student's t-test and the Mann–Whitney U test for quantitative variables, and the Pearson's chi-squared for categorical variables. The relationship between continuous variables was examined by the Pearson linear correlation test. To assess the best predictive clinical–genetic risk score, patients were distributed into two groups according to the BS response: **(i)** %EWL ≤ 50% (n = 113); **(ii)** %EWL > 50% (n = 301). On one side, the different SNPs were coded as 0, 1, or 2 according to the number of risk alleles associated with a favorable weight response. Clinical variables analyzed included gender, age at surgery, preoperative weight and BMI, type of surgery (RYGB or SG), EBW, and presence of type 2 diabetes. Univariate and multivariate logistic regressions were used to establish associations between the genetic and/or the clinical variables and the loss of weight.

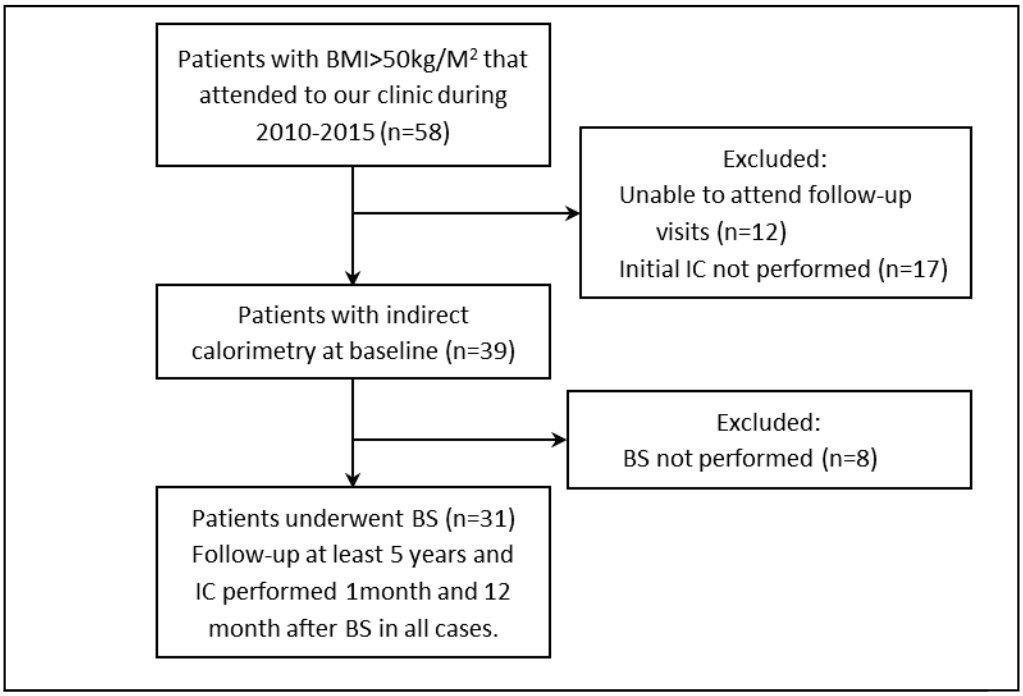
In this way, we generated three risk scores: **(i)** a clinical risk score, **(ii)** a genetic risk score, and **(iii)** the OBEGEN clinical-genetic risk score (OBEGENCGRS), which includes both the selected clinical and genetic variants in the multivariate logistic regression. Akaike Information Criterion (AIC)-based backward selection was used to remove not significant variables, from an initial model containing all the candidate predictors. The calibration of the logistic model's adequacy was determined using the test of fit by the Hosmer–Lemeshow. The accuracy of different scores/models in discriminating those who obtained the objective weight loss (%EWL > 50%) from those who did not achieve the objective weight loss (for evaluating the prediction performance of the models) was evaluated using a Receiver Operating Characteristic (ROC) curve analysis. The cut-offs to calculate the sensitivity and specificity of the developed algorithms were selected as the point which maximizes the Youden index. An odds ratio with its 95% confidence interval was finally calculated. The total area under the ROC (AUROC) curve was interpreted following guidelines: 0.9–1.0 excellent, 0.8–0.9, good; 0.7–0.8, fair; 0.6–0.7, poor; and 0.5–0.6, not useful. Comparisons between the obtained AUROC were compared using the method of Hanley and McNeil. All the contrasts were bilateral with a significance level of 0.05. The data were analyzed with the Statistical Package for the Social Sciences software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA).

4.3 Evaluation of resting energy expenditure in subjects with severe obesity and its evolution after bariatric surgery (109) (Annex 10.1.1.3).

4.3.1 Study design and population

A single-center observational study including consecutive patients with severe obesity and BMI >50 kg/m^2 attended the Morbid Obesity Unit of a third-level university hospital (Vall d´ Hebron University Hospital) that had performed IC between January 2010 and December 2015. The study flow chart is detailed in **Figure 3**. The study was approved by the Ethics Committee of our site and conducted following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines and the statements of the Declaration of Helsinki (98,102).

Figure 3. Flow chart of the study population.



BMI, Body mass index; *BS*, bariatric surgery; *IC,* indirect calorimetry; *RYGB*, Roux-en-Y gastric bypass; *SG*, sleeve gastrectomy.

The patients signed the informed consent form prior to inclusion in the study. All the patients underwent a complete medical history, anthropometric evaluation, and IC at baseline, 1 month, and 12 months after the BS.

4.3.2 Inclusion and exclusion criteria

The main inclusion criteria were: **a)** signed informed consent; **b)** age between 18 and 60 years (the limits for BS at our site); **c)** BMI >50kg/m^2; **d)** eligible for BS according to the standard of care protocol at our site.

The main exclusion criteria were: **a)** eating disorders; **b)** endocrine disease or treatment with potential influence on the REE (egg: systemic corticosteroids, untreated hyper/hypothyroidism); **c)** severe illness that can influence the outcomes; **d)** unable to perform the follow-up visits post BS at our site; **e)** other surgery than sleeve gastrectomy (SG) or Roux-en-Y gastric bypass (RYGB); **f)** second-step BS or revision surgery.

4.3.3 Procedures and variables collected for the study

Clinical and Anthropometric Variables

- ✓ Collected at Baseline: Age, gender, weight (kg), height (m), BMI (kg/m^2), excess of body weight (EBW) (kg), presence of comorbidities related to obesity. Excess body weight (EBW) was defined as follows: actual weight − ideal body weight (IBW) based on BMI 25 kg/m^2.
- ✓ Collected during Follow-up (1 Month, 12 Months, and 5 Years after BS): Weight (kg), BMI (kg/m^2), percentage of excess of weight loss (%EWL),

total weight loss (TWL), percentage of total weight loss (%TWL), and evolution of related comorbidities. Weight and BMI nadir were considered the minimum values reached after the BS; %EWL, TWL, and %TWL were calculated following standardized outcome reporting guidelines (82). The post-BS weight regain was defined as a 10% regain of the minimal weight after BS, as previously described in this document (97).

Energy Expenditure Determination (REE): The variables were collected at baseline, 1 month, and 12 months after the BS.

- ✓ Estimated Equations (eREE): Although the Harris-Benedict Equation (HBE) (110) is widely used in clinical practice, it appears to be less accurate when compared to the Mifflin-St Jeor equation (MSJ) in patients with obesity (78). In this study, we used the Mifflin-St Jeor Equation (MSJ) (111): 9.99*weight (kg) + 6.25*height (cm) − 4.92 x age + 166 x sex (M = 1; F = 0) −161.
- ✓ Indirect Calorimetry (mREE): was performed in supine position, on a neutral environment, and after resting for at least 20 min, using a Vmax 29 (Sensor Medics, Yorba Linda, CA, USA) portable metabolic monitor, available at our site. After the resting period, 15–20 min of calorimetric data was collected. The first 5 min of data was excluded in all cases. The equipment was calibrated prior to each measurement. The patients were instructed to avoid stimulating drinks, cigarette smoking, and exercise 24 hours prior to test and to be fasting at least 8 hours prior to the performance of IC. Oxygen consumption (VO2), carbon dioxide

production (VCO2), respiratory quotient (RQ), and resting energy expenditure (mREE) are generated in the final report.

4.3.4 Statistical Analysis

IBM SPSS statistical software version 24 was used. Continuous variables are expressed as means ± standard deviation (SD) for normal distributed variables and median ± interquartile range (IQR) for non-normal distributed variables. Categorical variables are expressed with percentages. For differences between groups in continuous variables, Student's t test or U-Mann-Whitney test was used while χ^2 was used for categorical variables. For differences between 3 and more time points, repeated measures ANOVA was used; if differences were found, a post hoc pairwise comparison was performed. Differences in weight loss rates at nadir and weight regain rates 5 years after surgery with predetermined definitions were explored using descriptive statistics. Correlation analysis was used to explore the associations between demographics (i.e., age, gender, and preoperative BMI), type of surgery (SG vs RYGB), presence of comorbidities, REE variables, and weight loss at nadir and weight regain 5 years after surgery according to the different definitions. Akaike Information Criterion (AIC)–based backward selection was used to remove insignificant terms from an initial model containing all the candidate predictors. A p-value < 0.05 was considered statistically significant.

5 RESULTS

5.1 Genetic testing to predict weight loss and diabetes remission and long-term sustainability after bariatric surgery: a pilot study (97) (Annex 10.1.1.1).

5.1.1 Clinical characteristics of patients

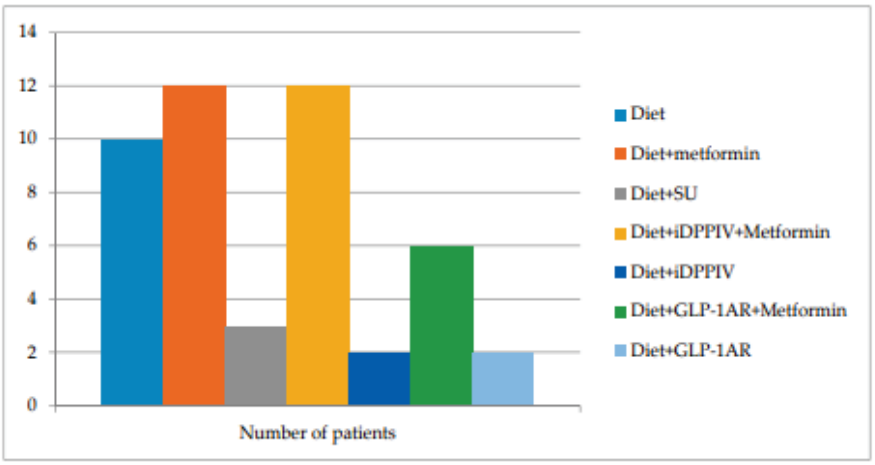
The clinical characteristics of the patients included in the study are shown in **Table 3**. Apart from age, we did not find any significant differences between diabetic and non-diabetic subjects before BS. The diabetic treatment received by subjects with diabetes is displayed in **Figure 4**. No other medication apart from AINEs occasionally and vitamin supplements as per protocol after bariatric surgery (cyanocobalamin 1000 mcg/month, cholecalciferol 25.000–100.000 UI/month) were administered.

Table 3. Baseline characteristics of the patients included in the study.

	NON-DIABETIC PATIENTS	TYPE 2 DIABETIC PATIENTS	P
N	50	47	
Age (years)	48.0 (37.5; 55.0)	52.0 (46.0; 58.8)	0.0016
Initial BMI (Kg/m2)	45.2 (43.0; 48.5)	42.5 (40.1; 46.4)	0.008
2 y post-BS BMI (Kg/m2)	31.8 (26.1; 35.6)	30.9 (26.8; 35.7)	n.s.
5 y post-BS BMI (Kg/m2)	32.63 (21; 52.14)	33.68 (21; 46.43)	n.s.
Hypertension (%)	48.3	49.5	n.s.
Dyslipidemia (%)	43.2	45.7	n.s.
Sleep apnea (%)	27.2	29.7	n.s.

The continuous variables are expressed as median (1st quartile; 3rd quartile) and the categorical data as percentages. BMI: body mass index. EWL: excess of weight loss. BS: bariatric surgery. Hypertension was defined by increased systolic (>140 mmHg) or increased diastolic (>90 mmHg) blood pressure or by the use of antihypertensive drugs, according to current guidelines. Dyslipidemia was defined by the use of lipid-lowering drugs, decreased values of HDL cholesterol (men < 0.9 mmol/L, women < 1.0 mmol/L) or by at least one increased value of total cholesterol (>5.2 mmol/L), LDL cholesterol or triglycerides (>1.7 mmol/L).

Figure 4. The diabetic treatment received before BS by the subjects with diabetes included in the study. SU: sulphonylurea, iDPPIV: DPPIV enzyme inhibitor, GLP-1AR: GLP-1 receptor agonists.



5.1.2 Weight loss

In the subgroup of non-diabetic patients, the multivariate logistic regression equation for predicting positive weight loss response (%EWL > 75%) after the BS (NiC-Bariatric-ND) includes SNPs associated with weight loss in response to a hypocaloric diet and SNPs associated to appetite regulation. The model showed an AUROC of 0.763 (95% CI 0.605 to 0.920; $p < 0.001$), a sensitivity of 86.49%, and a specificity of 57.14%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.679.

5.1.3 Weight regain

Weight regain after 5 years' follow-up was seen in 9.6% of the patients. The model to identify the patients who had presented weight regain after 5 years' follow-up showed an AUROC of 0.834 (95% CI 0.705 to 0.923; $p < 0.0001$), a sensitivity

and a specificity of 70.21%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.5148. In T2D patients, the multivariate logistic regression equation for the prediction of weight loss response (%EWL > 75%) after BS (NiC-Bariatric-D) included SNPs associated with weight loss in response to hypocaloric diet, SNPs associated to response to BS (52), and SNPs associated to response to lifestyle interventions (100). The model showed an AUROC of 0.929 (95% CI 0.850 to 0.99; $p < 0.001$), a sensitivity of 87.10% and a specificity of 93.33%. The calibration of the model's adequacy determined by the Hosmer–Lemeshow test was 0.291. Weight regain in subjects with diabetes was observed in 17.5% of them. The model to identify patients with diabetes who will regain weight after a follow-up of 5 years after bariatric surgery showed in this case an AUROC of 0.781 (95% CI 0.623 to 0.896; $p < 0.04$), a sensitivity of 71.43%, and a specificity of 84.85%. The calibration of the model's adequacy determined by the Hosmer–Lemeshow test was 0.8664. **Figure 5** shows the AUROC corresponding to weight regain in the whole (**Figure 5A**) population, non-diabetic subjects (**Figure 5B**), and T2D patients (**Figure 5C**).

5.1.4 Diabetes remission and relapse

Diabetes remission was seen in 73.91% of the type 2 diabetic patients included in the study (66.67% in the group of %EWL < 40% and 77.42% in the group of %EWL > 75%). Diabetes relapse was seen in 25% of the patients. The multivariate logistic regression equation for the prediction of diabetes remission and relapse after BS (NiC-Bariatric-DR) included SNPs associated with obesity, SNPs associated with weight loss in response to hypocaloric diet, SNPs associated with appetite regulation, and SNPs associated with genetic predisposition to diabetes. This

prediction model showed an AUROC of 0.868 (95% CI 0.709 to 0.976; p < 0.0001) for diabetes remission, with a sensitivity of 76.47% and a specificity of 83.33%. In our population, the AUROC for DiaRem was lower than obtained by genetic testing, (0.69 versus 0.86), and when both scores were combined, the AUROC was 0.87, with a sensitivity of 88.49% and a specificity of 80% (**Figure 6**).

Regarding diabetes relapse after 5 years, the model based showed an AUROC of 0.833 (95% CI 0.682 to 0.932; p < 0.0001), with a sensitivity of 90.00 and a specificity of 80.00 (**Figure 7**). The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.280.

Figure 5. The predictive capacity of the genetic score for weight regain after 5 years' follow-up in the whole (A) population, non-T2D subjects (B), and T2D patients (C).

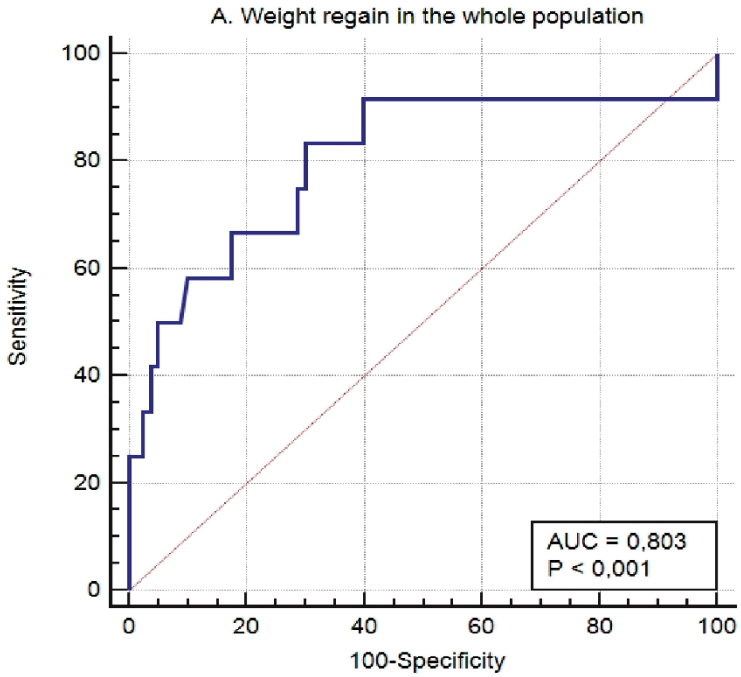
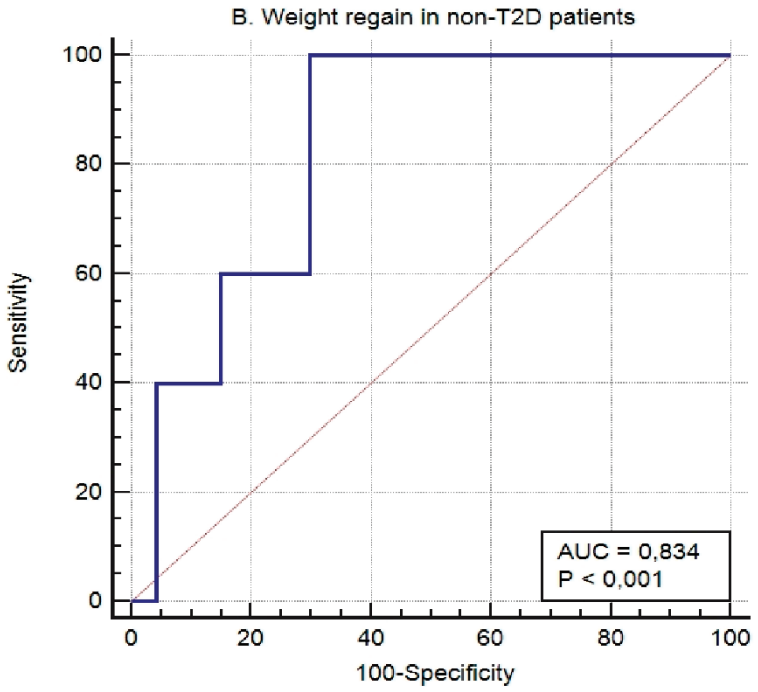


***Fig 5**. Cont.*


B. Weight regain in non-T2D patients
Sensitivity
100-Specificity
AUC = 0,834
P < 0,001
0
20
40
60
80
100

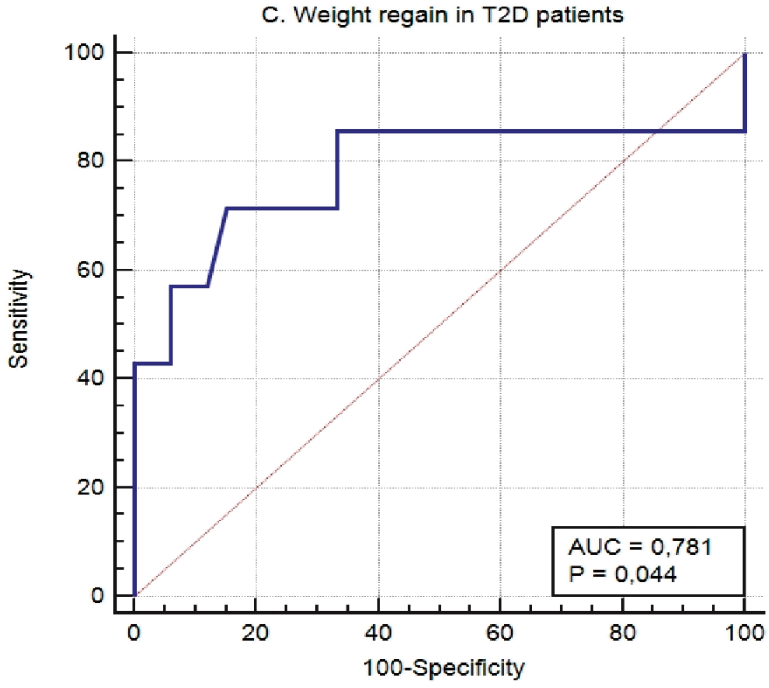

C. Weight regain in T2D patients
Sensitivity
100-Specificity
AUC = 0,781
P = 0,044
0
20
40
60
80
100

Figure 6. The predictive capacity of the DiaRem score and the combination between DiaRem and genetics in our study population. The AUROC for DiaRem was lower than obtained by genetic test (0.69 versus 0.86), and when both scores were combined the AUCROC was 0.87, with a sensitivity of 88.49% and a specificity of 80.00%.

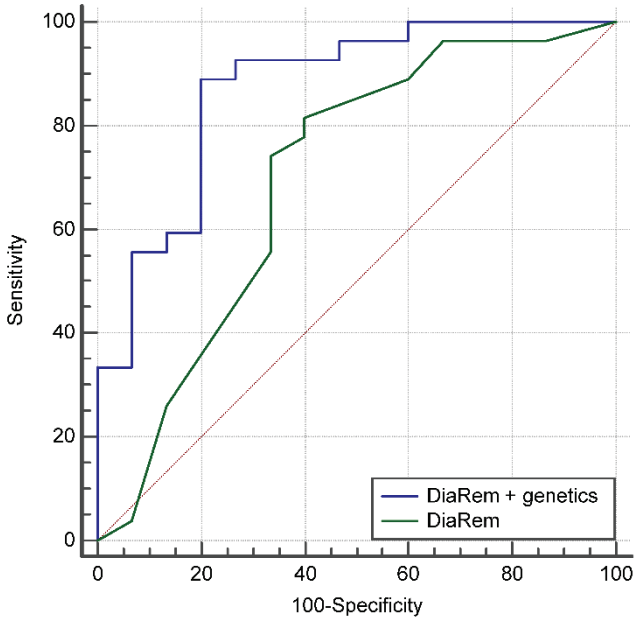
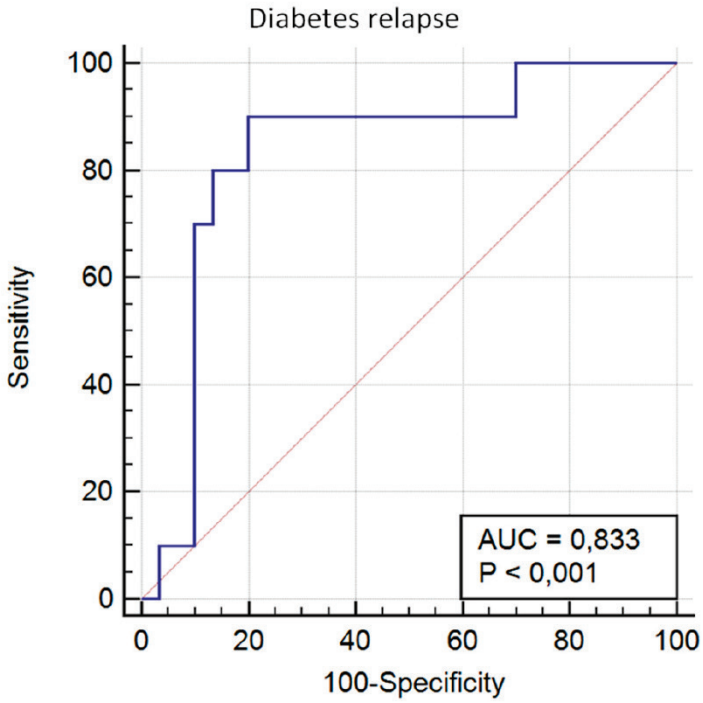


Figure 7. The predictive capacity of the genetic score for T2D relapse after 5 years' follow-up



5.2 A clinical-genetic score for predicting weight loss after bariatric surgery: the OBEGEN study (Annex 10.1.1.2).

5.2.1 Baseline Characteristics of Patients

The main baseline characteristics of patients included in the OBEGEN study are shown in **Table 4**. After a follow-up period of 14.6 ± 0.8 months, 301 (72.3%) patients achieved a %EWL higher than 50%. Patients with a favorable weight response were younger, mainly women, and underwent RYGB.

5.2.2 Construction of a Clinical Risk Score

When only available clinical data were evaluated, the multivariable logistic regression model showed than age at BS, type of BS and presence of type 2 diabetes were independent risk factors for predicting a favorable weight loss in the entire population (**Table 4**). Therefore, a clinical risk score was developed including these three variables that showed an AUROC for predicting a good response to BS of 0.775 [95% confidence interval (CI) 0.731 to 0.814, $p < 0.0001$], with a sensitivity of 93.0% and a specificity of 50.4%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.522.

5.2.3 Construction of a Genetic Risk Score

Additionally, when genetic data were analyzed alone, the multivariate logistic regression equation for predicting a favorable weight loss response after the BS included nine SNPs located in ADIPOQ, MC4R, IL-6, PPARG, INSIG2, CNR1, ELOVL6, PLIN1, and BDNF (**Table 4**). This genetic risk score showed an AUROC of 0.648 (95% CI 0.597 to 0.696, $p < 0.0001$), with a sensitivity of 48.7% and a

specificity of 75.0%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.922.

Table 4. Main clinical characteristics, metabolic, and anthropometry data of patients included in the study and according to the weight response to bariatric surgery.

	TOTAL	%EWL > 50%	%EWL ≤ 50%	P
Patients, n (%)	416	301 (72.3)	115 (27.6)	<0.001
Female, n (%)	348 (83.6)	260 (86.3)	88 (76.5)	<0.001
Age (yrs.)	48.3 ± 10.3	49.0 ± 10.4	51.5 ± 9.2	0.003
SG, n (%)	137 (32.9)	105 (34.8)	32 (27.8)	<0.001
RYGB, n (%)	280 (67.3)	218 (72.4)	62 (53.9)	<0.001
Initial BMI (Kg/m2)	44.3 ± 7.9	44.5 ± 9.6	44.2 ± 7.3	0.554
Initial weight (Kg)	113.0 ± 18.4	112.3 ± 20.3	113.4 ± 13.8	0.361
Excess weight (Kg)	49.3 ± 17.3	48.7 ± 19.2	51.3 ± 12.2	0.020
Nadir BMI (Kg/m2)	29.9 ± 5.8	28.5 ± 4.5	37.0 ± 6.8	<0.001
Type 2 diabetes, n (%)	173 (41.5)	128 (42.5)	45 (39.1)	<0.001
Hypertension (%)	286 (68.7)	204 (67.7)	82 (71.3)	<0.001
Dyslipidemia (%)	306 (73.5)	212 (70.4)	94 (81.7)	0.010
Sleep apnea (%)	116 (27.8)	88 (29.2)	28 (24.3)	<0.001

Data are mean ± SD, median (range) or n (percentage). SG: sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; BMI: body mass index. EWL: excess of weight loss. Hypertension was defined by increased systolic (≥140 mmHg) or increased diastolic (≥90 mmHg) blood pressure or using antihypertensive drugs, according to current guidelines. Dyslipidemia was defined using lipid-lowering drugs, decreased values of HDL cholesterol (men< 0.9 mmol/L, women 5.2 mmol/L), LDL cholesterol or triglycerides (>1.7 mmol/L).

5.2.4 Construction of the OBEGEN Clinical-Genetic Risk Score

Based on the clinical and genetic data from our population, we created the OBEGENCGRS, including age at surgery, type of surgery, presence of type 2 diabetes, and the nine SNPs associated with weight loss in response to BS (**Table 5**). The OBEGEN-CGRS score ranges from −4 to +4 points, with a cut-off point to define a good responder of 0.662. This predictive model showed an AUROC of 0.845 (95% CI 0.805 to 0.880, $p < 0.001$), with a sensitivity of 90.1% and a specificity of 65.5%. The calibration of the model's adequacy determined by the Hosmer–Lemeshow test was 0.927. An internal validation of this clinical- genetic algorithm

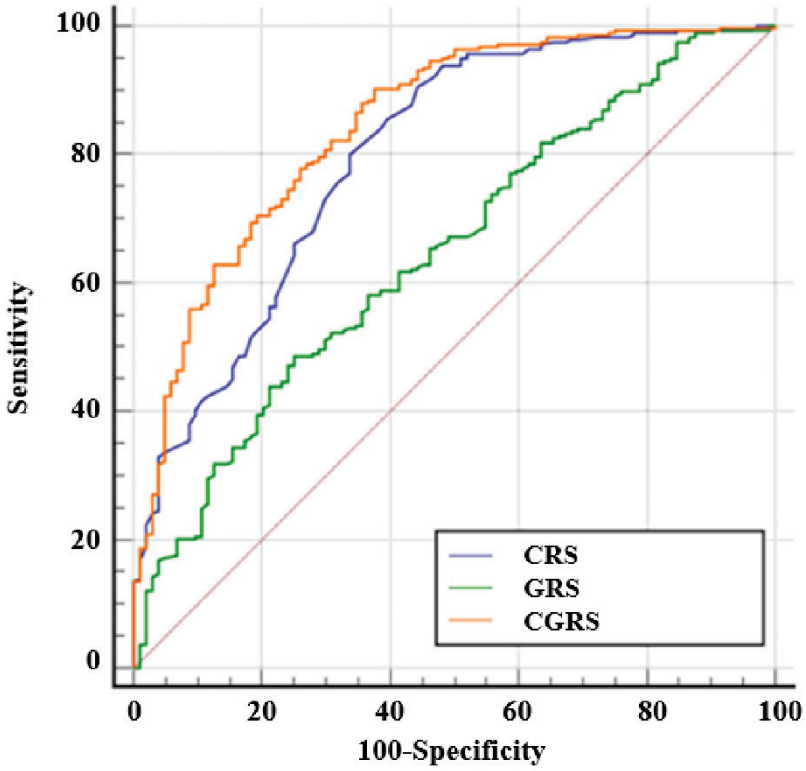
Table 5. Clinical and genetic variables that significantly predicts favorable weight loss after BS in the entire population of the OBEGEN project.

CLINICAL VARIABLES		**GENETIC VARIANTS**		**ALL SELECTED VARIANTS**	
	Coefficient		Coefficient†		Coefficient†
Age	−0.03458	rs16861209	−0.30388	CRS	1.13897
Type of surgery	0.69588	rs17782313	0.32234	GRS	1.30048
Type 2 Diabetes	3.05077	rs1800795	−0.33407	Constant	−1.34401
Constant	−3.75577	rs1801282	0.33407		
		rs3771942	−0.17997		
		rs6454674	0.24788		
		rs682447	0.41113		
		rs894160	0.28848		
		rs925946	0.28604		
		Constant	−0.30768		

†: Coefficients in multiple logistic regression model. CRS: clinical risk score; GRS: genetic risk score.

was performed using a Bootstrap method to quantify the uncertainty associated with the AUROC. The result was an AUC of 0.845 (95% CI: 0.800 to 0.888). The OBEGEN-CGRS score showed a significant higher AUROC than either the clinical score ($p = 0.0186$) or the genetic score ($p < 0.0001$) (**Figure 8**).

Figure 8. The predictive capacity of favorable weight loss (%EWL > 50%) obtained by the clinical risk score (CRS), the genetic risk score (GRS), and the clinical plus genetic risk score (CGRS) in our study population in the OBEGEN project.



5.3 Evaluation of resting energy expenditure in subjects with severe obesity and its evolution after bariatric surgery (Annex 10.1.1.3).

5.3.1 Baseline Characteristics of Patients

A total of 39 patients with SO and BMI >50 kg/m2 were included in the study as detailed in **Figure 3**. The baseline clinical and demographical characteristics of the patients are shown in **Table 6**. Measured REE was 2320.38 ± 750.81 kcal/day and significantly different to MSJ equation estimation (1994.44 ± 463.41 kcal/day, p= 0.035). Additionally, mREE directly correlated with initial weight; initial BMI and EW (0.792, 0.451, and 0.795 respectively $p < 0.0001$) indirectly correlated with age (−0.769, $p < 0.0001$). We found no difference in mREE between patients with or without associated comorbidities, including when stratified for number of comorbidities.

As expected, mREE was significantly different among men and women. Measured REE was higher in men compared to that in women (2761.0±122.0 kcal/day vs. 1964.0 ±622.0 kcal/day, $p <0.001$). One of the variables reported in the IC is the RQ (ratio of the amount of carbon dioxide produced to the amount of oxygen consumed), used to calculate rates of carbohydrate versus fat used to support energy metabolism. In this regard, when a molecule of glucose is metabolized, the RQ has a value of 1.0. Similarly, when one molecule of fat (tripalmitin) is completely metabolized, the RQ is 0.71 (112). In our cohort, RQ-baseline was 0.81±0.1, suggesting a fat oxidation–prone metabolism. In this regard, we found a negative statistically significant correlation with initial weight, initial BMI, and EW (r =−0.390, p= 0.01; r= −0.313, p= 0.05 and −0.423, p= 0.007, respectively).

Table 6. Baseline characteristics of patients with severe obesity.

DEMOGRAPHICS		**N = 39**
Gender, females % (n)		64.10 (25)
Age (years), Mean (SD)		46.5 ± 11.7
Initial Weight (kg), Mean (SD)		149.3 ± 30.36
BMI (kg/m2), Mean (SD)		56.2 ± 5.6
EW (kg), Mean (SD)		83.1 ± 22.3
Obesity- associated comorbidities		
Type 2 Diabetes, % (n)		30.8 (12)
Hypertension, % (n)		38.5 (15)
Dislipemia, % (n)		17.9 (7)
OSA, % (n)	Absent	20.5 (8)
	Mild	15.4 (6)
	Moderate	5.1 (2)
	Severe	59 (23)
Number of obesity related comorbidities, % (n)	None	10.3 (4)
	1	17.9 (7)
	2	35.9 (14)
	3	20.5 (8)
	4	15.3 (6)

BMI Body Mass Index, EW Excess of weight, NAFLD Non-Alcoholic Fatty Liver Disease, OSA Obstructive Sleep Apnea.

5.3.2 Evolution after BS

As reflected by **Figure 3**, 31 patients underwent BS and at least 5 years of follow-up: 22.6% underwent RYGB and 77.4% underwent SG. As per protocol, the SG is the recommended technique in almost all of the patients with SO. The data at 5 years follow-up is shown in **Table 7**. Patients achieved the minimum weight after the BS (nadir) after a mean follow-up of 17.1 ± 4.8 months after the BS: weight 80.2±20.5 kg, BMI 33.2±10.5 kg/m2. At this point, 87.01% (27/31) of the patients achieved >20%TWL and 80.6% (25/31) met a >50%EWL, regardless of the age, gender, or type of surgery.

Table 7. Follow-up subgroup characteristics.

N=31		**BASELINE**	**1-YEAR-FU**	**NADIR**	**5-YEARS-FU**
Age (years)		50.44± 7.52			
Sex, females % (n)		67.7 (21)			
Type of surgery % (n)		SG 77.4 (24) RYGB 22.6 (7)			
Weight (kg)		135.98±20.11[a]	92.08±23.22[a]	88.35±24.12[a]	94.37±24.67[a]
BMI (kg/m^2)		53.66±3.35[a]	36.13±6.06[a]	34.60±6.29[a]	37.04±6.02[a]
EW (kg)		72.51±11.98[a]	27.61±18.54[a]	24.89±18.54[a]	30.91±18.36[a]
Comorbidities					
Type 2 Diabetes, % (n)		45.2 (14)	6.4 (2)	6.4 (2)	19.3 (6)
Hypertension, % (n)		38.7 (12)	12.9 (4)	12.9 (4)	12.9 (4)
Dislipemia, % (n)		25.8 (8)	6.4 (2)	6.4 (2)	12.9 (4)
OSA, % (n)	Absent	45.2 (14)	61.3 (19)	61.3 (19)	51.6 (16)
	Mild	12.9 (4)	32.2 (10)	32.2 (10)	22.5 (7)
	Moderate	32.2 (10)	6.4 (2)	6.4 (2)	12.9 (4)
	Severe	12.9 (4)	0 (0)	0 (0)	6.4 (2)
Weight loss					
Percent of total weight loss (%TWL)			32.34±12.06 [a]	35.13±12.58[a]	30.74± 12.49[a]
%TWL> 20, % (n)			83.87 (26)	87.01 (27)	74.1 (23)
Percent excess weight loss (%EWL)			60.44±20.76 [a]	65.67±21.78[a]	57.32 ± 21.58[a]
%EWL >50, % (n)			64.5 (20)	80.6 (25)	67.7 (21)

BMI body mass index, *EW* excess of weight, *FU* follow-up, *OSA* obstructive sleep apnea, *RYGB* Roux –en- Y Gastric By-pass, *SG* Sleeve gastrectomy. Continuous variables expressed in mean ± SD. [a] Repeated measures ANOVA, $p < 0.001$.

At 5-year follow-up, weight was 94.37±24.67 kg and BMI 37.04±6.02 kg/m2, significantly increased from nadir (p<0.001), representing a significant weight regain in 32.25% (10/31) of the patients.

5.3.3 Changes in REE after BS

We found a significant reduction in mREE at least 1 month after the BS, achieving levels comparable to those of the Spanish population with normal weight (113), despite presenting BMI in morbid obesity range (BMI-1m after BS 45.67 ±3.80kg/m2). The mREE-12m remained significantly unchanged after the initial significant "drop-down" 1m after BS, while BMI-12m continued to significantly reduce (36.13 ± 6.06kg/m2, p<0.0001). **Figure 9** and **Table 8** show the evolution of the IC parameters after the BS. We found no statistically significant differences among techniques in REE at any time point.

Figure 9. Changes in measured resting energy expenditure and body mass index before and after bariatric surgery. *BMI*, body mass index; *mREE*, measured resting energy expenditure; *1m*, 1 month after bariatric surgery; *12m*, 12 months after bariatric surgery. Repeated measures ANOVA for: A BMI and B mREE before and after BS, p < 0.001. Results after Bonferroni correction are indicated if significant differences were found. *p < 0.05; **p < 0.01.

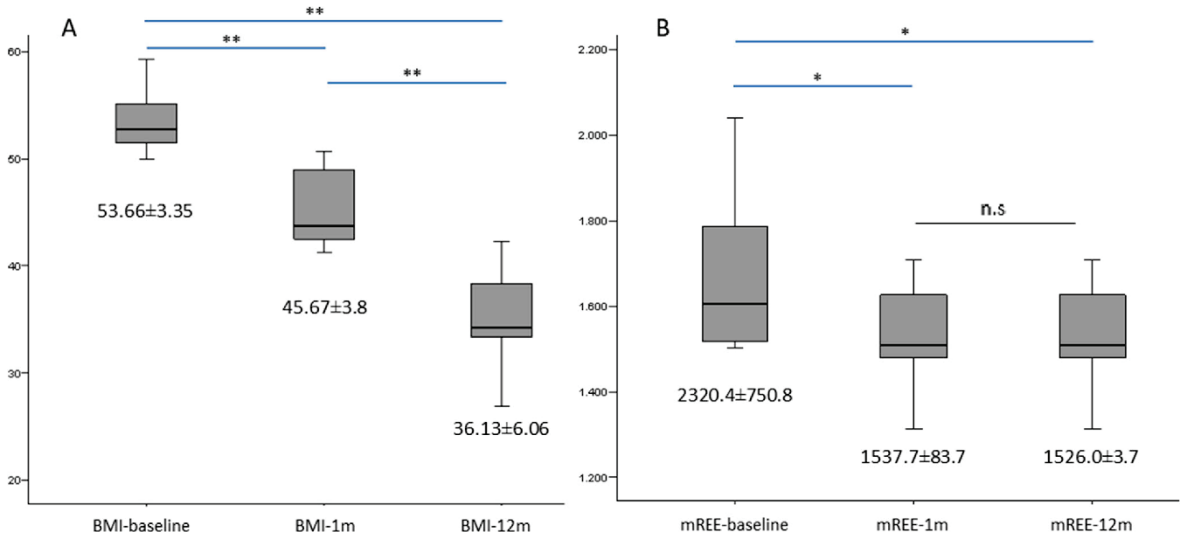


Table 8. Changes in Energy metabolism

N=31	BASELINE	1 MONTH AFTER BS	12 MONTHS AFTER BS	P VALUE ǂ
MREE (kcal/day)	2320.4 ± 750.8[A]	1537.7 ± 83.7[A, B]	1526.0 ± 3.7[B]	0.006
MSJ (kcal/day)	1994.4 ± 463.4[A]	1789.1 ± 307.5[A]	1551.1 ± 349.8[A]	0.001
RQ (V_{CO}^2/ V_O^2)	0.81 ± 0.13	0.79 ± 0.08	0.81 ± 0.07	N.S

MREE measured resting energy expenditure, *MSJ* Mifflin St-Jeor equation, *RQ* respiratory quotient, V_O^2 oxygen consumption (ml/min^{-1}), V_{CO}^2 carbon dioxide production (ml/min^{-1}). Continuous variables expressed in mean ± SD. ǂ repeated measures ANOVA, [A] Bonferroni correction p <0.005, [B] Bonferroni correction p =n.s

An inverse correlation was found between initial EW and mREE-1m and mREE-12m (r = −0.714, p = 0.047 and r = −0.681, p = 0.014, respectively). However, mREE-1m and mREE-12m did not correlate with any other weight-related variables (i.e., initial weight, 1m-weight, 1m-EW, nadir weight, nadir EW). An indirect correlation was observed between mREE-1m and mREE-12m and RQ-1m and RQ-12m, respectively, but not with mREE and RQ at baseline. Although we found a significantly difference between gender at mREE-baseline, these differences were no longer significant after BS, while MSJ showed differences between gender in all three time points, as reflected by **Table 9**.

We found no significant pre-BS predictors of reduction in m REE at 1m and 12m follow-up, among age, gender, BS technique, and obesity-related comorbidities. These parameters neither were predictors of significant weight regain at 5 years follow-up. Interestingly, the reduction of mREE at 12 months (calculated as mREE-baseline − mREE-12m) was a significant predictor of the following: (A) poor nadir weight loss after BS (%EWL<50%) and (B) weight regain at 5 years

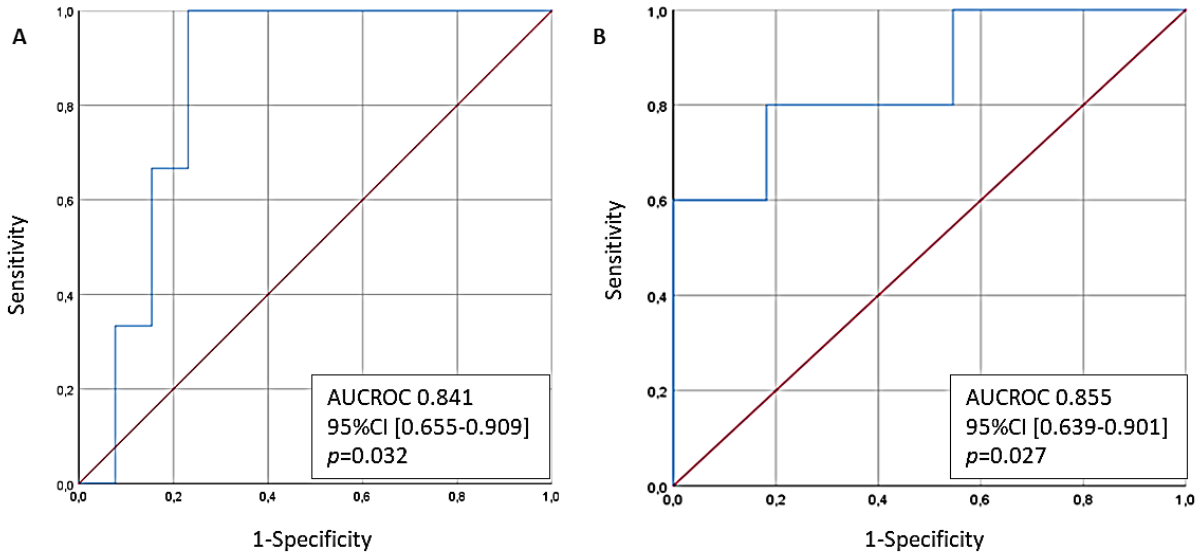
follow-up (AUCROC of 0.841 (95%CI [0.655–0.909], p=0.032) and AUCROC of 0.855 (95% CI [0.639–0.901]), p= 0.027, respectively) (**Figure 10**).

Table 9. Differences among gender in mREE and MSJ across three time points.

N=31		REE -baseline	REE-1m	REE-12m
mREE (kcal/day)	Female	1769.36±245.81[a]	1529.45±100.74	1526.0±95.55[b]
	Male	2561.0±449.40[a]	1555.73±40.40[b]	1548±72.32
MSJ (kcal/day)	Female	1778.36±97.92[a]	1604.73±97.62[a]	1393.91±107.31[a, b]
	Male	2469.80±245.79[a]	2194.80±177.32[a, b]	1896.80±461.10[a]
RQ	Female	0.92±0.19[a]	0.81±0.02	0.81±0.09
	Male	0.78±0.01[a]	0.79±0.10	0.80±0.01

[A] significantly difference between women and men p <0.05. [B] significantly differenc between gender and mree or msj p<0.05

Figure 10. The predictive capacity of the reduction in mREE at 12 months from baseline for: (A) EWL<50% at nadir and (B) weight regain after 5 years' follow-up.



6 DISCUSSION

6.1 Genetic testing to predict weight loss and diabetes remission and long-term sustainability after bariatric surgery: a pilot study. (Annex 10.1.1.1)

Bariatric surgery provides adequate and sustainable weight loss and T2D remission, but 15–20% of the subjects do not reach these targets (114). A recent study (115) showed a high inter-individual variability of the EWL response at mid-term after BS and that poor EWL could be illustrated by two different patterns: poor sustained weight loss or pronounced weight regain. At present, there are no reliable biomarkers for individual response to BS. Due to the increasing availability of BS around the world and the alarming prevalence of obesity and its associated comorbidities such as T2D, the discovery of biomarkers that will permit us to identify the best candidates for BS are urgently needed.

With this pilot study, we developed a genetic-based algorithm for the prediction of %EWL after BS and for T2D remission with high sensitivity and specificity. Still et al. (51) proposed a genetic score to predict the %EWL after BS, showing a non-statistically significant AUROC, a sensitivity of 48.39, and a specificity of 73.33. In addition, this score did not take into the account the presence of diabetes.

Regarding diabetes remission after BS, we analyzed for comparison purposes the predictive capacity of DiaRem scores (95) in our study population. In our population, the AUROC for DiaRem was lower than obtained by genetic test (0.69 versus 0.86), and when both scores were combined, the AUCROC was 0.87, with a sensitivity of 88.49% and a specificity of 80.00%. This finding supports the use of genetic testing in clinical practice.

It is worth mentioning that in diabetic patients, the rate of remission was not significantly different between the group with %EWL < 40% and the group with %EWL > 75% ($p = 0.674$). In addition, previous data showed that about 30% of T2D patients that are able to discontinue the medication after BS will present a relapse within the first 5 years (92–94). Some studies found weak correlation between weight regain, younger age or lower BMI before BS as predictors of T2D relapse after BS (94,116), while other studies found no association (92). Therefore, at present, there are no reliable predictors of T2D relapse after BS. In our study, the proposed score showed a high predictive value of T2D relapse after BS, thus, underlying the potential key role of genetic testing in precision medicine in order to assure better outcomes after BS. Interestingly, in our study, in the subgroup of T2D patients, the inclusion of SNPs associated to response to BS did not improve the prediction scores, suggesting that these genes are not critical or do not intervene in the remission and relapse of T2D. This finding suggests that the physiopathology of diabetes remission and relapse after RYGB might not be related with the %EWL in this population.

Overall, these results are intriguing and point to a genuine genetic background in the mechanisms involved in diabetes remission and relapse after BS, perhaps related to insulin resistance.

6.2 A clinical-genetic score for predicting weight loss after bariatric surgery: the OBEGEN study (Annex 10.1.1.2).

In this study we provide evidence that the combination of clinical plus genetic data is a reliable method to predict the weight response after BS. The OBEGEN-CGRS permits us to progress towards the personalization in the management of patients with severe obesity, seeking maximum efficiency with the least surgical damage. Although BS provides successful weight loss in most of the cases, 25–30% of patients who undergo BS may not achieve the desired weight reduction (117–119). This failure is considered multifactorial, with some preoperative factors associated with the hospital center (i.e., surgeons' experience, bariatric procedure, preoperative education, and recommended weight loss before BS), the patient (age, gender, ethnicity, preoperative BMI, and comorbidities associated with obesity), and psychosocial features (economic resources, household type, and personality disorders) (50,83,120–123).

The real factors that predict weight loss following BS are still far to be determined. This is due to the inconsistency in reporting and the methodological weaknesses in analysis, which include a little if any consideration of genetic factors (106,124). The development of new predictive tools for BS, based on some of the previous predictive factors, but at the same time fueled by new components, is a real need for clinicians who treat obesity worldwide. These instruments should help physicians to identify the best candidates who must undergo BS and to surgeons to optimize the surgical procedure. Few studies have addressed the influence of genetics on long-term dynamic changes in body weight with ambiguous results (76,125). The Swedish Obesity Study analyzed the impact of various SNPs from 11

genes in 1443 BS cases (53). After the evaluation of 20 gene SNPs in 249 morbidly obese subjects undergoing RYGB, Velázquez-Fernández et al. showed that POMC rs1042571 was the only associated with favorable weight loss (55).

In another group of 1011 subjects, an increasing number of SNPs alleles in or near the FTO, INSIG2, MC4R, and PCSK1 genes negatively influenced weight loss trajectories after RYGB in those with an initial BMI >50 kg/m2 (51). More recently, in a group of 146 individuals, carriers of another variant of the FTO gene (rs9939609) showed a lower success rate, as well as a greater and faster weight recovery beyond 2 years after BS (126). Nevertheless, the same SNP was not associated with different weight loss after 6 months of SG in 74 morbidly obese patients (54). Regarding the MC4R gene, among 1433 subjects with a follow-up period of 12 months after RYGB, carriers of the I251L variant lost 9% more weight compared with the noncarriers (52). Finally, a prospective observational study with 105 patients evaluated SNPs in the leptin receptor, FTO and FABP2 genes (127). This study showed that carriers of the LEP223 (rs1137101) experienced close to 25% lower excess weight at 12 and 24 months after bariatric surgery. Beyond the isolated study of one or the other gene, genetic risk scores composed by adiposity-related SNPs have been related with weight loss after RYGB or SG in Swiss, Danish, and Greek populations (56,128,129). It should be noted that only three of the nine genes included in the OBEGEN-CGRS appeared previously reported in association with the weight loss response after BS (51,52,129). This fact highlights the complexity of the genetic basis associated with the development of obesity, but also that the genes related to the therapeutic response may be different from those proposed so far.

In the OBEGEN study, including both genders and different bariatric procedures, the combination of clinic and genetic data enhanced the predictive capacity of the genetic risk score, with improved sensitivity and specificity. Altogether, our findings support the use of genetic testing in clinical practice. Among the multiple variants of genes that were evaluated, nine of them interact with clinical variables to modify the weight response to BS in the OBEGEN study. While low serum adiponectin levels have been associated with central obesity, insulin resistance, metabolic syndrome, and type 2 diabetes, ADIPOQ (rs16861209) has been significantly associated with elevated fasting serum adiponectin levels (130,131). Similarly, although IL-6 significantly increases the risk of obesity, in the PREDIMED trial carriers of the rs1800795 showed greater weight loss with the Mediterranean diet with supplements of olive oil compared to a Mediterranean diet low-fat diet than heterozygous and non-carrier carriers after 3 years of intervention (132,133). In addition, PPARG (rs1801282) was associated not only with short-term (6-month) and long-term (2-year) weight loss but also with weight regain in the Diabetes Prevention Program (134). PLIN1, a circadian lipid stabilizing protein in the adipocyte, has been associated with body weight regulation and PLIN1 (rs894160) with variability in weight loss (135,136). Elovl6, a microsomal enzyme involved in the elongation of saturated and monounsaturated fatty acids with 12, 14, and 16 carbons, regulates mitochondrial function and thermogenic capacity in brown adipose tissue (137). The BDNF (rs925946), INSIG2 (rs3771942) and CNR1 (rs6454674) variants are well stablished genetic determinants of obesity (120,138,139). Similarly, MC4R (rs17782313) is associated with high dietary intake and different obesity-related phenotypic traits, such as insulin resistance, type 2

diabetes, and hypertriglyceridemia (140). However, so far there are no data on the response to weight loss treatments regarding these last four variants.

The main differences between our CGRS and previous studies are located in the methodology assessment. Previous studies have been interested in analyzing the association between the number of genetic risk variables and the greater or lower weight loss following BS. However, we have focused our interest on the elaboration of predictive equations mixing clinical and genetic variables and the identification of predictive cut-off in those predictive equations. We have also calculated the prognostic capability of our score in identifying the “good” and “bad” responders to BS. We believe that this is a concept of great interest and more reliable in daily clinical practice. In this way, a patient who requires BS is exposed to an intervention that is difficult to reverse, so it is vital to be able to predict success or failure with a high degree of certainty. Currently, the choice of surgical technique is based on the baseline BMI and the presence of comorbidities. The introduction of a genetic predisposition score in the decision-making algorithms will bring us closer to the best selection of the patient, to choose the most convenient surgery, and to improve current health outcomes in our population. There are some potential limitations that need to be considered when contemplating the results of our study. First, we evaluated a selected population of patients who underwent bariatric surgery, excluding those who underwent to other surgical techniques other than RYGB and SG, as well as those who had used weight lowering pharmacotherapy or had required a second surgical intervention. However, we believe that the inclusion of these more extreme cases might increase rather than reduce the reliability of our score. Second, the OBEGEN project is a retrospective one, meaning

that no irrefutable clinical consequences can still be inferred to general population. Prospective long-term studies testing the genetic basis of patients with severe obesity before the recommendation of bariatric procedures are needed. Third, the characterization of favorable weight loss after BS is controversial (104). In the OBEGEN study we have used a classic definition (%EWL>50) to better confirm our previous pilot study, so the results could be different if the chosen definition had been another. Finally, our model needs to be validated in an untrained dataset to fully demonstrate its applicability in the real clinical practice.

6.3 Evaluation of resting energy expenditure in subjects with severe obesity and its evolution after bariatric surgery (Annex 10.1.1.3).

In the present study, we showed for the first time that an early and significant reduction in the REE (evaluated by means of IC-gold standard method) occurs in patients with SO that undergo bariatric surgery, up to levels comparable to those of the normoweighted Spanish population (113), despite the fact that 1 month after BS, their BMI is still in morbid obesity range. Furthermore, in our study, we showed that the reduction in REE at 12 months after the BS was a good predictor of a “good” or “poor” response to BS (“good” defined as %EWL nadir>50%) with a AUCROC of 0.841 (95%CI [0.655– 0.909], p=0.032) as well as for weight regain after 5 years of follow-up with an AUROC of 0.855 (95% CI [0.639– 0.901], p= 0.027). In other words, the greater the reduction in REE 1 year after BS, the less %EWL at nadir and the greater the weight regain after 5 years.

At present there is no reliable data on basal REE in patients with SO. We found in the literature only one study (141) that used IC, to compare our results at baseline and showed similar results (mean REE 2262 ± 122 kcal/day in patients with BMI 56kg/m2). Most of the studies published so far in patients with obesity and most of the few studies that were reported on SO used an estimated value of REE by means of equations (88). These equations were validated and calculated based on standard adults with normal weight (74). They might not be adequate for patients with obesity, and in particular with SO, and at present this represents an important gap in the personalized management of these patients. A recent external validation of REE predictive equations reported that the accuracy of the formulas decreases going from normal weight to class 3 obesity (142).

Having a real characterization of the REE in this population is necessary in order to personalize the diet (in particular calorie intake) and to assure a safe and effective weight loss and, more importantly, weight maintenance after successful weight loss, although compliance was shown to be limited in the case of long-lasting calorie-restriction intake (143). In order to shed light on this gap, in our study, we compared the values of the REE estimated by the standard recommended equations and the gold standard method, the indirect calorimetry. We found that at baseline the MSJ equation significantly underestimated the REE when compared to the gold standard (IC) (1994.44 ± 463.41 vs 2320.4 ± 750.8, p=0.031).

In exchange, after the BS, we found that the MSJ overestimated REE in males (2194.80±177.32 kcal/day vs 1555.73±40.40kcal/day, p<0.001), while in females showed no significand difference when compared to the REE measured by IC. Additionally, although we found a significant difference between genders at mREE-

baseline, these differences were no longer significant after BS, while MSJ showed differences between gender in all three time points (baseline, 1m, and 12m). This is an interesting finding and highlights the limitations of these equations that do not take into the account all the particularities of the patients with morbid obesity and in particular with SO. The MSJ estimates the REE by including the gender into the formula, but this formula was calculated using data from standard adults with normal weight and probably normal body composition (111). A possible explanation of this overestimation of eREE in males after the BS is that the formula of the equation does not include data on the changes that occur in body composition, in particular muscle mass loss after the BS (144). A significant reduction in muscle mass after the BS might explain the differences between the overestimated REE by equations and the real REE measured by IC.

Additionally, in our study, we found a significant reduction in mREE very early after the BS and 1 month and remained unchanged after 12 months, at similar levels with normoweighted Spanish population. Mean mREE-1m: 1537.67 ± 83.67 kcal/day, similar to the mREE of 1589±312 kcal/day found by *De la Cruz et al.* in healthy individuals with normal weight in Spain (113). Furthermore, the change in mREE from baseline to 12 months was a significant predictor of successful weight loss after BS and weight regain after 5 years follow-up (AUCROC of 0.841 (95%CI [0.655–0.909], p=0.032) and AUCROC of 0.855 (95% CI [0.639–0.901]), respectively), p= 0.027, respectively). No other factor included in the analysis showed a significant predictive value of evolution after BS (age, gender, BS technique, and obesity related comorbidities). Moreover, we found no differences in REE between the two types of surgery performed at any time points. However, it

should be noted that the study design was not powered to find these differences. Additionally, an indirect correlation was observed between mREE-1m and mREE-12m and between RQ-1m and RQ- 12m, but not with mREE and RQ at baseline. This finding suggests a metabolic adaptation after BS or a state of altered energy balance in the time points after surgery that can offer a partial explanation of the role of these changes in weight loss and weight regain after the BS. Metabolic adaptation (MA) is defined as the residual eREE after adjusting for changes in body composition and age (78). Although a negative energy balance, whether due to a decrease in caloric intake or an increase in energy consumption, would result in weight loss, it has been proposed that the weight loss activates compensatory mechanisms that condition the decrease observed in REE after surgery (145). Previous data in the literature suggested that a greater than predicted drop in mREE after an intervention induces a metabolic adaption, independently of the fat-free mass (146). These data, and data from our study, indicate that maybe significant changes in muscle mass that occur after BS can play a crucial role in the evolution of REE and evolution after the BS in terms of weight loss and maintenance. Our study has several limitations: (A) REE alone was measured, rather than total energy expenditure, which includes DIT and AEE. Although REE accounts for around 70% of total energy expenditure under normal circumstances, the changes in REE associated with weight loss parallel those in total energy expenditure (147). (B) Lack of body composition evaluation and (C) evaluation of dietary intake.

7 CONCLUSIONS

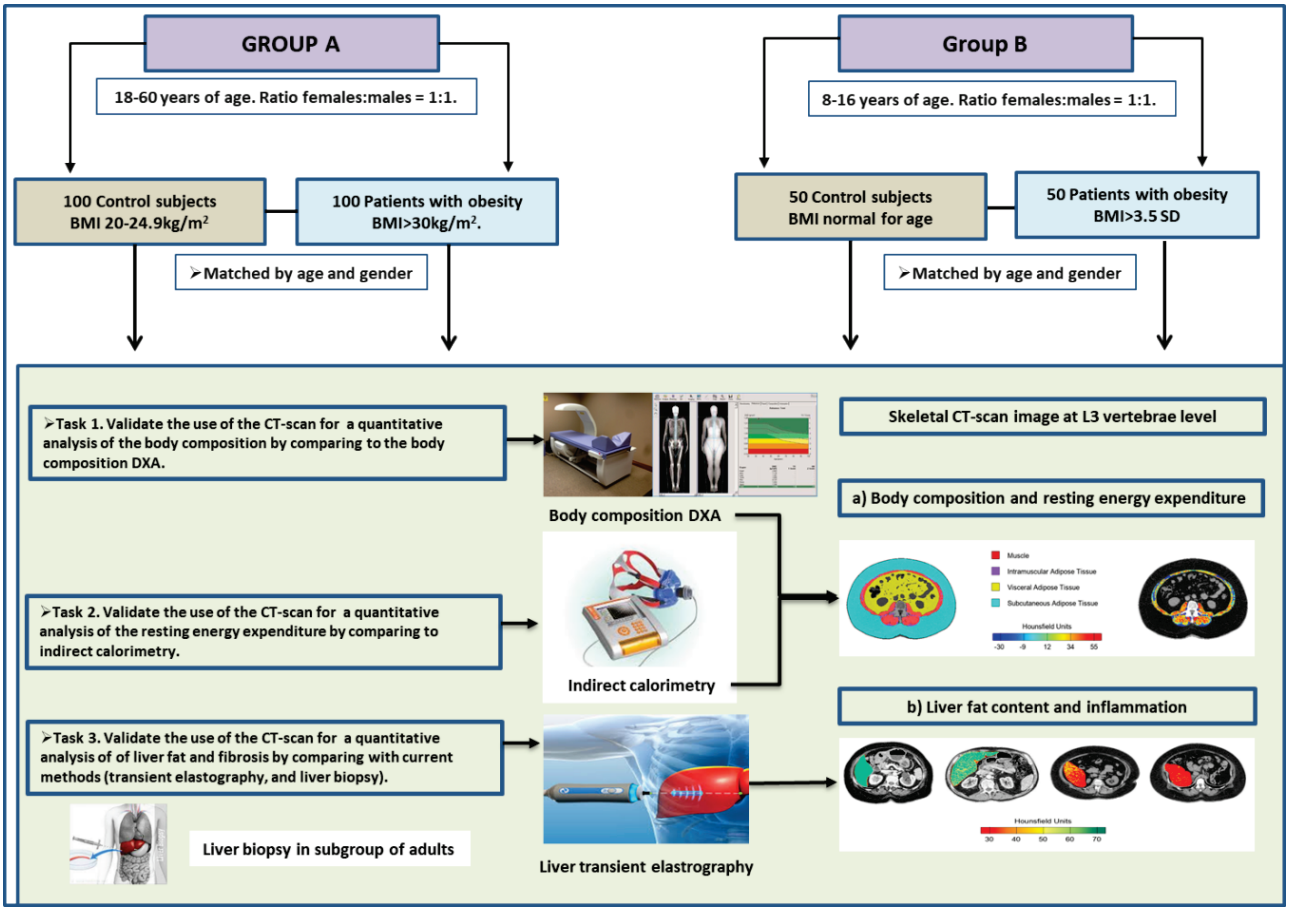
1. The genetic score developed in our studies is a highly sensitive and specific predictive marker of responses to BS in terms of weight loss and T2D remission and the long-term maintenance.
2. The combined score (clinical and genetic) significantly improved the accuracy of identifying patients with obesity that will be “good” or “bad” responders to BS in terms of weight loss and T2D remission.
3. In patients with obesity, a significant reduction of the REE occurs starting from 1 month after the BS; remains unchanged at 12 months- the metabolic adaptation.
4. The mathematical equations that are currently used to estimate the REE in the daily clinical practice are not accurate in patients with obesity.
5. The metabolic adaptation, evaluated as the reduction in REE at 12 months after the BS is one of the major conditioning factors of weight loss and maintenance 5 years after the BS.

8 FUTURE LINES OF INVESTIGATION

The results of the present thesis have opened new fields of research in our group. We have created new projects that are currently submitted for evaluation at competitive calls and are under review, aimed at developing and deepen our preliminary results.

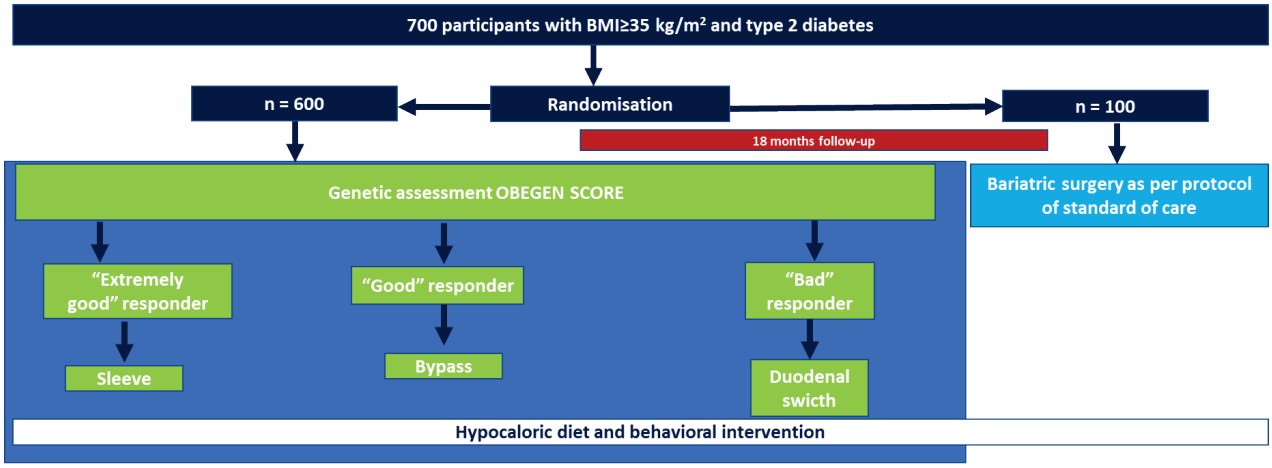
- ✓ **OBESCAN (Figure 11**). Aimed to: a) explore the underlying mechanisms of the metabolic adaptation after the BS; b) search for new simple and reliable methods to evaluate the REE in the daily clinical practice.

Figure 11. OBESCAN study design.



✓ **LOPESSOgen (Figure 12)**: Aimed at offering a personalized approach of the patients with obesity candidates for BS, based on our previous results of the genetic score.

Figure 12. LOPESSOgen study design.



WP1: Genetic testing and group asignation: all sites

WP2: Metabolism and body composition study: baseline, 6, 12, and 18 month
- Body composition by means of DXA, TC, BIA
- Metabolism study: indirect calorimetry

WP3: Bariatric surgery

WP4: Metabolomics

By facilitating the access to a complex metabolic and genetic evaluation, a personalized approach for the treatment of obesity can be provided in every case, and can strongly increase patients' adherence to treatment and follow-up, as well as the empowerment of both the patients and their families and care givers.

These two future studies, having as background the results of this thesis can have a great impact in the management of patients with obesity by changing the current clinical guidelines.

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10 ANNEXES

10.1 Scientific contributions derived from the thesis

10.1.1 Scientific Articles

10.1.1.1 Article 1.

Ciudin A, Fidilio E, Ortiz A, Pich S, Salas E, Mesa J, Hernández C, Simó-Servat O, Lecube A, Simó R. ***Genetic Testing to Predict Weight Loss and Diabetes Remission and Long-Term Sustainability after Bariatric Surgery: A Pilot Study***. J Clin Med. 2019 Jul 3;8(7):964. IF: 4.24. Q1.

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Article

Genetic Testing to Predict Weight Loss and Diabetes Remission and Long-Term Sustainability after Bariatric Surgery: A Pilot Study

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Abstract: Introduction: The aim of this pilot study was to assess genetic predisposition risk scores (GPS) in type 2 diabetic and non-diabetic patients in order to predict the better response to bariatric surgery (BS) in terms of either weight loss or diabetes remission. Research Design and Methods: A case-control study in which 96 females (47 with type 2 diabetes) underwent Roux-en-Y gastric by-pass were included. The DNA was extracted from saliva samples and SNPs were examined and grouped into 3 GPS. ROC curves were used to calculate sensitivity and specificity. Results: A highly sensitive and specific predictive model of response to BS was obtained by combining the GPS in non-diabetic subjects. This combination was different in diabetic subjects and highly predictive of diabetes remission. Additionally, the model was able to predict the weight regain and type 2 diabetes relapse after 5 years' follow-up. Conclusions: Genetic testing is a simple, reliable and useful tool for implementing personalized medicine in type 2 diabetic patients requiring BS.

Keywords: diabetes; obesity; bariatric surgery

1. Introduction

Obesity represents a major public health problem and it is associated with a significant economic burden on the health systems of developed countries, mainly due to the associated co-morbidities. Among these co-morbidities, type 2 diabetes (T2D) is one of the most important.

Bariatric surgery (BS) is a successful treatment for morbid obesity and leads to a dramatic improvement in obesity-related comorbidities [1]. The remission rate of T2D after BS is around 60–70% after 1 year of follow-up [2]. Therefore, there is a significant proportion of non-responders to BS in terms of diabetes remission. Additionally, after 5 years, there is about a 20–35% relapse of T2D after Y-de-Roux gastric by-pass (RYGB) [3–5]. A score based on clinical variables for the pre-operative prediction of T2D remission following RYGB surgery (DiaRem) was proposed [6]. However, this model has several limiting factors [7] and it has not been generally adopted in clinical practice. At present, there are no reliable predictors of T2D remission and relapse after BS.

In recent years, interest in the genetic influence on the response of different treatments for obesity has increased. Two retrospective studies [8,9] showed that several single nucleotide polymorphisms (SNPs) were associated with a poor response to BS. However, in these studies the discrimination capacity of the GPS was not significant, and the role of T2D in the response to BS and the impact of these genetic factors on diabetes remission were not evaluated.

On this basis, the aim of the present study was to evaluate whether genetic markers can be used for the prediction of adequate weight loss and diabetes remission after BS.

2. Material and Methods

A single-center, retrospective observational pilot study in a third-level university hospital (Vall d´Hebron University Hospital, Barcelona, Spain) was conducted following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. The study comprised patients that underwent RYBG surgery between January 2010 and December 2012. The inclusion criteria were women, stable weight in the prior 6 months before BS, and minimum of 5 years of follow-up after BS. In order to avoid heterogeneity and given that the vast majority of the patients under bariatric surgery were women, we decided to rule out the inclusion of men in this pilot study. The patients were informed about the study and they all signed the written informed consent form.

The exclusion criteria were male, marked mobility problems, a different BS technique apart from RYBG, and severe psychiatric or eating disorders. For the genetic study, a sample of saliva was collected. The characteristics of RYBG were food loop length: 150–180 cm, and bilio-pancreatic loop length: 120 cm, gastric pouch 30 cc^3. The technique was the same in all cases, performed by the same surgical team in our hospital.

Excess body weight (EBW) was defined as the amount of weight that was in excess of the ideal body weight (IBW). The percentage of excess weight loss (EWL) was calculated according to the formula: %EWL = (weight before BS (kg) − weight after BS (kg)/EBW(kg)) × 100. The post-BMI weight regain was defined as a 10% regain of the minimal weight after BS. The minimal weight after BMI was achieved at 2 years follow-up for all of the patients.

Diabetes remission was defined according to American Diabetes Association (ADA) criteria [10]. Relapse of T2D was defined as one or more of the following conditions: (a) restarting diabetes medication; (b) one or more HbA1c measures ≥ 6.5%; and/or (c) one or more fasting glucose measures ≥ 126 mg/dL [11].

The study was approved by the Local Ethics Committee and registered at Clinical.Trials.gov, NCT02405949.

2.1. Genotyping and Sequencing

The DNA was extracted from saliva samples and processed by GoldenGate® Genotyping Assay for VeraCode. The genetic predisposition was assessed using Nutri inCode (NiC) (Ferrer inCode) and selecting the 57 SNPs associated with susceptibility to diabetes, obesity, appetite regulation, weight loss in response to hypocaloric diet, and the response to BS. The details about the SNPs are reflected in the Supplementary Materials. The selected SNPs were grouped into three genetic predisposition risk scores (GPS): diabetes remission, weight loss in non-diabetic subjects, and weight loss in subjects with diabetes.

2.2. Statistical Analysis

In order to assess the best predictive GPS, patients were distributed into 4 subgroups according to the BS response (%EWL) and the presence of T2D: (1) %EWL < 40% without diabetes (n = 15); (2) %EWL < 40% with diabetes (n = 16); (3) %EWL > 75% without diabetes (n = 35); and (4) T2D and %EWL > 75% with diabetes (n = 31). Univariate and multivariate logistic regressions were used to establish associations. Akaike Information Criterion (AIC)-based backward selection was used to remove insignificant terms from an initial model containing all the candidate predictors. The calibration

of the model's adequacy was determined by the Hosmer–Lemeshow test. The area under the ROC curve (AUROC) was used for evaluating the prediction performance of the models. The cut-offs for the developed algorithms were selected as the point which maximizes the Youden index.

3. Results

The clinical characteristics of the patients included in the study are shown in Table 1. Apart from age, we did not find any significant differences between diabetic and non-diabetic subjects before BS. The diabetic treatment received by subjects with diabetes is displayed in Figure 1. No other medication apart from AINEs occasionally and vitamin supplements as per protocol after bariatric surgery (ciancobalamin 1000 mcg/month, colecalciferol 25.000–100.000 UI/month) were administered.

Table 1. Baseline characteristics of the patients included in the study.

	Non-Diabetic Patients	Type 2 Diabetic Patients	*p*
N	50	47	
Age (years)	48.0 (37.5; 55.0)	52.0 (46.0; 58.8)	0.0016
Initial BMI (Kg/m^2)	45.2 (43.0; 48.5)	42.5 (40.1; 46.4)	0.008
2 y post-BS BMI (Kg/m^2)	31.8 (26.1; 35.6)	30.9 (26.8; 35.7)	n.s.
5 y post-BS BMI (Kg/m^2)	32.63 (21; 52.14)	33.68 (21; 46.43)	n.s.
Hypertension (%)	48.3	49.5	n.s.
Dyslipidemia (%)	43.2	45.7	n.s.
Sleep apnea (%)	27.2	29.7	n.s.

In the subgroup of the non-diabetic patients, the multivariate logistic regression equation for predicting positive weight loss response (%EWL > 75%) after the BS (NiC-Bariatric-ND) includes SNPs associated with weight loss in response to a hypocaloric diet and SNPs associated to appetite regulation. The model showed an AUROC of 0.763 (95% CI 0.605 to 0.920; $p < 0.001$), a sensitivity of 86.49%, and a specificity of 57.14%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.679.

The continuous variables were median (1st quartile; 3rd quartile) and the categorical data were percentages. BMI: body mass index. EWL: excess of weight loss. BS: bariatric surgery. Hypertension was defined by increased systolic (≥140 mmHg) or increased diastolic (≥90 mmHg) blood pressure or by the use of antihypertensive drugs, according to current guidelines. Dyslipidemia was defined by the use of lipid-lowering drugs, decreased values of HDL cholesterol (men < 0.9 mmol/L, women < 1.0 mmol/L) or by at least one increased value of total cholesterol (>5.2 mmol/L), LDL cholesterol or triglycerides (>1.7 mmol/L).

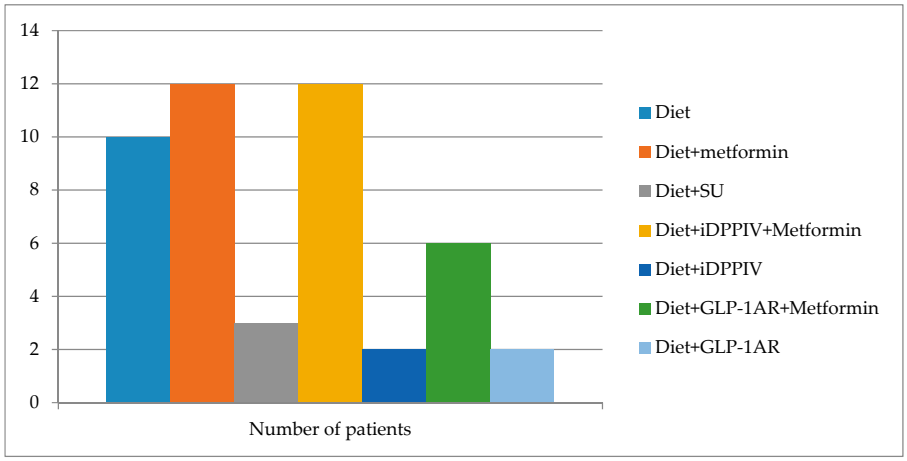

Figure 1. The diabetic treatment received before BS by the subjects with diabetes included in the study. SU: sulphonylurea, iDPPIV: DPPIV enzyme inhibitor, GLP-1AR: GLP-1 receptor agonists.

Weight regain after 5 years' follow-up was seen in 9.6% of the patients. The model to identify the patients who had presented weight regain after 5 years' follow-up showed an AUROC of 0.834 (95% CI 0.705 to 0.923; $p < 0.0001$), a sensitivity of 100%, and a specificity of 70.21%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.5148.

In T2D patients, the multivariate logistic regression equation for the prediction of weight loss response (%EWL > 75%) after BS (NiC-Bariatric-D) included SNPs associated with weight loss in response to hypocaloric diet, SNPs associated to response to BS [9], and SNPs associated to response to lifestyle interventions [11]. The model showed an AUROC of 0.929 (95% CI 0.850 to 0.99; $p < 0.001$), a sensitivity of 87.10% and a specificity of 93.33%. The calibration of the model's adequacy determined by the Hosmer–Lemeshow test was 0.291. Weight regain in subjects with diabetes was observed in 17.5% of them. The model to identify patients with diabetes who will regain weight after a follow-up of 5 years after bariatric surgery showed in this case an AUROC of 0.781 (95% CI 0.623 to 0.896; $p < 0.04$), a sensitivity of 71.43%, and a specificity of 84.85%. The calibration of the model's adequacy determined by the Hosmer–Lemeshow test was 0.8664. Figure 2 shows the AUROC corresponding to weight regain in the whole (Figure 2A) population, non-diabetic subjects (Figure 2B), and T2D patients (Figure 2C).

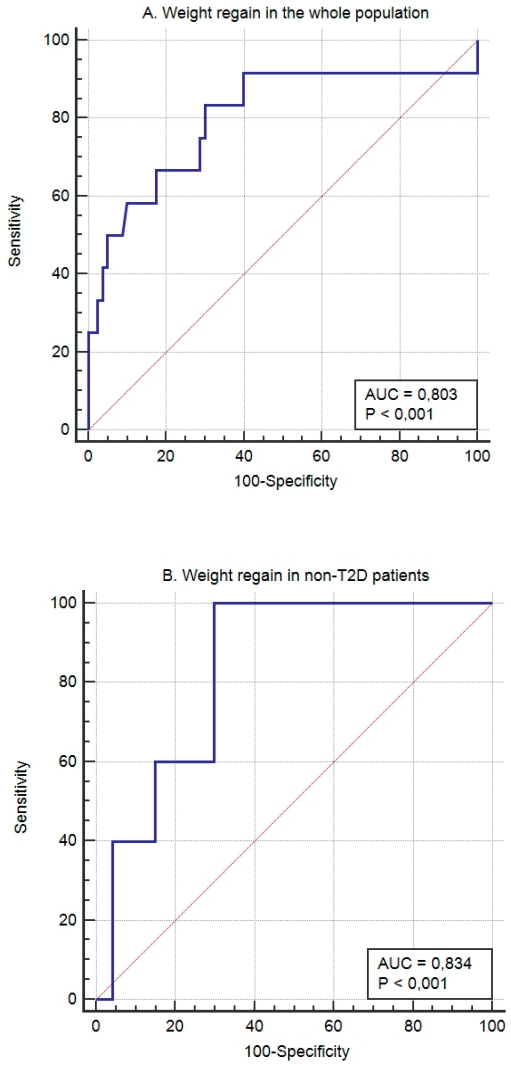

Figure 2. *Cont.*

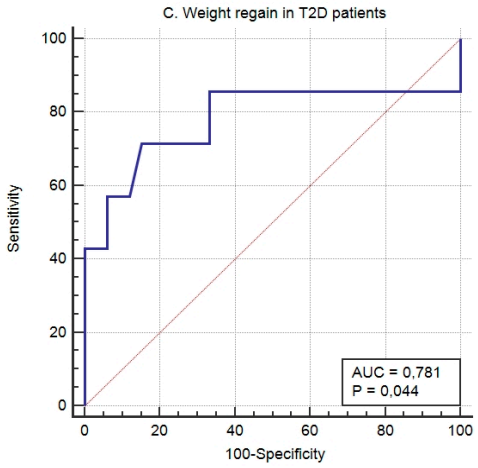

Figure 2. The predictive capacity of the genetic score for weight regain after 5 years' follow-up in the whole (**A**) population, non-T2D subjects (**B**), and T2D patients (**C**).

Diabetes remission was seen in 73.91% of the type 2 diabetic patients included in the study (66.67% in the group of %EWL < 40% and 77.42% in the group of %EWL > 75%). Diabetes relapse was seen in 25% of the patients.

The multivariate logistic regression equation for the prediction of diabetes remission and relapse after BS (NiC-Bariatric-DR) included SNPs associated with obesity, SNPs associated with weight loss in response to hypocaloric diet, SNPs associated with appetite regulation, and SNPs associated with genetic predisposition to diabetes. This prediction model showed an AUROC of 0.868 (95% CI 0.709 to 0.976; $p < 0.0001$) for diabetes remission, with a sensitivity of 76.47% and a specificity of 83.33%. In our population, the AEROC for DiaRem was lower than obtained by genetic testing, (0.69 versus 0.86), and when both scores were combined, the AUROC was 0.87, with a sensitivity of 88.49% and a specificity of 80% (Figure 3).

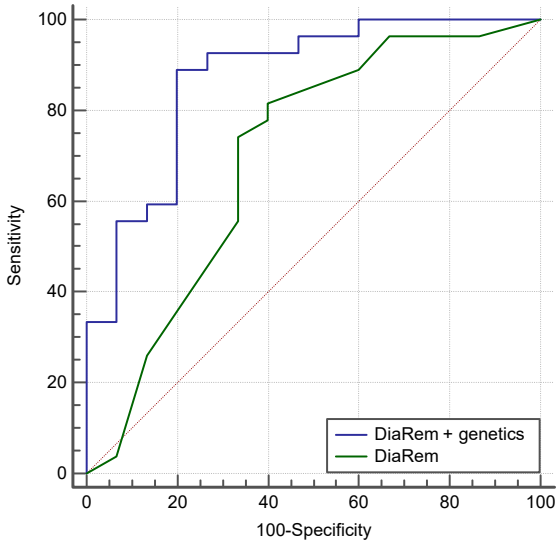

Figure 3. The predictive capacity of the DiARem score and the combination between DiARem and genetics in our study population. The AUROC for DiaRem was lower than obtained by genetic test (0.69 versus 0.86), and when both scores were combined the AUCROC was 0.87, with a sensitivity of 88.49% and a specificity of 80.00%.

Regarding diabetes relapse after 5 years, the model based showed an AUROC of 0.833 (95% CI 0.682 to 0.932; $p < 0.0001$), with a sensitivity of 90.00 and a specificity of 80.00 (Figure 4). The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.280.

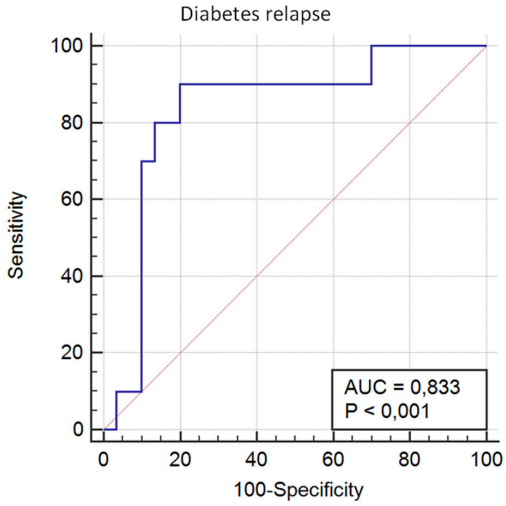

Figure 4. The predictive capacity of the genetic score for T2D relapse after 5 years' follow-up.

4. Discussion

Bariatric surgery provides adequate and sustainable weight loss and T2D remission, but 15–20% of the subjects do not reach these targets [12]. A recent study [13] showed a high inter-individual variability of the EWL response at mid-term after BS and that poor EWL could be illustrated by two different patterns: poor sustained weight loss or pronounced weight regain. At present, there are no reliable biomarkers for individual response to BS. Due to the increasing availability of BS around the world and the alarming prevalence of obesity and its associated co-morbidities such as T2D, the discovery of biomarkers that will permit us to identify the best candidates for BS are urgently needed.

In the present study, we developed genetic-based algorithms for the prediction of %EWL after BS and for T2D remission with high sensitivity and specificity. Still et al. [8] proposed a genetic score to predict the %EWL after BS, showing a non-statistically significant AUROC, a sensitivity of 48.39, and a specificity of 73.33. In addition, this score did not take into the account the presence of diabetes.

Regarding diabetes remission after BS, we analyzed for comparison purposes the predictive capacity of DiARem scores [8] in our study population. In our population, the AUROC for DiaRem was lower than obtained by genetic test (0.69 versus 0.86), and when both scores were combined, the AUCROC was 0.87, with a sensitivity of 88.49% and a specificity of 80.00%. This finding supports the use of genetic testing in clinical practice.

It is worth mentioning that in diabetic patients, the rate of remission was not significantly different between the group with %EWL < 40% and the group with %EWL > 75% ($p = 0.674$). In addition, previous data showed that about 30% of T2D patients that are able to discontinue the medication after BS will present a relapse within the first 5 years [3–5]. Some studies found weak correlation between weight regain, younger age or lower BMI before BS as predictors of T2D relapse after BS [5,14], while other studies found no association [3]. Therefore, at present, there are no reliable predictors of T2D relapse after BS. In our study, the proposed score showed a high predictive value of T2D relapse after BS, thus, underlying the potential key role of genetic testing in precision medicine in order to assure better outcomes after BS. Interestingly, in our study, in the subgroup of T2D patients, the inclusion of SNPs associated to response to BS did not improve the prediction scores, suggesting that these genes are not critical or do not intervene in the remission and relapse of T2D. This finding suggests that the

physiopathology of diabetes remission and relapse after RYGB might not be related with the %EWL in this population.

Overall, these results are intriguing and point to a genuine genetic background in the mechanisms involved in diabetes remission and relapse after BS, perhaps related to insulin resistance.

In conclusion, in this pilot study we have developed highly sensitive and specific genetic predictive scores of responses to BS in terms of weight loss and T2D remission and the long-term sustainability of these effects. These results would allow us not only to implement a more effective and personalized BS, but also to optimize healthcare resources. However, further studies with a larger sample size to confirm this pilot study are needed.

Supplementary Materials: The supplementary materials are available online at http://www.mdpi.com/2077-0383/8/7/964/s1.

Author Contributions: A.C. and E.F. contributed to the study design, obtained study data, assisted in data analysis, and drafted the initial manuscript. O.S. and A.O. obtained study data. S.P. and E.S. performed genetic and statistical analyses. J.M., C.H. and A.L. made substantial contributions to the acquisition, analysis and interpretation of data, and critically reviewed the manuscript. R.S. contributed to the study design, discussion, reviewed the manuscript, supervised the project, and is the guarantor of this work, and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version of the manuscript.

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10.1.1.2 Article 2.

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A Clinical-Genetic Score for Predicting Weight Loss after Bariatric Surgery: The OBEGEN Study

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Abstract: Around 30% of the patients that undergo bariatric surgery (BS) do not reach an appropriate weight loss. The OBEGEN study aimed to assess the added value of genetic testing to clinical variables in predicting weight loss after BS. A multicenter, retrospective, longitudinal, and observational study including 416 patients who underwent BS was conducted (Clinical.Trials.gov- NCT02405949). 50 single nucleotide polymorphisms (SNPs) from 39 genes were examined. Receiver Operating Characteristic (ROC) curve analysis were used to calculate sensitivity and specificity. Satisfactory response to BS was defined as at nadir excess weight loss >50%. A good predictive model of response [area under ROC of 0.845 (95% CI 0.805–0.880), $p < 0.001$; sensitivity 90.1%, specificity 65.5%] was obtained by combining three clinical variables (age, type of surgery, presence diabetes) and nine SNPs located in ADIPOQ, MC4R, IL6, PPARG, INSIG2, CNR1, ELOVL6, PLIN1 and BDNF genes. This predictive model showed a significant higher area under ROC than the clinical score ($p = 0.0186$). The OBEGEN study shows the key role of combining clinical variables with genetic testing to increase the predictability of the weight loss response after BS. This finding will permit us to implement a personalized medicine which will be associated with a more cost-effective clinical practice.

Keywords: obesity; bariatric surgery; weight loss; genetics; polygenic risk

1. Introduction

Obesity is a multifactorial and complex disease, caused by the contribution and interaction of environmental and genetic factors [1,2]. Its prevalence has increased dramatically in recent decades, and up to one-fifth of the whole world's population is expected to live with obesity by 2025 [3]. The discouraging results provided by the conventional treatment have led to the progressive use of the bariatric surgery (BS) as a main treatment for morbidly obesity in Western countries [4].

However, around 30% of the patients that undergo BS do not reach an appropriate weight loss and/or do not resolve the comorbidities associated with obesity [5–7]. This fail is associated with a decline in health-related quality of life and patients report feelings of frustration, anger, and even depression [8,9]. Therefore, the identification of new predictive factors of response to BS seems mandatory [10–13]. This strategy will permit us to identify the best candidates to BS and even to select the type of BS technique, thus optimizing the health care resources.

Inheritance is responsible for 40–75% of all the causes of obesity, a percentage modulated by the epigenetic influence [2,14]. Through genome-wide association studies (GWAS), a series of gene variants and single nucleotide polymorphisms (SNPs) in more than 120 genes have been linked with eating behavior, energy expenditure, response to diet, or lifestyle interventions [15,16]. As each variant alone has little effect on body weight, genetic predisposition is conditioned by the simultaneous presence of SNPs in multiple genes [11]. The possibility of elucidating the best combination of SNPs responsible for the variability of the response to BS in terms of weight loss offers the opportunity to design individualized therapy strategies. Our group has recently showed that an algorithm based on the selection of SNPs associated with predisposition to obesity, appetite regulation, and weight loss was able to predict the percentage of excess body weight loss (%EWL) after BS with high sensitivity and specificity [17]. It should be noted that this was a pilot study including only women who undergo a Roux-en-Y gastric bypass (RYGB), precluding the extrapolation of the results to the whole population.

The objective of the OBEGEN study is to confirm and extend our previous data regarding the added value of genetic testing to clinical variables in predicting the weight loss after BS. For this purpose, a total of 416 subjects (women and men) were evaluated, both RYGB and sleeve gastrectomy (SG) were included, and 50 SNPs were assessed.

2. Materials and Methods

2.1. Statement on Ethics

The OBEGEN study was approved by the human ethics committee of the University Arnau de Vilanova Hospital of Lleida (CEIC-1743). All potential participants gave written informed consent to join the study, which was conducted according to the Helsinki Declaration and the Good Clinical Practice Guidelines. The study was registered in Clinical.Trials.gov- NCT02405949 (accessed on 16 October 2021).

2.2. Study Design and Description of the Study Population

The OBEGEN project was a multicenter, retrospective, longitudinal, and observational study investigating the role of some genetic variants added to clinical variables to predict weight loss after BS. A total of 416 patients who underwent BS between January 2017 and August 2018 at the Obesity Units of four University Hospitals in Catalonia (Spain) were included.

Eligible patients were men and women ≥18 years old, who underwent BS at least 18 months prior to the study. Among the 449 patients who met these criteria, 33 were excluded because of the following reasons: current pregnancy (n = 2), development of drug or alcohol abuse or eating disorders after bariatric surgery (n = 5), markedly mobility problems (n = 4), a different bariatric surgery technique apart from RYGB or SG (n = 9), use of weight lowering pharmacotherapy (n = 7) or a second surgical intervention required during follow-up (n = 6). The study flow chart is displayed in Figure 1.

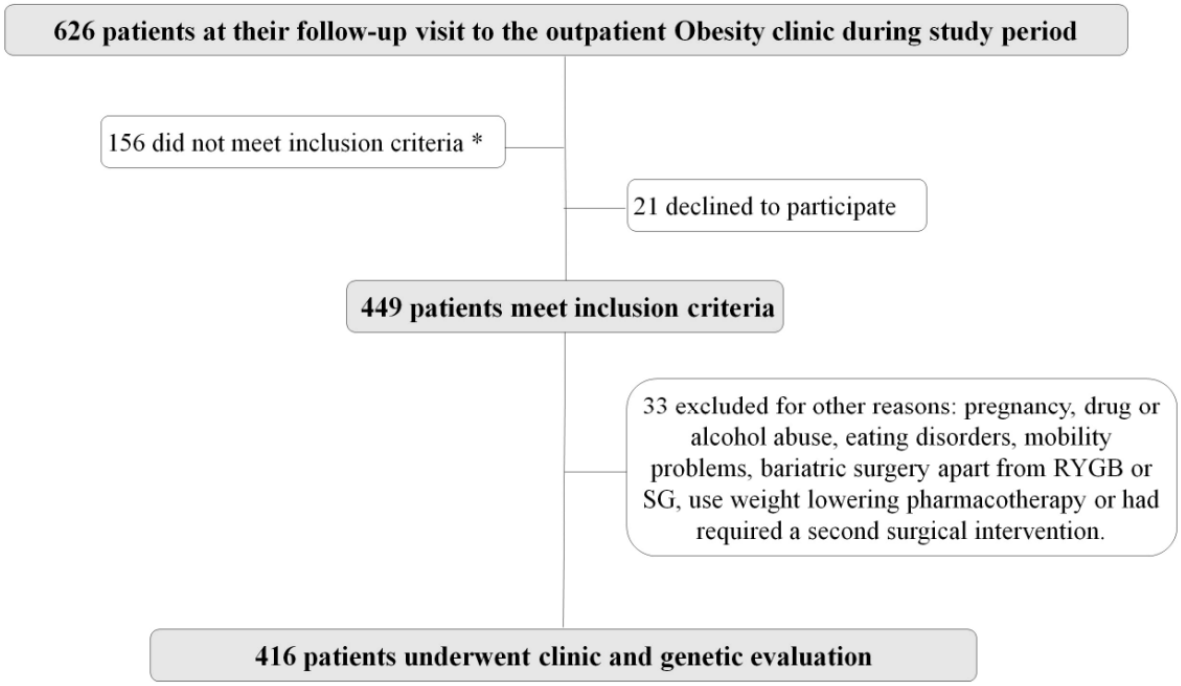

Figure 1. Flow chart of the OBEGEN study population.

All the patients agreed to participate in the study and signed the informed consent underwent complete medical history, anthropometric measurement, physical examination, and DNA sampling.

2.3. Outcome Weight Measures

The primary endpoint was the %EWL at nadir. Excess body weight (EBW) was defined as the amount of weight that was in excess from the ideal body weight (IBW). IBW was estimated according to the 1983 Metropolitan Life Insurance Tables (use the midpoint for medium frame) [18]. The %EWL was calculated according to the formula: [total preoperative weight (kg) − weight after bariatric surgery (kg)/EBW (kg)] × 100. Those with a reduction in their %EWL >50% at nadir were considered "good responders" [19].

2.4. Genotyping

DNA was extracted from saliva samples and processed by GoldenGate® Genotyping Assay for VeraCode. The genetic predisposition was assessed using the 50 SNPS in 39 genes included in a commercial nutrigenomic product, the Nutri inCode (NiC) (Ferrer inCode, Barcelona, Spain). This product includes SNPs that had previously been associated with susceptibility to weight loss, both in response to lifestyle intervention and BS [8,20,21]. In addition, Nutri inCode also includes selected variants of published GWAS studies or replication studies related to genetic susceptibility to regulate appetite and develop type 2 diabetes and obesity [22,23]. Finally, in the present study, the panel has been enriched with new 11 SNPs compared to our pilot study (Table 1) [17].

Table 1. Selected genes and single nucleotide polymorphisms evaluated in the OBEGEN study.

Gene	Name	Chromosome Allocation of Human Ortholog	SNP
ACE	angiotensin I converting enzyme	17q23.3	rs4343
ADIPOQ	adiponectin, C1Q and collagen domain containing	3q27.3.	rs16861209 rs2241766
ADRB3	adrenoceptor beta 3	8p11.23.	rs4994 rs9693898
AGRP	Agouti related neuropeptide	16q22.1	rs11575892 *
AGTR1	angiotensin II receptor type 1	3q24.	rs5186
APOA2	apolipoprotein A2	11q23.3.	rs5082
APOA5	apolipoprotein A5	11q23.3.	rs651821
APOC3	apolipoprotein C3	11q23.3.	CD010
BDNF	brain derived neurotrophic factor	11p14.1.	rs6265 rs925946
CCDC93	coiled-coil domain containing 93	2q14.1	rs10490628 *
CDKAL1	CDK5 regulatory subunit associated protein 1 like 1	6p22.3.	rs7754840
CDKN2B	cyclin dependent kinase inhibitor 2B	9p21.3.	rs10811661
CLOCK	clock circadian regulator	4q12.	rs4580704 rs4864548
CNR1	cannabinoid receptor 1	6q15	rs6454674 *
ELOVL6	ELOVL fatty acid elongase 6	4q25.	rs682447
ESR1	estrogen receptor 1	6q25.1	rs3778099 *
FTO	fat mass and obesity associated	16q12.2.	rs9939609
GHRL	ghrelin and obestatin prepropeptide	3p25.3.	rs696217
IGF2	insulin like growth factor 2	11p15.5	rs680 *
INSIG2	insulin induced gene 2	2q14.1.	rs7566605 rs3771942 *
IL-1B	interleukin 1 beta	2q14.1	rs1143643 *
IL6	interleukin 6	7p15.3.	rs1800795
LEP	leptin	7q32.1.	rs12535708
LEPR	leptin receptor	1p31.3.	rs1137100
LPL	lipoprotein lipase	8p.22.	rs328
MC4R	melanocortin 4 receptor	18q21.32.	rs12970134 rs52820871 rs17700633 rs2229616 rs17782313
MTCH2	mitochondrial carrier 2	11p11.2.	rs10838738
NEGR1	neuronal growth regulator 1	1p31.1	rs2568958 *
PLIN1	perilipin 1	15q26.1.	rs1052700 rs894160
PPARA	peroxisome proliferator activated receptor alpha	22q13.31.	rs1800206
PPARG	peroxisome proliferator activated receptor gamma	3p25.2.	rs1801282
PCSK1	proprotein convertase subtilisin/kexin type 1	5q15.	rs6235
PON1	paraoxonase 1	7q21.3.	CD014
SIRT1	sirtuin 1	10q21.3	rs7069102 *
TCF7L2	transcription factor 7 like 2	10q25.2.	rs7903146
TMEM18	transmembrane protein 18	2p25.3	rs2867125 *
UCP1	uncoupling protein 1	4q31.2	rs45539933 *
UCP2	uncoupling protein 2	11q13.4.	rs659366
UCP3	uncoupling protein 3	11q13.4.	rs1800849
WFS1	wolframin ER transmembrane glycoprotein	4p16.1.	rs10010131

SNP: single nucleotide polymorphism. *: Genetic variants that have been added from the pilot study [17].

2.5. Statistical Methods

A normal distribution of the variables was established using the Kolmogorov–Smirnov test, and data are expressed as the mean ± SD, median (interquartile range), or as a percentage. Comparisons between groups were made using the Student's *t*-test and

the Mann–Whitney U test for quantitative variables, and the Pearson's chi-squared for categorical variables. The relationship between continuous variables was examined by the Pearson linear correlation test.

To assess the best predictive clinical–genetic risk score, patients were distributed into two groups according to the BS response: (i) %EWL $\leq$ 50% (n = 113); (ii) %EWL > 50% n = 301). On one side, the different SNPs were coded as 0, 1, or 2 according to the number of risk alleles associated with a favorable weight response. Clinical variables analyzed included gender, age at surgery, preoperative weight and BMI, type of surgery (RYGB or SG), EBW, and presence of type 2 diabetes. Univariate and multivariate logistic regressions were used to establish associations between the genetic and/or the clinical variables and the loss of weight. In this way, we generated three risk scores: (i) a clinical risk score, (ii) a genetic risk score, and (iii) the OBEGEN clinical-genetic risk score (OBEGEN-CGRS), which includes both the selected clinical and genetic variants in the multivariate logistic regression.

Akaike Information Criterion (AIC)-based backward selection was used to remove not significant variables, from an initial model containing all the candidate predictors. The calibration of the logistic model's adequacy was determined using the test of fit by the Hosmer–Lemeshow. The accuracy of different scores/models in discriminating those who obtained the objective weight loss (%EWL > 50%) from those who did not achieve the objective weight loss (for evaluating the prediction performance of the models) was evaluated using a Receiver Operating Characteristic (ROC) curve analysis. The cut-offs to calculate the sensitivity and specificity of the developed algorithms were selected as the point which maximizes the Youden index. An odds ratio with its 95% confidence interval was finally calculated. The total area under the ROC (AUROC) curve was interpreted following guidelines: 0.9–1.0 excellent, 0.8–0.9, good; 0.7–0.8, fair; 0.6–0.7, poor; and 0.5–0.6, not useful. Comparisons between the obtained AUROC were compared using the method of Hanley and McNeil.

All the contrasts were bilateral with a significance level of 0.05. The data were analyzed with the Statistical Package for the Social Sciences software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA).

3. Results

3.1. Baseline Characteristics of Patients

The main baseline characteristics of patients included in the OBEGEN study are shown in Table 2. After a follow-up period of 14.6 $\pm$ 0.8 months, 301 (72.3%) patients achieved a %EWL higher than 50%. Patients with a favorable weight response were younger, mainly women, and underwent RYGB.

3.2. Construction of a Clinical Risk Score

When only available clinical data were evaluated, the multivariable logistic regression model showed than age at BS, type of BS and presence of type 2 diabetes were independent risk factors for predicting a favorable weight loss in the entire population (Table 3). Therefore, a clinical risk score was developed including these three variables that showed an AUROC for predicting a good response to BS of 0.775 [95% confidence interval (CI) 0.731 to 0.814, $p < 0.0001$], with a sensitivity of 93.0% and a specificity of 50.4%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.522.

Table 2. Main clinical characteristics, metabolic, and anthropometry data of patients included in the study and according to the weight response to bariatric surgery.

	Total	%EWL > 50%	%EWL ≤ 50%	*p*
Patients, *n* (%)	416	301 (72.3)	115 (27.6)	<0.001
Female, *n* (%)	348 (83.6)	260 (86.3)	88 (76.5)	<0.001
Age (yrs)	48.3 ± 10.3	49.0 ± 10.4	51.5 ± 9.2	0.003
SG, n (%)	137 (32.9)	105 (34.8)	32 (27.8)	<0.001
RYGB, n (%)	280 (67.3)	218 (72.4)	62 (53.9)	<0.001
Initial BMI (Kg/m^2)	44.3 ± 7.9	44.5 ± 9.6	44.2 ± 7.3	0.554
Initial weight (Kg)	113.0 ± 18.4	112.3 ± 20.3	113.4 ± 13.8	0.361
Excess weight (Kg)	49.3 ± 17.3	48.7 ± 19.2	51.3 ± 12.2	0.020
Nadir BMI (Kg/m^2)	29.9 ± 5.8	28.5 ± 4.5	37.0 ± 6.8	<0.001
Type 2 diabetes, n (%)	173 (41.5)	128 (42.5)	45 (39.1)	<0.001
Hypertension (%)	286 (68.7)	204 (67.7)	82 (71.3)	<0.001
Dyslipidemia (%)	306 (73.5)	212 (70.4)	94 (81.7)	0.010
Sleep apnea (%)	116 (27.8)	88 (29.2)	28 (24.3)	<0.001

Data are mean ± SD, median (range) or n (percentage). SG: sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; BMI: body mass index. EWL: excess of weight loss. Hypertension was defined by increased systolic (≥140 mmHg) or increased diastolic (≥90 mmHg) blood pressure or using antihypertensive drugs, according to current guidelines. Dyslipidemia was defined using lipid-lowering drugs, decreased values of HDL cholesterol (men< 0.9 mmol/L, women <1.0 mmol/L) or by at least one increased value of total cholesterol (>5.2 mmol/L), LDL cholesterol or triglycerides (>1.7 mmol/L).

Table 3. Clinical and genetic variables that significantly predicts favorable weight loss after BS in the entire population of the OBEGEN project.

Clinical Variables		Genetic Variants		All selected Variants	
	Coefficient		Coefficient †		Coefficient †
Age	−0.03458	rs16861209	−0.30388	CRS	1.13897
Type of surgery	0.69588	rs17782313	0.32234	GRS	1.30048
Type 2 Diabetes	3.05077	rs1800795	−0.33407	Constant	−1.34401
Constant	−3.75577	rs1801282	0.33407		
		rs3771942	−0.17997		
		rs6454674	0.24788		
		rs682447	0.41113		
		rs894160	0.28848		
		rs925946	0.28604		
		Constant	−0.30768		

†: Coefficients in multiple logistic regression model. CRS: clinical risk score; GRS: genetic risk score.

3.3. Construction of a Genetic Risk Score

Additionally, when genetic data were analyzed alone, the multivariate logistic regression equation for predicting a favorable weight loss response after the BS included nine insSNPs located in ADIPOQ, MC4R, IL-6, PPARG, INSIG2, CNR1, ELOVL6, PLIN1, and BDNF (Table 3). This genetic risk score showed an AUROC of 0.648 (95% CI 0.597 to 0.696, $p < 0.0001$), with a sensitivity of 48.7% and a specificity of 75.0%. The calibration of the adequacy of the model determined by the Hosmer–Lemeshow test was 0.922.

3.4. Construction of the OBEGEN Clinical-Genetic Risk Score

Based on the clinical and genetic data from our population, we created the OBEGEN-CGRS, including age at surgery, type of surgery, presence of type 2 diabetes, and the nine SNPs associated with weight loss in response to BS (Table 3). The OBEGEN-CGRS score ranges from −4 to +4 points, with a cut-off point to define a good responder of 0.662. This predictive model showed an AUROC of 0.845 (95% CI 0.805 to 0.880, $p < 0.001$), with a sensitivity of 90.1% and a specificity of 65.5%. The calibration of the model's adequacy determined by the Hosmer–Lemeshow test was 0.927. An internal validation of this clinical-

genetic algorithm was performed using a Bootstrap method to quantify the uncertainty associated with the AUROC. The result was an AUC of 0.845 (95% CI: 0.800 to 0.888).

The OBEGEN-CGRS score showed a significant higher AUROC than either the clinical score ($p = 0.0186$) or the genetic score ($p < 0.0001$) (Figure 2).

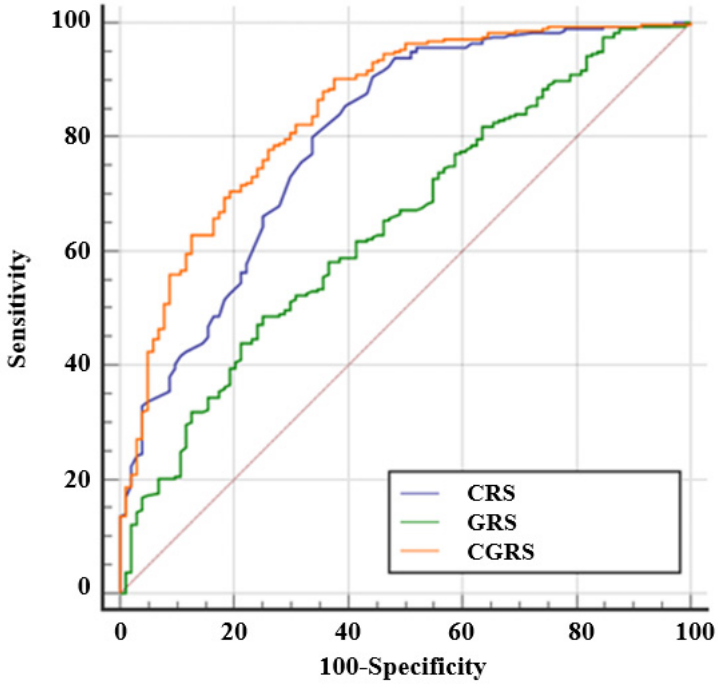

Figure 2. The predictive capacity of favorable weight loss (%EWL > 50%) obtained by the clinical risk score (CRS), the genetic risk score (GRS), and the clinical plus genetic risk score (CGRS) in our study population in the OBEGEN project.

4. Discussion

In this study we provide evidence that the combination of clinical plus genetic data is a reliable method to predict the weight response after BS. The OBEGEN-CGRS permits us to progress towards the personalization in the management of patients with severe obesity, seeking maximum efficiency with the least surgical damage.

Although BS provides successful weight loss in most of the cases, 25–30% of patients who undergo BS may not achieve the desired weight reduction [5–7]. This failure is considered multifactorial, with some preoperative factors associated with the hospital center (i.e., surgeons' experience, bariatric procedure, preoperative education, and recommended weight loss before BS), the patient (age, gender, ethnicity, preoperative BMI, and comorbidities associated with obesity), and psychosocial features (economic resources, household type, and personality disorders) [11–13,24–26]. The real factors that predict weight loss following BS are still far to be determined. This is due to the inconsistency in reporting and the methodological weaknesses in analysis, which include a little if any consideration of genetic factors [21,27].

The development of new predictive tools for BS, based on some of the previous predictive factors, but at the same time fueled by new components, is a real need for clinicians who treat obesity worldwide. These instruments should help physicians to identify the best candidates who must undergo BS and to surgeons to optimize the surgical procedure.

Few studies have addressed the influence of genetics on long-term dynamic changes in body weight with ambiguous results [16,28]. The Swedish Obesity Study analyzed the impact of various SNPs from 11 genes in 1443 BS cases [29]. After the evaluation of 20 gene SNPs in 249 morbidly obese subjects undergoing RYGB, Velázquez-Fernández et al. showed that POMC rs1042571 was the only associated with favorable weight loss [30].

In another group of 1011 subjects, an increasing number of SNPs alleles in or near the FTO, INSIG2, MC4R, and PCSK1 genes negatively influenced weight loss trajectories after RYGB in those with an initial BMI >50 kg/m^2 [10]. More recently, in a group of 146 individuals, carriers of another variant of the FTO gene (rs9939609) showed a lower success rate, as well as a greater and faster weight recovery beyond 2 years after BS [31]. Nevertheless, the same SNP was not associated with different weight loss after 6 months of SG in 74 morbidly obese patients [32]. Regarding the MC4R gene, among 1433 subjects with a follow-up period of 12 months after RYGB, carriers of the I251L variant lost 9% more weight compared with the noncarriers [20]. Finally, a prospective observational study with 105 patients evaluated SNPs in the leptin receptor, FTO and FABP2 genes [33]. This study showed that carriers of the LEP223 (rs1137101) experienced close to 25% lower excess weight at 12 and 24 months after bariatric surgery.

Beyond the isolated study of one or the other gene, genetic risk scores composed by adiposity-related SNPs have been related with weight loss after RYGB or SG in Swiss, Danish, and Greek populations [34–36]. It should be noted that only three of the nine genes included in the OBEGEN-CGRS appeared previously reported in association with the weight loss response after BS [10,20,36]. This fact highlights the complexity of the genetic basis associated with the development of obesity, but also that the genes related to the therapeutic response may be different from those proposed so far. In the OBEGEN study, including both genders and different bariatric procedures, the combination of clinic and genetic data enhanced the predictive capacity of the genetic risk score, with improved sensitivity and specificity. Altogether, our findings support the use of genetic testing in clinical practice.

Among the multiple variants of genes that were evaluated, nine of them interact with clinical variables to modify the weight response to BS in the OBEGEN study. While low serum adiponectin levels have been associated with central obesity, insulin resistance, metabolic syndrome, and type 2 diabetes, ADIPOQ (rs16861209) has been significantly associated with elevated fasting serum adiponectin levels [37,38]. Similarly, although IL-6 significantly increases the risk of obesity, in the PREDIMED trial carriers of the rs1800795 showed greater weight loss with the Mediterranean diet with supplements of olive oil compared to a Mediterranean diet low-fat diet than heterozygous and non-carrier carriers after 3 years of intervention [39,40]. In addition, PPARG (rs1801282) was associated not only with short-term (6-month) and long-term (2-year) weight loss but also with weight regain in the Diabetes Prevention Program [41]. PLIN1, a circadian lipid stabilizing protein in the adipocyte, has been associated with body weight regulation and PLIN1 (rs894160) with variability in weight loss [42,43]. Elovl6, a microsomal enzyme involved in the elongation of saturated and monounsaturated fatty acids with 12, 14, and 16 carbons, regulates mitochondrial function and thermogenic capacity in brown adipose tissue [44]. The BDNF (rs925946), INSIG2 (rs3771942) and CNR1 (rs6454674) variants are well stablished genetic determinants of obesity [45–47]. Similarly, MC4R (rs17782313) is associated with high dietary intake and different obesity-related phenotypic traits, such as insulin resistance, type 2 diabetes, and hypertriglyceridemia [48]. However, so far there are no data on the response to weight loss treatments regarding these last four variants.

The main differences between our CGRS and previous studies is located in the methodology assessment. Previous studies have been interested in analyzing the association between the number of genetic risk variables and the greater or lower weight loss following BS. However, we have focused our interest on the elaboration of predictive equations mixing clinical and genetic variables and the identification of predictive cut-off in those predictive equations. We have also calculated the prognostic capability of our score in identifying the "good" and "bad" responders to BS. We believe that this is a concept of great interest and more reliable in daily clinical practice. In this way, a patient who requires BS is exposed to an intervention that is difficult to reverse, so it is vital to be able to predict success or failure with a high degree of certainty. Currently, the choice of surgical technique is based on the baseline BMI and the presence of comorbidities. The introduction of a

genetic predisposition score in the decision-making algorithms will bring us closer to the best selection of the patient, to choose the most convenient surgery, and to improve current health outcomes in our population.

There are some potential limitations that need to be considered when contemplating the results of our study. First, we evaluated a selected population of patients who underwent bariatric surgery, excluding those who underwent to other surgical techniques other than RYGB and SG, as well as those who had used weight lowering pharmacotherapy or had required a second surgical intervention. However, we believe that the inclusion of these more extreme cases might increase rather than reduce the reliability of our score. Second, the OBEGEN project is a retrospective one, meaning that no irrefutable clinical consequences can still be inferred to general population. Prospective long-term studies testing the genetic basis of patients with severe obesity before the recommendation of bariatric procedures are needed. Third, the characterization of favorable weight loss after BS is controversial [19]. In the OBEGEN study we have used a classic definition (%EWL>50) to better confirm our previous pilot study, so the results could be different if the chosen definition had been another. Finally, our model needs to be validated in an untrained dataset to fully demonstrate its applicability in the real clinical practice.

5. Conclusions

In conclusion, the OBEGEN project shows how the addition of genetic testing to the currently used clinical variables significantly improve our capability to identify patients with obesity who will be "good" or "bad" responders to BS in terms of weight loss. This information should help us both to personalize the therapeutic approach in severe obesity (e.g., select beforehand surgical techniques with a higher degree of malabsorption in those patients identified as "bad" responders), thus optimizing the limited health resources.

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Institutional Review Board Statement: The OBEGEN study was approved by the human ethics committee of the University Arnau de Vilanova Hospital of Lleida (CEIC-1743). Written informed consent was obtained from all subjects involved in the study, which was conducted according to the Helsinki Declaration and the Good Clinical Practice Guidelines. The study was registered in Clinical.Trials.gov- NCT02405949.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

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10.1.1.3 Article 3.

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ORIGINAL CONTRIBUTIONS

Evaluation of Resting Energy Expenditure in Subjects with Severe Obesity and Its Evolution After Bariatric Surgery

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Abstract

Purpose One major determinant of weight loss is resting energy expenditure (REE). However, data regarding REE is scarce in patients with severe obesity (SO)—BMI>50kg/m^2. Most studies used equation in order to estimate REE and not indirect calorimetry (IC) (gold standard). Additionally, there is no reliable data on the impact of bariatric surgery (BS) on REE.

Objectives (a) To evaluate the REE in patients with SO; (b) to compare REE measured by IC (mREE) to that calculated by Mifflin St-Jeor equation (eREE); (c) to evaluate the impact of BS on REE and the relationship with evolution post-BS.

Material and Methods Single-center observational study including consecutive patients with SO between January 2010 and December 2015, candidates for BS. mREE was determined at baseline, and 1 and 12 months post-BS by IC, using a Vmax metabolic monitor.

Results Thirty-nine patients were included: mean age 46.5±11.77 years, 64.1%women. Preoperative mREE was 2320.38 ±750.81 kcal/day. One month post-BS, the mREE significantly decreased (1537.6 ± 117.46 kcal/day, p = 0.023) and remained unchanged at 12 months (1526.00 ± 123.35 kcal/day; p =0.682). Reduction in mREE after the BS was a predictor of reaching successful weight loss (nadir) and weight regain (5 years follow-up) (AUCROC of 0.841 (95%CI [0.655–0.909], p=0.032) and AUCROC of 0.855 (95% CI [0.639–0.901]), p= 0.027, respectively). eREE was not valid to identify these changes.

Conclusion In patients with SO, a significant reduction of mREE occurs 1 month post-BS, unchanged at 12 months, representing the major conditioning of successful weight loss and maintenance post-BS.

Keywords Bariatric surgery · Severe obesity · Resting energy expenditure

Key Points
• Bariatric surgery impacts energy expenditure of patients with severe obesity.
• Significant reduction in energy expenditure occurs at least 1 month after bariatric surgery.
• The reduction in resting energy expenditure is a good predictor of weight regain.

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Introduction

The physiology of weight gain and weight loss is complex, multifactorial, and by far to be completely elucidated. A recent systematic review identified 124 determinants of weight loss maintenance. Of those, reducing energy intake, increasing energy expenditure, and monitoring behaviors showed the strongest level of evidence [1]. One of the major determinants is the balance between energy intake and energy expenditure. Weight variations are associated with variations in total energy expenditure (TEE) [2]. TEE is influenced by factors such as age, gender, weight, body composition, diet, and physical activity [3]. TEE is defined as the amount of heat energy used by the human body for daily physiological functions and is divided into 3 main components: (a) resting energy expenditure (REE)—accounting for around 70% of TEE; (b) diet-induced thermogenesis (DIT); and (c) activity energy expenditure (AEE) [4].

Historically, several methods have been developed for assessing TEE. However, each approach has its advantages and disadvantages. If the purpose is to assess free-living TEE, doubly labelled water (DLW) is recommended. DLW provides information on TEE for a 4–20-day period, likely to reflect the normal energy requirement of individuals. DLW is proven to be safe and useful in all age groups and in several clinical settings. On the other hand, it is highly expensive, and proper equipment and specialized expertise are required to analyze isotope concentration in body fluids by mass spectrometry [5].

Direct calorimetry measures total heat loss from the body while the participant is isolated in a thermally controlled chamber. Although very accurate, it is unpractical for measuring TEE in a free-living population context. On the other hand, indirect calorimetry measures CO_2 production and VO_2 consumed in a controlled environment (closed-circuit) to calculate the amount of energy expended. It should be noted that if performed in a resting state, IC will allow the measurement of REE, which is not provided by other techniques. For this reason, IC is considered the gold standard for REE measure [4]. Additionally, the technique for REE measure is time-saving and requires minimal training, making it feasible and practical for study populations. Furthermore, in order to assess exercise metabolism, open-circuit portable indirect calorimetry techniques are more suitable. More recently, heart rate monitoring portable devices may be useful for assessment of physical activity rather than TEE. Finally, questionnaires of activity recall and motion sensors, such as pedometers and accelerometers, may have a role in evaluating interventions aimed at increasing physical activity; instead, its use to quantify REE is very limited [5].

Some data in the literature suggested that REE is increased in patients with morbid obesity [6]. Reliable data on REE in these cases is necessary to personalize calorie intake in order to assure a safe and effective weight loss and, more importantly, weight maintenance after successful weight loss. Nevertheless, REE is calculated in the daily clinical practice by means of estimation equations. Although widely used in clinical settings, it should be noted that these equations were validated based on data from healthy normoweighted subjects. These estimations are not always accurate for REE in subjects with overweight or obesity [7]. Actual published evidence is reflecting great disparities between predicted and measured energy expenditure values in patients with obesity [8–10]. Additionally, at present, there is no reliable data regarding their accuracy in estimating REE in patients with severe obesity (SO).

Bariatric surgery (BS) has proven to be an effective treatment for obesity resulting in sustainable and substantial weight loss and improvement of related comorbidities [11]. Furthermore, BS was proven to be safe and effective in patients with SO at short-medium follow-up, representing the preferred treatment for obesity in these patients [12]. Some authors suggested that BS can modify the REE and have proposed that the greater long-term success of BS as a treatment for obesity could be partially explained by the effects of BS on REE [13]. Nevertheless, others have found no influence of REE on outcomes after BS, rather a compensatory adaptive thermogenesis mechanism that occurs in response to a decreased energy intake [14]. Whether the changes in REE after BS act as determinants of weight loss maintenance is still under investigation. One possible mechanism comes from evidence in rodents. In obese mice, bariatric surgery seems to increase brown adipose tissue activity postoperatively resulting in increased energy consumption and decreased respiratory exchange frequency. These effects deteriorated when mice experienced weight regain 8 weeks after surgery [15]. In humans, evidence supporting a "browning" of adipose tissue after BS is increasing. However, evidence in the literature is contradictory [16].

It should be noted that around 20–25% of patients that undergo BS do not achieve successful weight loss [17], or, more importantly, about 30–35% fail to maintain weight loss [18], experiencing significant weight regain starting from 3 years after the BS [19]. Additionally, weight loss is often less significant than would be expected for a given degree of caloric restriction or BS technique [20]. While it is clear that individuals differ in the susceptibility to weight loss (and their subsequent ability to sustain this lower body weight), robust predictors of response to a weight loss intervention remain unclear. Data regarding REE is very scarce in patients with SO, and practically there is no reliable data on the impact of BS on REE in this population [21].

On these bases, the aims of the present study were as follows: (a) to evaluate the REE in patients with SO by means of the *gold standard* method (IC); (b) to compare the values of the REE measured by IC (mREE) to the estimated value

calculated by equation (eREE); (c) to evaluate the impact of BS on the REE and the relationship with the evolution post-BS (in terms of weight loss, weight regain, and resolution of comorbidities).

Material and Methods

A single-center observational study including consecutive patients with SO and BMI >50 kg/m^2 attended the Morbid Obesity Unit of a third-level university hospital (Vall d´ Hebron University Hospital) that had performed IC between January 2010 and December 2015. The study was approved by the Ethics Committee of our site and conducted following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines and the statements of the Declaration of Helsinki. The patients signed the informed consent form prior to inclusion in the study.

All the patients underwent a complete medical history, anthropometric evaluation, and IC at baseline, 1 month, and 12 months after the BS.

Inclusion criteria: (a) signed informed consent; (b) age between 18 and 60 years (the limits for BS at our site); (c) BMI >50kg/m^2; (d) eligible for BS according to the *standard of care* protocol at our site.

Exclusion criteria: (a) eating disorders; (b) endocrine disease or treatment with potential influence on the REE (egg: systemic corticosteroids, untreated hyper/hypothyroidism); (c) severe illness that can influence the outcomes; (d) unable to perform the follow-up visits post BS at our site; (e) other surgery than sleeve gastrectomy (SG) or Roux-en-Y gastric bypass (RYGB); (f) second-step BS or revision surgery.

Procedures and variables collected for the study:

Clinical and Anthropometric Variables

Collected at Baseline Age, gender, weight (kg), height (m), BMI (kg/m^2), excess of body weight (EBW) (kg), presence of comorbidities related to obesity. Excess body weight (EBW) was defined as follows: actual weight − ideal body weight (IBW) based on BMI 25 kg/m^2.

Collected During Follow-up 1 Month, 12 Months, and 5 Years After the BS Weight (kg), BMI (kg/m^2), percentage of excess of weight loss (%EWL), total weight loss (TWL), percentage of total weight loss (%TWL), and evolution of related comorbidities. Weight and BMI nadir were considered the minimum values reached after the BS; %EWL, TWL, and %TWL were calculated following standardized outcome reporting guidelines [22]. The post-BS weight regain was defined as a 10% regain of the minimal weight after BS, as previously described [23].

Energy Expenditure Determination (REE) Variables

Collected at baseline, 1 month, and 12 months after the BS:

Estimated Equations (eREE) Although the Harris-Benedict Equation (HBE) [24] is widely used in clinical practice, it appears to be less accurate when compared to the Mifflin-St Jeor equation (MSJ) in patients with obesity [13]. In this study, we used the Mifflin-St Jeor Equation (MSJ) [25]: 9.99*weight (kg) + 6.25*height (cm) − 4.92 * age + 166 * sex (M = 1; F = 0) −161.

Indirect Calorimetry (mREE) IC was performed in supine position, on a neutral environment, and after resting for at least 20 min, using a Vmax 29 (Sensor Medics, Yorba Linda, CA, USA) portable metabolic monitor, available at our site. After the resting period, 15–20 min of calorimetric data was collected. The first 5 min of data was excluded in all cases. The equipment was calibrated prior to each measurement. The patients were instructed to avoid stimulating drinks, cigarette smoking, and exercise 24 h prior to and to be fasting at least 8 h prior to the performance of IC. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ), and resting energy expenditure (mREE) are generated in the final report.

Statistical Analyses

IBM SPSS statistical software version 24 was used. Continuous variables are expressed as means ± standard deviation (SD) for normal distributed variables and median ± interquartile range (IQR) for non-normal distributed variables. Categorical variables are expressed with percentages. For differences between groups in continuous variables, Student's t test or U-Mann-Whitney test was used while χ^2 was used for categorical variables. For differences between 3 and more time points, repeated-measures ANOVA was used; if differences were found, a post hoc pairwise comparison was performed. Differences in weight loss rates at nadir and weight regain rates 5 years after surgery with predetermined definitions were explored using descriptive statistics. Correlation analysis was used to explore the associations between demographics (i.e., age, gender, and preoperative BMI), type of surgery (SG vs RYGB), presence of comorbidities, REE variables, and weight loss at nadir and weight regain 5 years after surgery according to the different definitions. Akaike Information Criterion (AIC)–based backward selection was used to remove insignificant terms from an initial model containing all the candidate predictors. A p-value < 0.05 was considered statistically significant.

Results

A total of 39 patients with SO and BMI >50 kg/m^2 were included in the study as detailed in Figure 1. The baseline clinical and demographical characteristics of the patients are shown in Table 1. Measured REE was 2320.38 ± 750.81 kcal/day and significantly different to MSJ equation estimation (1994.44 ± 463.41 kcal/day, p= 0.035). Additionally, mREE directly correlated with initial weight; initial BMI and EW (0.792, 0.451, and 0.795 respectively $p < 0.0001$) indirectly correlated with age (−0.769, $p < 0.0001$). We found no difference in mREE between patients with or without associated comorbidities, including when stratified for number of comorbidities. As expected, mREE was significantly different among men and women. Measured REE was higher in men compared to that in women (2761.0±122.0 kcal/day vs. 1964.0 ±622.0 kcal/day, p <0.001).

One of the variables reported in the IC is the RQ (ratio of the amount of carbon dioxide produced to the amount of oxygen consumed), used to calculate rates of carbohydrate versus fat used to support energy metabolism. In this regard, when a molecule of glucose is metabolized, the RQ has a value of 1.0. Similarly, when one molecule of fat (tripalmitin) is completely metabolized, the RQ is 0.71 [26]. In our cohort, RQ-baseline was 0.81±0.1, suggesting a fat oxidation–prone metabolism. In this regard, we found a negative statistical significant correlation with initial weight, initial BMI, and EW (r =−0.390, p= 0.01; r= −0.313, p= 0.05 and −0.423, p= 0.007, respectively)

Evolution After BS

As reflected by Figure 1, 31 patients underwent BS and at least 5 years of follow-up: 22.6% underwent RYGB and 77.4% underwent SG. As per protocol, the SG is the recommended technique in almost all of the patients with SO. The data at 5 years follow-up is shown in Table 2. Patients achieved the minimum weight after the BS (nadir) after a mean follow-up of 17.1 ± 4.8 months after the BS: weight 80.2±20.5 kg, BMI 33.2±10.5 kg/m^2. At this point, 87.01% (27/31) of the patients achieved >20%TWL and 80.6% (25/31) met a >50%EWL, regardless of the age, gender, or type of surgery.

At 5-year follow-up, weight was 94.37±24.67 kg and BMI 37.04±6.02 kg/m^2, significantly increased from nadir (p<0.001), representing a significant weight regain in 32.25% (10/31) of the patients.

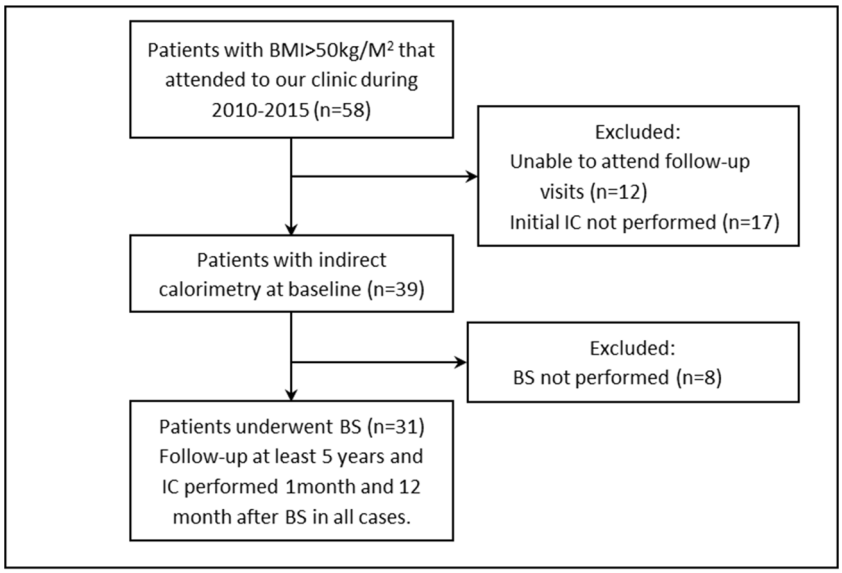
Changes in REE After BS

We found a significant reduction in mREE at least 1 month after the BS, achieving levels comparable to those of the Spanish population with normal weight [35], despite presenting BMI in morbid obesity range (BMI-1m after BS 45.67 ±3.80kg/m^2). The mREE-12m remained significantly

Table 1 Baseline characteristics of patients with severe obesity

Demographics		n = 39
Gender, females, % (n)		64.10 (25)
Age (years), mean (SD)		46.5 ± 11.7
Initial weight (kg), mean (SD)		149.3 ± 30.36
BMI (kg/m^2), mean (SD)		56.2 ± 5.6
EW (kg), Mean (SD)		83.1 ± 22.3
Obesity-associated comorbidities		
Type 2 diabetes, % (n)		30.8 (12)
Hypertension, % (n)		38.5 (15)
Dyslipidemia, % (n)		17.9 (7)
OSA, % (n)	Absent	20.5 (8)
	Mild	15.4 (6)
	Moderate	5.1 (2)
	Severe	59 (23)
Number of obesity-related comorbidities, % (n)	None	10.3 (4)
	1	17.9 (7)
	2	35.9 (14)
	3	20.5 (8)
	4	15.3 (6)

BMI body mass index, *EW* excess of weight, *NAFLD* non-alcoholic fatty liver disease, *OSA* obstructive sleep apnea



BMI, Body mass index; *BS*, bariatric surgery; *IC*, indirect calorimetry; *RYGB*, Roux-en-Y gastric bypass; *SG*, sleeve gastrectomy.

Fig. 1 Flowchart of the inclusion of the patients in the study. BMI, body mass index; BS, bariatric surgery; IC, indirect calorimetry; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy

unchanged after the initial significant "drop-down" 1m after BS, while BMI-12m continued to significantly reduce (36.13 ±6.06kg/m^2, p<0.0001). Figure 2 and Table 3 show the evolution of the IC parameters after the BS. We found no statistical significant differences among techniques in REE at any time point.

An inverse correlation was found between initial EW and mREE-1m and mREE-12m (r = −0.714, p = 0.047 and r = −0.681, p = 0.014, respectively). However, mREE-1m and mREE-12m did not correlate with any other weight-related variables (i.e., initial weight, 1m-weight, 1m-EW, nadir weight, nadir EW). An indirect correlation was observed

Table 2 Follow-up subgroup characteristics

N=31		*Baseline*	*1-year FU*	*Nadir*	*5-years FU*
Age (years)		50.44± 7.52			
Sex, females, % (*n*)		67.7 (21)			
Type of surgery, % (*n*)		SG 77.4 (24) RYGB 22.6 (7)			
Weight (kg)		135.98±20.11[a]	92.08±23.22[a]	88.35±24.12[a]	94.37±24.67[a]
BMI (kg/m^2)		53.66±3.35[a]	36.13±6.06[a]	34.60±6.29[a]	37.04±6.02[a]
EW (kg)		72.51±11.98[a]	27.61±18.54[a]	24.89±18.54[a]	30.91±18.36[a]
Comorbidities					
Type 2 diabetes, % (*n*)		45.2 (14)	6.4 (2)	6.4 (2)	19.3 (6)
Hypertension, % (*n*)		38.7 (12)	12.9 (4)	12.9 (4)	12.9 (4)
Dyslipidemia, % (*n*)		25.8 (8)	6.4 (2)	6.4 (2)	12.9 (4)
OSA,% (*n*)	Absent	45.2 (14)	61.3 (19)	61.3 (19)	51.6 (16)
	Mild	12.9 (4)	32.2 (10)	32.2 (10)	22.5 (7)
	Moderate	32.2 (10)	6.4 (2)	6.4 (2)	12.9 (4)
	Severe	12.9 (4)	0 (0)	0 (0)	6.4 (2)
Weight loss					
Percent of total weight loss (%TWL)			32.34±12.06[a]	35.13±12.58[a]	30.74± 12.49[a]
%TWL> 20, % (*n*)			83.87 (26)	87.01 (27)	74.1 (23)
Percent excess weight loss (%EWL)			60.44±20.76[a]	65.67±21.78[a]	57.32 ± 21.58[a]
%EWL >50, % (*n*)			64.5 (20)	80.6 (25)	67.7 (21)

BMI body mass index, *EW* excess of weight, *FU* follow-up, *OSA* obstructive sleep apnea, *RYGB* Roux-en-Y gastric bypass, *SG* sleeve gastrectomy. Continuous variables expressed in mean ± SD. [a] Repeated-measures ANOVA, p < 0.001

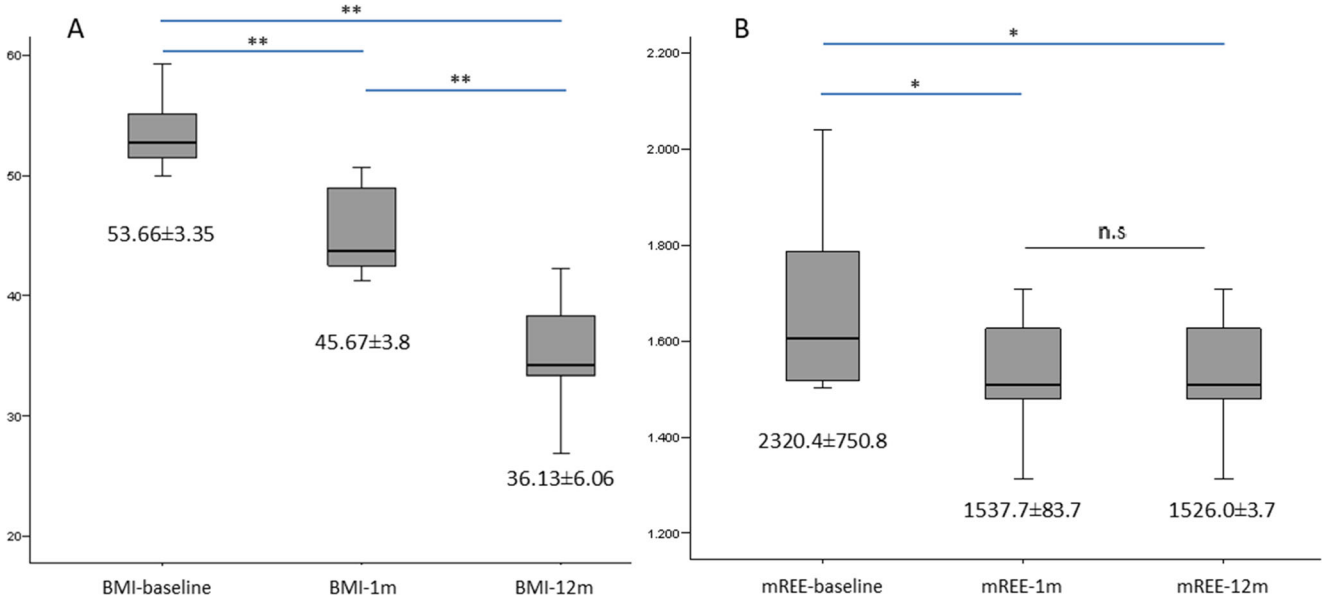


Figure 2 Changes in measured resting energy expenditure and body mass index before and after bariatric surgery. BMI, body mass index; mREE, measured resting energy expenditure; 1m, 1 month after bariatric surgery; 12m, 12 months after bariatric surgery. Repeated-measures ANOVA for: **A** BMI and **B** mREE before and after BS, $p < 0.001$. Results after Bonferroni correction are indicated if significant differences were found. *$p < 0.05$; **$p < 0.01$

between mREE-1m and mREE-12m and RQ-1m and RQ-12m, respectively, but not with mREE and RQ at baseline.

Although we found a significantly difference between gender at mREE-baseline, these differences were no longer significant after BS, while MSJ showed differences between gender in all three time points, as reflected by Table 4.

We found no significant pre-BS predictors of reduction in m REE at 1m and 12m follow-up, among age, gender, BS technique, and obesity-related comorbidities. These parameters neither were predictors of significant weight regain at 5 years follow-up. Interestingly, the reduction of mREE at 12 months (calculated as mREE-baseline − mREE-12m) was a significant predictor of the following: (A) poor nadir weight loss after BS (%EWL<50%) and (B) weight regain at 5 years follow-up (AUCROC of 0.841 (95%CI [0.655–0.909], p=0.032) and AUCROC of 0.855 (95% CI [0.639–0.901]), p= 0.027, respectively) (Figure 3).

Discussion

In the present study, we showed for the first time that an early and significant reduction in the REE (evaluated by means of IC-gold standard method) occurs in patients with SO that undergo bariatric surgery, up to levels comparable to those of the normoweighted Spanish population [27], despite the fact that 1 month after BS, their BMI is still in morbid obesity range. Furthermore, in our study, we showed that the reduction in REE at 12 months after the BS was a good predictor of a "good" or "poor" response to BS ("good" defined as %EWL nadir>50%) with a AUCROC of 0.841 (95%CI [0.655–0.909], p=0.032) as well as for weight regain after 5 years of follow-up with an AUCROC of 0.855 (95% CI [0.639–0.901], p= 0.027). In other words, the greater the reduction in REE 1 year after BS, the less %EWL at nadir and the greater the weight regain after 5 years.

Table 3 Changes in energy metabolism

N=31	*Baseline*	*1 month after BS*	*12 months after BS*	*p value* ǂ
mREE (kcal/day)	2320.4 ± 750.8[a]	1537.7 ± 83.7[a, b]	1526.0 ± 3.7b	0.006
MSJ (kcal/day)	1994.4 ± 463.4[a]	1789.1 ± 307.5[a]	1551.1 ± 349.8[a]	0.001
RQ (V_{CO}^2/ V_O^2)	0.81 ± 0.13	0.79 ± 0.08	0.81 ± 0.07	n.s

mREE measured resting energy expenditure, *MSJ* Mifflin St-Jeor equation, *RQ* respiratory quotient, V_O^2 oxygen consumption (ml/min^{-1}), V_{CO}^2 carbon dioxide production (ml/min^{-1}). Continuous variables expressed in mean ± SD

ǂRepeated-measures ANOVA

[a] Bonferroni correction p <0.005

[b] Bonferroni correction p =n.s

Table 4 Differences among gender in mREE and MSJ across three time points

N=31		*REE-baseline*	*REE-1m*	*REE-12m*
mREE (kcal/day)	Female	1769.36 ±245.81[a]	1529.45±100.74	1526.0±95.55[b]
	Male	2561.0±449.40[a]	1555.73±40.40[b]	1548±72.32
MSJ (kcal/day)	Female	1778.36±97.92[a]	1604.73±97.62[a]	1393.91±107.31[a, b]
	Male	2469.80 ±245.79[a]	2194.80±177.32[a, b]	1896.80±461.10a
RQ	Female	0.92±0.19[a]	0.81±0.02	0.81±0.09
	Male	0.78±0.01[a]	0.79±0.10	0.80±0.01

[a] Significant difference between women and men $p < 0.05$ [b] Significant difference between gender and mREE or MSJ $p<0.05$

As explained in the "Introduction," at present there is no reliable data on basal REE in patients with SO. We found in the literature only one study [28] that used IC, to compare our results at baseline and showed similar results (mean REE 2262 ± 122 kcal/day in patients with BMI 56kg/m^2). Most of the studies published so far in patients with obesity and most of the few studies that were reported on SO used an estimated value of REE by means of equations [20]. These equations were validated and calculated based on standard adults with normal weight [9]. They might not be adequate for patients with obesity, and in particular with SO, and at present this represents an important gap in the personalized management of these patients. A recent external validation of REE predictive equations reported that the accuracy of the formulas decreases going from normal weight to class 3 obesity [29]. Having a real characterization of the REE in this population is necessary in order to personalize the diet (in particular calorie intake) and to assure a safe and effective weight loss and, more importantly, weight maintenance after successful weight loss, although compliance was shown to be limited in the case of long-lasting calorie-restriction intake [30].

In order to shed light on this gap, in our study, we compared the values of the REE estimated by the standard recommended equations and the gold standard method, the indirect calorimetry. We found that at baseline the MSJ equation significantly underestimated the REE when compared to the gold standard (IC) (1994.44 ± 463.41 vs 2320.4 ± 750.8, p=0.031). In exchange, after the BS, we found that the MSJ overestimated REE in males (2194.80±177.32 kcal/day vs 1555.73±40.40kcal/day, p<0.001), while in females showed no significand difference when compared to the REE measured by IC. Additionally, although we found a significant difference between gender at mREE-baseline, these differences were no longer significant after BS, while MSJ showed differences between gender in all three time points (baseline, 1m, and 12m). This is an interesting finding and highlights the

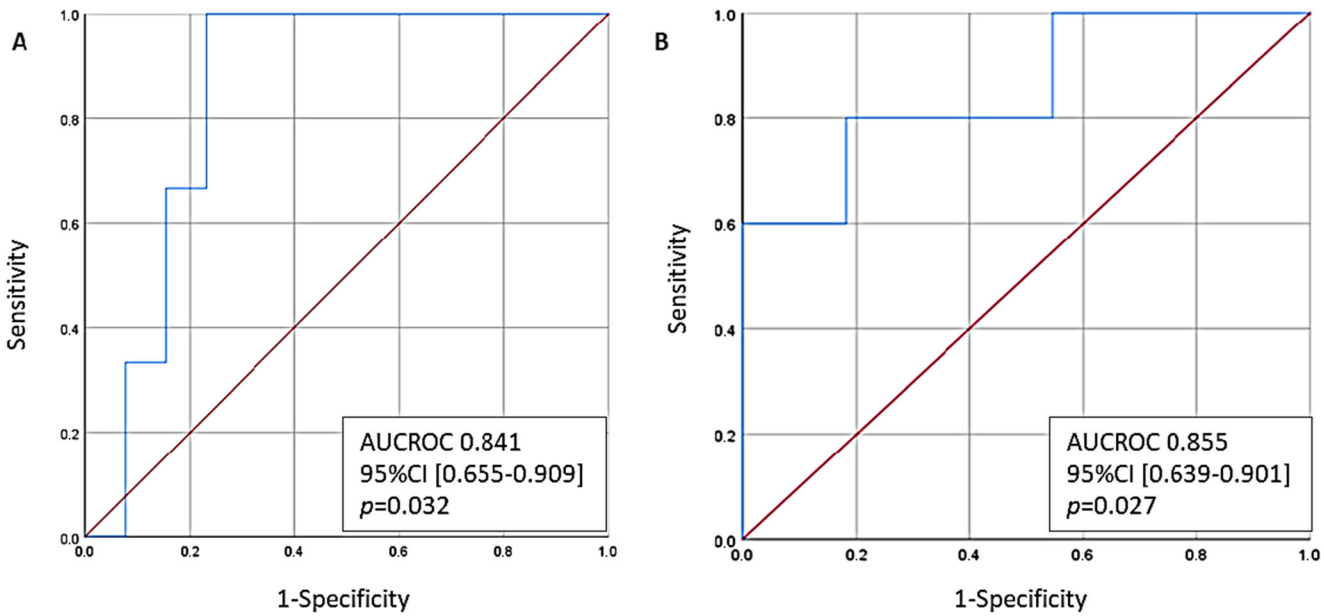


Figure 3 The predictive capacity of the reduction in mREE at 12 months from baseline for: **A** EWL<50% at nadir and **B** weight regain after 5 years follow-up

limitations of these equations that do not take into the account all the particularities of the patients with morbid obesity and in particular with SO. The MSJ estimates the REE by including the gender into the formula, but this formula was calculated using data from standard adults with normal weight and probably normal body composition [25]. A possible explanation of this overestimation of eREE in males after the BS is that the formula of the equation does not include data on the changes that occur in body composition, in particular muscle mass loss after the BS [31]. A significant reduction in muscle mass after the BS might explain the differences between the overestimated REE by equations and the real REE measured by IC.

Additionally, in our study, we found a significant reduction in mREE very early after the BS and 1 month and remained unchanged after 12 months, at similar levels with normoweighted Spanish population. Mean mREE-1m: 1537.67 ± 83.67 kcal/day, similar to the mREE of 1589±312 kcal/day found by De la Cruz et al. in healthy individuals with normal weight in Spain [27]. Furthermore, the change in mREE from baseline to 12 months was a significant predictor of successful weight loss after BS and weight regain after 5 years follow-up (AUCROC of 0.841 (95%CI [0.655–0.909], p=0.032) and AUCROC of 0.855 (95% CI [0.639–0.901]), respectively), p= 0.027, respectively). No other factor included in the analysis showed a significant predictive value of evolution after BS (age, gender, BS technique, obesity-related comorbidities). Moreover, we found no differences in REE between the two types of surgery performed at any time points. However, it should be noted that the study design was not powered to find these differences.

Additionally, an indirect correlation was observed between mREE-1m and mREE-12m and between RQ-1m and RQ-12m, but not with mREE and RQ at baseline. This finding suggests a metabolic adaptation after BS or a state of altered energy balance in the time points after surgery that can offer a partial explanation of the role of these changes in weight loss and weight regain after the BS. Metabolic adaptation (MA) is defined as the residual eREE after adjusting for changes in body composition and age [13]. Although a negative energy balance, whether due to a decrease in caloric intake or an increase in energy consumption, would result in weight loss, it has been proposed that the weight loss activates compensatory mechanisms that condition the decrease observed in REE after surgery [32]. Previous data in the literature suggested that a greater than predicted drop in mREE after an intervention induces a metabolic adaption, independently of the fat-free mass [33]. These data, and data from our study, indicate that maybe significant changes in muscle mass that occur after BS can play a crucial role in the evolution of REE and evolution after the BS in terms of weight loss and maintenance.

Our study has several limitations: (A) REE alone was measured, rather than total energy expenditure, which includes DIT and AEE. Although REE accounts for around 70% of total energy expenditure under normal circumstances, the changes in REE associated with weight loss parallel those in total energy expenditure [34]. (B) Lack of body composition evaluation and (C) evaluation of dietary intake.

Concluding Remarks The validated equations used widely in the clinical practice are not reliable for the REE estimation in patients with SO. We showed for the first time that in patients with SO, a significant reduction of the REE occurs at 1 month after the BS, remains unchanged at 12 months, and is the major conditioning of successful weight loss and maintenance after the BS. Further studies are needed in order to shed light on these data, and to explore the underlying mechanisms.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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10.1.2 Oral Communications in Congresses

10.1.2.1 Oral Communication 1

Liliana Gutiérrez-Carrasquilla, Andreea Ciudin, Eduardo Salas, Enric Sánchez, Assumpta Caixàs, Nuria Vilarrasa, Enzamaria Fidilio, Raquel Martí, Rafael Simó, Albert Lecube. ***Estudio clínico aleatorizado para evaluar el impacto de la predisposición genética en la eficacia de la cirugía bariátrica.*** Oral communication at the 62nd Congress of SEEN, 13-15 October 2021, Seville. Endocrinol Diabetes Nutr. 2021;68(Espec Cong 2):1-21

45. ESTUDIO CLÍNICO ALEATORIZADO PARA EVALUAR EL IMPACTO DE LA PREDISPOSICIÓN GENÉTICA EN LA EFICACIA DE LA CIRUGÍA BARIÁTRICA

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Introducción: Un 25-30% de los pacientes sometidos a cirugía bariátrica (CB) presentan una respuesta ponderal insuficiente [pérdida del exceso de peso (%PEP) < 50%]. Nuestro grupo ha elaborado un *genetic predisposition score* (GPS) basado en la combinación de 5 polimorfismos de un solo nucleótido y modulado por variables clínicas capaz de predecir la correcta pérdida ponderal tras CB [rango entre -4 (la "peor") y 4 (la "mejor" respuesta), con un punto de corte de 0,662, sensibilidad 90,1% y especificidad 62,5%].

Objetivos: Evaluar si conocer la predisposición genética permitirá una mejor selección de los candidatos a CB y mejorar así su eficacia respecto a la pérdida de peso.

Métodos: Estudio que incluye 416 pacientes sometidos a CB (bypass gástrico o gastrectomía vertical) en 4 centros con un seguimiento mínimo de 18 meses. Aleatorización 1:1 por bloques a 2 grupos: (i) Grupo A: 208 pacientes de los que excluiremos para los análisis aquellos con un GPS< 0,662 (potenciales malos respondedores); (ii) Grupo B: 208 pacientes en los que conoceremos pero no haremos uso del estudio genético.

Resultados: El 72,3% de los 416 pacientes consiguieron una buena respuesta a la CB (%PEP≥ 50%), siendo más frecuente entre mujeres, sujetos más jóvenes y sometidos a bypass gástrico (71,6% en Grupo A y 73,2% en Grupo B; p = 0,720). Cuando excluimos del Grupo A a los 76 pacientes cuya base genética y características clínicas les identificaba como "malos respondedores", el porcentaje de buenos respondedores se incrementó hasta el 85,6% (p = 0,007). Si evaluamos a los 280 pacientes sometidos a BPG, el %PEP≥ 50 pasa del 42,6% entre aquellos con un GPS desfavorable (GPS< 0,662) hasta el 85,3% entre aquellos con un GPS favorable (p < 0,001).

Conclusiones: Predecir mediante la combinación de variables genéticas y clínicas la respuesta ponderal a la CB permite mejorar su eficacia, permitiendo una atención más personalizada del tratamiento de la obesidad.

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