UNIVERSITAT DE BARCELONA

Chronobiological related factors and weight loss evolution in severe obese patients undergoing bariatric surgery

Tania Pamela Ruiz Lozano

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FACULTAT DE FARMACIA I CIÈNCIA DELS ALIMENTS

PROGRAMA DE DOCTORAT ALIMENTACIÓ I NUTRICIÓ

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Tania Pamela Ruiz Lozano, 2016

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Life is about timing.

Carl Lewis

Abbreviations

%EWL	Excess weight loss
BMAIL-1	Brain and muscle Arnt-like protein-1
BMI	Body mass index
BS	Bariatric surgery
CD	Chronodisruption
CLOCK	Circadian Locomotor Output Cycles Kaput
CR	Calorie restriction
CRY	Cryptochrome Circadian *Clock*
EE	Early evening
HFD	High fat diet
LE	Late evening
LFD	Low fat diet
MetS	Metabolic Syndrome
PER	Period
PPWL	Primary poor weight loss
RYGB	Roux-en-Y gastric bypass
SG	Sleeve gastrectomy
SNC	Suprachiasmatic nucleus
SNP	Single nucleotide polymorfism
SOS	Swedish Obese Subjects trial
SPWL	Secundary poor weight loss
T2DM	Type 2 diabetes mellitus
WHO	World Health Organization
TTFL	Transcriptional/translational feedback loops
Kg/m2	Kilograms divided by the square of height in meters

Contents

Chapter 1

Presentation

Over the past years, obesity has become a major public health concern because of its prevalence increasing at epidemic rates. Importantly, among the different degrees of obesity, severe obesity is the one that has recently grown at the highest rates. Since obesity is associated with increased rates of comorbidities such as type 2 diabetes, dyslipidemia, and cardiovascular diseases, among others, there is need for better ways to treat and prevent it. Currently, the most efficient weight loss treatment for severe obesity is bariatric surgery (BS). Nevertheless, weight loss after bariatric surgery is variable with a sizable portion of patients not achieving or sustaining marked weight loss. The reasons why patients do not achieve success in terms of weight loss after a bariatric surgery are still largely unknown. It has been hypothesized that several factors pre and post-surgery may influence the weight loss outcome. Clinical, hormonal, socioeconomic, psychological and nutritional factors have been studied. Recent evidence has shown an association between weight loss-resistance in obesity and factors related with chronobiology such as the individual chronotype; a set of polymorphisms of the *CLOCK*; and external synchronizing factors such as the timing of food intake. Chronobiology is a relatively new area of research that studies the biological rhythms, which are found throughout the living organism. Despite the increasing body of literature on this field, there is no evidence that chronobiology related factors may influence the weight loss outcome in subjects undergoing bariatric surgery.

The following work aims to elucidate the role of chronobiological related factors in obesity and weight loss evolution in patients that underwent the two currently more common bariatric surgery techniques: Roux-en-Y Gastric Bypass (RYGB) and Sleeve Gastrectomy (SG)

The main objectives were: I) Identify weight loss patterns depicting the high inter-individual variability weight loss response among patients that underwent RYGB and SG. II) To examine whether evening chronotype is related with obesity and weight loss among severe obese patients after six years of bariatric surgery and to examine potential interactions between chronotype and *CLOCK 3111T/C* for obesity in this population. III) To evaluate the role of food-timing in the evolution of weight loss in subjects that underwent bariatric surgery

Chapter 2

General Introduction

Obesity

Over the past decades the prevalence of obesity and overweight has increased reaching epidemic levels, and hence representing a major health problem in both developed and developing world (Ng, M et al. 2013). Global estimates of obesity between 1980 and 2008 indicate that the prevalence of obesity doubled in every region of the world (Finucane, MM et al. 2011). In 2014 more than 1.9 billion adults were overweight, out of this number; 600 million were obese (WHO, 2015). Furthermore, severe obesity was extremely rare in the early 1970s but since then it has increased faster than obesity, with no evidence of slowing (Ogden, CL et al.2007).Obesity is defined as abnormal or excessive fat accumulation that may impair health (WHO, 2015). Nonetheless because of its simplicity and good correlation with body fatness, body mass index (BMI) is commonly used to classify overweight and obesity adults. BMI is defined as a person´s weight in kilograms divided by the square of his height in meters (Kg/m^2). According to the World Health Organization (WHO) overweight is defined as having a BMI ≥ 25 kg/m^2 and < 30 kg/m^2, obesity is defined by having a ≥ 30 kg/m^2; while severe or morbid obesity is defined as having a BMI ≥ 40 kg/m^2 (WHO, 2015). Obesity is a complex disorder and may result from a number of variables (Shukla, AP et al. 2015) that may be summarize as the result from the imbalance between energy intake and expenditure which associates with alterations of many metabolic pathways (Milagro,FI et al. 2013). Moreover, obesity represents an increased risk for coronary artery disease, hypertension, hyperlipidemia, type 2 diabetes mellitus (T2DM), dyslipidemia, certain forms of cancer, sleep apnea, and stroke among others (Smith, BR et al. 2011). The central issue of obesity is excess energy consumption (dietary intake) relative to energy expenditure (energy loss *via* metabolic and physical activity). Several factors, including genetic, physiologic, environmental, psychological, social and economic, are currently considered to interact in varying degrees to promote the development of obesity in a particular individual (Aronne, LJ et al. 2009).

Obesity treatment

Effective treatment of obesity is clearly a major health priority. Clinical management of obesity and severe obesity is mainly focused on *lifestyle interventions, pharmacological treatment and bariatric surgery* (BS) (Shukla, AP et al. 2015). Although evidence exists that even a modest weight loss up of 5% from initial weight is likely to result in clinically meaningful reduction in impaired biochemical parameters such as triglycerides and blood glucose (Wing, RR et al. 2011), it has been suggested that larger weight loss could be required to achieve health benefits in subjects with severe obesity. Obesity treatments require great level of commitment from both the patient and the healthcare provider. *Lifestyle interventions* including diet modification, physical activity, and behavioral therapy are the first line of treatment for obesity. Traditionally, as part of the diet modification, calorie restriction (CR) is recommended to achieve weight loss through negative energy balance (Blackburn, GL et al. 2010). Diet modification could include adjusting the macronutrient composition, restricting the consumption of certain foods, or encouraging the consumption of others (Sacks, FM 2009). Moreover, physical activity and cognitive behavioral therapy have shown to help optimize and maintain weight loss (Jeffery, RW et al. 2003) (Butryn, ML et al. 2007; Corbalán, MD et al. 2009). Eventually, patients may require adjunctive therapies to meet their weight loss and health goals, particularly *pharmacotherapy* (Sweeting, AN et al. 2015). Pharmacological treatment for obesity is considered in patients with a BMI ≥30 kg/m^2 or BMI ≥27 kg/m^2 if weight-related comorbidities such as hypertension, T2DM, dyslipidemia, and/or obstructive sleep apnea are present. Currently there are several drugs approved in the United States and Europe for long-term management of obesity: Orlistat, phentermine/topiramate, locarserin, naltrexone/bupropion, and liraglutide 3.0 mg/d (Shukla, AP et al. 2015; Yumuk, V et al. 2015). Nevertheless, lifestyle modification with or without pharmacotherapy often does not result in large enough weight loss, especially in the severe obese. Particularly in this last group the third treatment modality mentioned above, bariatric surgery (BS), is the most effective treatment. Overall, surgical procedures to treat obesity aim to reduce body weight through restricting food intake or causing malabsorption of food or a combination of both (Colquitt, JL et al. 2014). Moreover, BS patients are expected to modify their eating behavior and encouraged to adopt a healthier lifestyle by including daily exercise. Thus, obesity treatment in the severely obese should be based on a combination of bariatric surgery and changes in lifestyle.

Bariatric Surgery

According to data from the Swedish Obese Subjects (SOS) trial, surgery is the only treatment for obesity resulting, on average, in more than 15% weight loss over 10 years (Sjöström, L 2008). Moreover, beyond weight loss, it also helps to significantly ameliorate obesity-attributable comorbidities in the majority of bariatric surgery patients. In an observational 2-cohort study, patients that underwent bariatric surgery have reduced 89% the risk of 5-year mortality compared to controls matched for age and gender (Christou, NV et al. 2004). Furthermore, BS improves and cause resolution of comorbid conditions such as T2DM (Sjöström, L et al. 2004), hypertension (Flores, L et al. 2014), dyslipidemia (Sjöström, L et al. 2004), metabolic syndrome (MetS) (Batsis, JA, 2008) among others. In addition, regardless of the procedure type, is generally considered as safe and efficacious procedure, with 30-day mortality rates ranging from 0.1% to 1.1% depending on the procedure (Buchwald, H et al. 2004). Currently the two most common techniques in bariatric surgery are Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) (Shukla, AP et al. 2015). Those surgical techniques can be classified based on their restrictive and/or malabsorptive effect on weight loss (Schroeder R et al.2011).

Roux-en-Y gastric bypass (R) This surgical procedure combines restriction and malabsorption and is currently considered the gold-standard bariatric surgery technique. Restriction is achieved by the creation of a small gastric pouch. Mild malabsorption derives from the bypass of the duodenum and proximal jejunum that prevents full absorption of nutrients (Jaunoo, SS et al. 2010). In brief, the procedure entails partition of the upper part of the stomach using surgical staples to create a small gastric pouch of about 20 mL along the lesser curvature of the stomach the division of the jejunum 40 cm distal to the ligament of Treitz, and end-to-side jejunojenjunostomy 150 cm distal to the gastrojejunostomy (Vidal, J et al. 2007;Moringo, R et al. 2008). Complications that may occur following RYGB include failure of the staple partition, leaks at the junction of the stomach and small intestine, acute gastric dilation, and delayed gastric emptying either spontaneously or secondary to blockage (Colquitt, JL et al. 2014). Other possible complications associated with this surgery include: wound hernias, vomiting caused by narrowing of the stoma due to scar tissue, and intestinal obstruction (Colquitt, JL et al. 2014). Also, an adverse event caused by eating refined sugar called dumping syndrome may occur after RYGB, which symptoms include: rapid heart rate, nausea, tremor, faint feeling and diarrhea (Colquitt, JL et al. 2014). In addition, nutritional requirements should be monitored since nutritional deficiencies such as calcium, vitamin D, vitamin B12, and some iron deficiency anemia may happen because of decreased intake, limited digestions and absorption, and food intolerances. Therefore supplementation should be considered based on individual requirements.

Sleeve Gastrectomy (SG). This procedure was originally used as the first part of a two surgical procedure, being followed at a later date by a conversion to either gastric bypass or duodenal switch (Nguyen, NT et al. 2013). In SG the stomach is divided vertically to reduce its size about 80%

from its original sized. In short, the greater curvature including the complete fundus is resected from the distal antrum (5cm proximal to the pylorus) to the angle of His, resulting in a sleeved or tubular shape. (Vidal, J et al. 2007;Moringo, R et al. 2008). SG results in impaired mechanical and chemical digestion accelerated gastric emptying, and lesser production of the intrinsic factor required for vitamin B12 absorption. Nonetheless digestion and absorption are altered to a lesser extent than following RYGBP. Thus, although this commonly performed procedure is not reversible its nutritional complication rate is lower as compared to RYGB. This operation is relatively quick to perform, which reduces the risk of complications (Colquitt, JL et al. 2014).

Regarding weight loss, bariatric surgery produces substantial weight loss in the majority of patients, with most of the change occurring between 18 and 24 months post-surgery (Schroeder R et al.2016).A successful weight loss outcome in bariatric surgery is described as a loss of >50% excess weight loss (%EWL), loss of >20-30% of initial body weight, and achieving a BMI <35kg/m^2 (Halveson, JD et al. 1981). RYGB and SG procedures present very similar outcomes when compared with each other regarding weight loss effectiveness and improvement in weight associated comorbidities. However, SG presents larger weight regain as compared with RYGB (Schauer, PR et al. 2014). Nevertheless, there is a sizable portion of the patients that experience a relative poor weight loss response with great variability in the amount and trajectory of weight loss (Christou, NV et al. 2006;Courcoulas, AP et al. 2013). A large body of research has addressed the association of poor weight loss response post-surgery with a set of factors involved in the variation of response to the surgery. Clinical variables such as higher pre-surgical BMI, pre-surgical T2DM medication, and aged greater than 50 years have been identified as predictors of weight loss response (Still, CD et al. 2014). Moreover, a number of psychiatric conditions have been associated with weight loss after a bariatric surgery (Pataky, Z et al. 2011). In addition, dietary habits such as poor eating factors (Fox, B et al. 2015), the technical procedure (larger gastric pouch) (Campos, GM et al. 2008), endocrine (Pedersen, SD et al. 2013; De Hollanda et al. 2015), and genetics factors (Sarzynski, M et al. 2011;Balasar, O et al. 2015) have been also involved in the variation of postsurgical response.

Chronobiology

Chronobiology studies the biological rhythms that are found throughout the living organism at the different levels of organization (Garaulet, M et al. 2013). Circadian rhythms are those biological rhythms around the day (24h rhythms) that are generated by clocks that are endogenous in nature (Garaulet, M, et al 2013). The circadian timing system is a complex network of circadian clocks that generate the circadian rhythmicity. Circadian rhythms influence a broad range of biological processes, including neuronal, endocrine, metabolic and behavioral function (Johnston, JD et al. 2016). In mammals, the suprachiasmatic nucleus (SNC) located in the hypothalamus is the controlling clock component (Sadacca, LA et al. 2011). In addition, other clocks, called peripheral clocks, are found within all major tissues such as heart, liver, adipose tissue, and muscle, and possibly within most individual cells of the body (Bass, J &Takahashi, JS 2010). Clocks must be synchronized to each other and to the outside environment in order to benefit an organism (Johnston, JD et al. 2016). The synchronization of this system is caused by *zeitgeber* factors, which are factors external to a given clock (Johnston, JD et al. 2016) such as changes from light to darkness, from activity to resting and from eating to fasting. Social contacts are also considered zeitebergs (Garaulet, M et al. 2010).

The circadian systems can be conceptualized as having three components: the inputs, the 24-h oscillators, and the outputs (**Figure 1**) (Garaulet, M et al. 2010). The central pacemaker or SNC receives inputs from the environment that serve as stimuli to synchronize its rhythms to the outside world: *zeitgeber* factors (Garaulet, M et al.2013). Among those, **light** is the most powerful *zeitgeber* in nature. Under natural environmental conditions, the SCN reset every day by the periodical light/dark signal (Lax, P et al. 1998). Light/dark cycles entrain the central clock in the SNC where it mainly dominates activity related rhythms such as sleep/wake cycles, the autonomic nervous system, core body temperature, and endocrine rhythms (outputs) (Oike, H et al. 2014). On the other hand, feeding/fasting cycles entrain peripheral clocks that are located in most tissues including a part of the brain (Tahara, Y & Shibata, S. 2013). **Feeding time** is a dominant factor in determining the phase of circadian clocks. However, the central clock is not essential for food anticipatory activity (Oike, H et al. 2014). Circadian clocks enable the anticipation of daily events, conferring a considerable advantage for saving time and the efficient use of energy (Tahara, Y and Shibata, S. 2013).

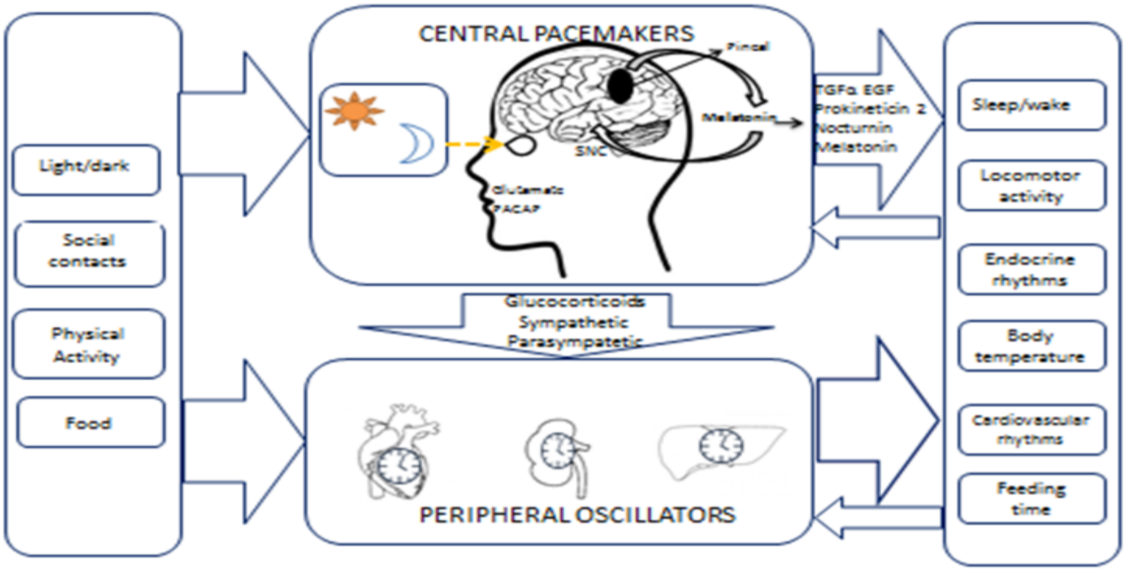

Figure 1. A general overview of the functional organization of the circadian system in mammals. (Garaulet, M et al. 2010).

At the molecular level circadian clocks are composed of a set of proteins that generate self-sustained circadian oscillations through positive and negative transcriptional/translational feedback loops (TTFL) (Garaulet, M et al. 2010). The core of the circadian clock is composed by a positive limb of the molecular clock that comprises two transcription factors: Circadian Locomotor Output Cycles Kaput (*CLOCK*) and brain and muscle ARNT-like protein 1 (BMAL1), while Period (*PER*) and Cryptochrome (*CRY*) are responsible for the negative limb (Garaulet, M 2013). *CLOCK* and BMAIL1 stimulate the transcription of 3 *PER* genes and 2 *CRY* genes. The translated PER and CRY proteins then form protein complexes that translocate into the nucleus and repress the transcriptional activation of their own genes by *CLOCK* and *BMAIL1*. Posttranscriptional and posttranslational modifications regulate the precise temporal dynamics of this loop (Partch, CL et al. 2014)(Lim, C et al. 2013). In addition, interlinked with the primary loop are multiple secondary loops, which involve important biochemical components of cellular metabolism (Johnston JD, et al. 2016). Those secondary loops involves the circadian transcription of the nuclear receptor REV-ERB-α (NR1D1) by *CLOCK-BMAIL1* dimer acting through E-box regulatory elements; the resulting protein then inhibits *BMAIL-1* transcription through retinoic acid-related orphan receptor response elements, resulting in circadian rhythms of *BMAL1* mRNA expression (Preitner, N et al.

2002). Since *CLOCK* is also expressed in peripheral tissues that are metabolic tissues, circadian genes are involved in metabolic regulation including glucose homeostasis (Kalsbeek, A et al. 2014).

Chronodisruption.

A breakdown in the normal phase relationship between the internal circadian rhythms and period of around 24-h environment cycles is known as *chronodisruption* or circadian disruption (Erren, T et al. 2009). Chronodisruption is defined as a relevant disturbance of the internal temporal order of physiological, biochemical, and behavioral circadian rhythms (Garaulet, M et al. 2013). As the central clock organizes local clocks through neuronal and humoral signals, desynchornization among clocks is believed to result in the development of pathologic conditions such as metabolic syndrome, obesity, cancer, psychiatric disorders and premature ageing ((Wirz-Justice, A 2006 ;Garaulet, M & Madrid JA. 2009; Garaulet, M et al.2013 ;Erren, T et al. 2009).

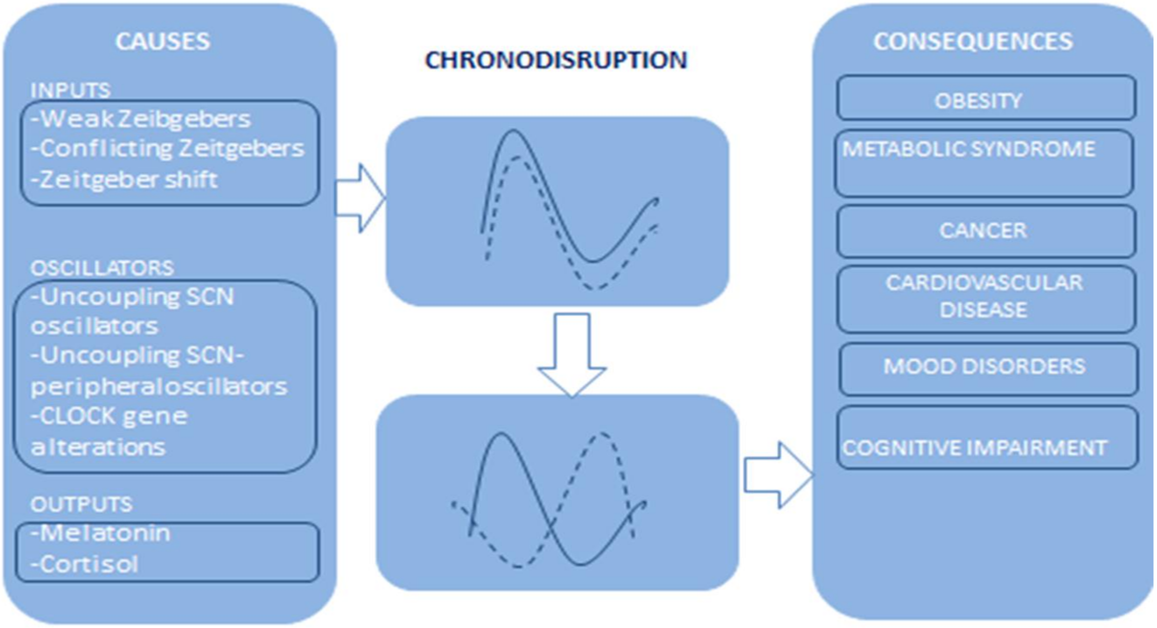

Figure 2 Causes and consequences of chronodisruption. (Garaulet, M et al. 2010). The result from phase shift in the oscillation of the circadian (solid line) and activity-controlled physiological processes (dotted line) is known as chronodisruption (Garulet, M et al. 2010).

Chronodisruption may be common in several conditions such as jet lag, shift work, light at night, or social jet lag (Garaulet, M et al. 2010) and it can be induced by factors related to the following (**Figure 2**):

- Impairment of *inputs* to the circadian system:
  - Weak *Zeitgebers*: Low contrast between day and night synchronizing signals.
  - Conflicting *Zeitgebers*: Exposure to *zeitgebers* of different periods or unusual phasing
  - *Zeitgeber* Shift: Frequent shifts in the time provided by *zeitgebers*.
- Circadian oscillators: The uncoupling between different subpopulations of oscillators caused by aging or clock gene alteration and the uncoupling between central pacemaker and peripheral oscillators.
- Outputs: The suppression of nocturnal melatonin and the loss of daily cortisol rhythm are also chronodisrupters (Garaulet, M et al. 2010; Garaulet, M et al. 2013).

Traditionally, research on obesity has focused on cellular functions related with energy homeostasis, biochemical pathways that regulate nutrient absorption, distribution, metabolism, as well gene-environment interaction, among others (Martinez, JA 2014). Recently, notable research has been done on the relationship between obesity and factors related with chronobiology, especially on chronodisruption (Garaulet, M et al. 2010;Garaulet, M et al. 2013). The mechanisms that associate obesity and circadian disruption are not well unknown. The most well-known studies on the subject have been performed on shift workers (Lund, J et al.2001;Croce, N et al.2007). There is epidemiological evidence that shift work is related with obesity, diabetes, and cardiovascular disease, abdominal obesity, low high-density lipoprotein and hypertriglyceridemia (Karlsson, B et al. 2001). Further research has been done using experimental models and observational data to study the interaction between chronodisruption and obesity (Ando, H et al.2005;Garaulet, M et al. 2013). Chronobiological factors such as sleep, food timing, circadian preference, and clock genes seem to have a role in obesity and/or poor weight loss in obese subjects.

CLOCK genes

A large number of studies in humans and animals have shown that genetic factors are important contributors to obesity and its development (Walley, AJ et al. 2006;Choquet, H et al. 2011). The *CLOCK*, a component of the circadian system that regulates the expression of other integral circadian genes, seems to be involved in altered metabolic functions (Garaulet, M et al. 2010).

Studies of polymorphisms in *CLOCK* in humans have revealed that the molecular clock may play a role in cardiovascular diseases, obesity and diabetes (Scott, EM et al. 2008). C carriers of *CLOCK 3111T/C* single nucleotide polymorphism (SNP) (rs1811260) present sleep reduction, increased values of plasma ghrelin, and disturbances of eating behavior (Garaulet, M et al. 2011). Also, C carriers display a less robust circadian rhythm than TT carriers and a delay in the acrophase that characterizes evening-type subjects (Bandin, C et al. 2012). Moreover, it has been demonstrated that in subjects with binge eating disorder, that 3111T/C SNP of the CLOCK gene could be a biological vulnerability factor predisposing obese individuals to reach a higher BMI value (Monteleone P et. al 2008). Those findings suggest that the 3111T/C SNP predisposes obese individuals to a higher BMI. Thus, we could hypothesize that 3111T/C SNP of the *CLOCK* gene may have a part in the genetic susceptibility that contributes to obesity. Regarding to weight loss, it has been shown that *CLOCK* gene is implicated in weight reduction in obese patients participating in a dietary program based on the Mediterranean diet. Indeed, the genetic variation in the rs1801260 CLOCK (*CLOCK 3111T/C*) was associated with obesity at baseline and also affected weight loss. Patients with the variant allele (C) lost significantly less weight compared with wild type. Repeated measures analysis showed that weight loss over time was significantly different between rs1801260 CLOCK variations. Carriers of the C allele displayed greater difficulty in losing weight than non-carriers (Garaulet, M et al. 2010)

Sleep

Several studies have shown self-reported poor sleep as an independent risk factor for obesity and limited weight loss (Nedeltcheva, AV et al. 2011;Beccuti, G et al.2011). Most of the evidence is based on studies focused on duration of sleep; however timing and regularity of sleep also may be important (Patel, SR et al.2014). Poor sleep quality has also been associated with an increased risk of metabolic syndrome and several of its core components such as higher waist circumference)(Jennings, JR et al. 2007;Biggi, N et al. 2008). Sleep duration is also linked with increased odds ratio of having metabolic syndrome o some of its components. (Hall et al.2008). In an interesting review was postulated that both lower energy expenditure and an excess of energy intake could be implicated in this interaction (Van Cauter, E & Knutson, Kl, 2008). Indeed, studies in adults and adolescents have shown associations between inadequate sleep and alterations in leptin and/or ghrelin, which may affect hunger and appetite, increasing the risk of overeating and consequently weight gain (Garaulet, M et al. 2011). Moreover, short sleep duration could lead to obesity by increasing the time available to eat, and also has been suggested to decrease energy expenditure by increasing fatigue as well changes in thermoregulation as shown in **figure 3**.Furthermore, individual chronotype has significant effects on sleep parameters (Soehner, AM et al.2011). Therefore sleep is an important chronobiological factor that could influence not only body weight but also weight loss outcomes.

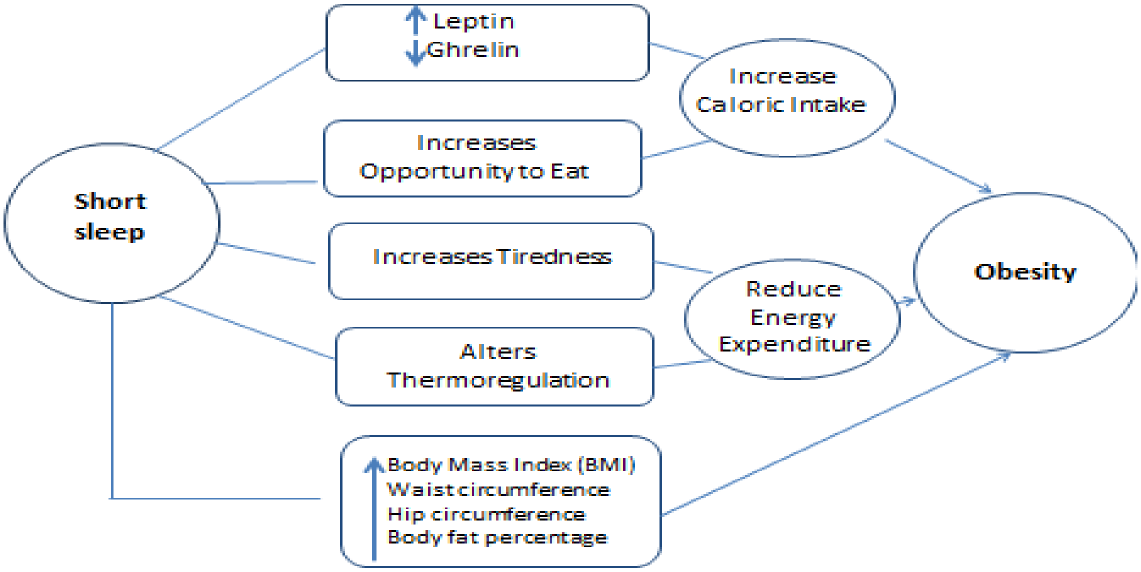

Figure 3. Potential mechanisms by which sleep deprivation may predispose to obesity .Adapted from Patel & Hu, 2008 and Garaulet, M et al. 2011.

Time of food intake

Recent research has shown that not only what we eat but also the time when we eat may have a significant role in obesity treatment (Arble, DM et al. 2009;Garaulet, M et al. 2013) Garaulet, M et al. 2013;Sofer, S et al. 2015). In animal models, a change in the timing of feeding has been associated with obesity. Mice that were fed in a high-fat diet during their usual sleeping time gained more weight than controls, despite similar energy intake and estimated energy expenditure (Arble, DM et al. 2009). Also, evidence from animals has shown that restricting feeding times can prevent diet-induced obesity and improve metabolic markers (Sherman, H et al. 2012;Hatori, M et al. 2012). In human subjects, recent studies have reported that the timing of meals has implications in the prevention of obesity, weight loss and regain, appetite, insulin resistance and metabolic syndrome (Jakubowicz D et al. 2013;Garaulet, M et al. 2013;Sofer, S et al.

2015). In the same line, it has been demonstrated that the timing of food consumption influence weight loss and can induce a disruption of the circadian system which might produce unhealthy consequences in humans (Bandin, C et al. 2015). In a prospective longitudinal study with an overweight and obese population, those who ate their main meal (lunch) later in the day loss significantly less weight than those who eat lunch early (Garaulet, M et al. 2013). Surprisingly, there were no significant differences in total energy consumption, macronutrient distribution, sleep, and hormone levels between early and late eaters (Garaulet, M et al. 2013). Thus, current evidence suggests that eating late may impair the success of weight loss therapies.

Chronotype

To identify chronotype preference, Horne and Østberg developed the morningness-eveningness questionnaire (MEQ) where individuals can be stratified as morning, evening and neutral type (Horne, J & Ostberg, O 1976). Chronotype features are thought to conform a stable trait with a large genetic component (Klei, L et al. 2005). Circadian preference has been related to several metabolic disturbances (Lucassen, EA et al.2013;Yu, JH et al.2015). Evidence from studies in humans has shown that eveningness as compared to morningness is significantly linked with the metabolic syndrome (Yu, JH et al.2015). Also evening chronotype was associated with eating later and a tendency towards fewer and larger meals and lower HDL-cholesterol levels. Evening subjects had more sleep apnea and higher stress hormones (Lucassen, EA et al.2013). A late chronotype has also been related with poorer dietary habits and increased BMI (Arora, T et al. 2015). In addition, evidence suggests that chronotype with marked evening shift in activities and delayed sleep onset is particularly present in patients with bipolar disorder (Mansour, HA et. al 2005). Individuals with the evening type may have inborn circadian fragility that facilitates the desynchornization of endogenous rhythms, or may be more subject to "social jet lag" (Wittmann, M et al. 2006). Social jet lag implies that individuals with evening chronotype are constantly required to adjust to external demands that are out of synchrony with their circadian rhythms (Wittmann, M et al. 2006). Hence, evening subjects may require to continuously adjusting to daytime occupational and social demands (Wittmann, M et al. 2006). Those findings suggest that that the individual chronotype preference specifically evening type subjects makes the subject more prone to chronodisruption and thus more prone to metabolic disturbances such as obesity.

Chapter 3

Hypothesis and Objectives

In spite of preliminary studies that demonstrate the implication of the circadian system in obesity, most of these theories have been demonstrated in animal models and in obese subjects that are submitted to dietary treatments to lose weight. Nevertheless, to our knowledge these new hypotheses have not been tested yet in severe obese patients undergoing bariatric surgery.
In the current work **we hypothesize** that similar to what happens in obese subjects who follow a dietary treatment to lose weight, the circadian system is also connected to weight loss in severe obese subjects undergoing bariatric surgery.

In order to test our hypothesis, three specific **aims** will be treated in three different chapters:

1) To describe the inter-individual variability in the weight loss response up to 5 years of follow up after the two most commonly performed bariatric surgery techniques **(Chapter 4)**.
2) To examine whether the individual chronotype is related to obesity and weight loss evolution in severe obese after bariatric surgery and to examine potential interactions between circadian preferences and CLOCK 3111T/C for obesity in this population (**Chapter 5**).
3) To evaluate if the timing of food intake is associated with weight loss evolution in severe obese patients after bariatric surgery (**Chapter 6**).

Chapter 4

Patterns of weight loss response following Gastric Bypass and Sleeve Gastrectomy

Patterns of Weight Loss Response Following Gastric Bypass and Sleeve Gastrectomy.

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ABSTRACT

Introduction: Indeed, bariatric surgery is the best available therapy to achieve significant weight loss in severely obese subjects. Nevertheless several studies have shown that after a bariatric surgery the weight loss can vary among subjects and there are a sizable proportion of patients that do not achieve a successful weight loss. The aim of the present work was to describe the presence of different patterns of weight loss up to 4.5 years follow up after RYGB and SG. In addition, we examined pre-surgical predictors of such postsurgical weight loss outcomes.

Subjects and Methods: Data from 658 subjects that underwent BS surgery at Hospital Clinic between 2005 and 2009 were analyzed. Patients were classified based on three patterns of weight loss expressed as excess weight loss (%EWL). Subjects with EWL > 50% at nadir weight and throughout subsequent follow-up were considered as good weight loss responders. Patients with EWL <50% at nadir weight and up to the end of follow up were considered as primarily poor weight loss (1-PWL) responders. Patients with EWL ≥50% at nadir weight but EWL <50 % at last follow up at visit were classified as secondarily poor weight loss (2-PWL) responders. Predictors associated with different weight loss outcomes such as higher pre-surgical BMI, pre-surgical T2DM, and lower numbers of postsurgical appointments were ascertained using regression analysis.

Results: Median follow-up was 4.5 years (55.7 months). Good weight loss was found in 75.7% of the cohort while 1-PWL response in 4.7%. In addition, 1-PWL was associated with larger BMI and T2DM at baseline (prior to surgery). 2-PWL response was found in 19.6% of the studied population, with larger weight regain compared to the other groups and more common following SG.

Conclusions: Our analysis shows the high inter-individual variability of the weight loss response at mid-term following bariatric surgery. Poor weight loss after RYGB and SG can be illustrated by two patterns: 1) A primarily poor weight loss response characterized by limited weight loss

throughout follow up and 2) a secondarily poor weight loss pattern characterized by significant weight loss but subsequent weight regain leading to a final EWL <50 %.

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ORIGINAL CONTRIBUTIONS

Patterns of Weight Loss Response Following Gastric Bypass and Sleeve Gastrectomy

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Abstract

Background Despite the health benefits of bariatric surgery (BS) extend beyond WL, better understanding of the WL response may help improve the outcomes of BS. In this context, we aimed to assess patterns within the variability of weight loss (WL) after Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG).

Methods WL data from 658 subjects that underwent RYGB (n=464) or SG (n=194) as first BS were analyzed. Based on excess WL (EWL), subjects were categorized as good WL responders (EWL≥50 % at nadir weight and throughout follow-up), primarily poor WL responders (1-PWL:EWL<50 % at nadir weight and thereafter), and secondarily poor WL responders (2-PWL:EWL≥50 % at nadir weight, but <50 % at last follow-up visit). Predictors associated with different WL outcomes were ascertained using regression analysis.

Results Median follow-up was 55.7 months. Nadir EWL ranged 12.4–143.6 %; last follow-up visit EWL ranged −22.1–143.6 % and weight regain (WR) ranged 0–64.1 kg. Good WL was found in 75.7 of the cohort. 1-PWL response (4.7 %) was characterized by lesser WL but similar WR as compared to good WL and was associated with larger BMI and diabetes prior to surgery. 2-PWL response (19.6 %) was characterized by larger WR as compared to the other groups and was more common following SG. Lesser percentage of medical appointments kept was associated with 1-PWL and 2-PWL.

Conclusion Our data show the high inter-individual variability of the WL response at mid-term after RYGB and SG and that poor WL after BS could be illustrated by two different patterns, characterized either by sustained limited WL (1-PWL), or pronounced weight regain (2-PWL).

Keywords Gastric bypass · Sleeve gastrectomy · Weight loss

Introduction

It is well established that the health benefits of bariatric surgery (BS) extend beyond weight loss (WL) [1, 2]. Nonetheless, several lines of evidence suggest that WL is an important contributor to the health outcomes associated with BS [3, 4]. Thus, it is conceivable that better understanding of the WL response to BS techniques may help delineate strategies to optimize this therapeutic approach.

Undoubtedly, BS is the best available therapy to achieve and sustain significant WL in morbidly obese subjects [2]. However, several studies have shown that postsurgical WL varies widely and a sizable proportion of subjects present a relatively poor response [2, 5–8]. In the landmark Swedish Obese Subjects (SOS) study, maximum WL after surgery averaged approximately 34 kg but ranged between −95.5 and +2.0 kg [5]. Furthermore, in that study, nadir weight was ensued by gradual weight regain averaging 11.8 kg but ranging between 0.0 and 51.4 kg by 6 years after surgery. Similarly, the 95 % confidence interval for excess WL (EWL) ranged between 70.1 and 84.9 % at 3 years and between 42.7

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and 63.9 % at 6 years in a small cohort of subjects that underwent sleeve gastrectomy (SG), another commonly performed BS technique [7]. The above mentioned studies nicely describe the substantial variability in weight change following BS. Nonetheless, description of patterns of weight change within this variability has seldom been attempted [2]. It could be hypothesized that characterization of different weight loss patterns may help advance the identification of factors associated with variable weight loss following BS.

Against this background, the primary aim of our study was to describe the presence of different patterns of WL up to 5 years of follow-up after two commonly performed BS techniques, namely Roux-en-Y gastric bypass (RYGB) and SG. As secondary aim, we examined pre-surgical predictors of such postsurgical WL outcomes.

Subjects and Methods

Participants in our retrospective analysis of prospectively collected data were selected among the 945 subjects that underwent BS surgery at our institution between 2005 and 2009. Eligibility criteria included age ≥18 years, first-time RYGB or SG surgery, and ≥30 months of available follow-up. Patients were considered for BS based on the current guidelines [9]. The technical aspects and the criteria for selection of RYGB or SG at our institution have previously been reported [10, 11]. In brief, laparoscopic RYGB included the creation of a small proximal gastric pouch of about 20 mL along the lesser curvature of the stomach, the division of the jejunum 40 cm distal to the ligament of Treitz, an end-to-side gastrojejunostomy of about 1.5 cm in diameter using a circular stapler, and a side-to-side jejunojejunostomy 150 cm distal to the gastrojejunostomy. For the SG, the greater curvature including the complete fundus was resected from the distal antrum (5 cm proximal to the pylorus) to the angle of His. A laparoscopic stapler, EndoGIA (Autosuture, Norwalk, CT, USA) with a 60-mm cartridge (3.5-mm staple height, blue load), was used to divide the stomach alongside a 34 French bougie (placed against the lesser curvature of the stomach). Following approval by the local ethics committee, written informed consent was obtained from all study participants.

Data was prospectively collected prior to surgery and at 4, 8, 12, 18, 24, 30, 36, 48, and 60 months in the postsurgical period. A diagnosis of type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia, sleep apnea syndrome, and tobacco use was based on medical history and laboratory data. Body weight, height, and waist circumference were measured as previously described [10]. Postoperative WL was expressed as a percentage of the pre-surgical excess weight (% EWL=[100×(weight prior to surgery−weight at the time of evaluation)/(weight prior to surgery−weight corresponding to body mass index (BMI)=25 kg/m^2)]). Maximum WL was described as the maximum EWL recorded at postsurgical checkup visits. Weight regain was defined as the difference between body weight at last follow-up and nadir weight and was expressed in kilograms or as percent of maximum WL. Medical appointments kept were calculated as the percent of visits attended out of the nine scheduled postsurgical medical visits.

Three different patterns of WL were pre-specified based on the EWL Reinhold criteria modified by Christou et al. [8]. Patients with EWL>50 % at nadir and throughout subsequent follow-up were considered as good WL responders. Patients with EWL<50 % at nadir weight and up to the end of follow-up were considered as primarily poor WL (1-PWL) responders. Subjects with EWL≥50 % at nadir weight but EWL<50 % at last follow-up at visit were considered as secondarily poor WL (2-PWL) responders. Time to adjudication of a 2-PWL response was defined as the time elapsed between surgery and the study visit at which EWL<50 % was first recorded following nadir weight. Patients that underwent SG as primary BS procedure but went through revisional BS were classified as 1-PWL responders or 2-PWL responders based on the WL trajectory up to the time of the second surgery.

All data are expressed as mean±SD unless stated otherwise. Differences between groups were evaluated using parametric or non-parametric test as appropriate. Predictive factors of the different WL outcomes were ascertained by logistic regression analysis. Clinical features associated with 2-PWL response were evaluated by means of logistic and Cox regression analysis, the latter to take into account the time of follow-up. Survival analysis was used to compare occurrence of a 2-PWL response over time following RYGB and SG. Statistical analyses were performed using the SPSS 20.0 statistical package, and significance was set at a *p* value of <0.05.

Results

Table 1 displays the clinical characteristics of the study of 658 participants. Out of the original 945 potentially eligible patients, 50 (5.0 %) were excluded as current BS that was not a primary procedure, 23 (2.4 %) as revisional surgery for SG was performed <30 months (mainly because of severe gastroesophageal reflux), and 237 (25.0 %) because lack of follow-up beyond 30 months. At the time of surgery, age ranged from 18 to 69 years and BMI from 35 to 84 kg/m^2. RYGB and SG were performed, respectively, in 70.5 and 29.5 % of the cohort. Because of our criteria for the selection of the surgical technique, subjects that underwent SG presented larger BMI, waist circumference, and more commonly a diagnosis of T2DM, hypertension, and dyslipidemia. Moreover, male gender ($p<0.001$) and older age ($p<0.01$) were found in SG subjects.

Table 1 Clinical characteristics of the study participants at baseline

	Whole cohort	Gastric bypass	Sleeve gastrectomy
n	658	464	194
Gender (% female)	74.5	78.4	64.9[c]
Age (years)	45.3±11.0	44.6±10.1	47.1±12.4[b]
BMI (kg/m^2)	47.1±6.5	45.6±5.0	50.7±8.2[c]
Waist circumference (cm)	132±15	128±13	140±17[c]
Diabetes mellitus (%)	27.8	25.6	33.0 ($p=0.057$)
Hypertension (%)	41.5	37.2	51.5[a]
Dyslipidemia (%)	25.5	23.0	31.4
Tobacco use (%)	22.2	22.6	21.1
Sleep apnea syndrome (%)	18.1	16.8	21.1

Data are expressed as mean±SD

BMI body mass index

[a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$ (for the comparison between gastric bypass and sleeve gastrectomy groups)

In the entire cohort, median postoperative follow-up was 55.7 months (range 30–68 months). Weight loss was maximal (nadir weight) at 23.7±15.7 months after surgery, and at that time, EWL was 81.7±19.2 %. At last evaluation, EWL was 65.3±22.8 % (corresponding to a weight regain of 9.2±8.4 kg or 20.9±11.9 % relative to nadir weight). The three WL parameters showed high inter-individual variability with maximum EWL ranging 12.4 to 143.6 %, EWL at last checkup −22.1 to 143.6 %, and weight regain 0 to 64.1 kg. Length of follow-up was larger in RYGB as compared to SG subjects (respectively 54.3±9.2 and 48.8±10.8 months; $p<0.001$). Analysis of covariance with gender, age, BMI, prevalence of T2DM, hypertension, and length of follow-up as covariates showed EWL at nadir (adjusted marginal mean±standard error; RYGB 81.3±0.8 versus SG 83.0±1.4 %) and time to nadir weight (RYGB 24.4±0.7 versus SG 22.2±1.2 months) were not significantly different between surgical cohorts (respectively $p=0.952$ and $p=0.136$). Weight regain was smaller after RYGB (RYGB 8.6±0.4 kg or 19.5±0.9 % versus SG 10.6±0.6 kg or 24.1±1.4 %; both $p<0.01$).

At last follow-up visit, 498 (75.7 %) of the study participants presented EWL≥50 % and were thus considered as good WL responders. In contrast, EWL<50 % was encountered in 160 (24.3 %) subjects, with 31 (4.7 %) and 129 (19.6 %) being classified, respectively, as 1-PWL responders or 2-PWL responders according to the pre-specified criteria. The EWL trajectories of these three groups of subjects are presented in Fig. 1. Of note, the EWL=50 % at maximum WL corresponded to the 5th percentile of the EWL distribution of the whole cohort at nadir weight. The EWL=50 % corresponded to the 12th, 15th, 23rd, or 27th percentile of the WL distribution, respectively, at 30, 36, 48, or 60 months follow-up. As shown in Table 2, differences in EWL between the good WL responders group and the 2-PWL responder group were already apparent at maximum WL ($p<0.001$). Weight regain in the good WL responders group ranged between 0 and 64.1 kg and was significantly less as compared to the 2-PWL responder group (range 4.3 to 57.6; $p<0.001$) (Table 2). Weight regain in the 1-PWL responder group ranged from 0 to 25.8 kg ($p=0.265$ and <0.001, respectively, compared to good WL and 2-PWL responder groups) (Table 2).

Comparison (ANOVA analysis) of the clinical characteristics of study subjects based on WL outcomes is shown in Table 2. Logistic regression analysis showed that 1-PWL response was independently associated with higher BMI [OR 1.060 (95 % confidence interval, CI, 1.060–1.060), $p=0.024$], pre-surgical diagnosis of T2DM [OR 2.407 (95 % CI 1.047–5.532), $p=0.039$], and the percentage of postsurgical medical appointments kept [OR 0.963 (95 % CI 0.943–0.983), $p<0.001$]. Since weight regain over time characterized 2-PWL response, Cox regression analysis was performed to evaluate the independent contribution of clinical variables to this WL pattern. The analysis demonstrated that SG [OR 1.775 (95 % CI 1.167–2.700), $p<0.01$], albeit marginally, a lower BMI [OR 0.970 (95 % CI 0.943–0.998), $p=0.035$], and the percentage of postsurgical medical appointments kept [OR 0.953 (95 % CI 0.940–0.966), $p<0.001$] were significant predictors of 2-PWL response. Survival analysis with Kaplan-Meier as estimate showed that adjudication of a 2-PWL response occurred not only more often but also earlier in subjects that underwent SG (time to EWL<50 % of subjects in the secondary WL failure group: SG, 44.6±2.2 versus RYGB, 52.7±2.1 months; $p=0.01$).

Discussion

Our data obtained at a median of 4.5 years after surgery illustrate the high inter-individual variability in the WL response to RYGB and SG, two commonly and currently performed BS techniques, that could be depicted in three distinct patterns of WL. First, a good WL response pattern characterized by EWL>50 % both at maximum WL and last follow-up visit, occurring in three out of four of the study participants.

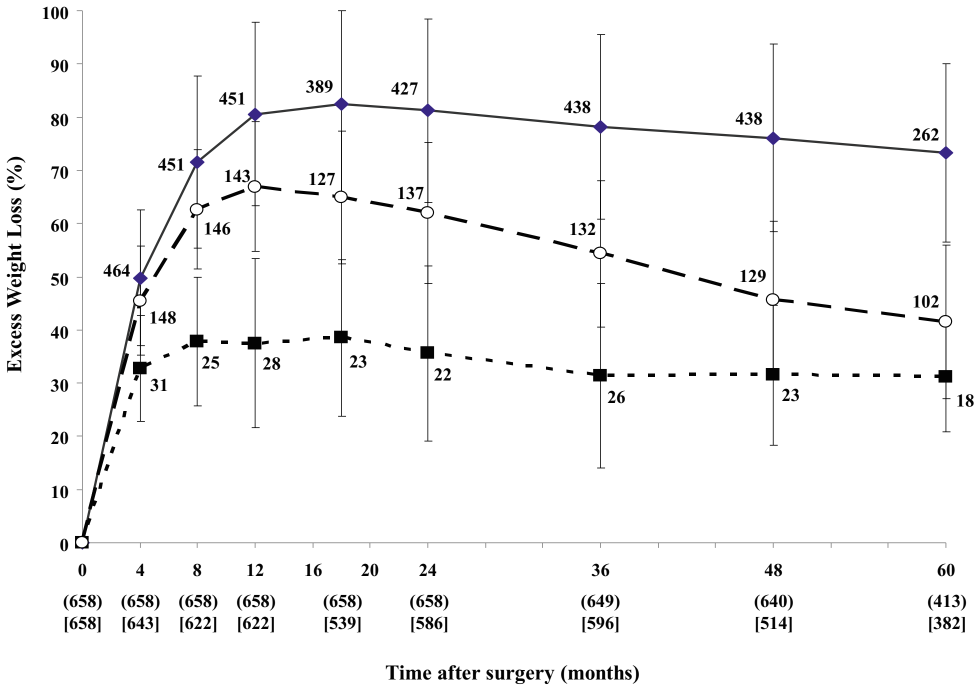

Fig. 1 Excess weight loss over 5 years in subjects with good weight loss (WL) response, primarily poor WL response, and secondarily poor WL failure. *Blue diamonds*, *solid line*: good WL group; *open circles*, *dashed line*: secondarily poor WL responders group; *black squares*, *dashed line*: primarily poor WL responders group. The number of patients in each category that contributed to each time point is shown next to each point on the graph. The number of patients that were eligible for follow-up and the number of patients evaluated at each time point are shown, respectively, *between parentheses* and *squared brackets* below the *X*-axis

Second, a less common 1-PWL response pattern was characterized by poor WL and no major weight regain, resulting in EWL<50 % throughout follow-up. Third, a 2-PWL response pattern was characterized by limited but larger than 50 % EWL at nadir and progressive subsequent weight regain, occurring in about 1 out of 5 study subjects.

Table 2 Clinical characteristics at baseline and weight loss (WL) parameters of study subjects according to the three different WL patterns

	Good WL response	Primarily poor WL response	Secondarily poor WL response	*p* value
n	498	31	129	
Gender (% female)	76.1	70.9	69.0	0.330
Age (years)	44.7±11.0	47.5±10.5	47.2±10.6	0.037
BMI (kg/m^2)	47.0±6.8	49.7±6.1	46.9±5.6	0.068
Waist circumference (cm)	132±15	137±15	132±13	0.118
Type of surgery (% GBP)	72.6	61.2	70.6	0.323
Diabetes mellitus (%)	24.7$^{a, 1}$	45.1	35.7	0.005
Hypertension (%)	38.4$^{a, 1}$	51.6	50.4	0.022
Dyslipidemia (%)	24.6	25.8	27.4	0.857
Tobacco use (%)	24.5	12.9	15.5	0.034
Sleep apnea syndrome (%)	15.5 $^{a, 1}$	16.1	28.7	0.013
Maximum EWL (%)	87.7±16.3$^{b, 2}$	43.4±13.1$^{\#}$	68.5±11.5	<0.001
Time to maximum EWL (months)	24.5±13.2^{a,1}	17.3±11.8	15.3±7.2	<0.001
EWL at last follow-up visit (%)	74.6±16.9$^{b, 2}$	31.7±14.2	38.4±12.9	<0.001
Weight regain (Kg from BW at nadir)	7.3±7.$^{b, 2}$	7.9±7.1$^{\#}$	16.7±9.3	<0.001
Postsurgical medical appointments kept (%)	87.3±15.4^{a}	77.4±23.3$^{\#}$	88.9±13.5	<0.001

Data are expressed as mean±SD. *p* value for the comparison among the three WL groups

BMI body mass index, *EWL* excess weight loss, *BW* body weight

$^{a}p<0.05$; $^{b}p<0.001$ (for the post hoc comparison between the good WL response and primarily poor WL response groups); $^{1}p<0.001$; $^{2}p<0.01$ (for the post hoc comparison between the good WL response and secondarily poor WL response groups); $^{\#}p<0.001$ (for the post hoc comparison between the primarily poor WL response and secondarily poor WL response groups)

The high inter-individual variability in the long-term WL response following BS found in our study confirms previous data following RYGB and expands this finding to the increasingly performed SG. The overall WL response to RYGB found in our study is similar to that previously reported in studies including data beyond 5 years of follow-up [5, 6, 8, 12–14]. Unfortunately, although increasingly performed, long-term data following SG is limited. A recent systematic review of randomized clinical trials including a SG-arm showed %EWL that ranged from 49 to 81 % at 6 months to 3 years follow-up [15]. Similarly, systematic review of SG series with longer follow-up showed %EWL that averaged between 43 and 86 % [16]. However, the number of patients was small ($n \leq 60$) in all the contributing studies to that of systematic review. Of note, Prevot et al. recently reported variable EWL (43±25 %) at 5 years follow-up in a series of SG only patients ($n=84$), with 42 % of them presenting EWL <50 % at last follow-up visit [17]. Our findings of no difference in maximum WL but larger weight regain following SG as compared to RYGB are in agreement with the 3-year follow-up data from a recently reported RCT in subjects with T2DM comparing these two surgical techniques with medical therapy [18].

As mentioned above, our data suggest that variable WL response following RYGB and SG could be depicted in three different patterns: good WL response, 1-PWL response, and 2-PWL response. We acknowledge the EWL criteria chosen to define these trajectories were arbitrary though based in current literature and have recently been challenged [19]. As expected from its static nature, the EWL<50 % criteria corresponded to different percent values when applied to the distributions of maximum or last follow-up visit WL. Nonetheless, we used this criterion because of lack of consensus on how insufficient WL following BS should be defined. Considering the limitations above that the WL response in our poor WL groups was limited is demonstrated by their positioning in the poorest quartile of the WL distribution in our cohort. Interestingly, although 1-PWL responders presented by definition lower EWL at maximum WL, they presented with similar weight regain as compared to good WL responders. In contrast, 2-PWL responders were characterized by larger weight regain as compared to good WL and 1-PWL responders. Thus, we would suggest that our pre-specified definition of the two poor WL trajectories was clinically meaningful as it discriminated between subjects that did no achieve adequate postsurgical WL throughout follow-up from those in whom the long-term outcome was determined mainly by marked weight regain. Interestingly, using mathematical modeling up to five distinct WL trajectories have recently been reported in subjects that had undergone RYGB [2]. Although different criteria to those reported herein were used, approximately 24 % of the subjects included in the study presented with a WL<25 % relative to baseline. Interestingly, 2 % of the whole cohort presented no further WL after 6 months of follow-up and WL of approximately 10 % after 3 years.

Reviews on the large body of available research on clinical predictors of WL response as continuous variable following BS have previously been reported [20–22]. Studies in the literature differ in how WL was assessed, the clinical predictors tested, and the length of follow-up used for the assessment. Although our study aimed primarily at providing a framework for future assessments of factors associated with postsurgical WL as trajectories, analysis of a limited set of factors in our series yielded consistent results with previous literature in the field [20–23]. Higher pre-surgical BMI, presurgical T2DM, and lower number of postsurgical appointments kept have been identified as associated to lesser EWL (assessed as continuous variable) after BS. Importantly, Cox regression analysis in our cohort showed that SG was associated with increased odds of 2-PWL response as compared to RYGB. Of note, this effect was not apparent when data was analyzed only up to 4 years follow-up (data not shown). Thus, our data underscore the importance of long-term follow-up when comparing the WL results of these two commonly performed BS techniques. Interestingly, except for the number of appointments kept, the set of factors associated with 1-PWL or 2-PWL in our series was distinct. In this context, we consider that our data may provide a framework that may help advance in the identification of factors associated with variable WL after BS. We would hypothesize factors associated with resistance to WL would potentially underlie the 1-PWL response. In contrast, factors facilitating weight regain would largely lie beneath 2-PWL response. We acknowledge that, unfortunately, we evaluated a very limited set of clinical factors precluding definite testing of these hypotheses. Thus, future studies are warranted to evaluate the association of these phenotypes with a comprehensive set of clinical [20–23], genetic [5, 24], or hormonal factors [22] potentially involved in the variable postsurgical response.

We acknowledge that our study has several limitations. As mentioned above, while we used criteria based on the literature, these criteria could be viewed as arbitrary in defining the WL response ensuing BS [19]. In fact, several clinicians in the field of BS would argue that resolution of comorbidities and quality of life is of greater relevance to the outcomes of BS than a WL above certain threshold [20]. Undoubtedly, we acknowledge the many health benefits of BS beyond WL [1, 25]. We fully endorse that multiple rather than single outcomes need to be considered when evaluating the overall health impact of BS [20]. Nonetheless, herein, we rather focused in WL as single outcome as WL after BS because of the relevance of sustained weight reduction for the resolution of obesity-associated comorbidities [2, 3], and the potential health burden associated with persistent obesity resulting from poor WL response or weight regain after BS. Second, albeit the proportion of missing data in our series was comparable to that in previous studies in the field [13, 16, 17], we recognize this as limitation of our observational study. Importantly, at baseline, those lost to follow-up were not significantly different to those included in the study in the clinical characteristics that were independently associated with the different WL patterns. Third, we acknowledge that lack of randomization precludes definite conclusion of the comparisons between RYGB and SG. Finally, we acknowledge that we failed to provide a comprehensive analysis of the many factors potentially associated with the different WL phenotypes proposed in our analysis.

In summary, our analysis further illustrates the high inter-individual variability of the WL response at mid-term following BS. Within the limitations of lack of consensus definition, our data show that poor WL after RYGB and SG could be illustrated by two different patterns: (1) a primarily poor WL response pattern characterized by limited WL throughout follow-up encountered in approximately 5 % of subjects and at comparable rates following the two types of surgeries and (2) a secondarily poor WL pattern characterized by significant WL but subsequent weight regain leading to a final EWL<50 % encountered in approximately 20 % of subjects. Importantly, our data suggest that at a median follow-up of 4.5 years, the 2-PWL response is more commonly associated with SG as compared to RYGB. Our data on the occurrence of poor WL by no means should be interpreted as overall poor outcome of BS. Nonetheless, we propose further studies aiming at better understanding of the different WL trajectories after BS may foster maximization of the health benefits of this therapeutic approach.

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Authors' Contributions A. de H. and J.V. designed the study, analyzed the data, and wrote the manuscript. T.R. and A.J. analyzed the data and reviewed and edited the manuscript. A.L. and L.F. reviewed and edited the manuscript. J.V. is the guarantor of this work and as such, had full access to all the data in the studies, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of Interest The authors have no conflict of interest to report relevant to this article.

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Chapter 5

Evening-chronotype associates with obesity in severe obese subjects: Interaction with *CLOCK 3111T/C*

Evening-chronotype associates with obesity in severe obese subjects: Interaction with CLOCK 3111 T/C

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ABSTRACT

Introduction: Notable amounts of research have suggested an association between obesity and factors related with chronobiology such as chronotype preference, food timing, and sleep. Individuals classified as evening type have been associated with increase BMI, unhealthy eating pattern and several metabolic disturbances. Remarkably chronotype features have a large genetic component and research has been focused on *CLOCK,* a component of the circadian system. In this line, *CLOCK 3111T/C* SNP C carriers present sleep reduction increased values of plasma ghrelin, and alterations of eating behaviors. However, little is known about the relationship between obesity parameters, weight loss, chronotype and genetic background in *CLOCK 3111T/C* among severe obese subjects who have undergone to bariatric surgery. The aim of the present study is to examine if evening chronotype was related to obesity parameters and weight loss evolution in a cohort of severe obese subjects after a bariatric surgery.

Subjects and Methods: A total of 252 patients that underwent bariatric surgery at the Hospital Clinic of Barcelona between 2006-2011 participated in our study. Data were prospectively collected prior to BS and at 12, 18, 24, 36, 48, 60, and 72 months after surgery. Participants were grouped according their circadian preference in either morning or evening type. Obesity and weight loss parameters, dietary intake (energy and macronutrient intake), chronotype, meal timing, sleep duration and *CLOCK* genotype were studied.

Results: Subjects classified as evening chronotype were significantly associated with higher BMI ($p=0.014$), and higher body weight ($p=0.024$) at baseline. Also, evening-type subjects had a lower weight loss than morning-type subjects after a bariatric surgery and differences were maintained after adjustments by gender, age, body weight at baseline, and type of surgery ($p<0.05$).In addition, the weight-loss progression between morning and evening chronotype differed significantly ($p<0.05$) from the 4th till 6th year towards a higher weight regain among evening-types. Moreover, a significant interaction between *3111T/C* SNP and chronotype for body weight at baseline ($p<0.001$). Specifically among carriers of the risk allele C, late-type showed higher body

weight than early-type (p=0.012). Interestingly, *CLOCK 3111T/C* SNP significantly associated with obesity and sleep duration in older subjects.

Conclusions: We have shown for the first time in a severe obese cohort that evening chronotype are associated with a higher degree of obesity (body weight and BMI at baseline). Also, evening-type subjects tend to lose less weight than morning types after a bariatric surgery. Furthermore, carriers of the risk allele C in *CLOCK 3111T/C* with evening preference presented a higher body weight than those with morning preference. In older subjects *CLOCK 3111T/C* was associated with obesity and sleep duration. Our results suggested that circadian preference and CLOCK have a potential role on obesity and weight loss therapies.

Evening-chronotype associates with obesity in severe obese subjects: interaction with CLOCK 3111T/C

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EVENING-CHRONOTYPE ASSOCIATES WITH OBESITY IN SEVERE OBESE SUBJECTS: INTERACTION WITH *CLOCK 3111T/C.*

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ABSTRACT

BACKGROUND: Chronotype has been related to obesity and metabolic disturbances. However, little is known about the relationship between circadian preferences and genetic background in *CLOCK* genes with obesity and weight loss among severe obese patients after bariatric surgery.

OBJECTIVE: The research goals were (1) to examine whether evening chronotype is related to obesity and weight loss evolution in severe obese followed during six years after bariatric surgery and (2) to examine potential interactions between circadian preferences and *CLOCK 3111T/C* for obesity in this population.

SUBJECTS/METHODS: Participants (n=252, 79% female; age [mean+/−SD]: 52±11 years; BMI: 46.4±6.0 kg/m^2) were grouped in evening-type and morning-types. Obesity and weight loss parameters, energy and macronutrients intake, energy expenditure, chronotype, meal timing, sleep duration, and *CLOCK* genotype were studied.

RESULTS: Evening-type subjects showed significantly higher initial body weight (p=0.015) and BMI (p=0.014) than morning-types. Moreover, evening-type, when compared with morning-types, lost less weight (% of excess weight loss) after bariatric surgery (p=0.015). Weight-loss progression between the two chronotype groups differed significantly from the 4th year after the bariatric surgery towards a higher weight regain among evening-types (p<0.05). We also detected a significant interaction between *CLOCK 3111T/C* SNP and chronotype for body weight at baseline (p<0.001). Specifically, among carriers of the risk allele C, evening-types showed higher body weight than morning types (p=0.012). In addition, *CLOCK 3111T/C* SNP significantly associated with obesity and sleep duration in the older subjects.

CONCLUSIONS. Evening chronotype is associated with higher obesity in severe obese subjects and with lower weight loss effectiveness after bariatric surgery. In addition, circadian preferences interact with *CLOCK 3111T/C* for obesity. The circadian and genetic assessment could provide tailored weight loss recommendations in subjects that underwent bariatric surgery.

KEYWORDS: Chronotype, Severe obesity, *CLOCK*, weight loss, bariatric surgery

INTRODUCTION

Recent studies suggest the association between obesity and factors related with our chronobiology such as morning-evening preferences, food timing, and sleep characteristics[1-3]. Circadian rhythms are oscillators with a period of approximately 24 hours that are generated in the suprachiasmatic nucleus of the hypothalamus. These endogenous rhythms orchestrate most physiological functions and are synchronized to the environment mainly by sunlight, physical activity, and food timing.[4] Chronodisruption is a relevant disturbance of the circadian organization of physiology, endocrinology, metabolism, and behavior, which links light and biological rhythms to the development of several diseases.[5] To identify individual differences in chronotype, Horne and Ostberg[6] developed a self-assessment questionnaire identified as the Morningness-Eveningness Questionnaire (MEQ). In this questionnaire, individuals can be stratified by circadian preference subjectively reported as morning, evening, and neutral type. This circadian preference has been related to several metabolic disturbances[7,8]. Indeed, Yu *et al.*[7] has recently reported that eveningness is significantly linked with metabolic syndrome when compared to morningness. Other authors, in a prospective randomized controlled study, demonstrated that eveningness is associated with: an increase in body mass index (BMI), an unhealthy eating pattern (characterized by eating late), a trend towards a decreased number of eating occasions with larger portion sizes, and a decrease in HDL-C.[8]

Interestingly, chronotype features have a large genetic component. In fact, in a study performed using classical twin models, phase parameters which characterize morning or evening type subjects, showed strong genetic influences.[9] In this sense, our group of research has focused particularly in *CLOCK* gene (Circadian Locomotor Output Cycles

Kaput).[10-13] *CLOCK* is a component of the circadian system that regulates the expression of other integral circadian genes, and is involved in altered metabolic function[12]. Previous studies have shown that C carriers of *CLOCK 3111T/C* single nucleotide polymorphism (SNP) (rs1801260) display a less robust circadian rhythm than TT and a delayed acrophase that characterizes evening-type subjects.[14] Moreover, sleep reduction, increased values of plasma ghrelin, and alterations of eating behaviors, which characterize C carriers of *CLOCK 3111T/C*, could partly explain the increase in BMI and the reduced weight loss effectiveness of a dietary treatment showed by C carrier subjects.[11] Indeed, it has been demonstrated that *CLOCK 3111T/C* may predict the outcome of weight loss strategies that are based on low-energy diets.[1,12]

Nevertheless, little is known about the relationship between obesity parameters, weight loss, individual chronotype and genetic background in *CLOCK 3111T/C* among severe obese subjects who have undergone to bariatric surgery. Considering that the individual chronotype has been related to obesity and to other metabolic disturbances, the aim of our study was to examine, for the first time, whether evening chronotype was related to obesity parameters and weight loss evolution in a severe obese cohort followed during six years after bariatric surgery. Furthermore, potential interactions between circadian preferences and *CLOCK 3111T/C* for obesity have been also tested.

SUBJECTS AND METHODS

Participants and procedures

Participants in our observational prospective study were among the 1,135 subjects that underwent bariatric surgery at the Hospital Clinic of Barcelona (Spain) between 2006 and 2011. Eligibility criteria included age ≥18 years, first-time bariatric surgery, and 72 months of available follow up. Among of the 320 patients who agreed to participate, 21% of the initial volunteer subjects dropped out of the trial. Finally a total of 252 patients (79% women) participated in this study. Patients were considered for bariatric surgery based on the current guidelines,[15] which include: a body mass index (BMI) ≥ 40 kg/m^2 or a BMI ≥ 35 kg/m^2 with 2 or more health risk factors, such as high blood pressure or diabetes. Two commonly performed surgery techniques were performed, namely Roux-en-Y gastric bypass (RYGB; n=194) and sleeve gastrectomy (SG; n=58). The technical aspects of those bariatric surgery techniques and the criteria for selection of RYGB or SG at the Hospital Clinic of Barcelona (Spain) have previously been reported.[16,17] Data were prospectively collected prior to bariatric surgery and at 12, 18, 24, 36, 48, 60 and 72 months (6 years) in the postsurgical period. All procedures were in accordance with good clinical practice. Patient data were codified to guarantee anonymity.

All subjects attended both group and individual sessions, which included nutritional counseling according to the current guidelines for the bariatric patient prior the surgery.[15] Dietary advice was given to the patients after surgery: at 2 and 6 weeks, and then at 4, 8, and 12 months, emphasizing to sustain a hypocaloric and protein-rich diet, rather than a recommendation of specific timetable. During the first year after the surgery, the patients were advised to eat 5-6 meals per day and after this first year, to eat 3-4 meals per day. No different nutritional education was given according to the type of surgery.

Ethics

The study was undertaken following the ethical guidelines of the Declaration of Helsinki 1961 (revised Edinburgh 2000) and the current legislation concerning clinical research in humans. The Ethics Committee of the Hospital Clinic of Barcelona approved the protocol and the written informed consent was obtained from all the participants of the study.

Obesity parameters

Subjects were weighed wearing light clothes and without shoes to the nearest 0.1kg (Seca 703 scale, Hamburg, Germany). Height was determined using a fixed wall stadiometer (Seca 217, Hamburg, Germany) to the nearest 0.1cm. Waist circumference was measured to the nearest 0.5cm, at the level of the iliac crest, and hip circumference was measured to the nearest 0.1cm to the maximum extension at the buttocks level. All measurements were made with a standard flexible and inelastic measuring tape. Body mass index (BMI) was calculated as weight (kg) divided by squared height (meters). Bariatric weight loss results can be expressed using different relative measures. Thus, postoperative weight loss is expressed as a percentage of excess weight loss (%EWL) following the formula: %EWL= [100×(weight prior to surgery − weight at the time of evaluation)/ (weight prior to surgery − weight corresponding to a BMI = 25 kg/m^2)]. Maximum weight loss is defined as the maximum EWL recorded at postsurgical checkup visits. Nadir weight is the lowest weight achieved while weight regain is the difference between body weight at last follow-up and nadir weight.

Energy and dietary intake before and after bariatric surgery

To evaluate the dietary intake, we analyzed 4-days food records (one of which was a non-working day) that were collected at every follow up prior and after surgery. For the purpose of our study we have included: a) prior to surgery (baseline values), b) at nadir weight, and c) at the last follow up. Instructions about how to fill the 4-days record were explained by a registered dietitian during the clinical evaluations. Patients were instructed to complete the dietary records the week prior to the nutritional interview. Total energy intake and macronutrient composition were analyzed using the software Dietsource 2.0® (Novartis). During the follow-up period of each subject, patients also recorded the time of day when each meal was started (for example, breakfast, lunch and dinner) with the questionnaire developed by Bertéus-Forslund *et al*.[18]

Energy expenditure

The total expenditure was calculated by multiplying each individual's basal metabolic rate with the individual physical activity level (PAL). Basal metabolic rate was estimated by the Harris-Benedict equation and physical activity level was self-reported as either "sedentary or light activity" (PAL=1.53) or "moderate activity" (PAL=1.76).[19]

Chronotype questionnaire

Subjects completed the 19-item morningness/eveningness questionnaire (MEQ; score range: 16-86) of Horne and Ostberg[6] at the follow-up period. It contains 19 questions such as: "At what time would you like to get up?", "At what time do you feel tired?", "What would be the best time to perform hard physical work?", "During the first half-hour after you wake up in the morning, how tired do you feel?", or "If you have no commitments the next day, what time would you go to bed compared to your usual bedtime?" among other questions. According to this score, individuals are categorized as neutral (53-64 of score), morning (above 64 of score) or evening (under 53 of score) types.[20] Morning-eveningness typology is a way to characterize subjects depending on individual differences of wake/sleep patterns and the time of the day people report to perform best. Some people are night "owls" and like to stay up late in the night and sleep late in the morning (evening-types), whereas others are "early birds" and prefer to go to bed early and arise with the break of dawn (morning-types).

Sleep duration

Habitual sleep duration was estimated by questionnaire[11] including the questions ‘During week days: How many hours (and minutes) do you usually sleep?’ and ‘During weekend days: How many hours (and minutes) do you usually sleep?’ A total weekly sleep duration was calculated as [(min weekdays x 5) + (min weekend days x 2)]/7.

DNA isolation and *CLOCK* genotyping

DNA extraction from blood samples was performed with the automated Chemagic Separation Module I based in magnetic bead technology (Chemagic MSM I, Perkin Elmer, Chemagen Technology GmbH, Germany) at the Biobank HCB-IDIBAPS (Hospital Clinic de Barcelona-IDIBAPS, Spain). The genotyping of *CLOCK 3111T/C* SNP (C___8746719_20; rs1801260) was performed using the TaqMan allelic discrimination assay according to the standardized laboratory protocols of the manufacturer Life Technologies on the Applied Biosystems® 7900HT sequence detection system (Applied Biosystems, California, EEUU) at the Human Genotyping Unit of CeGen (Spanish National Cancer Research, Spain).

Statistics

Subjects were dichotomized in evening-type and in morning-type using the median of the population as the cutoff point[11]. Pearson correlations were used to investigate the relationship between weight parameters and MEQ score. Any significance relationship between variables was further investigated with regression analysis. We applied the Student t-test to compare crude means, and further statistical adjustment was performed for gender, age, type of surgery and body weight baseline when needed (ANCOVA).

A discriminant function analysis[21] was performed in order to determine whether chronotype characteristics could reliably classify subjects in two obesity groups according to their obesity degree ($\leq$ 120 or >120 kg of initial weight). In this study, chronotype characteristics included in the MEQ (chronotype scale items) were treated as predictors. Taking into account that gender significantly affected the model, the population was divided into men and women. Univariate F tests were then calculated in order to determine the importance of each independent variable in forming the discriminant functions. Examining the Wilk's Lambda values for each of the predictors revealed how important the independent variable was to the discriminant function, with smaller values representing greater importance.

For the genetic analyses, a dominant genetic model was applied for the *CLOCK 3111T/C* SNP as previously described.[11] Then, minor C allele carriers (CC+CT) were compared against major T allele homozygotes (TT). To study *CLOCK 3111T/C**chronotype interactions, *CLOCK 3111T/C**age interactions for body weight at baseline, and *CLOCK 3111T/C**age interactions for sleep, we used univariate linear regression models adjusted for age and gender when needed (ANCOVA). The statistical power was: 83.4%, 80.5% and 61.2%, respectively, considering a type I error of 5%: $Z_{\alpha/2} = 1.96$ which is a sufficient statistical power (higher than 50%). [22,23]

All data were expressed as mean ± standard deviation (SD) unless stated otherwise. For all the analyses in which MEQ score was involved, one outlier was detected and eliminated using the Outlier Labeling Rule[24] with a g factor of 2.2. Statistical analyses were performed using SPSS 21.0 Software (SPSS). A two-tailed P-value of <0.05 was considered statistically significant.

RESULTS

General characteristics

Table 1 summarizes the general characteristics of the population studied, including initial anthropometric values and weight loss parameters, circadian characteristics (sleep duration, chronotype, and timing of food intake) and distribution of the 3111T/C genotypes. The half of the population studied (53.4%) was short-sleeper (<7h) with a predominance of eveningness (26.2%) *versus* morningness (14.3%) according to the MEQ.

Eveningness is associated with obesity and weight loss parameters in the subjects studied.

In the current population of severe obese subjects our results show that at baseline evening-type subjects (scoring lower levels of the MEQ score) showed significantly higher obesity parameters than morning-types with higher body weight (p=0.015, after adjusting by gender and age p=0.024) and higher BMI (p=0.014, after adjusting by gender and age p=0.018) (**Figure 1**). In the same line, when patients were divided into evening and morning-types according to the median of the population of the MEQ score (median of the population = 57), evening-type subjects (≤57 MEQ score) had a significantly higher obesity degree at baseline (both body weight and BMI) than morning types (>57 MEQ score) (**Table 2**). Statistical differences were also found between the morning and evening chronotypes in several weight loss parameters after bariatric surgery: evening-types had a lower weight loss (expressed as % of excess weight loss at last follow-up visit and as maximum % of excess weight loss) than morning-types and differences were maintained after adjustments by gender, age, body weight at baseline, and type of surgery (p<0.05). In addition, we observed that the weight-loss progression between the two chronotype groups differed significantly ($p<0.05$) from the 4th year after the bariatric surgery until the end (5th and 6th year), towards a higher weight regain among evening-types (after adjusting by age, gender, initial body weight and type of surgery (**Figure 2**). Evening-types also displayed a higher nadir weight ($p=0.045$) and a higher body weight ($p=0.043$) at the last follow-up visit than the morning-types (**Table 2**), even though those differences were not maintained after adjusting by gender, age, body weight at baseline, and type of surgery.

Further analysis using a discriminant model demonstrated, only in women, that three specific questions related to the chronotype characteristics could reliably classify subjects in two obesity groups depending to the obesity degree. The questions were the following: 1) “You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own internal “clock”. Which one of the following time would you choose?”, 2) “During the first half-hour after you wake up in the morning, how tired do you feel?”, and 3) “If you have no commitments the next day, what time would you go to bed compared to your usual bedtime?”

Timing of food intake, specifically lunch and dinner time, differed between evening and morning chronotypes. Indeed, evening-type patients had lunch and dinner significantly later than morning-types ($P<0.05$) while no differences were found in sleep duration. Moreover, no significant differences were found in total energy and dietary intake at baseline, nadir weight (usually achieved 18-24 months after the surgery) and the last follow-up **(Table 2)** or at other time points analyzed between both chronotypes.

CLOCK interacts with the individual chronotype for obesity parameters.

We examined *CLOCK 3111T/C* in the context of morning-evening chronotype. When patients were classified into the three different chronotypes (morning, evening and neutral) with MEQ, a significant interaction was found between *CLOCK 3111T/C* and the individual chronotype for body weight at baseline (p<0.001). Further stratified analyses between morning- and evening-type subjects showed that among carriers of the risk allele C, evening-type subjects were more obese at baseline (body weight) than morning-type patients (p=0.012) (**Figure 3)**. Differences were maintained after adjusting for age and gender (p=0.022). However, among T carriers no significant differences were found between both morning and evening chronotypes attending to obesity parameters (p=0.414, and after adjusting for age and gender p=0.327)**.**

CLOCK associates with obesity only in the older group.

No significant associations were found between obesity characteristics (initial body weight and BMI) and *CLOCK 3111T/C* in the total population studied. However, further analyses showed a significant *CLOCK 3111T/C**age interaction for total body weight at baseline (p=0.018). In this line, subsequent stratified analyses according to median age (median=52 years) indicated that *CLOCK 3111T/C* was significantly associated with obesity among the older subjects (> 52 years), C carriers showed higher initial body weight at baseline (124.8±19.3kg) than TT carriers (115.5±15.7kg) (p=0.003, after adjusting for gender p=0.010) (**Figure 4A**). No association between *CLOCK 3111T/C* and obesity was found in the younger group (*P*>0.05). Interestingly, C carriers slept significantly less than T carriers in the older group (**Figure 4B**).

Discussion

To our knowledge, this is the first prospective observational study to demonstrate that evening chronotypes are associated with a higher degree of obesity (body weight and BMI at baseline) in a severe obese population. Our results also reveal that evening-type subjects lose less weight than morning types after bariatric surgery. More importantly, weight-loss progression between the two chronotypes differed from the 4th year after the bariatric surgery until the end (5th and 6th year), towards a higher weight regain among evening-types. A gen-chronotype interaction was found for obesity in the total population studied. Thus, among carriers of the risk allele C in *CLOCK 3111T/C*, those who were evening-type showed a higher body weight at baseline than those who morning-type. Moreover, *CLOCK 3111T/C* was significantly associated with obesity and sleep duration in the older subjects. Results suggest that both circadian preference and *CLOCK* have a potential role on obesity and weight loss therapies based on bariatric surgery.

Previous studies have shown that evening chronotypes, also referred as late chronotypes, are associated with several health risks such as diabetes, cardiovascular diseases, and obesity.[7,25-27] Different reasons may be driving those associations such as: unfavorable dietary habits, meal timings, sleep habits.[2,7] In the current work, we have demonstrated for the first time that, in a population consisting exclusively of severe obese subjects, evening-types were more obese than morning-types. Our results are in line with previous findings that showed that evening chronotype was related to higher BMI and neck circumference in a population that included both obese and severe obese subjects (BMI ranging from 30 to 55 kg/m^2).[8]. Recently, similar outcomes have been found in studies performed in other groups of age, such as young adolescents, which showed positive and significant associations between evening chronotypes and obesity.[2]

Attending to the effectiveness in weight loss of the bariatric surgery, our results have shown that the surgery was less effective for weight loss in evening-type patients than in morning-types. Indeed, evening-type individuals lost a lower percentage of excess weight than morning-types, results that were still present after adjusting by gender, age, type of surgery, and initial body weight. More importantly, weight-loss progression between the two chronotypes differed from the 4th year after the bariatric surgery until the end towards a higher weight regain among evening-types. These data suggest that during the first 3 years after the surgery, it is the treatment *per se* what is driving the weight loss effectiveness; however, from the 4th year, it appears that behavior-related characteristics of the subjects such as being morning or evening type, get more relevant in the long-term maintenance of the weight loss achieved. Until now, very little research has been done on the association between subjects´ chronotype and total weight loss and weight loss maintenance in dietary treatment [8,28] and none, to our knowledge, after bariatric surgery in severe obese patients. Ross *et al.*[28] have recently demonstrated that moderate morning-type obese subjects were more successful at both weight loss and long-term maintenance than obese evening-types under a weight-loss dietary program.

Dietary energy intake, macronutrient distribution, timing of food intake, and sleep characteristics may influence weight loss effectiveness.[1,29,30] Several studies have reported that evening-type individuals have a less healthy lifestyle than morning-types, with unfavorable dietary habits.[2,28] Nevertheless, in the current population, we did not find significant differences in dietary intake characteristics (total energy intake and macronutrient distribution) between evening and morning chronotypes.

Timing of food intake has been proposed as a novel factor that might be involved in energy homeostasis and variability in response to weight loss treatments, consistent with the link between circadian rhythm and energy regulation.[31] In the current study, those patients with circadian preference towards eveningness did have lunch and dinner significantly later than morning types (15 min later for lunch and 25 min later for dinner). Our results are consistent with other author's findings who have also reported that evening types tend to eat later.[8,32] In this line, it has been suggested that "when" food is eaten is an influential factor in weight loss effectiveness beyond "what" is eaten, and food timing could be accounting for the weight loss differences between chronotypes the current severe obese population .[1,32-34] Thus, current results agree with two recent studies performed by our group [32,33], the first, in a cohort of overweight and moderately obese Mediterranean population and the second in a cohort of severe obese subjects after bariatric surgery. In both studies, it was demonstrated that eating lunch late was predictive of decreased weight loss success of a dietary weight loss therapy. Similarly to the current data, early lunch eaters and late lunch eaters showed similar energy intake, macronutrient distribution and estimated energy expenditure.[32, 33] In a randomized, crossover trial performed also by our group, we also demonstrated that decreased resting-energy expenditure, decreased fasting carbohydrate oxidation, decreased glucose tolerance, blunted daily profile in free cortisol concentrations and decreased thermal effect of food may be accounting for differences in weight loss between early and late lunch eaters.[35]

Other factor that may be accounting for the differences in weight loss effectiveness of the bariatric surgery between evening- and morning-type patients may be sleep duration and quality. It has been pointed out that the individual chronotype has significant effects on sleep parameters.[28,36] Although our data indicate similar sleep duration among evening- and morning-types, we should bear in mind the fact that evening-type subjects might tend to accumulate sleep on weekdays that eventually could recovery at the weekend, as suggested by some authors.[7,37] It could happen that the sleep loss occurring, at least during weekdays, could contribute to unbalanced metabolic profiles of evening-type patients. Nevertheless, no overall differences in sleep duration were found between late and early eaters in the current severe obese population that showed differences in chronotype score and exhibited different pattern of weight loss, results that are consistent with the findings of a previous study of our group. [32]

One remarkable result from the current work is that the individual chronotype interacts with the genetic background (*CLOCK 3111T/C*) for obesity parameters. Our results indicate that evening-type subjects who were carriers of the risk allele C at *CLOCK 3111T/C* showed a higher body weight at baseline than a) C carriers morning-types, and than b) total T carriers. This is the first study to demonstrate that the individual chronotype interacts with *CLOCK 3111T/C* for severe obesity, which suggests that to be an evening-type person and also to be a carrier the risk allele C at *CLOCK 3111T/C*, increases the risk of obesity. Previously, our group has found significant associations between *CLOCK SNPs* and individual chronotype, showing that C carriers in general are more evening-type[13]. Those results have been replicated in other populations, such as Caucasian[38] and Japanese[39] both healthy subjects and in a population of Korean bipolar patients.[40] Likewise, in a study conducted by Bandin *et al*., it was found that obese women with C genetic variants in *CLOCK 3111T/C* displayed a delayed phase in peripheral temperature which characterizes evening-type subjects.[14]

Likewise, associations between *CLOCK* SNPs and obesity have been demonstrated.[12,42] We have replicated this association for *CLOCK 3111T/C* but only in the older group (>52y). Specifically, it has been reported that individual *CLOCK* genotypes may affect several variables associated to behavior such as eating behaviors[12,41]. Previous research in *CLOCK* mutant mice showed that mice developed obesity, hyperphagia and exhibited changes in eating behavior, mood and sleep pattern.[43] Indeed, in the current work and only among the older subjects carriers of minor C allele of *CLOCK 3111T/C* SNP was reported shorter daily sleep duration.

Our results share the findings of other authors[44] who demonstrated that several *CLOCK* variants are associated with sleep duration. However, the association between *CLOCK* and sleep duration is not consistent throughout studies[45] and age may be implicated in this lack of agreement. Current results suggest that the impact of the circadian system alterations (i.e. genetic variations in *CLOCK* genes) on obesity or on sleep may be higher among older (>52y) than among younger subjects, and that this specific genetic variation in *CLOCK* may partly explain the high interindividual variability that exists in obesity and sleep characteristics with aging. In this line, we have previously shown that sleep abnormalities are more common in old ages and they increase together with a loss of circadian robustness in menopausal women compared with premenopausal women.[46]

Interestingly, we might provide tailor advice to severe obese women based in the results of our discriminant analyses which suggest that these women should try a) to schedule their physical activity in the morning hours and b) to go to bed earlier at night that they usually do in order to avoid tiredness. Both recommendations might help to decrease the propensity to severe obesity and reinforce the recommendation that clinicians should consider the individual chronotype to potentiate the effectiveness of the bariatric surgery.

One strength of our study is that is the first observational study to assess chronotype and *CLOCK 3111T/C SNP* in a large sample of severe obese subjects that underwent bariatric surgery including long-term data (6 years). However, some study limitations should be noted. One limitation is the fact that dietary intake, food timing, sleep data and chronobiological preferences were obtained from patients using validated questionnaires.[6,11,20] Although self-reported data has many important uses in research, caution must be accepted when interpreting it. Hence further investigation focused in circadian preference and *CLOCK* gene is needed using a reference method such a dietary biomarkers, sleep polysomnography and accelerometers to corroborate the accuracy of the data and avoid bias. Second, we cannot rule out the possibility that along the follow up period other factors could affect weight parameters such as energy expenditure, body composition, and hormones (i.e. plasma ghrelin), chronic diseases or medication use. Thus, future work is needed to assess the direct effect of circadian preference and *CLOCK* gene on weight loss therapies including the factors mentioned above not only at baseline but also during the follow up. Third and last, we are aware that the sample size was relatively small and a relative high variability was observed. However, to obtain a population of such characteristics and to follow it for a long period of time (6 years) is difficult and laborious. Further studies are needed to replicate the data that we have found in the current study.

In summary, our results have revealed that evening chronotype is associated with initial obesity and to weight loss evolution in severe obese patients after bariatric surgery. Indeed, weight loss effectiveness was better in morning-types as compared to evening-types after surgery treatment. Furthermore, we found that circadian preferences interact with *CLOCK 3111T/C* for obesity in our population. Our findings suggest that genetic and circadian assessment could provide tailored weight loss recommendations even in subjects that underwent bariatric surgery. This study is the first step towards enhancing our understanding of the potential influence of circadian preference and *CLOCK* genotype on severe obesity and it has potential implications for future research and clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

MIP, MG designed the research; TRL, MIP, JV, AdH conducted the research; TRL, MIP, MG analyzed data; MC contributed to the statistical analysis. TRL, MG, MIP wrote the paper. All authors read and approved the final manuscript.

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FIGURE LEGENDS

Figure 1. Scatterplots depicting correlations between body weight at baseline (kg) and MEQ chronotype (**A**) and body mass index (BMI) at baseline and MEQ chronotype (**B**).

Figure 2. Evolution of weight-loss, expressed as percentage of excess of weight loss (mean and s.e.m.) in evening and morning patients over 6 years after bariatric surgery. * Indicates significant differences ($p<0.05$) after adjusting by age, gender, initial body weight and type of surgery.

Figure 3. *CLOCK3111 T/C* interacts with chronotype assessed by MEQ (evening-type versus morning-type) for obesity (body weight at baseline, kg) in severe obese subjects (p-value for the interaction < 0.001). Among minor C allele carriers, “evening-types” had significantly higher body weight at baseline than “morning-types” (p=0.012, after adjusting by age and gender p=0.022) while no differences among T allele carriers (P=0.414, after adjusting by age and gender p=0.327). Values are means±SD. * Indicates significant difference ($p<0.05$).

Figure 4. *CLOCK3111 T/C* interacts with age for obesity (body weight at baseline, kg) (p-value for interaction=0.018) and with age for sleep duration (hours) (p-value for interaction=0.020). (**A**) In <u>older patients</u>: minor C allele carriers had significantly a higher body weight at baseline than TT carriers (p=0.003, after adjusting by gender p=0.010). (**B**) In <u>older patients</u>, minor C allele carriers sleep lesser hours than TT carriers (p=0.045, after adjusting by gender p=0.042). Data are presented as mean±SD.

Table 1. General characteristics of the severe obese population studied.

	Mean	**SD**
Age (years)	52.0	11.0
Anthropometric		
BMI (kg/m^2)	46.4	6.0
Body weight (kg)	123.1	19.6
Height (m)	1.62	0.09
Waist (cm)	130.4	12.9
Hip (cm)	139.3	13.1
Weight loss parameters		
EWL at last follow-up visit (%)	65.8	33.4
Maximum EWL (%)	80.4	23.0
Nadir weight (kg)	78.0	15.5
Body weight at last follow-up visit (kg)	87.6	18.1
Weight regain (kg)	9.2	10.6
Sleep and Circadian-related characteristics		
Sleep duration (hours per day)	6.98	1.42
Morning-evening score	56.2	8.5
Morning evening classification	**n**	**(%)**
Neutral-type	150	59.5
Morning-type	36	14.3
Evening-type	66	26.2
Meal times		
Breakfast	8:51	1:12
Lunch	14:12	0:46
Dinner	21:19	0:51
CLOCK polymorphism (n=252)	**n**	**(%)**
TT	129	51.2
TC	99	39.3
CC	24	9.5

Note: BMI: body mass index; EWL: excess weight loss

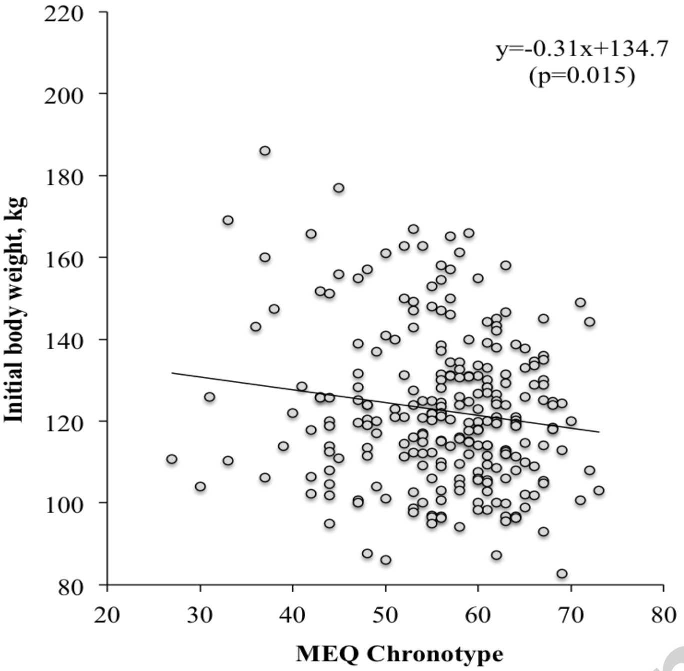
Table 2. Anthropometric characteristics, weight loss parameters after bariatric surgery, energy and dietary intake, meal times, and sleep duration of the severe obese population classified according to the MEQ questionnaire.

	Morning-types (n=124)		**Evening-types** (n=128)		**P value**
	Mean	SD	Mean	SD	
Baseline body weight (kg)	119.8	15.9	126.0	22.3	**0.020**[1]
Baseline BMI, (kg/m^2)	45.2	5.0	47.5	6.7	**0.003**[1]
Weight loss parameters after bariatric surgery					
EWL at last follow-up visit (%)	66.7	29.0	58.3	27.9	**0.015**[2]
Maximum EWL (%)	82.9	22.6	77.9	23.3	**0.041**[2]
Nadir weight (kg)	76.0	13.3	80.0	17.2	0.241[2]
Body weight at last follow-up visit (kg)	85.2	16.8	90.0	19.0	0.214[2]
Weight regain (kg)	9.2	11.5	9.4	10.14	0.975[2]
Baseline energy intake and expenditure energy expenditure and dietary intake					
Total energy (kcal/day)	2444.4	1031.5	2475.4	1045.7	0.925[2]
Total energy expenditure (kcal/day)	2105.2	225.0	2117.3	337.1	0.807[2]
Proteins (%)	17.01	4.2	16.8	4.1	0.881[2]
g/day	97.0	33.2	97.7	32.4	0.942[2]
Carbohydrates (%)	39.3	8.7	38.5	9.4	0.661[2]
g/day	233.3	96.9	233.4	100.7	0.989[2]
Fats (%)	43.5	9.1	44.4	9.8	0.655[2]
g/day	122.1	64.7	124.7	64.1	0.891[2]
Energy and dietary intake at nadir					
Total energy (kcal/day)	1538.9	405.3	1516.6	250.4	0.603[2]
Proteins (%)	20.5	4.9	21.0	12.9	0.804[2]
g/day	77.4	22.5	72.5	18.1	0.153[2]
Carbohydrates (%)	38.8	8.5	38.3	9.9	0.860[2]
g/day	148.2	47.5	144.7	45.2	0.597[2]
Fats (%)	40.4	7.3	42.3	8.9	0.248[2]
g/day	70.9	27.1	71.9	20.5	0.849[2]
Energy and dietary intake last follow up					
Total energy (kcal/day)	1630.4	486.3	1599.3	454.1	0.790[2]
Proteins (%)	18.8	4.7	18.5	5.1	0.617[2]
g/day	73.6	19.9	81.5	73.8	0.410[2]
Carbohydrates (%)	40.1	8.9	40.9	10.6	0.531[2]
g/day	165.2	64.0	163.9	61.4	0.912[2]
Fats (%)	41.0	7.8	41.1	10.0	0.979[2]
g/day	73.6	24.9	74.0	30.4	0.885[2]
Meal times					
Breakfast time	8:45	1:12	8:57	1:12	0.264[2]
Lunch time	14:04	0:45	14:19	0:46	**0.017**[2]
Dinner time	21:06	0:50	21:31	0:50	**<0.001**[2]
Sleep duration					
Sleep (hours)	7.0	1.5	6.9	1.5	0.380[2]

Note. BMI: body mass index; EWL: excess weight loss. [1]p-values adjusted for gender and age; [2]p-values adjusted for gender, age, type of surgery, and body weight at baseline.

Figure 1. Scatterplots depicting correlations between body weight at baseline (kg) and MEQ chronotype (**A**) and body mass index (BMI) at baseline and MEQ chronotype (**B**).

A



B

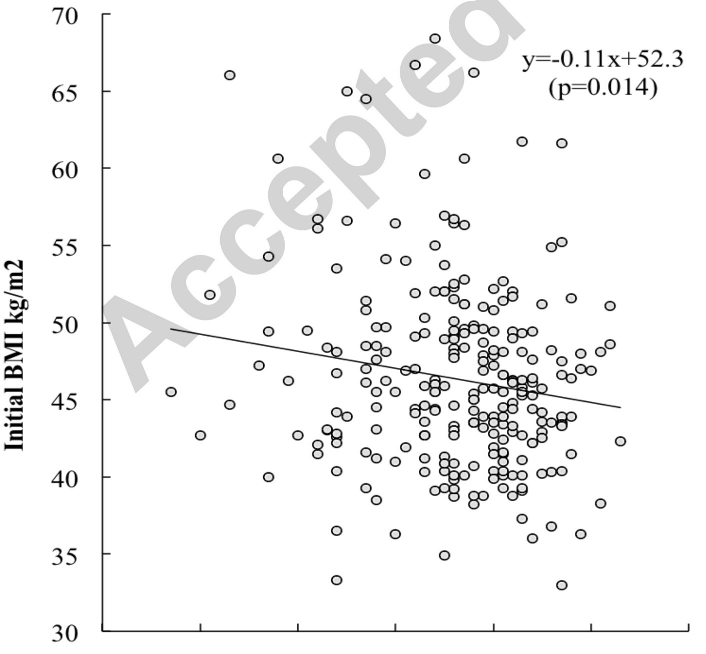
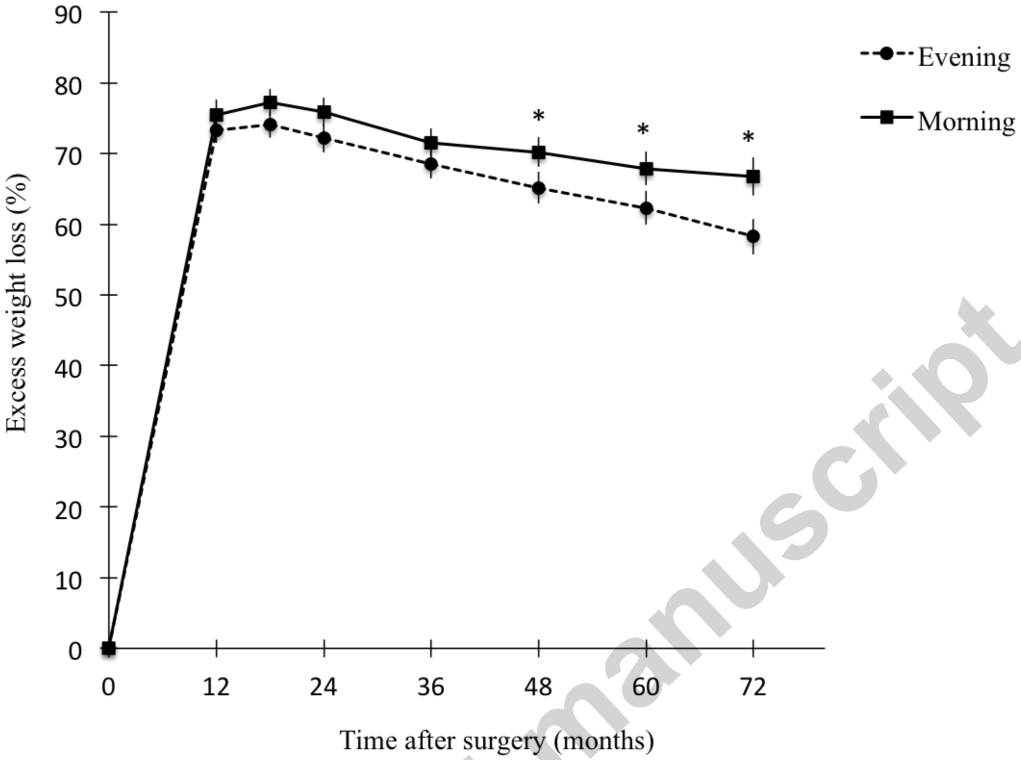


Figure 2. Evolution of weight-loss, expressed as percentage of excess of weight loss (mean and SEM) in evening and morning patients over 6 years after bariatric surgery. * Indicates significant differences (p<0.05) after adjusting by age, gender, initial body weight and type of surgery.



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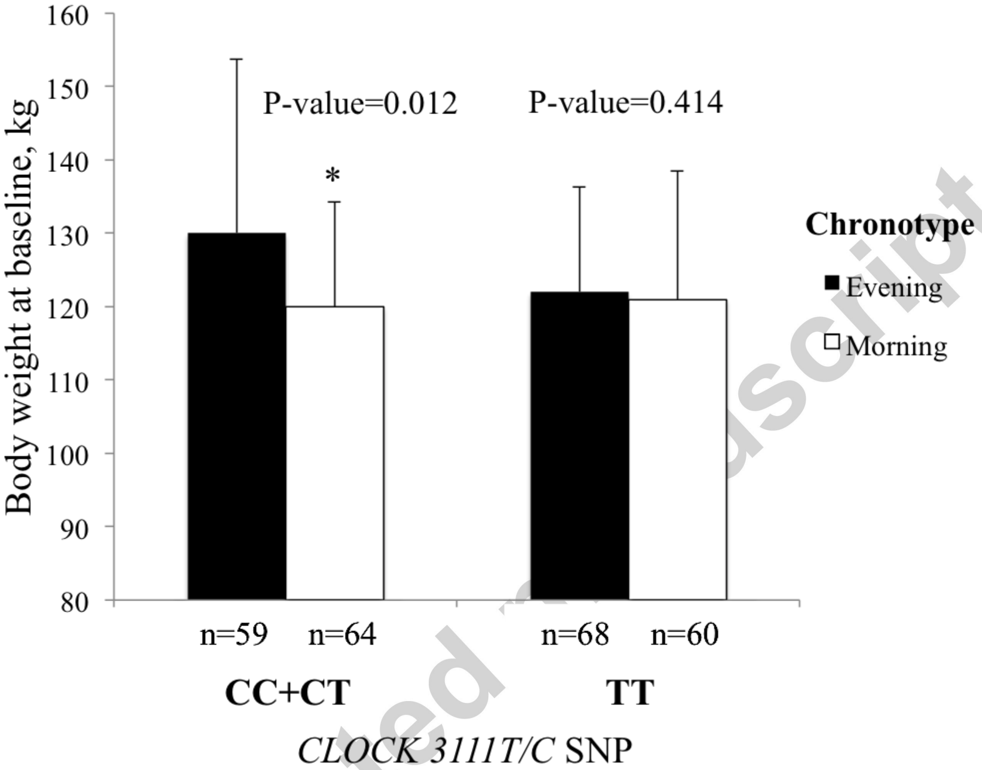

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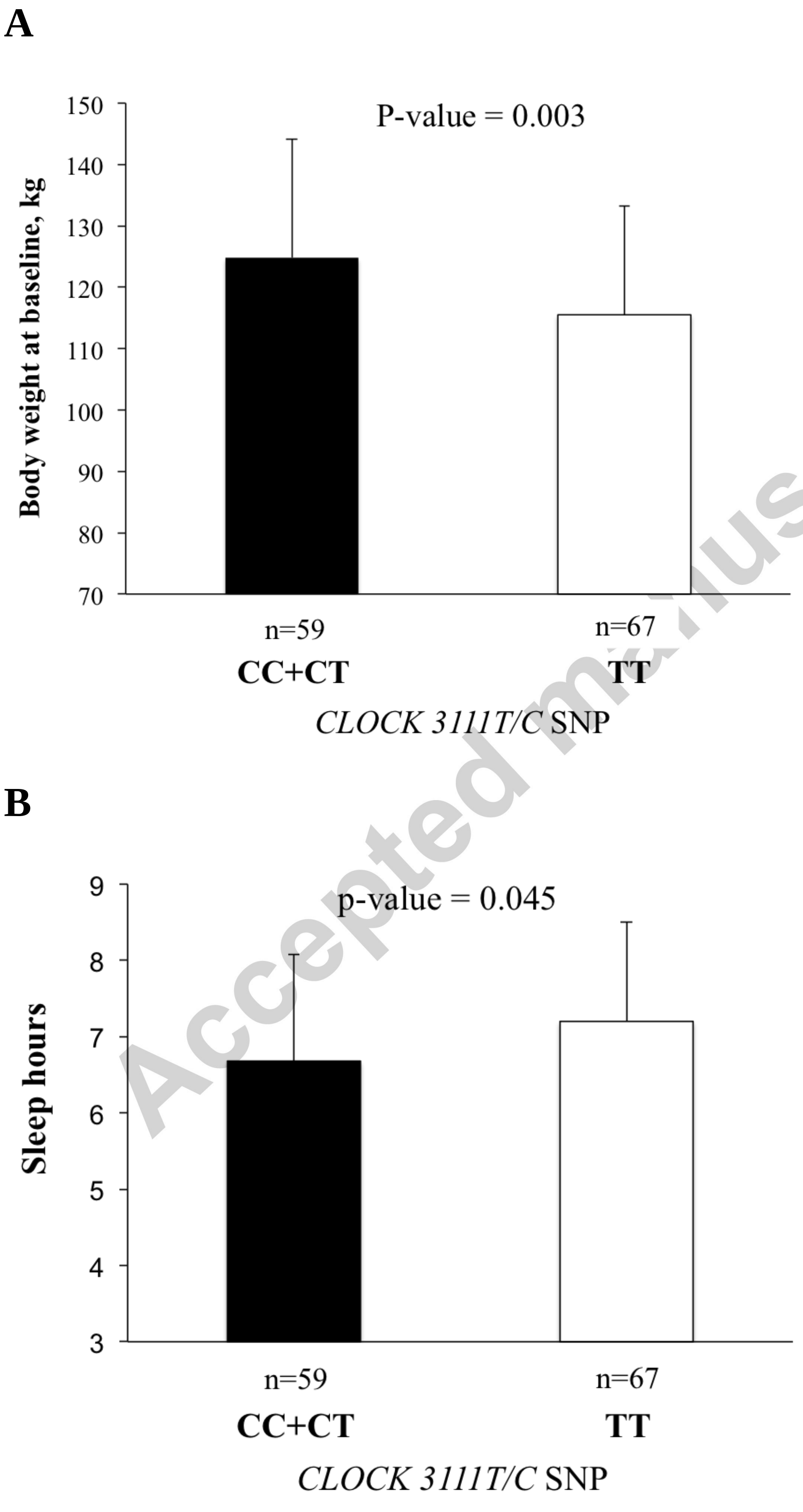

Chapter 6

Timing of food intake is associated with weight loss evolution in severe obese patients after bariatric surgery.

Timing of food intake is associated with weight loss evolution in severe obese patients after bariatric surgery.

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ABSTRACT

Introduction: Previously, we have reported that poor weight loss after a bariatric surgery at med-term could be illustrated by two different patterns. Primarily poor weight loss (PPWL) response characterized by sustained limited weight loss and secondarily poor weight loss (SPWL) response characterized by a successful initial weight loss but subsequent weight regain Therefore, a large amount of research has been focused on the potentially factors involved in the variability of weight loss outcomes after a BS. Recently several studies have suggested that food timing have a significant role on obesity treatment. However, the relationship between food timing and weight loss after a bariatric surgery is unknown. The aim of our observational prospective study was to analyze if food timing is associated with weight loss effectiveness following a bariatric surgery in a cohort of severe obese subjects.

Subjects and Methods: A final cohort of 270 patients (79% women) that underwent bariatric surgery at the Hospital Clinic of Barcelona between 2006 and 2011 participated in our study. Subjects were classified according their weight loss trajectory after bariatric surgery based on de Hollanda et al. classification (2014). Later, they were grouped according to the food time intake of their main meal in either early (before 15:00 h) or late (after 15:00 h) eaters. Moreover, we also analyzed obesity and biochemical parameters, dietary intake (energy intake and macronutrients), energy expenditure, sleep duration and chronotype.

Results: The percentage of late eaters was significantly higher in the primarily poor weight loss responders (~70%) than in both secondarily poor weight loss responders (~42%) and good weight loss responders (~37%) (p=0.011). Consistently, primary poor weight loss responders had lunch later as compared to good and secondarily weight loss responders (p=0.034). No significant differences were found in pre-surgical biochemical parameters, dietary intake (at baseline and follow up), energy expenditure, sleep duration and morning-evening score (chronotype).

Moreover, no significant differences were observed after adjusting for gender, age and type of surgery.

Conclusions: Weight loss effectiveness after a bariatric surgery is associated with timing of the main meal. Indeed, weight loss effectiveness was better in early eaters as compared to late eaters. Our results suggest that eating at the right time may be a relevant factor to considerer in weight loss therapy even after a bariatric surgery.

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Original article

Timing of food intake is associated with weight loss evolution in severe obese patients after bariatric surgery

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SUMMARY

Background: Recent research has demonstrated a relationship between the timing of food intake and weight loss in humans. However, whether the meal timing can be associated with weight loss in patients treated with bariatric surgery is unknown.
Objective: To evaluate the role of food-timing in the evolution of weight loss in a sample of 270 patients that underwent bariatric surgery with a follow-up of 6 years.
Methods: Participants (79% women; age [mean ± SD]: 52 ± 11 years; BMI: 46.5 ± 6.0 kg/m^2) were classified according their weight loss response patterns after bariatric surgery: good weight-loss-responders (67.8%), primarily poor weight-loss-responders (10.8%) or secondarily poor weight-loss-responders (21.4%). Then, they were grouped in early-eaters and late-eaters, according to the timing of the main meal (before or after 15:00 h). Obesity and biochemical parameters, energy and macronutrients intake, energy expenditure, sleep duration, and chronotype were studied.
Results: The percentage of late eaters (after 15:00 h) was significantly higher in the primarily poor weight-loss-responders (~70%) than in both secondarily poor weight-loss-responders (~42%) and good weight-loss-responders (~37%) ($p = 0.011$). Consistently, primarily poor weight-loss-responders had lunch later as compared to good and secondarily poor weight-loss-responders ($p = 0.034$). Age, gender and type of surgery were not determining. Surprisingly, obesity-related variables, biochemical parameters, pre-surgical total energy expenditure, sleep duration, chronotype, calorie intake and macronutrients distribution, were similar among groups.
Conclusions: Weight loss effectiveness after bariatric surgery is related to the timing of the main meal. Our preliminary results suggest that the timing of food intake is important for weight regulation and that eating at the right time may be a relevant factor to consider in weight loss therapy even after bariatric surgery.

1. Introduction

Treatment for severe obesity includes life style changes, such as dietary interventions and exercise, and bariatric surgery [1]. From those approaches, bariatric surgery is the most successful weight loss strategy for severe obesity and its health benefits are beyond weight loss [2]. In terms of weight outcomes in bariatric surgery, "success" is described as loss of >50% excess weight (% EWL), loss of

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>20–30% of initial weight, and achieving a BMI <35 kg/m^2, with the maximum weight loss being observed typically during the post-operatively period between 18 and 24 months [3]. Nonetheless, weight loss after bariatric surgery varies widely and a significant proportion of patients respond poorly [4–6]. Description of patterns of weight change within this variability has seldom been attempted [5]. Recently, de Hollanda et al. [5] have reported the high inter-individual variability of the weight loss response following surgery in a Mediterranean population. Interestingly, poor weight loss after bariatric surgery could be illustrated by two different patterns: primarily poor weight-loss-response (approximately 5% of the patients) characterized by sustained limited weight loss, and secondarily poor weight-loss-response (approximately 20% of the subjects) characterized by a successful initial weight loss but subsequent weight regain leading to a final EWL <50%.

A substantial amount of research has addressed the association of poor weight loss response after bariatric surgery with a complete set of factors, potentially involved in the variation of postsurgical responses, such as: clinical [6], genetic [7], hormonal [8], and nutritional [9]. However, the role and relative importance of all these factors in the variability of weight loss outcomes after bariatric surgery is not well understood. Current studies suggest that not only “what” we eat, but also “when” we eat may have a significant role in obesity treatment [10–14]. Moreover, recent research links energy metabolism to the circadian clock at different levels: behavioral, physiological and molecular, concluding that the timing of food intake itself have a major role in obesity [10,13]. Our group, in a longitudinal study with an overweight and obese Mediterranean population, recently found that those who ate their main meal later in the day (lunch for this population) lost significantly less weight than those who ate lunch early, although early eaters and late eaters showed similar intake and physical activity, dietary consumption, macronutrient distribution, sleep duration and hormone levels [13]. These results suggest that eating late may weaken the achievement of weight loss therapies [12,13]. Furthermore, we have demonstrated in a randomized, crossover trial that eating late lunch is associated with a decreased of a) resting-energy expenditure, b) fasting carbohydrate oxidation and c) glucose tolerance [12]. Moreover, eating late lunch flattened daily profile in levels of free cortisol and decreased thermal effect of food on wrist temperature [12]. Also, a recent human study has shown that the time of food intake affects both the energy expenditure and the metabolic responses to meals [14]. Nevertheless, there is currently no evidence that food timing can predict weight loss in severe obese patients submitted to bariatric surgery. Therefore, the aim of our observational prospective study (6 years of follow up) was to evaluate if food timing is associated with the weight loss effectiveness following bariatric surgery in a cohort of severe obese.

2. Subjects and methods

2.1. Participants and procedures

Participants in our observational prospective study were selected from the 1135 subjects that underwent bariatric surgery at the Hospital Clinic of Barcelona (Spain) between 2006 and 2011. Inclusion criteria included age ≥18 years, first-time bariatric surgery, and 60 months of available follow up. From those who fulfilled the eligibility criteria, a total of 320 patients agreed to participate. Fifteen % of the initial volunteer subjects dropped out of the trial. Finally, a total of 270 patients (79% women) participated in this study. Patients were considered for bariatric surgery based on the current guidelines, which include to have a body mass index (BMI) ≥40 kg/m^2 or to have a BMI ≥35 kg/m^2 with 2 or more health risk factors, such as high blood pressure or diabetes [15]. Two commonly performed surgery techniques were performed, namely Roux-en-Y gastric bypass (RYGB; n = 203) and sleeve gastrectomy (SG; n = 67). The technical aspects of those surgery techniques and the criteria for selection of RYGB or SG at the Hospital Clinic Barcelona have previously been reported [16]. Data were prospectively collected prior to the surgery and at 12, 18, 24, 36, 48, 60 and 72 months (6 years) in the postsurgical period. All procedures were in accordance with good clinical practice. Patient data were codified to guarantee anonymity.

All subjects attended both group and individual sessions, which included nutritional counseling according to the current guidelines for the bariatric patient prior the surgery [15]. Dietary advice was given to the patients after surgery: at 2 and 6 weeks, and then at 4, 8, and 12 months, emphasizing to sustain a hypocaloric and proteinrich diet, rather than a recommendation of specific timetable. During the first year after the surgery, the patients were advised to eat 5–6 meals per day and after this first year, to eat 3–4 meals per day. No different nutritional education was given according to the type of surgery.

2.2. Ethics

The study followed the ethical guidelines of the Declaration of Helsinki 1961 (revised Edinburgh 2000) and the current legislation concerning clinical research in humans. Ethics Committee of the Hospital Clinic Barcelona approved the protocol and the written informed consent was obtained from all the participants of the study.

2.3. Obesity and biochemical parameters

Participants were weighed wearing light clothes and without shoes to the nearest 0.1 kg (Seca 703 scale, Hamburg, Germany). Height was determined using a fixed wall stadiometer (Seca 217, Hamburg, Germany) to the nearest 0.1 cm. Waist circumference was measured to the nearest 0.5 cm, at the level of the iliac crest, and hip circumference was measured to the nearest 0.1 cm to the maximum extension at the buttocks level. All measurements were made with a standard flexible and inelastic measuring tape. Body mass index (BMI) was calculated as weight (kg) divided by squared height (meters). Postoperative weight loss (WL) was expressed as a percentage excess of weight loss (%EWL) following the formula: EWL = [100 × (weight prior to surgery − weight at the time of evaluation)/(weight prior to surgery − weight corresponding to body mass index (BMI) = 25 kg/m^2)]. Plasma cholesterol, triglycerides, lipoproteins' concentrations were determined by automated chemical analysis at the Hospital Clinic of Barcelona.

2.4. Energy and dietary intake before and after bariatric surgery

The dietary intake was analyzed through 4-days food records (one of which was a non-working day) that were collected at every follow up prior and after surgery. For the purpose of our study we have included: a) prior to surgery (initial values), b) at nadir weight, and c) at the last follow up. Instructions about how to fill the 4-days record were explained by a registered dietitian during the clinical evaluations. Patients were instructed to complete the dietary records the week prior to the nutritional interview. Total energy intake and macronutrient composition were analyzed using the software Dietsource 2.0® (Novartis). During the follow-up period of each subject, patients also registered the time (hour) when each meal began (for example, breakfast, lunch and dinner) with the questionnaire developed by Bertéus-Forslund et al. [17] The cohort was divided in early and late Spanish lunch eaters (before or after 15:00 h) following Garaulet et al. [13].

2.5. Energy expenditure

The total expenditure was calculated by multiplying each individual's basal metabolic rate with the individual physical activity level (PAL). Basal metabolic rate was estimated by the Harris–Benedict equation and physical activity level was self-reported as either “sedentary or light activity” (PAL = 1.53) or “moderate activity” (PAL = 1.76) [18].

2.6. Weight loss classification criteria

The criterion of weight loss response following bariatric surgery proposed by de Hollanda et al. [5], which establishes three different patterns of weight loss, was used to classify the patients. Those three patterns were: 1) Patients with EWL ≥50% at nadir and throughout subsequent follow-up were considered as good weight-loss-responders; 2) Patients with EWL <50% at nadir weight and up to the end of follow up were considered as primarily poor weight-loss-responders; and 3) Patients with EWL ≥50% at nadir weight but EWL <50% at last follow up were considered as secondarily poor-weight-loss responders.

2.7. Morningness/eveningness questionnaire

Subjects completed the 19-item morningness/eveningness questionnaire (MEQ) of Horne and Ostberg [19] at the follow-up period. According to this score, individuals were categorized as neutral (53–64 of score), morning (above 64 of score) or evening types (under 53 of score) [20]. Morningness–eveningness typology is a procedure to characterize individuals depending on individual differences of wake/sleep patterns and the time of the day people report to better performance. Some people are night ‘owls’ and like to stay up late in the night and sleep late in the morning (evening type), whereas others are ‘early birds’ and prefer to go to bed early and arise with the break of dawn (morning types).

2.8. Sleep duration

Habitual sleep duration was evaluate by questionnaire, including the questions ‘During week days: How many hours (and minutes) do you usually sleep?’, and ‘During weekend days: How many hours (and minutes) do you usually sleep?’. A total weekly sleep duration was calculated as ((min weekdays*5) + (min weekend days*2))/7 [21].

2.9. Statistics

All data are expressed as mean ± standard deviation (SD) unless stated otherwise. Differences in the general characteristics of the population, in daily energy and macronutrient intake and in meal times between the subjects grouped by the three different weight loss patterns were analyzed by analysis of variance (ANOVA). Levene's test to assess variance homogeneity and Tukey's post hoc tests were performed. Then, subjects were grouped in early and late eaters for Spanish lunch using the median values of the population as the cutoff point, as previously reported [13]. Chi-square tests were used to test differences in percentages between early or late lunch eaters. Statistical analyses were performed using SPSS 21.0 software (SPSS). A two-tailed p-value of <0.05 was considered statistically significant.

3. Results

In the population studied, 67.8% of participants were considered good weight-loss-responders (presented EWL ≥50% at nadir and last follow up) according to the criteria proposed by de Hollanda et al. [5] On the other hand, 10.8% of subjects were classified as primarily poor weight-loss-responders (showing EWL <50% at nadir) and 21.4% of the participants as secondarily poor weight-loss-responders. The EWL trajectories of our whole cohort according to de Hollanda et al. [5] patterns of weight loss response following bariatric surgery are shown in Fig. 1.

Table 1 includes the initial characteristics of the patients according to the pattern of weight loss response following bariatric surgery. No significant differences were found in obesity-related variables neither in biochemical parameters such as pre-surgical blood lipids values, pre-surgical total energy expenditure, sleep duration and individual chronotype (morning–evening score) as assessed by the morningness–eveningness questionnaire, among the three weight loss groups. Moreover, no significant differences were found for energy intake and the macronutrients distribution at the periods of time studied (Table 2). No significant differences were observed also after adjusting for gender, age and type of surgery ($p > 0.05$).

Our results indicate that weight loss effectiveness was related to the timing of the meals. The percentage of late eaters (after 15:00 h) was significantly higher in the primarily poor weight-loss-responders (~70%) than in both the secondarily poor weight-loss-responders (~42%) and the good weight-loss-responders (~37%) ($p = 0.011$), after adjusting for gender, age and type of surgery (Fig. 2). Consistently, primarily poor weight-loss-responders had lunch later (by approximately 22 min) compared to the other two groups, while no differences were found in the timing of the other two main meals of the day (breakfast and dinner) among the three weight loss groups (Table 3).

4. Discussion

As far as we are aware, this is the first observational prospective study to show a relationship between meal timing and weight loss response in a cohort of severe obese after bariatric surgery. We found that the percentage of late lunch eaters was significantly higher in the primarily poor weight-loss-responders and their lunch was an average of 22 min later than the secondarily poor and the good weight-loss-responders. Interestingly, the difference of the evolution of weight loss among the three groups: good, secondary poor, and primary poor weight-loss-responders was not explained by differences in caloric intake, macronutrient distribution, sleep characteristics, chronotype or estimated energy expenditure during the time period studied.

Previously, our research group proved **that eating late was predictive of decreased weight loss success** in overweight and moderately obese subjects following a dietary weight loss therapy [13]. Also, in an interventional study, we have also shown that delaying the timing of the main meal of the day may create metabolic disturbances such as decreased resting-energy expenditure, decreased glucose tolerance and carbohydrate oxidation, among others [12]. Recently, it has been shown that time-restricted feeding (TRF), with food access limited to daytime 12 h every day and on a high fat diet, prevented body weight gain in *Drosophila*. [22] Authors concluded that the daily rhythm of feeding and fasting *per se* (without any change in caloric intake and activity) could improve sleep, prevent body weight gain, and deceleration of cardiac aging under TRF, benefits that appear to be mediated by the circadian clock [22]. Moreover, Bo et al., in a recent study conducted on healthy subjects, have shown that the time of the food intake

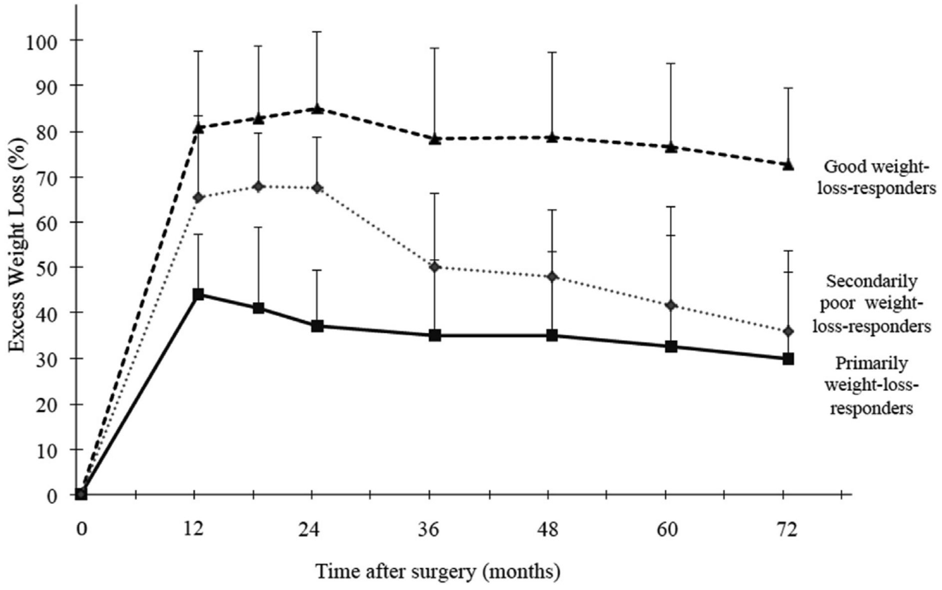

Fig. 1. Excess weight loss over 6 years according to the three different weight loss patterns following bariatric surgery.

Table 1
Characteristics[a] of the population grouped according to the three different weight loss patterns following bariatric surgery.

	Good WL[b] response	Primarily poor WL response	Secondarily poor WL response	p-Value
n	183	29	58	–
Age (y)	50.4 (11.0)	57.3 (8.7)	54.1 (11.5)	0.002
Gender (% female)	81.9	86.2	65.5	0.014
Type of surgery (% GBP[c])	76.9	86.2	63.8	0.045
Initial weight (kg)	123.1 (18.8)	123.3 (37.4)	123.9 (16.5)	0.995
Initial BMI (kg/m^2)	46.3 (5.3)	47.6 (8.9)	46.5 (6.3)	0.549
Initial waist (cm)	130.8 (12.3)	128.6 (17.9)	131.2 (10.8)	0.658
Initial waist hip ratio	0.94 (0.08)	0.89 (0.08)	0.96 (0.12)	0.078
Initial triglycerides (mg/dl^{-1})	138.0 (57.5)	124.8 (54.8)	159.0 (91.8)	0.362
Initial cholesterol (mg/dl^{-1}	200.3 (40.9)	192.3 (32.5)	200.3 (31.4)	0.799
Initial LDL (mg/dl^{-1})	125.8 (30.9)	122.0 (23.1)	124.8 (30.3)	0.942
Initial HDL (mg/dl^{-1})	47.5 (10.1)	46.8 (8.6)	41.5 (8.4)	0.153
Initial total energy expenditure (kcal/day)	2082.9 (268.8)	2053.5 (424.2)	2013.4 (265.5)	0.274
Morning–evening score[d]	56.2 (8.2)	53.6 (10.1)	57.5 (8.4)	0.127
Sleep duration (hrs)	6.9 (1.2)	7.0 (1.5)	7.1 (1.8)	0.737

[a] Data are shown as percentage or mean (SD).
[b] WL: weight loss.
[c] GBP: gastric bypass.
[d] Morningness – eveningness typology: evening types<53, neutral types 53–64, morning types >64.

itself affects both the thermogenic and the metabolic responses to meals [14].

It is important to consider that in our severe obese population, weight loss effectiveness after bariatric surgery was associated with the timing of the main meal (lunch for the Spanish population), with no significant association with breakfast and dinner. Moreover, no significant differences in the percentage of breakfast skipping (~10%) among the three groups were found. Thus, it is hypothesized that this relative important intake of energy (lunch comprises ~40% of daily energy intake in Spanish populations [20]) could be resetting peripheral clocks by itself or indirectly through changes in timing of the other meals [10,13].

In our study, the caloric intake of the severe obese subjects followed was similar to that described in other severe obese populations for both pre- and post-surgery [23,24]. However, interestingly, there were no significant differences in energy intake and macronutrient distribution among the three weight loss groups at any of the points studied (baseline, nadir and last follow up), suggesting that the time "**when**" food is eaten is an influential factor in weight loss effectiveness beyond "**what**" is eaten (in terms of energy intake and macronutrient distribution) in our population. Several previous studies done in mice and rats had similar outcomes concluding that the time of food intake is crucial in weight evolution regardless of energy intake [11].

Furthermore, we investigated different **clinical factors at baseline** that could potentially affect the weight loss response to bariatric surgery such as anthropometric and metabolic parameters. Unexpectedly, obesity degree or metabolic parameters did not predict the weight loss outcome among the different weight loss patterns. Several studies have reported that baseline BMI [25,26] could be considered predictor of success in terms of weight loss after bariatric surgery. A possible explanation for the differences found between the current study and previous ones could be that most of these studies were performed in a short-term follow up while our study presents a **mean of 6 years of follow-up**.

Another factor to considerer for weight loss **is sleep duration** because several studies have associated short sleep duration as an increased risk for obesity and impaired weight loss [27,28].

Table 2
Daily energy and macronutrient intake[a] of the population grouped according to the three different weight loss patterns following bariatric surgery.

	Good WL[b] response	Primarily poor WL response	Secondarily poor WL response	p-Value
n	183	29	58	–
Dietary initial values				
Energy intake (kcal)	2507.9 (1108.6)	2152.1 (746.4)	2448.5 (850.9)	0.332
Protein intake (g)	96.8 (34.2)	96.3 (25.6)	99.4 (30.6)	0.906
Carbohydrate intake (g)	242.3 (105.7)	200.0 (78.5)	230.1 (81.5)	0.183
Fat intake (g)	125.5 (67.9)	106.2 (48.1)	120.9 (54.5)	0.429
Protein intake (%)	16.5 (3.8)	18.8 (4.8)	17.3 (4.8)	0.062
Carbohydrate intake (%)	39.7 (8.8)	36.6 (7.3)	38.9 (10.4)	0.377
Fat intake (%)	43.8 (9.2)	43.6 (9.7)	43.7 (9.8)	0.995
Dietary values at nadir weight[c]				
Energy intake (kcal)	1492.6 (301.5)	1570.8 (361.9)	1593.5 (355.2)	0.282
Protein intake (g)	74.5 (20.4)	79.4 (18.8)	72.7 (22.7)	0.552
Carbohydrate intake (g)	142.3 (46.9)	148.8 (40.1)	154.9 (57.3)	0.464
Fat intake (g)	69.5 (21.5)	73.1 (31.4)	74.7 (22.0)	0.544
Protein intake (%)	21.4 (11.5)	20.4 (4.1)	18.6 (5.1)	0.375
Carbohydrate intake (%)	38.1 (9.2)	38.8 (10.7)	39.8 (9.5)	0.699
Fat intake (%)	41.5 (8.3)	40.7 (10.2)	41.7 (7.4)	0.909
Dietary values at last follow-up[d]				
Energy intake (kcal)	1614.0 (498.1)	1519.6 (330.1)	1616.7 (418.1)	0.708
Protein intake (g)	80.1 (22.9)	68.2 (16.4)	71.6 (21.4)	0.580
Carbohydrate intake (g)	164.0 (62.3)	160.6 (54.7)	169.1 (66.4)	0.888
Fat intake (g)	74.3 (28.9)	67.2 (18.3)	71.4 (25.6)	0.557
Protein intake (%)	18.6 (4.9)	18.4 (4.2)	18.6 (5.3)	0.983
Carbohydrate intake (%)	40.4 (9.7)	41.9 (7.9)	41.8 (11.5)	0.695
Fat intake (%)	41.3 (8.8)	40.1 (8.0)	39.7 (9.5)	0.667

[a] Data are shown as percentage or mean (SD).
[b] WL: weight loss.
[c] Nadir weight was achieved at 18–24 months after surgery.
[d] Last follow-up was at 60 months after surgery.

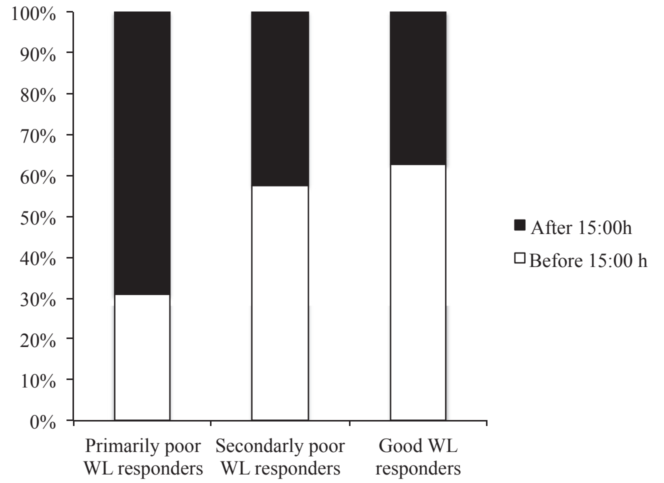

Fig. 2. Percentages of early eaters (before 15:00) and late eaters (after 15:00) in the population grouped according to the three different weight loss patterns following bariatric surgery. WL: weight loss. Differences among percentages were statistically significant (p = 0.011) after adjusting by gender, age and type of surgery.

However, in the current work the self-reported data on sleep duration indicate similar sleep duration (~7 h) among the different weight loss patterns. Our results agree with the data of Garaulet et al. [13], which indicated no overall differences in sleep duration between late and early eaters who showed different patterns of weight loss. Moreover, in a previous study with human subjects Baron et al. found that the caloric consumption in the evening (after 8:00 PM) was associated with a higher BMI independently of sleep timing and duration [29].

Our study supports the efficacy of bariatric surgery on severe obesity treatment since the current population showed a high proportion of good weight loss responders (67.8%). Our results are comparable to other studies carried out in Dutch [30] and in American population [4]. We further provide novel data on the effect of the timing of food intake in bariatric surgery effectiveness. It is worth pointing out the use of the weight loss patterns proposed by de Hollanda et al. [5] since they define two distinct poor weight loss trajectories that can be clinically meaningful. As a result, Hollanda's patterns can help to discriminate among subjects who did not achieve adequate postsurgical weight loss throughout follow up (primarily poor-weight-loss response) from

Table 3
Meal times (hours: minutes)[a] of the population grouped according to the three different weight loss patterns following bariatric surgery.

	Good WL[b] response	Primarily poor WL response	Secondarily poor WL response	p-Value[c]
Breakfast	08:52^{d} (01:16) (n = 165)	08:45^{d} (01:07) (n = 26)	09:01^{d} (01:07) (n = 52)	0.496
Lunch	14:09^{e} (00:46) (n = 183)	14:31^{f} (00:43) (n = 29)	14:10^{e} (00:50) (n = 58)	**0.034**
Dinner	21:22^{g} (00:52) (n = 183)	21:09^{g} (00:49) (n = 29)	21:07^{g} (00:46) (n = 58)	0.090

Bold face representing statistical differences with $P < 0.05$.
Different letters indicate significant differences between groups after post hoc analysis (Tukey's post hoc test).
[a] Data are expressed as mean (SD).
[b] WL: weight loss.
[c] Adjusted by gender, age and type of surgery.

those in whom long-term outcome was determined mainly by a pronounced weight regain (secondarily poor-weight-loss response). In the current work the timing of food intake was particularly useful to discriminate between "good" and "primarily poor-weight-loss responders" but not secondarily poor-weight-loss responders. It has been hypothesized that factors linked with resistance to weight loss would potentially underlie the primarily poor-weight-loss responders. On the contrary, factors facilitating weight regain would largely lie beneath secondarily poor-weight-loss response [5].

Our study explores the important subject of the food timing in weight-loss therapies. An important strength is that includes a long-term data (6 y) with a relatively large sample considering clinical and anthropometrical factors and unique information on meal timing, chronotype and sleep duration. However, our study has several limitations. First, we want to highlight the fact that is an observational prospective study. Therefore, although the association between timing of the main meal and weight loss response to bariatric surgery is an important observation, further research is needed to demonstrate the causality of and potential mechanisms underlying this relationship in bariatric surgery patients. Several potent mechanisms could be implicated in this association such as changes in energy expenditure and metabolic disturbances as have been demonstrated by our group [12,31]. Second, we cannot rule out the possibility that the energy expenditure differed between the three weight loss groups after surgery, even though we found no significant differences in energy expenditure at baseline among the three groups. Moreover, energy expenditure was estimated using Harris and Benedict equation during the pre-operative phase. Therefore, more research is needed to measure the effect of meal timing on energy expenditure through calorimetry, as it has been previously done in normal weight subjects [12]. Furthermore, another limitation is the fact that dietary intake, physical activity and sleep data were self-reported by the patients using validated questionnaires. Self-report data has many important uses but caution must be accepted when interpreting it. Hence further investigation in food timing is needed using a reference method such a biomarkers, accelorometers and sleep polysomnograpy to corroborate the accuracy of the data and avoid bias. Finally, the dietary intake assessment only includes global total energy and macronutrient distribution *per* day but not by each meal. Nonetheless, as mentioned before, lunch is the most important meal of the day in this Spanish population and did not differ in size between early and late eaters in our previous study [13]. In addition, Spanish lunchtime intake could be considered late when compared to other cultures. However, it should bear in mind that lunch is the main meal in Spain; therefore our results open a door to investigate the influence of the time of the main meal on weight evolution in other cultures.

To summarize, we have found for the first time an association between the timing of food intake and weight-loss response after a bariatric surgery treatment. Indeed, weight loss effectiveness was better in early eaters as compared to late eaters. Age, gender and type of surgery were not determining in our results. Moreover, differences in weight loss evolution could not be explained by differences in energy intake, dietary composition and sleep duration. These preliminary results stress the importance of not just what we eat, but also when we eat. Our data furthermore suggest that eating at the right time may be a relevant factor to consider in weight loss therapy even in bariatric surgery.

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

MIP, MG designed the research; TRL, MIP, JV, AdH conducted the research; TRL, MIP, MG analyzed data; TRL, MG, MIP, FAJLS wrote the paper. All authors read and approved the final manuscript.

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Chapter 7

General discussion

The **main objective** of this thesis was to examine the influence of a set of chronobiological related factors, endogenous and exogenous with the initial obesity and weight loss evolution following gastric bypass and sleeve gastrectomy. To achieve this goal, it was necessary to describe the high inter-individual variability of the weight loss response at mid-term after the most commonly performed bariatric surgery techniques: RYGB and SG. After such identification we sought to study the relationship between obesity and weight loss evolution with circadian-related factors such as the individual chronotype and the genetic bases at clock, mainly the *CLOCK 3111T/C* SNP already connected with obesity and weight loss. Finally, we wanted to know if an external synchronizer such as the timing of food intake, previously associated with weight loss evolution in obese subject who follow a dietary treatment, is also a predictor factor for total weight loss in severe obese subjects undergoing bariatric surgery. In this discussion section, we will consider the results of the papers included in the thesis in more general terms, we will also provide an update on current research in this area, and we will present a broader interpretation of the main findings.

1. Identification of two patterns of poor weight loss after Gastric Bypass and Sleeve Gastrectomy.

Bariatric surgery is the most effective treatment for severe obesity resulting in a large and marked weight loss (Courcoulas, AP et al. 2013).However, several studies have shown that weight loss outcomes after bariatric surgery can vary widely among patients and a sizable portion of them present a relative poor weight loss response (Christou, NV et al. 2006;Courcoulas, AP et al. 2013). Therefore, we aimed to describe the presence of different patterns of weight loss up to 4.5 years of follow-up after RYGB and SG **(Chapter 4).** Thus, we have analyzed data from 685 severe obese patients that underwent BS surgery at Hospital Clinic in Barcelona between 2005 and 2009. Three different patterns of weight loss were pre-specified based on the Reinhold criteria modified by Christou et al.(Reinhold (Christou, NV et al. 2006). Patients with EWL >50% at nadir weight and throughout subsequent follow-up were considered as" **good weight loss responders**". Patients with EWL <50% at nadir weight and up to the end of follow-up were considered as "**primarily-poor weight loss responders**" (1-PWL) responders, and "**secondarily poor weight loss responders**" (2-PWL) were those subjects with EWL ≥50% at nadir weight but EWL <50% at last follow-up visit.

From this classification three out of four participants were considered as **good weight loss responders** (~76%). The current results are similar to previous research on the high inter-individual variability in the long-term weight loss response following RYGB and SG. In 2006, Christou *et al*. have found that 34% of their cohort had lost at least 80% of EWL after more than 10 years follow-up after a RYGB (Christou, NV et al. 2006). Also, Valezi et al. have performed prospective study to study weight loss effectiveness after RYGB in an American cohort. Patients' %EWL was of 70% after 5 years, and 67% after 10 years postoperatively (Valezi, AC et al. 2013). Similar data can be found on SG; however long-term data is limited. In a retrospective study with 30 subjects, mean EWL was of 56% after 5 years post-surgery (Alexandrou, A et al. 2015). In addition, in a small cohort of around 60 patients' EWL averaged between 43 and 86% (Diamantis, T et al. 2014). On the other hand, ~5% of our cohort were classified as **primary poor weight loss responders** presenting poor weight loss and no major weight regain resulting in EWL <50% throughout follow-up. Weight loss failure after a bariatric surgery has been previously described with high variability in the reported rates occurring in around 5-35% of cases (Capella, JF, et al, 1996;Christou, NV et al. 2006;Brolin, RE et al. 2007;Cazzo, E et al. 2014). Similar rates to our cohort were found by *Capella*, et al. where 7% of subjects presented less than 50% of excess weight loss less after 5 years of follow-up (Capella, JF, et al, 1996). Moreover, *Christou* et al. have reported that 18% of the population was classified as failure at 5 years of follow-up after a RYGB, and a 35% of the patients were considered as failure at 10 years of follow-up (Christou, NV et al. 2006). Surgical/anatomical factors and hormonal/metabolic factors are associated with primary weight loss failure (Cazzo, E et al. 2014). Interesting, one out five study subjects (~20%) was classified as **secondary poor weight loss responders** according to our pre-specified criteria. Although one of the main purpose of bariatric surgery is to achieve long-term weight loss, it seems that patients my face weight regain during the post-surgery period (McGrice, M et al. 2015). Weight regain, also known as recidivism or secondary weight gain is a medium-late term occurring after the weight nadir (Sjöström, L 2004;Adams, TD et al. 2012;Lauti, M et al. 2016). Although weight regain following bariatric surgery is well recognized is poorly reported on the literature (Karmali, S et al. 2013;Kushner RF, 2015).Similar to our results, in a small cohort with a follow-up of 5 years after SG, weight regain was found in around 20% of the population (Bohdjalian et al. 2010).Recently *Liu* et al. (2015) reported a 29% regain rate after a follow-up of 5 years in a cohort from Hong Kong (Liu et al. 2015). Weight regain following bariatric surgery have been attributed to nutritional noncompliance, hormonal/metabolic imbalance, mental health, physical inactivity and anatomical/surgical factors (Karmali, S et al. 2013;Lauti M, et al. 2016).

Of note, we acknowledged that the % EWL criteria chosen to define the three categories of weight loss response were arbitrary though based on the current literature. Nevertheless, we used this criterion because lack of consensus on how insufficient weight loss following bariatric surgery should be defined. Moreover, poor weight loss trajectories showed a clear tendency: Primary poor-weight loss responders presented by definition lower % EWL at nadir and similar weight regain as compared with good weight loss responders. In contrast, secondarily poor-weight loss responders were characterized by larger weight regain as compared with good and poor-weight loss responders. Thus, poor weight loss after bariatric surgery could be illustrated by two different patterns that could help to discriminate between subjects that do not achieve adequate postsurgical weight loss throughout follow up from those in whom long-term was determined mainly by a pronounced weight regain. Therefore, patients classified as primary poor weight loss responders may be considered as resistant to weight loss whilst secondary poor weight loss responders could be viewed as poor weight loss maintainers. Overall, our data was based on criteria used in the literature in the field (%EWL) and customized to depict weight loss trajectories with clinical significance. Interestingly, an additional report has been published on trajectories of weight loss after bariatric surgery (Courcoulas, AP et al. 2013). Direct comparison is difficult as the criteria used to define weight loss trajectories varied when compared. Nevertheless, we can find a similar proportion of subjects identified with poor weight loss among the different reports.

Finally, we also analyzed pre-surgical predictors of such postsurgical weigh loss outcomes. Previously variables such as higher pre-surgical BMI, pre-surgical T2DM medication, and aged greater than 50 years have been identified as predictors of weight loss response (Still, CD et al. 2014). Our data have shown that a higher pre-surgical BMI, pre-surgical T2DM, and lower number of postsurgical appointments kept have been associated to lesser %EWL after bariatric surgery. Moreover, our results showed that SG was associated with increased odds of 2-PWL response as compared with RYGB. It is important to mention that this effect did not appear until up to 4 years follow up, which highlights the importance of long-term data follow-up when comparing the weight loss response between RYBG and SG.

Our results may provide a framework that may help advance in the identification of factors associated with variable weight loss after bariatric surgery. However the number of factors evaluated in our study was limited. We hypothesized that factors associated with weight loss resistance would be potentially related to primarily poor weight loss responders. Whilst factors related with weight regain would be associated with secondarily poor weight loss responders. We suggest that further research is needed to evaluate the association of resistance to weight loss and weight gain with different factors involved in the high inter-individual variability in the weight loss response to RYGB and SG.

2. Circadian preference, CLOCK 3111 T/C SNP in obesity and weight loss.

In humans, **chronotype** is an attribute that reflects individual circadian phase. Circadian phases or achrophases refer to the timing of the day in which a certain physiological variables, achieve their maximum value (Garaulet, M et al. 2010).Therefore, circadian phases reveal **at what time** of the day the individual´s physical functions, hormone levels, body temperature, cognitive faculties, eating and sleeping patterns **are active** (Levandovski, R. et al 2013).Inter-individual differences in the phases of these main physiological variables reflect different preferences among individuals (Levandovski, R. et al 2013). In this line, some individuals are more morning-type subjects, indicating that the achrophases of some of these physiological variables, such as body temperature or physical activity patterns are in the morning, and other individuals are more evening-type, who are characterized of having the phases of these physiological variables in the evening. Interestingly, chronotype features have a large genetic component (López-Minguez, J et al. 2015).Most of the research has particularly focused on Circadian Locomotor Output Cycles Kaput (*CLOCK)* gene. A component of the circadian system that has been involved in altered metabolism function and may predict the outcome of weight loss based on a low-energy diet (Garaulet, M et al. 2010). Due to the evidence pointing towards an association between chronotype, *CLOCK 3111T/C* SNP, and obesity, we decided to analyze **whether evening chronotype was related with initial obesity and weight loss evolution** in a cohort of severe obese patients. Moreover, we also wanted to examine potential interactions between circadian preferences and *CLOCK 3111T/C* for obesity in this severe obese sample (**chapter 5**). Our current study performed in a cohort of 252 severe obese subjects with a mean of 52 years old, reveals for the first time that the evening chronotype is associated with severe obesity. Thus, individuals with a more evening chronotype showed a higher degree of obesity measured by body weight and BMI. Our results are in agreement with previous studies that showed that eveningness is associated with several health risks, such as diabetes, cardiovascular diseases and obesity (Yu, JH et al.

2015)(Merikanto, I et al. 2013)(Soreca, I et al. 2009). In a previous prospective, randomized, controlled study of obese subjects, evening chronotype was associated with lower HDL-C levels, and a trend towards higher both BMI and neck circumference (Lucanssen EA et al 2013). Moreover, in a study performed in a sample of lean subjects, evening chronotype tended to have a large BMI when compared morning and neutral types (Sato-Mito, N et al, 2011). Also, Arora & Taheri (2015) have found in a large sample of young adolescents an association between evening chronotype and increased BMI (Arora & Taheri, 2015).

Regarding **the effectiveness of weight loss after bariatric surgery and circadian preference**, the results of our study showed that evening subjects displayed a higher weight at nadir and at the last follow up than morning subjects after bariatric surgery. Moreover, late chronotype patients had a lower percentage of excess weight loss than morning chronotype and this was maintained after adjusting by sex, age, and type of surgery. Several studies have previously described an association between weight loss resistance and evening preference. However, to our knowledge this is the first study to unveil an association between chronotype, weight loss and weight loss maintenance after a bariatric surgery in severely obese patients. These results obtained in severe obese subjects are in agreement with previous results obtained in a cohort of 500 obese patients, showing that CLOCK 3111C carriers who show an evening preference are less successful losing weight in response to a dietary treatment based in a Mediterranean diet (Garaulet, M et al 2011). Moreover, in a large cohort of 1465 participants in a weight loss program subjects, C risk carriers of *CLOCK 3111T/C* that also had a late circadian preference were associated with fewer adherence to the weight loss plan, resistance to weight loss and higher levels of plasma ghrelin (Garaulet, M et al 2010). Moreover, Ross et al. have demonstrated that individuals who had reached a successful weight loss and long-term maintenance were more likely to be characterized as "morning type", with longer sleep duration (Ross, KM et al 2015). Interestingly, our current results in severe obese subjects indicate that weight loss progression between morning and evening subject types significantly differed from the 4th year after bariatric surgery until the end towards a higher weight regain among evening types. This result suggests that from the 4th year post-surgery, it appears that behavior-related characteristics of the subjects such as chronotype classification (morning/evening type) become more relevant for the maintenance of the weight loss achieved.

There is evidence that individuals with **evening type** have less healthy lifestyle **compared** with **morning type**, including unfavorable dietary habits (Arora, T et al. 2015). However, in the same line that previous finding (Garaulet, M et al. 2013; **Chapter 6),** no significant differences were found for **total energy intake** and dietary **macronutrients** distribution at the periods of time studied between both chronotypes. Nevertheless **timing of food intake** differed between chronotypes. Evening patients had both lunch (p=0.017) and dinner (p=<0.017) later than morning types. It has been pointed out that "evening type" tends to have an irregular schedule, which might influence food consumption (Wittmann, M et al. 2006). In addition, no differences were found in **sleep duration** between both chronotypes. Our results are consistent with our previous findings where no overall differences in sleep duration were found between late and early eaters which showed no differences in chronotype score (**chapter 6**). In the same way, Garaulet et al. have found no differences in sleep duration between late and early eaters (Garaulet, M et al. 2013). However, we should take in account the fact that evening-type tend to accumulate sleep on weekdays that eventually could recover at the weekend (Roepke, SE et al. 2010). Although we have used validated questionnaires, our data from sleep duration is self-reported. Therefore, further investigation focused in circadian preference and *CLOCK* gene is needed using a reference method for sleep such as sleep polysomnograpy to corroborate the accuracy of the data and avoid bias of the present study.

In humans, *CLOCK 3111T/C* SNP (rs1801260) has been associated with mood disorders, metabolic syndrome, obesity, and diurnal preference. In a Caucasian (Katzenberg, D et al. 1998) and Japanese (Mishima, K et al. 2005) healthy population *CLOCK 3111 C* allele was found a positive association with evening preference. Likewise the *CLOCK 3111 C* allele was related with evening chronotype in a sample of Korean bipolar patients (Lee, KY et al. 2010). In the current study we have detected a significant interaction between *CLOCK 3111T/C SNP* with evening chronotype for obesity parameters. Thus, among carriers of the risk allele C in *CLOCK 3111T/C*, those who were more evening-type showed a higher body weight at baseline than C carriers morning types and T carriers. To our knowledge this is the first study to demonstrate a gen-chronotype (evening-type) interaction for obesity in the total population studied. Previously it has been shown a positive correlation between weight loss resistance, evening chronotype and genetic variants of *CLOCK* gene (Garaulet, M et al. 2010;Garaulet, M et al. 2011)(Garaulet, M et al. 2012; López-Guimerà,G et al 2014). Recently, Garaulet, M et al. (2011) have found that overweight and obese subjects carrying the minor C allele lost significantly less weight than TT subjects. Moreover, in a large cohort was demonstrated that the combination of minor alleles at the *SIRT1* and *CLOCK 3111TC* is associated with evening preferences and that each minor allele exerts an additive effect on weight loss (Garaulet, M et al. 2012). In addition, a study conducted by Bandin et al. found that obese women with C genetic variants in *CLOCK 3111T/C* display a less robust circadian rhythm than TT and a delayed acrophase (Bandín, C et al. 2013). Furthermore, it has been shown that rs1801260 may predict the outcome of weight loss strategies that are based on low-energy diets (Garaulet, M et al. 2010). Those results suggest that the circadian system and specifically this genetic variant may have an impact on weight loss and C carriers of *CLOCK 3111T/C* may be more prone to obesity.

Finally, we also found an association between obesity and sleep duration, but only after we stratified our population according to the median age (52 years old). In the older subjects, C carriers showed higher initial body weight at baseline (124.8±19.3 kg) than TT carries (115.5±15.7 kg) (p=0.003), after adjusting for gender (p=0.010). Moreover, C carriers slept significantly less than T carriers in the older group. Our results share the findings of other authors that demonstrated that several *CLOCK* variants are associated with sleep duration. A previous study has found that after following a weight loss plan differences in weight loss among C and TT carriers were only significant for the older group (Garaulet, M et al. 2011). In the current study, *CLOCK 3111T/C* was also associated with MEQ score for initial weight and sleep duration in the older group. Genotyped based differences in chronotype and sleep patterns have been shown previous in a Mediterranean population. Carriers of minor C allele of *CLOCK 3111T/C SNP* reported shorter daily sleep duration. Also, TT subjects have reported feeling best at morning while C carriers were more represented in the feeling best in the evening (Garaulet, M et al. 2011).

In summary, our results confirm an association between evening preference, *CLOCK 3111T/C* SNP and obesity. The differences in weight between morning and evening subjects could not be explained by differences in energy intake, dietary composition and sleep duration. Our results imply that genetic and circadian assessment could provide tailored weight loss recommendations even in subjects that undergo bariatric surgery. Shall our data be confirmed in future studies, we deem findings from the current study will have potential implications for future research and clinical practice.

3. Not only what we eat but also when we eat? Food timing in obesity and weight loss.

Chronobiology is a relatively new field of research with a large number of studies showing an association between circadian disruption and metabolic disorders (Ribas-Latre, A. 2015). From animal models and human studies, it has been demonstrated that **feeding at the wrong time** can lead to **obesity** or **reduced weight loss**. Due to the growing evidence, we wanted to evaluate the role of food-timing in the evolution of weight loss in a sample of 270 patients that underwent bariatric surgery (RYGB or SG) with a follow up of 6 years (**Chapter 6**). In our prospective observational study we have shown, for the first time, in severe obese subjects an association between the timing of the main meal and weight loss response after a bariatric surgery treatment. We have found that the percentage of late lunch eaters was significantly higher in the primarily poor weight loss responder (~70%) than in both the secondarily poor weight loss responders (~42%) and the good weight loss responders (~37%) (p=0.011), after adjusting for gender, age and type of surgery. Primarily poor weight loss responders had lunch 22 minutes later than secondarily poor and good weight loss responders while no differences were found in the timing of breakfast and dinner among the three groups. Alike to our results in a prospective longitudinal study, Garaulet et al. have previously found, in a Mediterranean cohort of 420 overweight/obese patients, that those who ate their main meal late lost significantly less weight than early eaters (Garaulet, M et al. 2013). Those results suggest that **eating late was predictive of decreased weight loss success**. It is worthy to point out that the study conducted by Garaulet et. al was the first to investigate the relationship between the timing of the main meal and long term effectiveness (20 weeks) of a weight loss dietary treatment in a large cohort. Our study (**Chapter 6**) replicates the results found first by Garaulet et al. (2013) but this time in a sample of severe obese subjects that undergo bariatric surgery. Moreover, insulin sensitivity was lower in late eaters compared with early eaters (Garaulet et al. 2013). Previously, Jakubowicz et al have shown, in a cohort of obese sedentary and non-diabetic population; that a high carbohydrate and protein breakfast may prevent weight regain by reducing diet induced compensatory changes in hunger, cravings and ghrelin suppression. They have concluded that daily changes in food consumption, i.e., the distribution of energy and along the day towards a lower energy intake in the evening as compared to the morning could help to achieve long-term weight loss (Jakubowicz, D et al. 2012). Recently, Bo et al. have demonstrated in a cohort of healthy subjects that the time of food intake *per se* affects both the thermogenic and metabolic response to meal. This study suggests that the time to eat should be taken in to account when planning a healthy diet (Bo, S et al. 2015). Moreover, in a randomized, crossover trial eating late was associated with a decreased of resting energy expenditure, fasting carbohydrate oxidation and glucose tolerance. In addition, eating late lunch blunted daily profile in free cortisol concentrations and decreased thermal effect of food on wrist temperature (Bandin, C et al. 2014).

Body weight can change when **energy intake** is not equal to energy expenditure over a given period of time (Hill, JO et al. 2012). However, in our study the **caloric intake** and **macronutrient intake** among the three different patterns-groups of weight loss showed no significant differences at any of the points studied. These results suggest that the time when food is eaten is an influential factor in weight loss effectiveness beyond what is eaten in our cohort. Similarly, Garaulet et al. found no differences in caloric intake and macronutrient distribution in a Mediterranean population where eating late weakened the achievement of weight loss therapy (Garaulet, M et al. 2013). Moreover, it has been shown that mice fed at day gain more weight compared with mice fed at night with no statistical significant differences between caloric intake and locomotor activity between the light and dark-fed conditions (Arble, DM et al. 2009). Furthermore, also in mice fed with a restricted high fat diet (HFD) during light time for 4 hours resulted in a lower body weight compared to mice fed a low fat diet (LFD) ad lib, although they consumed the same amount of calories (Sherman, H et al. 2012). Taken together, this evidence suggests that the **time of food intake is crucial in weight loss evolution regardless of energy intake.**

Regarding to the total **energy expenditure** at baseline (pre-surgical), no significant differences were found among the different weight loss trajectories. Similar results were found in a cohort of overweight/obese patients undergoing a 20 weeks weight loss diet based were early eaters lost more weight than late eaters (Garaulet, M. et al. 2013). We should bear in mind that both studies are observational and have used the Harris-Benedict equation to estimate energy expenditure, which could be seen as a limitation. This equation is not very accurate to assess energy expenditure (especially during weight loss) (Garaulet, M. et al. 2013). Furthermore, although we did not find differences in either energy intake or expenditure among the different weight loss -trajectory groups, we cannot rule out the possibility that the groups had differences in resting energy expenditure not predicted by their weight, height, gender and age. Therefore we suggest more research to measure the effect of meal timing on energy expenditure through calorimetry. In this sense, recently Bandin et al. had addressed specifically those aspects in an interventional study of 32 women, which aimed to investigate the effects of changes in lunch timing on metabolic measurements, such as energy expenditure and glucose tolerance. Resting and postprandial energy expenditure was measured by indirect calorimetry in two meal-timing conditions, late eating (LE) (13:00 h) and early eating (EE) (16:30h), with identical meals in a crossover design. Their results showed that a delay in lunch timing by three and half hours resulted in decreased resting energy expenditure prior to the mail. Nevertheless, no significant differences were found in postprandial energy expenditure(Bandín, C et al.2015) . Indeed further research is required to measure the effect of early and late eating on energy expenditure.

Another factor linked with chronobiology that has been related with obesity is **sleep duration**. As previous research has pointed out, short sleep duration is an increased risk for obesity impaired weight loss (Beccuti, G. et al. 2011;Nedeltcheva, Av. et al. 2014). In the present study, our classified population into the different weight loss patterns showed similar sleep duration (around 7 hours). Similar to our data, in an overweight and obese Mediterranean population, no overall differences was found in sleep duration between late and early eaters who showed different patterns of weight loss after following a weight loss program (Garaulet, M. et al 2013).Moreover, Baron et al. have conducted a study to evaluate the role of sleep timing in dietary patterns and BMI in 52 subjects divided into normal and late sleepers. They found that the caloric consumption after 8:00 pm was associated with higher BMI independently of sleep timing and duration (Baron, KG et al. 2011).

Although our study mainly focuses on the weight loss outcome after a bariatric surgery, we should take in to account that the health benefits from the bariatric surgery extend beyond weight loss (Sjöström, L et al. 2013;Courculas, AP et al. 2013). Concerning to the weight loss evolution of our population after a bariatric surgery, we found that 67.8% of our studied population was good weight loss responders. On the other hand, 10.8% of subjects were classified as primary poor weight loss responder and 21.4% of the participants as secondarily poor weight loss responders. These numbers are comparable to other studies carried out in a Mediterranean, Dutch and, American population (Mathus-Vliegen, EMH et al. 2006; De Hollanda, A et al. 2014;Morris, CJ et al. 2015).

We have decided to assess the weight loss evolution after a bariatric surgery of our population using the weight loss patterns after a bariatric surgery proposed in 2014 by De

Hollanda et al. We have considered using this classification for the following reasons: 1) Our studied populations presents similarities (Mediterranean population) 2) Their follow up was up 4 years with a considerable number of subjects (658 patients) 3) This new classification can be clinically meaningful since it help to discriminate among subjects who did not achieve adequate post-surgical weight loss through follow up (primary poor weight loss response) from those in whom long-term outcome was determined by a marked weight regain (secondarily poor weight loss response). It is important to note that because of the design of our study, we couldn't address directly the mechanisms linking meal timing, weight loss and/or obesity. However, it has been proposed that unusual feeding time may produce chronodisruption. Moreover, hormones, genetic background and chronotype may be involved therefore more research is needed.

To summarize, this is the first observational prospective study to show a relationship between meal timing and weight loss response in a cohort of severe obese patients that underwent a bariatric surgery (RYGB or SG). Weight loss effectiveness was better in early eaters as compared with late eaters. Age, gender, and type of surgery were not determining in our results. Interestingly, the difference in weight loss trajectories could not be explained by differences in energy intake, dietary composition and sleep duration. Our results stress the importance of not only what you eat but also when you eat even in bariatric surgery. Therefore eating at the right time may be a relevant factor to consider in weight loss therapies.

Chapter 8

Conclusions

1. We developed a new classification for weight loss response patterns at mid-term following bariatric surgery (RYGB and SG) in a severely obese population, which discriminate, for the first time, between subjects who present weight loss resistance (**primary poor weight loss responders**) and subjects who present large weight regain after a sustained weight loss (**secondary poor weight loss responders**).

2. **Primary poor weight loss responders** represented approximately 5% of subjects and showed an excess weight loss lower than 50% while **secondary poor weight loss responders** represented around 20% of subjects. Those individuals were characterized by a large weight regain presenting an EWL higher than 50% at nadir weight but lower than 50% at last follow up visit. Poor weight loss should not be interpreted as an overall poor outcome of bariatric surgery.

3. We confirmed for the first time that the individual chronotype is associated with a higher degree of obesity (body weight and body mass index at baseline) in a cohort of severely obese subjects. More importantly, weight-loss progression after bariatric surgery differed between morning and evening chronotypes from the fourth year toward a higher weight regain among evening types.

4. We demonstrated for the first time that the individual chronotype interacts with *CLOCK 3111T/C* for severe obesity, which suggests that to be an evening-type person and also to be a carrier the risk allele C at *CLOCK* 3111T/C increases the risk of obesity. In addition, *CLOCK 3111T/C* was significantly associated with obesity and sleep duration in older subjects.

5. We confirmed that weight loss effectiveness after bariatric surgery is related to the timing of the main meal. We found that the percentage of late lunch eaters was significantly higher in the primarily poor weight-loss-responders and their lunch later than the secondarily poor and the good weight-loss-responders. Interestingly, the difference of the evolution of weight loss among the three groups: good, secondary poor, and primary poor weight-loss-responders was not explained by differences in caloric intake, macronutrient distribution, sleep characteristics, chronotype or estimated energy expenditure during the time period studied.

6. Our studies support that the circadian preferences and genetic variants of *CLOCK* may have a potential role in severe obesity and weight loss therapies. Moreover, the timing of food intake also is important for weight regulation and eating at the right time, and may be a relevant factor to considerer in weight loss therapies including bariatric surgery.

Chapter 9

Summary

Firstly, we tried to describe the presence of different patterns of weight loss up to 4.5 years of follow-up after RYGB **(Chapter 4).** Therefore we examined in a retrospective analysis data from severe obese patients that underwent BS surgery at Hospital Clinic in Barcelona between 2005 and 2009. We suggest that the high inter-individual variability of the weight loss response to RYGB and SG at mid-term following could be depicted by three distinct patterns of weight loss : 1) good weight loss response pattern characterized by EWL >50% both at maximum weight loss and last follow-up visit. While poor weight loss can be illustrated by two different patterns: 1) primary poor weight loss characterized by poor weight loss response throughout follow up and 2) secondarily poor weight loss pattern characterized by significant weight loss but subsequent weight regain leading to a final EWL <50% encountered in approximately 20% of the subjects. Then, we aimed to determine if more chronobiological related factors were involved in the poor weight loss response after a bariatric surgery. We have examined whether morning-evening chronotype is related with obesity and weight loss evolution in the studied population. In addition, we wanted to examine potential interactions between chronotype and *CLOCK 3111T/C* for obesity in this population (**Chapter 5**). We show that evening chronotype is associated with initial obesity and to weight loss evolution in severe obese patients after BS. In addition, we found that carriers of the risk allele C in *CLOCK 3111T/C* with more evening type showed a higher body weight compared with more morning type subjects. Moreover, *CLOCK 3111T/C* was associated with obesity and sleep duration in older subjects. Since our previous data have shown two poor weight loss trajectories we subsequently wanted to evaluate the association of different factors that could potentially being involved in the variable postsurgical response. Because eating late can be considered as a disruptor of the circadian organization that could lead to chronodisruption, we next evaluate the role of food-timing in the evolution of weight loss in a sample of 270 patients that underwent BS with divided by the three different weight loss patterns above mentioned. We have found that the percentage of late lunch eaters was significantly higher in the primarily poor weight loss responders. Interestingly these differences in weight loss evolution could not being explained in differences in energy intake, dietary composition, circadian preference and sleep duration. Because of these findings, we suggest that eating at the right time may be a relevant factor to considerer in weight loss therapy even in patients that underwent bariatric surgery **(Chapter 6)**

Chapter 7 provides an overall discussion regarding to this PhD-thesis.

Chapter 10
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